Risk factors and transmission of healthcare-related pathogens

Anne F. Voor in 't holt

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Risk Factors and Transmission of Healthcare-Related Pathogens

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Chapter 1

General introduction

HEALTHCARE-ASSOCIATED INFECTIONS

Healthcare-associated infections (HAIs) are infections patients get in healthcare facilities while being treated for another disease (1). These infections appear during admission or after discharge and are therefore mostly referred to as nosocomial infections or hospital infections. Most commonly, they are defined as occurring between 48 hours after admission and 30 days after discharge (2, 3). However, there are many other definitions, one more applicable than the other. It was estimated that in the United States (U.S.) about 721,800 patients developed HAIs in 2011, and about 75,000 patients (10.4%) with a HAI died (4). Additionally, a U.S. survey showed that the percentage of HAIs in 2011 was 4.0% (95% confidence interval [CI] =3.7% to 4.4%) (4). Pneumonia (21.8%) and surgical site infections (SSI, 21.8%) were the leading HAIs in the U.S., followed by gastrointestinal tract infections (17.1%), urinary tract infections (12.9%) and primary bloodstream infections (9.9%) (4). More than half of these HAIs occurred outside the intensive care unit (ICU). The Centers for Disease Control and Prevention (CDC) reports that efforts to prevent HAIs are successful, as for example central line-associated bloodstream infections (CLABSIs) were shown to be reduced in the U.S. by 50% between 2008 and 2014 (5). Annual financial losses by HAIs have been estimated at $6.5 billion in the U.S., and €7 billion in Europe (2).

In the Netherlands, since 2007, the Dutch National Nosocomial Surveillance Network (PREZIES) has monitored HAIs by prevalence surveys based on voluntary participation of hospitals. In 2017, 66 out of 78 Dutch hospitals participated (6). The prevalence of HAIs in Dutch hospitals in 2017 was 5.0% (95% CI= 4.6% to 5.3%) - 624 HAIs in absolute numbers (6, 7). Similar to the U.S. surveys, the most prevalent HAIs in the Netherlands were pneumonia and SSI (7).

HAIs may be caused by a variety of microorganisms, including bacteria, viruses, fungi and parasites. In this thesis we will focus on bacteria. Globally, the most common microorganisms causing HAIs are bacteria - with as most frequently isolated *Staphylococcus aureus* and *Escherichia coli* (2). However, geographical differences do occur as for example in Italy *Klebsiella* species were most frequently isolated in HAIs, followed by *E. coli* and *Pseudomonas aeruginosa* (8). In the Netherlands, the picture mimics the global situation with *E. coli* and *S. aureus* as most common bacteria in HAIs (8).

The microorganisms that cause HAIs may come from endogenous or exogenous sources. Endogenous sources are sites in or on the human body that are normally inhabited by microorganisms, such as the skin and the gastrointestinal tract (1). Preventive measures that can be installed to prevent HAIs from an endogenous source include for example *S. aureus* decolonization of nasal and extranasal body sites to prevent SSI, or selective digestive tract decontamination (SDD) for patients admitted to the ICU to prevent pneumonia (9, 10). Additionally, skin antiseptics before surgery and attention to personal hygiene of patients are also to prevent endogenous infections (11, 12).

Exogenous sources refer to all sources outside the patients' body, such as the hospital environment, healthcare workers and other patients (1). In the innate environment of hospitals, including patients' rooms, microorganisms can survive from a few days up to months, depending on the microorganisms involved (13). Therefore, washbasins, tables, door handles, etc. can act as a continuous source for transmission of microorganisms, which after successful transmission leads to colonization, and can then subsequently cause HAIs in patients (14-16). Also, HAIs can be associated with devices used for medical procedures, such as catheters, ventilators or endoscopes (14-16). Especially when devices are used into sterile or organic spaces in the body, exogenous infections can be detected. Prevention of HAIs from exogenous sources includes for example thoughtful use of medical devices and thorough cleaning and disinfection, the latter if appropriate, of the hospital environment (14, 16). A measure that prevents infections from endogenous sources as well as from exogenous sources is hand hygiene.

Questions to be addressed in this thesis; chapter 2: Which infection prevention measures can be installed and are proven to be effective to prevent HAIs in patients?

HIGHLY-RESISTANT MICROORGANISMS

In recent years there has been a worldwide increase of HAIs caused by highly-resistant microorganisms (HRMO) and of patients colonized with HRMO. These HRMO are of great concern since there is no parallel progression in the development of novel antibiotics. Examples are extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae (*i.e.* Gram-negative bacteria resistant to third-generation cephalosporin antibiotics), carbapenemase-producing bacteria such as oxacillinase (OXA)-48 *K. pneumoniae*, or Verona Integron-encoded Metallo-β-lactamase (VIM)-positive *P. aeruginosa* (*i.e.* Gram-negative bacteria resistant to carbapenem antibiotics), and vancomycin-resistant enterococci (VRE – Gram-positive bacteria resistant to the antibiotic vancomycin). In February 2017, the World Health Organization (WHO) classified carbapenem-resistant Enterobacteriaceae, ESBL-producing Enterobacteriaceae, and carbapenem-resistant *P. aeruginosa* and *Acinetobacter baumannii* as priority 1; critical (17). Enterobacteriaceae include *K. pneumoniae*, *E. coli*, *Enterobacter* spp., *Serratia* spp., *Proteus* spp., *Providencia* spp., and *Morganella* spp. (17). Global health experts agreed that these bacteria pose the greatest threat to human health and new antibiotics are urgently needed. The burden of disease caused by HRMO is high in terms of morbidity and mortality in affected patients, and extra costs for healthcare (18). Worldwide, the prevalence of HRMO varies from less than one percent to above 50 percent and differs between countries and per HRMO. In 2011 and 2012, the European Centre for Disease Control and Prevention (ECDC) conducted an EU-wide point prevalence survey to determine antimicrobial resistance

of microorganisms reported in HAIs (19). The results showed alarming rates of third-generation cephalosporin resistance in Enterobacteriaceae and carbapenem resistance in *A. baumannii* and *P. aeruginosa* (19).

Two large Dutch studies showed that at admission in a hospital, 6.4% to 7.4% of patients carried an ESBL-producing Enterobacteriaceae, and at discharge 8.7% to 10.1%, respectively (20). This means that 2.3% to 2.7% is possibly hospital acquired (20). Possibly, because bacteria which were undetected at admission can proliferate and become predominant due to antibiotic selection pressure (21).

If patients are identified as being either colonized or infected with HRMO in the hospital, measures to prevent transmission of these HRMO to other patients should be installed. Colonized means presence of a HRMO on a body surface (*e.g.* skin, mouth, intestines or airway) without causing disease. Infection means multiplication of bacteria in the human body, causing disease (22). Regarding HRMO, it is not only important to prevent infections, but also colonizations and its spread; because colonization can lead to an invasive infection. Specific measures can differ per HRMO involved, but most often it involves a single-occupancy room and wearing gloves and gowns when entering the room. In a large Dutch study, the rate of transmission of ESBL-producing Enterobacteriaceae to other patients despite the use of contact-isolation measures was 5.4%, of which 61% was attributable to ESBL-producing *E. coli* (23). In this multicenter cluster-randomized study, acquisition to roommates and/or to patients admitted to the same department was assessed by taking perianal swabs at admission and at discharge from all patients hospitalized for more than 2 days (20). Given the above facts, there is an ongoing discussion about the indications for isolation and in case of isolation, to what extent preventive measures are absolutely necessary (24). Of course, not only costs, but also the setting (*e.g.* case mix), patient outcome and difficulty of treatment needs to be taken into consideration.

Questions to be addressed in this thesis; chapter 3: What are the risk factors for acquisition of HRMO? How are HRMO transmitted?

OUTBREAKS

The CDC definition of an outbreak is: "the occurrence of more cases of disease than expected in a given area or among a specific group of people over a particular period of time" (www.cdc.gov). Outbreaks can be caused by all microorganisms possible, but outbreaks by HRMO are especially of great concern, since they pose the greatest threat to human health (18, 25). Outbreaks can be small and contained quickly, however, transmission can also be ongoing with ultimately involvement of hundreds of patients (26, 27). A hospital outbreak is most often uncovered by (i) analyzing surveillance data by the

infection control department, or (ii) by an alert by a concerned clinician to the infection control department. Often, after the alert an epidemiological timeline will be created to visualize patient movements throughout the hospital, and to unravel epidemiological relationships between patients identified with the same microorganism (Figure 1).

When an outbreak is detected, exposed patients need to be screened and the source needs to be eliminated in order to halt the outbreak. Also, for a full understanding and investigation of the outbreak, data needs to be collected about all patients involved. Patient information that needs to be collected includes: (i) full admission history (including departments and room numbers) of colonized and infected patients, (ii) information about contact-isolation measures installed and at which date(s), (iii) dates of all infection prevention measures installed at the department(s) of interest, (iv) all laboratory results of the microorganism(s) of interest, including susceptibility pattern, minimal inhibitory concentrations (MICs) of the antibiotics tested, resistance genes, and phenotypic and/ or genotypic typing results, and (v) all patient information about known risk factors. Known risk factors are for example antibiotic use, ICU admission, mechanical ventilation and length of hospital stay.

Questions to be addressed in this thesis, chapter 2 and 3: Which risk factors, environmental sources and effective infection prevention strategies have been identified in other outbreaks? What is the best way to describe and study outbreaks?

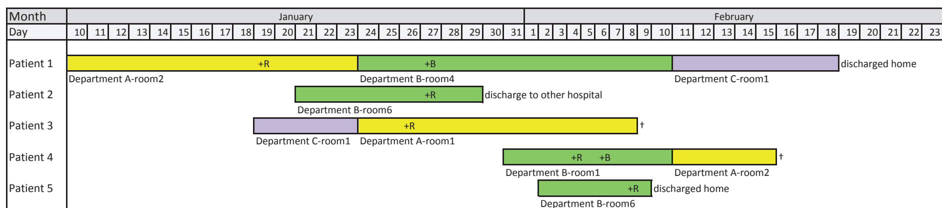

Figure 1. Epidemiological timeline of 5 individual patients. The different colors are different departments. +R; rectal swab positive for the specific microorganism, +B, blood culture positive for the specific microorganism.

MOLECULAR TYPING

Because of the increase in HRMO and the subsequent hospital outbreaks sophisticated laboratory typing techniques are needed (28). Molecular typing techniques help to identify different bacterial strains and clones and are therefore important in infection prevention and control. Currently, a wide range of genotypic and phenotypic typing techniques are available, each with advantages and disadvantages (29-31). Important aspects of typing techniques to consider are: (i) stability, (ii) typeability, (iii) discriminatory power, (iv) epidemiological concordance, (v) reproducibility, (vi) appropriate and

well-defined test population, (vii) flexibility, (viii) rapidity, (ix) accessibility, (x) ease of use, (xi) costs, (xii) amenability to computerized analysis, and (xiii) incorporation of typing results in an electronic databases (32). An overview of the most commonly used genotypic typing techniques in hospital settings is presented in Table 1.

The choice of genotyping method depends on the microorganism involved, the availability of the method, and knowledge and local or national expertise about the method. It is also important to consider whether you want to compare isolates only within your hospital setting, or also between hospitals and even between different countries, and if you want to compare strains identified over a short or long period of time (Table 1). Pulsed-field gel electrophoresis (PFGE) is still considered as the golden standard for many important healthcare-related pathogens (29, 33). However, PFGE is technically demanding, time consuming and labor intensive (29). It is difficult to apply this technique in routine diagnostics as a tool for detection of an outbreak and is therefore not widely used. Whole-genome sequencing (WGS) is a technology providing full genetic information on the entire bacterial genome (34). However, this technique is still costly and time consuming. As alternative, conventional Multilocus sequence typing (7 or 8 genes), is extended to whole genome MLST (wgMLST) (35). In this way, 1500-4000 genes can be considered. In some microbiological laboratories in the world, including the Netherlands, wgMLST is already implemented as a routine technique to monitor HRMO and to detect outbreaks in an early phase (36).

Questions to be addressed in this thesis; chapter 4: Is routine, rapid typing needed in a non-outbreak situation? Can recent transmission events be detected by a combination of phenotypic and genotypic typing techniques?

Table 1. An overview of most commonly used genotypic typing techniques in hospital laboratories and its application in local outbreak investigations and surveillance.

Technique	Abbreviation	Costs per isolate[1]	Local outbreak investigation[2]	Surveillance[2]
Amplified Fragment Length Polymorphism	AFLP	+	++	-
Multilocus Sequence Typing	MLST	+	+	++
Multilocus Variable-Number Tandem Repeat Analysis	MLVA	+	+	++
Polymerase Chain Reaction – Ribotyping	PCR Ribotyping	+	+	+
Single Locus Sequence Typing	SLST	-	+	+
Whole Genome Sequencing	WGS	++	++	++

[1] -,low; +, medium; ++, high.
[2] -, not applicable; +, applicable; ++, highly applicable.

OUTLINE OF THIS THESIS

The literature reviews and observational studies in this thesis are about the role of epidemiology in describing, identifying and controlling transmission of healthcare-related pathogens. The ultimate goal of conducting these studies is to optimize care and to provide safer care for patients admitted to the Erasmus MC. In chapter 2 three literature reviews are described about (i) ESBL-producing *Klebsiella* species, (ii) carbapenem-producing Enterobacteriaceae and (iii) VIM-positive *P. aeruginosa*. The most important risk factors, effective infection prevention strategies and sources have been identified. In chapter 3 the theoretical knowledge from the systematic reviews has been applied in different outbreak scenarios in the Erasmus MC. (i) A case-control study on a long-lasting outbreak of VIM-positive *P. aeruginosa*. (ii) A nationwide study about contamination of duodenoscopes; following an outbreak report on a duodenoscope as source of VIM-positive *P. aeruginosa* published by Verfaillie *et al.* (37). (iii) An outbreak investigation of a *Clostridium difficile* outbreak at a gastro-intestinal surgical ward. Finally, in chapter 4 the role of epidemiology when using genotypic and phenotypic typing techniques is described, (i) for ESBL-producing *Klebsiella* species, and (ii) for ESBL-producing *E. coli*.

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Chapter 2

Healthcare-related pathogens: risk factors

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Chapter 2.1

A systematic review and meta-analyses of the clinical epidemiology of carbapenem-resistant Enterobacteriaceae

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Anne F. Voor in 't holt
Margreet C. Vos

Antimicrobial Agents and Chemotherapy, 2018; 62(1)

ABSTRACT

Carbapenem-resistant Enterobacteriaceae (CRE) are major healthcare-associated pathogens and responsible for hospital outbreaks worldwide. To prevent a further increase in CRE infections and to improve infection prevention strategies, it is important to summarize the current knowledge about CRE infection prevention in hospital settings. This systematic review aimed to identify risk factors for CRE acquisition among hospitalized patients. In addition, we summarized the environmental sources/reservoirs and the most successful infection prevention strategies related to CRE. A total of 3,983 potentially relevant articles were identified and screened. Finally, we included 162 studies in the systematic review, of which 69 studies regarding risk factors for CRE acquisition were included in the random-effects meta-analysis studies. The meta-analyses regarding risk factors for CRE acquisition showed that the use of medical devices generated the highest pooled estimate (odds ratio [OR] = 5.09; 95% confidence interval [CI] = 3.38 to 7.67), followed by carbapenem use (OR = 4.71; 95% CI = 3.54 to 6.26). To control hospital outbreaks, bundled interventions, including the use of barrier/contact precautions for patients colonized or infected with CRE, are needed. In addition, it is necessary to optimize the therapeutic approach, which is an important message to infectious disease specialists, who need to be actively involved in a timely manner in the treatment of patients with known CRE infections or suspected carriers of CRE.

INTRODUCTION

Over the last two decades, a global dissemination of carbapenem-resistant Enterobacteriaceae (CRE) has been observed (1, 2). Currently, CRE are responsible for hospital outbreaks worldwide. Infections with these resistant bacteria are associated with high rates of morbidity and mortality, especially in patients with serious underlying disorders or patients admitted to the intensive care unit (ICU) (3).

Carbapenem resistance in Enterobacteriaceae is mainly mediated by the horizontal transfer of genes encoding carbapenem-hydrolyzing carbapenemase enzymes, although porin mutations or the overexpression of efflux pumps can also lead to carbapenem resistance, especially in combination with the hyperproduction of β-lactamase enzymes (4, 5). The production of carbapenemase enzymes is plasmid mediated and can be found in multiple different species of Enterobacteriaceae, such as *Klebsiella pneumoniae* and *Escherichia coli* (1, 5-7). These conjugative plasmids often carry additional genes conferring resistance to other antibiotics, such as fluoroquinolones and aminoglycosides, limiting the treatment options even more (3, 8).

To prevent a further increase in CRE infections in patients by improving infection prevention strategies, it is important to summarize the current knowledge about CRE in hospital settings. This systematic review and meta-analyses aimed to evaluate the clinical epidemiology of CRE by answering the following questions. First, what are risk factors associated with CRE acquisition among hospitalized patients? Second, which environmental sources/reservoirs were identified in CRE outbreaks? Third, what were the essential components of effective infection control in preventing or ending hospital outbreaks?

METHODS

This systematic review and meta-analyses followed the guidelines presented in the PRISMA statement (supplement 1) (9). Protocol details were submitted to the PROSPERO International Prospective Register of Systematic Reviews (registration number: CRD42017055455).

Study selection

Articles related to our research questions were identified through a search of the literature in multiple databases (until 11 January 2017): Embase, Medline Ovid, Cochrane, Web of Science, and Google Scholar (supplement 2). The search was not limited by language, date of publication, country of publication, carbapenem resistance mechanism, study design, or patient characteristics.

We used the following inclusion criteria during the study selection: (i) studies reporting risk factors for the acquisition of CRE, (ii) studies mentioning environmental sources/reservoirs for CRE, and (iii) studies describing effective infection prevention strategies to halt nosocomial outbreaks. Risk factors for acquisition could include risk factors for infection as well as risk factors for colonization with CRE. Enterobacteriaceae were considered resistant to carbapenem antibiotics when this was shown using phenotypic tests and/or when carbapenemase genes could be identified.

We excluded studies related to nonhuman infections, nonhospital studies, conference abstracts, letters to the editor, commentaries, weekly reports, and editorials. Studies were also excluded if patients with CRE infections were compared to patients who were colonized with CRE. First, the titles and abstracts of all retrieved citations were screened independently by K.V.L. and A.F.V. After this screening, K.V.L. and A.F.V. performed a second screening based on the full text.

Data extraction

We designed a data abstraction form, pilot-tested it on three randomly selected articles, and redefined it according to the outcomes. The following data were extracted: first author, journal, year published, country, study design, study setting, patient characteristics, the carbapenem-resistant microorganism(s) studied, risk factors for acquisition/mortality, site of colonization/infection, protective factors for acquisition/mortality, potential reservoirs for CRE, and effective infection prevention strategies for CRE. The extracted data were sent to the corresponding author of the original article to verify the extracted data and to gain additional information if relevant. When we did not receive any response after the given deadline (*i.e.* 2 weeks), a reminder was sent. If no response was received and crucial information was missing, the study was excluded.

Data analysis

Risk factors for CRE acquisition

All risk factors associated with the acquisition of CRE for which an odds ratio (OR) with 95% confidence interval (95% CI) was reported were divided into two groups: those related to antibiotic exposure and other. Risk factors that were reported as a hazard ratio or relative risk were not included in a random-effects meta-analysis and were therefore only summarized.

The first category, related to antibiotic exposure, was further divided into the following nine categories: (i) carbapenem use, (ii) cephalosporin use, (iii) quinolone use, (iv) use of other β-lactam antibiotics or β -lactam use in general, (v) glycopeptide use, (vi) antibiotic exposure (in general), (vii) number of antibiotics administered, (viii) duration of exposure, and (ix) other. The second category, other, was also divided into nine categories, as follows: (i) underlying disease or condition, (ii) invasive procedures, (iii) medi-

cal devices, (iv) ICU admission, (v) exposure to hospital care, (vi) demographic patient characteristics, (vii) mechanical ventilation, (viii) CRE exposure, and (ix) other.

Studies reporting protective factors for the acquisition of CRE were summarized and included in a meta-analysis if they could be categorized into one of the previously described categories.

Meta-analysis

The meta-analyses were performed using StatsDirect statistical software (Altrincham, United Kingdom) including the random-effects model of DerSimonian and Laird (10). A P value of <0.05 was considered statistically significant. A meta-analysis was performed only if ≥3 studies reported the same risk factor and if the risk factors within the category were not too diverse. Publication bias was examined visually with the use of funnel plots and assessed with the indicators of Egger *et al.* and Begg-Mazumdar (11, 12). When both indications showed a significant result, it was assumed that publication bias was present.

Eight additional meta-analyses were performed for each risk factor category: 1a, studies including only *K. pneumoniae* isolates; 1b, other studies; 2a, studies with an ICU setting; 2b, studies with a different study setting; 3a, studies describing only carbapenemase production as the carbapenem resistance mechanism; 3b, studies describing another resistance mechanism or did not investigate the resistance mechanism involved; 4a, studies with a moderate/high study quality; 4b, studies with a low study quality.

Infection prevention strategies and environmental sources/reservoirs

All effective infection prevention strategies mentioned in the included articles were categorized, and a top 10 was created on the basis of the number of studies that reported these infection prevention strategies. In addition, studies describing sources and/or reservoirs for CRE in a hospital setting were reviewed and summarized.

Study quality

A quality assessment was performed for all studies included in a meta-analysis using the strengthening the reporting of observational studies in epidemiology (STROBE) guideline (supplement 3) (13). Studies with a score of ≤15 points were considered to be of relatively low methodological quality, studies receiving a quality score of 16, 17, or 18 points were rated to be of moderate quality, and studies with a score of ≥19 points were considered to have a relatively high study quality. Study quality was not considered an exclusion criterion.

RESULTS

During our literature search we identified 3,983 potentially relevant articles (Figure 1). All titles and abstracts of the retrieved articles were screened against our inclusion and exclusion criteria, resulting in the exclusion of 3,720 publications. The remaining 263 articles underwent a second screening based on the full text. Seven full-text articles were received by e-mail after we contacted the corresponding authors. Finally, 162 articles were included in the systematic review (Figure 1). For these studies, the data were extracted and the corresponding author was contacted with a request to check our completed data extraction form. Finally, the corresponding authors of 100 out of 162 articles (61.7%) responded to our request and provided feedback and additional information if necessary.

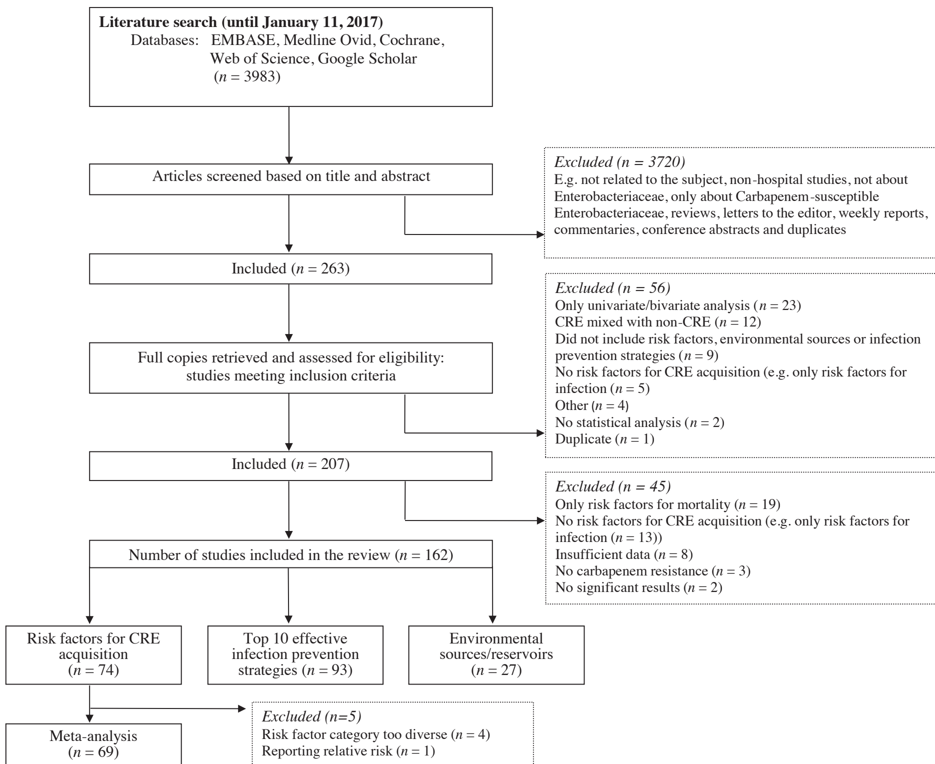


Figure 1. Flow diagram of study selection for the systematic review of studies on carbapenem-resistant Enterobacteriaceae.

Table 1. Summary of studies reporting protective factors for acquisition of CRE, based on multivariable analysis[a]

			Risk estimate			
Authors, yr (reference)	**Country**	**Risk factor**	**OR**	**95% CI**	***P* value**	**Quality[b]**
Akgul et al., 2016 (19)	Turkey	Nonuse of glycopeptide	0.143	0.031-0.674	<0.05	14
Akgul et al., 2016 (19)	Turkey	Nonuse of steroids	0.244	0.072-0.822	<0.05	14
Akgul et al., 2016 (19)	Turkey	Absence of tracheostomy	0.06	0.006-0.614	<0.05	14
Garbati et al., 2016 (20)	Saudi Arabia	Not being in the ICU	0.027	0.001-0.496	0.015	18
Gasink et al., 2009 (21)	USA	Blood isolate (compared to an isolate from other body sites)	0.33	0.12-0.86	0.02	17
Giuffrè et al., 2013 (22)	Italy	Administration of ampicilin-sulbactan plus gentamicin	0.20	0.03-0.97	0.004	16
Kwak et al., 2005 (23)	South Korea	Use of a fluoroquinolone[c]	0.26	0.07-0.97	0.045	18
Madueño et al., 2017 (24)	Spain	Corticosteroid use	0.33	0.15-0.74	0.007	16
Madueño et al., 2017 (24)	Spain	Antibiotic use	0.20	0.65-0.62	0.01	16
Mittal et al., 2016 (25)	India	Use of aminoglycosides	0.257	0.068-0.975	0.046	13
Mittal et al., 2016 (25)	India	Use of a ventilator[c]	0.291	0.097-0.871	0.027	13
Schwartz-Neiderman et al., 2016 (26)	Israel	Use of cephalosporins[c]	0.2	0.1-0.6	0.005	18
Torres-Gonzalez et al., 2015 (27)	Mexico	Admission to the ICU[c]	0.42	0.20-0.88	<0.05	19

[a]Abbreviations: CRE, carbapenem resistant Enterobacteriaceae; OR, odds radio; CI, confidence interval; ICU, intensive care unit. [b]According to the STROBE quality assessment scale (13). [c]Risk factor included in a random-effects meta-analysis study.

All included studies were published between 2005 and 2017. Two articles were written in Spanish, one article was written in Chinese, one article was written in Greek, and one article was written in Slovak. All other articles were written in English (n=157, 96.9%). Most studies were conducted in Europe (n=62; 38.3%), mainly in Greece (n=14) and Italy (n=11). A total of 52 studies (32.1%) were conducted in Asia, mainly in Israel (n=18) and China (n=16). The remaining 48 studies were conducted in North America (n=31), South America (n=12), Australia (n=3), and Africa (n=2). Thirty-seven (22.8%) out of the 162 studies used a study design involving only the ICU. The majority of studies focused on a single species of the Enterobacteriaceae family; a *Klebsiella* spp. (n=103; 63.6%), an *Enterobacter* spp. (n=5), *E. coli* (n=4), *Citrobacter freundii* (n=3), and *Providencia stuartii* (n=2). The remaining 45 studies (27.8%) involved multiple Enterobacteriaceae species.

Carbapenemase production was described by 124 studies (76.5%), and these mainly involved KPC (n=91), NDM (n=24), and OXA (n=22) carbapenemases. Nine studies (5.6%) mentioned the production of β-lactamase enzymes in combination with porin mutations. In addition, one study detected only porin mutations and two studies detected only β-lactamase production in their carbapenem-resistant Enterobacteriaceae isolates. Thirty-two studies (19.8%) did not mention or investigate the carbapenem resistance mechanism involved.

Factors associated with CRE acquisition

We identified 74 studies describing factors associated with CRE acquisition with a statistically significant odds ratio (OR) or hazard ratio (HR) obtained from a multivariable analysis. All reported protective factors for CRE acquisition are summarized in Table 1. All reported risk factors were divided into two groups: related to antibiotic exposure and other. In addition, five studies reported risk factors associated with mortality among CRE carriers, including nine risk factors and four protective factors (14-18). The highest odds ratio was reported for the risk factor ICU stay (OR = 11.10, 95% CI = 1.85 to 66.95) (17).

Risk factors related to antibiotic exposure

All factors related to antibiotic exposure were further divided into nine smaller categories (Table 2). Carbapenem exposure (n=26) and cephalosporin exposure (n=15) were the most frequently mentioned risk factors associated with CRE acquisition.

For five out of the nine categories a random-effects meta-analysis was performed (Table 3 and Figure 2). For the risk factor carbapenem exposure, one study was excluded because it reported a hazard ratio instead of an odds ratio. The five meta-analyses included 43 studies, reporting 63 risk factors (OR>1) and 2 protective factors (OR<1). Carbapenem use (OR = 4.71, 95% CI = 3.54 to 6.26) and cephalosporin use (OR = 4.49, 95% CI = 2.42 to 8.33) generated to highest pooled ORs. Both publication bias indicators showed a significant result for risk factors carbapenem use, cephalosporin use, and glycopeptide use.

Table 2. Antibiotic exposure as a risk factor for the acquisition of CRE, based on multivariable analysis[c]

Associated risk factor	Frequency	RE	RE range	No. of cases (range)	Studies
Carbapenem use	25	OR	1.83-29.17	9-100	(28);(29);(30);(31);(32);(33);(20);(34);(35);(36);(37);(23);(38);(39);(40)[d];(41);(42);(17);(43);(44);(27);(18);(45);(46)
	1	HR	2.68	19	(27)
Cephalosporin use	15	OR	2.24-49.56	15-100	(47);(30);(21);(48);(16);(23);(49);(38);(50);(40)[d];(17);(51);(18);(44)
Quinolone use	9	OR	1.18-28.9	18-88	(28);(52);(53);(21);(35);(44);(54);(55);(56)
Antibiotic exposure (in general)[a,b]	9	OR	1.66-13.37	26-464	(57);(58);(33);(59);(35);(60);(61);(62);(54)
Other β-lactam use	9	OR	1.08-11.71	34-464	(58);(63);(64);(53);(65);(66);(50);(41);(44)
Other[a]	7	OR	1.02-33	25-103	(67);(36);(65);(68);(39);(51);(44)
Glycopeptide use	5	OR	2.94-43.84	20-203	(16);(66);(39);(69);(46)
No. of antibiotics administered[a,b]	3	OR	1.6-12.60	59-164	(31);(70);(42)
Duration of exposure[a,b]	3	OR	1.04-9.8	25-104	(67);(71);(72)

[a]This category was not included in a random-effects meta-analysis. [b]Exposure to any antibiotic.
[c]Abbreviations: CRE; carbapenem-resistant Enterobacteriaceae; RE, risk estimate; OR, odds ratio; HR, hazard ratio.
[d]This risk factor was identified two times in the study of Orsi *et al.* (40)

In total, 26 additional meta-analyses were performed to access the effect of the Enterobacteriaceae species studied, ICU study setting, the carbapenem resistance mechanism involved, and the study quality on the overall risk estimates (supplement 4). In the additional meta-analyses, all risk factors remained significantly associated with CRE acquisition (pooled OR > 1).

Other risk factors for CRE acquisition

Other risk factors associated with the acquisition of carbapenem-resistant Enterobacteriaceae were divided into nine categories and are summarized in Table 4. The risk factor underlying disease or condition (n=32 times identified) was the most frequently found. For eight out of nine categories, a meta-analysis including 59 studies was performed (Table 3 and Figure 3). In the categories underlying disease or condition and CRE exposure, one study was excluded because it reported a hazard ratio instead of an odds ratio. In the categories exposure to hospital care and mechanical ventilation, one study was excluded because it reported relative risk instead of an odds ratio.

From the eight different random-effects meta-analyses, the highest pooled OR was found for medical devices (OR = 5.09, 95% CI = 3.38 to 7.67), followed by invasive procedures (OR = 4.67, 95% CI = 3.59 to 6.07) and ICU admission (OR = 4.62, 95% CI = 2.46

Table 3. Random-effects meta-analyses of antibiotic exposure and other risk factors and/or protective factors for acquisition of CRE[a]

Associated risk factor	No. of times identified	Pooled OR (95%CI)	*P* value for risk of publication bias by use of the indicator of: Egger	Begg-Mazumdar
Antibiotic exposure				
Carbapenem use	25	4.71 (3.54-6.26)	<0.05	<0.05
Cephalosporin use	16	4.49 (2.42-8.33)	<0.05	<0.05
Quinolone use	10	2.46 (1.44-4.23)	<0.05	0.29
Other β-lactam use	9	2.00 (1.49-2.70)	<0.05	0.26
Glycopeptide use	5	4.18 (2.30-7.60)	<0.05	<0.05
Other risk factors				
Underlying disease or condition	31	2.54 (2.08-3.09)	<0.05	0.12
Invasive procedures	20	4.67 (3.59-6.07)	<0.05	<0.05
Medical devices	17	5.09 (3.38-7.67)	<0.05	<0.05
ICU admission	15	4.62 (2.46-8.69)	<0.05	<0.05
Demographic patient characteristics	13	1.08 (1.03-1.14)	<0.05	<0.05
Exposure to hospital care	12	1.05 (1.02-1.08)	<0.05	<0.05
Mechanical ventilation	11	1.96 (1.42-2.69)	<0.05	<0.05
CRE exposure	5	4.10 (1.46-11.52)	<0.05	0.23

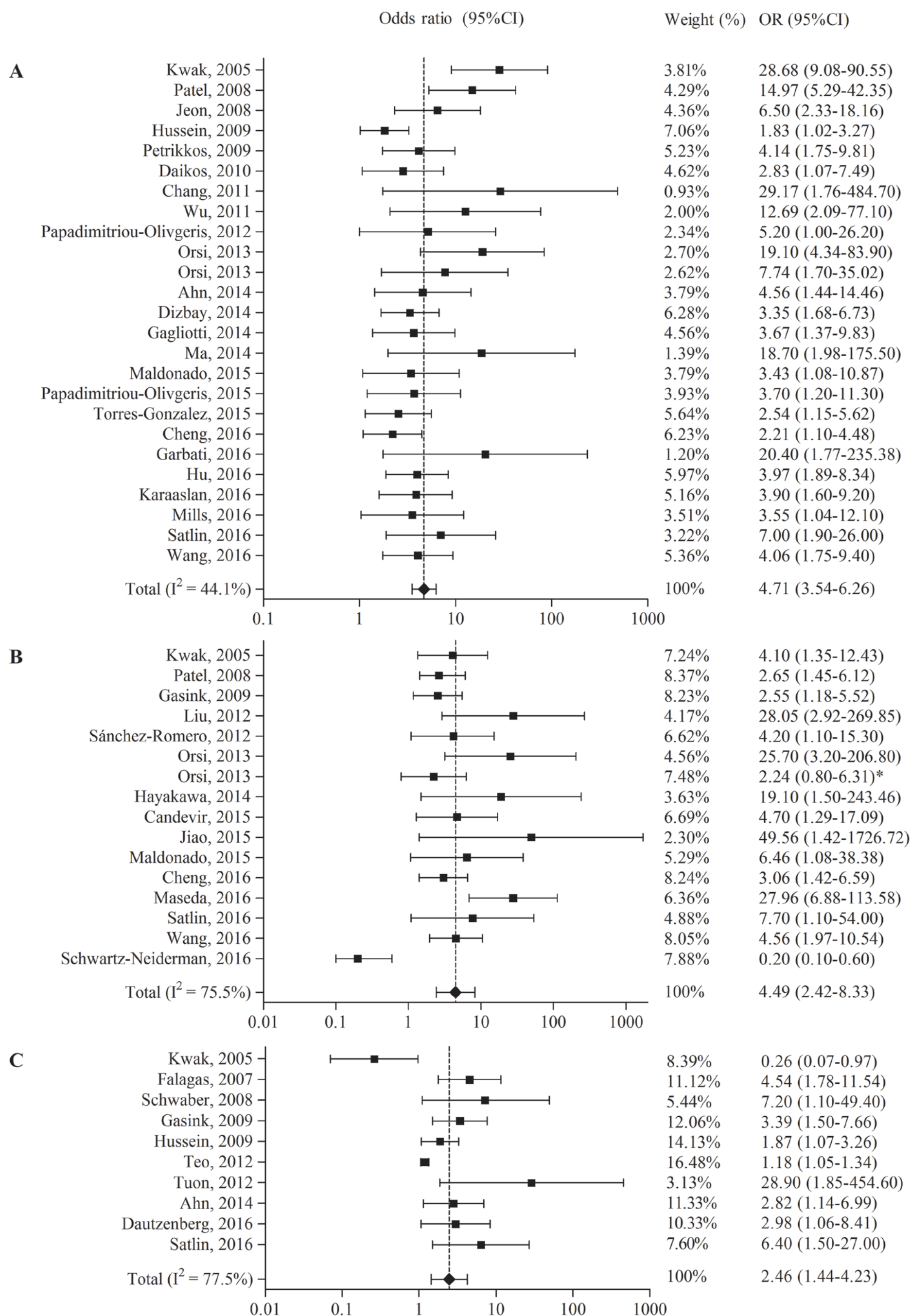
[a]Abbreviations: CRE, carbapenem-resistant Enterobacteriaceae; OR, odds radio; CI, confidence interval; ICU, intensive care unit.

to 8.69. Both publication bias indicators showed a significant result for all risk factors, except underlying disease or condition and CRE exposure.

The effects of the different variables (*e.g.*, the CRE species studied, ICU study setting and the mechanisms of carbapenem resistance) were reviewed by performing 47 additional meta-analyses. Surprisingly, all risk factors showed a decreased (or equal) pooled OR when only studies in which carbapenemase production was shown were included, with the OR difference ranging from 0 to -1.29 (supplement 5, figure C). The meta-analyses of the remaining studies that described another resistance mechanism (*e.g.*, porin mutations) or that did not investigate the resistance mechanism involved showed a large increase in the reported pooled ORs for all tested risk factors with the mean change being +2.89.

Effective infection prevention strategies

We identified 95 studies describing effective infection prevention strategies used to control the spread of carbapenem-resistant Enterobacteriaceae in a hospital setting. These were converted to the top 10 most successful intervention strategies (Table 5). The use of barrier and/or contact precautions was found to be the most successful in-



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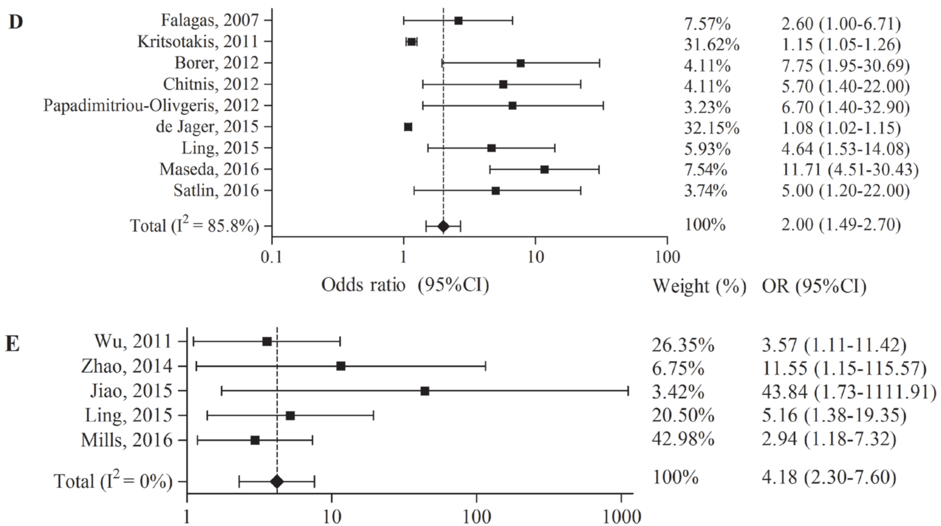


Figure 2. Forest plots of random-effects meta-analyses of antibiotic exposure as a risk factor and/or protective factor for the acquisition of carbapenem-resistant Enterobacteriaceae.
(A) Carbapenem use; (B) cephalosporin use; (C) quinolone use; (D) β-lactam use; (E) glycopeptide use. *non-significant confidence interval (Orsi *et al.* were contacted multiple times to receive the correct numbers; unfortunately, the authors did not respond).

tervention strategy (n=71), followed by patient cohorting (n=68) and active surveillance (n=56). Control of antibiotic use was mentioned in only 17 studies and could be found in ninth place. Besides these 10 strategies, some other interventions were described in the literature, such as restricted/no admission to the affected wards (n=9) and the use of chlorhexidine for patient disinfection (n=9).

Environmental sources and reservoirs

Twenty-seven studies provided information about the environmental sources and reservoirs identified within their hospitals. All hospital locations in which carbapenem-resistant Enterobacteriaceae were identified are summarized in Table 6. Contaminated sinks were the most frequently described (n=10), followed by patient beds (n=6) and mechanical ventilation equipment (n=5).

DISCUSSION

Summary of evidence

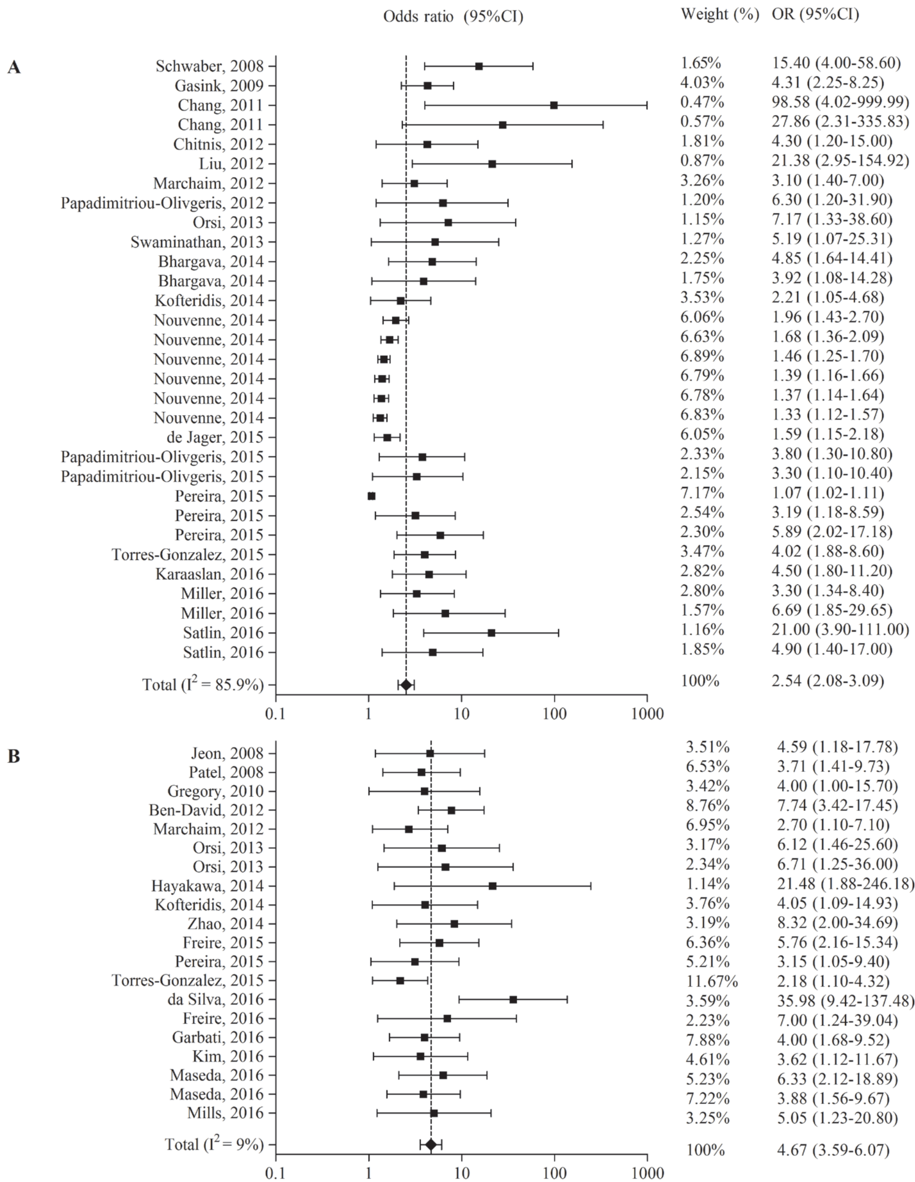
In this systematic review, we identified 13 risk factors associated with the presence of carbapenem-resistant Enterobacteriaceae. These risk factors were, in order of those with

Table 4. Other risk factors associated with the acquisition of CRE, based on multivariable analysis[c]

Associated risk factor	Frequency	RE type	RE range	No. of cases (range)	Study reference(s) (no. of different risk factors per reference
Underlying disease or condition	31	OR	1.07-98.58	17-133	(73)[2x];(29)[2x];(63);(64);(21);(37);(74);(49);(60);(61)[2x];(75)[6x];(40);(41);(42)[2x];(76)[3x];(44)[2x];(54);(72);(27)
	1	HR	5.74	19	(27)
Other [a]	19	OR	1.35-45.904	20-464	(57);(77);(58)[2x];(30);(32);(78)[2x];(79);(49);(60)[2x];(39);(75);(42);(80);(27)[2x];(69)
	1	RR	5.94	149	(81)
	1	HR	19.0	26	(78)
Invasive procedures	20	OR	2.18-35.98	15-99	(82);(83);(14);(84);(20);(78);(48);(36);(85);(74);(60);(50)[2x];(39);(40)[2x];(17);(76);(27);(69)
Medical devices[b]	17	OR	1.67-677.82	15-203	(73);(77);(47);(30);(82)[2x];(14);(84);(86);(16);(37)[2x];(66);(87);(70);(51);(55)
ICU admission	14	OR	1.13-17.4	25-88	(47);(31);(32);(35);(74);(71);(41);(42);(43);(44);(54);(80);(88);(46)
Patient demographic characteristics	13	OR	1.03-10.53	10-164	(77);(30);(52)[2x];(83);(84)[2x];(22);(37);(89);(70);(62);(56)
Exposure to hospital care	12	OR	1.014-58.067	15-99	(82);(52);(59);(20);(36);(85);(24)[2x];(41);(17);(55);(69)
	1	RR	1.36	149	(81)
Mechanical ventilation	10	OR	1.2-17.80	18-164	(63);(82);(64);(39);(70);(17);(51);(26);(72);(56)
	1	RR	1.99	149	(81)
CRE exposure	5	OR	1.15-11.9	53-165	(70)[2x];(26);(72);(27)
	1	HR	5.03	19	(27)

[a]This category was not included in a random-effects meta-analysis. [b]Mechanical ventilation is excluded from this category.
[c]Abbreviations: CRE, carbapenem-resistant Enterobacteriaceae; RE, risk estimate; OR, odds ratio; HR, hazard ratio; RR, relative risk; ICU, intensive care unit.

the highest to those with the lowest pooled OR, (i) medical devices, (ii) carbapenem use, (iii) invasive procedures, (iv) ICU admission, (v) cephalosporin use, (vi) glycopeptide use, (vii) CRE exposure, (viii) underlying disease or condition, (ix) quinolone use, (x) β-lactam use, (xi) mechanical ventilation, (xii) demographic patient characteristics, and (xiii) exposure to hospital care (Table 3). Medical devices, antibiotic use, ICU admission, exposure to hospital care, and underlying diseases were also identified to be risk factors in systematic reviews regarding the acquisition of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella* spp. (176,) and carbapenem-resistant *Pseudomonas aeruginosa* (177).



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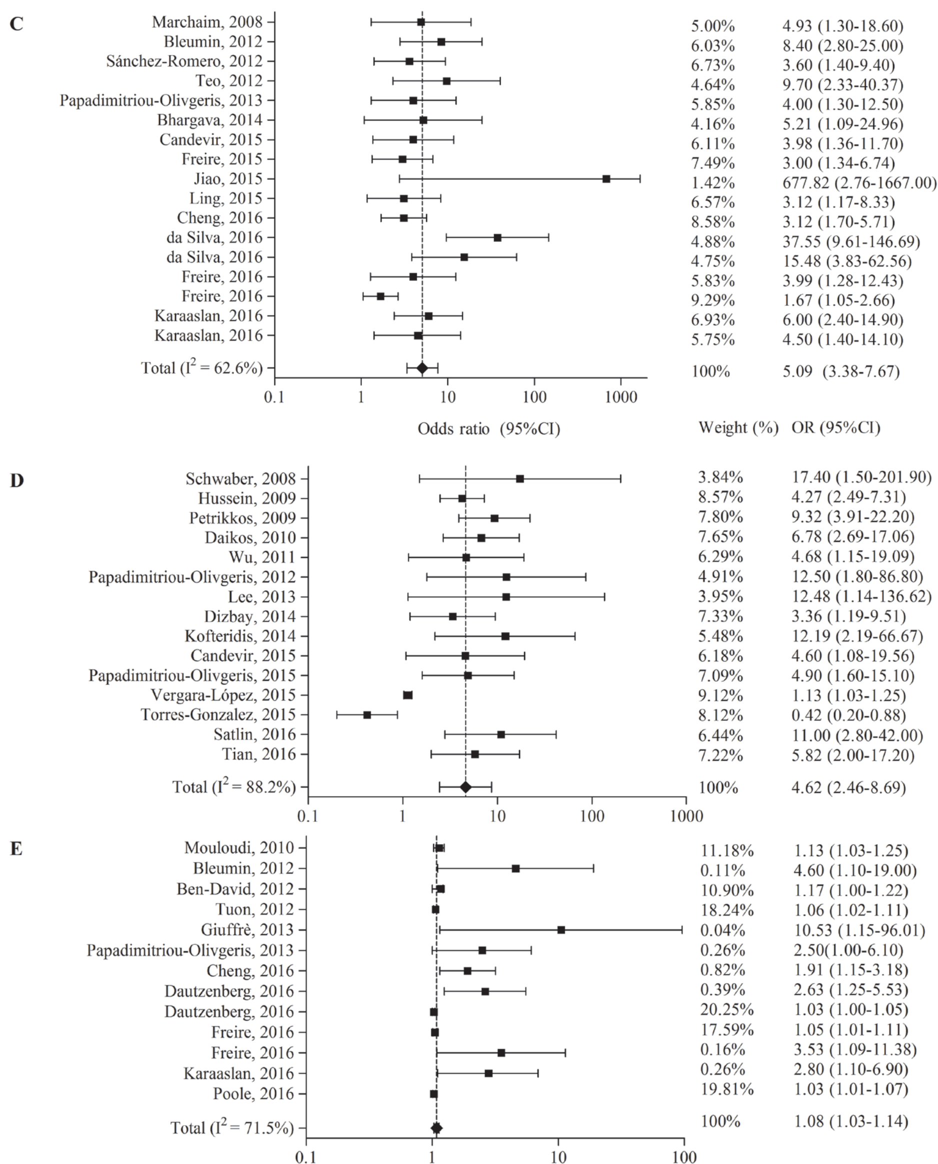
Plasmids responsible for carbapenem resistance often carry additional genes conferring resistance to other antibiotics, such as fluoroquinolones and aminoglycosides. This can explain why the use of these antibiotic classes is found to be a risk factor for CRE acquisition. However, this explanation cannot be used for glycopeptide antibiotics. Wu *et al.* (46) and Jiao *et al.* (16) supposed that vancomycin treatment disrupts the intestinal



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microflora, promoting the colonization of Enterobacteriaceae. Glycopeptide use was also identified to be a risk factor for carbapenem-resistant *P. aeruginosa* (177, 178) and ESBL-producing bacteria (179) acquisition.

On the contrary, 4 out of 13 significant risk factors were also described to be protective against CRE acquisition by other authors: quinolone use (23), mechanical ventilation (25),

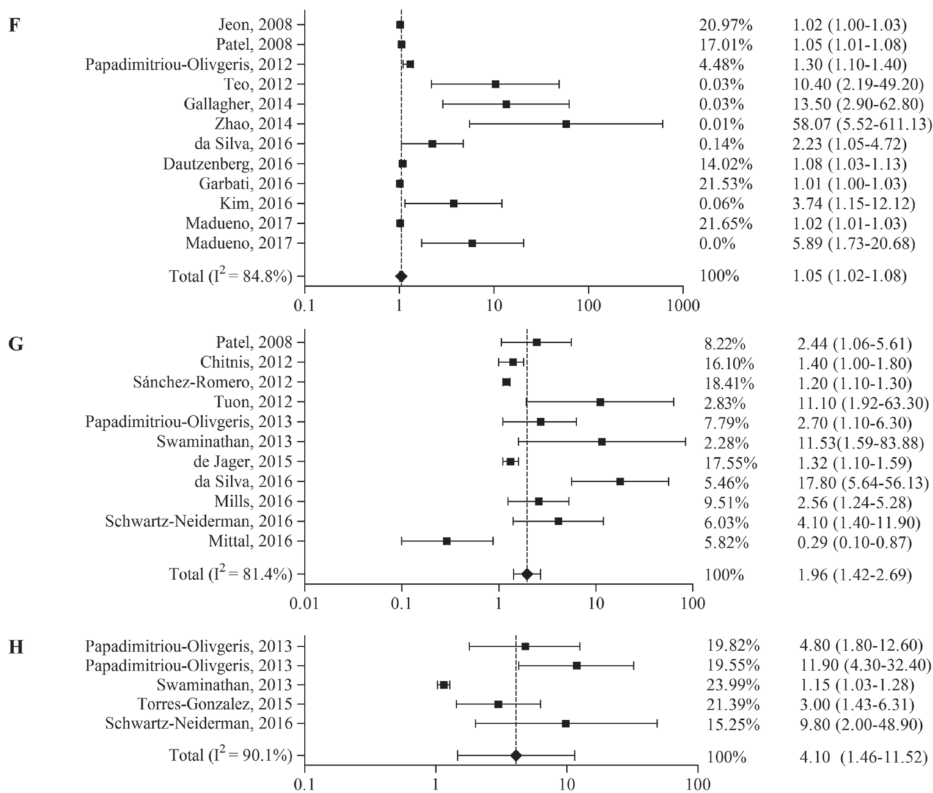

Figure 3. Forest plots of random-effects meta-analyses of other risk factors and/or protective factors for the acquisition of carbapenem-resistant Enterobacteriaceae. (A) Underlying disease or condition; (B) invasive procedures; (C) medical devices; (D) ICU admission; (E) demographic patient characteristics; (F) exposure to hospital care; (G) mechanical ventilation; (H) CRE exposure.

cephalosporin use (26), and ICU admission (27). Kwak *et al.* speculated that fluoroquinolone use was found to be a protective factor because this antibiotic was often given as a substitute for carbapenem or cephalosporin antibiotics (23). Torres-Gonzalez *et al.* reported that ICU admission was protective against CRE acquisition. This observation could be explained by the fact that their CRE outbreak was initially detected in the ICU and a successful bundle of infection prevention measures was initiated in that area (27).

We also performed additional meta-analyses to estimate the influence of the following variables on the overall risk estimate: the Enterobacteriaceae species studied, the ICU study setting, the carbapenem resistance mechanism involved, and the study quality. The carbapenem resistance mechanism was found to have the highest influence on the risk estimates, especially in the meta-analyses of non-antibiotic-related risk factors for CRE acquisition (supplement 4, figure C). We observed that our risk factors

Table 5. Top 10 strategies to control hospital outbreaks with CRE[a]

Intervention	No. of studies	Study references
1. Barrier/contact precautions	71	(90);(91);(92);(93);(94);(95);(96);(97);(98);(99);(30);(63);(100);(101);(83);(102);(103);(104);(105);(106);(107);(108);(109);(22);(78);(48);(110);(111);(112);(113);(114);(115);(116);(117);(118);(87);(119);(120);(121);(122);(123);(75);(124);(125);(126);(127);(128);(129);(130);(131);(132);(133);(134);(135);(72);(136);(137);(27);(138);(139);(140);(141);(142);(143);(144);(145);(146);(147);(45);(84);(148)
2. Patient transfer to single room or cohorting	68	(90);(91);(149);(95);(96);(150);(98);(151);(99);(30);(63);(100);(152);(101);(153);(83);(154);(155);(103);(104);(105);(106);(84);(107);(108);(156);(22);(78);(110);(111);(157);(112);(113);(114);(158);(115);(159);(117);(87);(119);(120);(121);(160);(123);(75);(124);(125);(161);(127);(129);(130);(131);(132);(133);(134);(162);(136);(27);(139);(141);(142);(143);(144);(145);(146);(163);(147);(45)
3. Active surveillance/screening for CRE	56	(90);(93);(149);(94);(95);(96);(97);(150);(98);(164);(151);(63);(100);(152);(153);(154);(155);(103);(104);(105);(106);(107);(108);(110);(112);(113);(158);(116);(117);(118);(68);(119);(121);(122);(160);(123);(75);(124);(126);(161);(128);(129);(130);(132);(133);(135);(162);(72);(137);(138);(139);(141);(142);(143);(147);(45)
4. Enhanced hand hygiene	52	(165);(90);(91);(92);(93);(95);(96);(150);(98);(166);(30);(63);(102);(103);(105);(106);(84);(148);(108);(156);(109);(22);(78);(110);(111);(112);(113);(158);(159);(117);(118);(87);(119);(120);(121);(122);(124);(125);(126);(161);(128);(133);(135);(136);(27);(139);(140);(143);(145);(163);(147);(45)
5. Enhanced environmental cleaning	51	(165);(90);(91);(92);(93);(149);(96);(150);(99);(166);(30);(63);(167);(101);(64);(154);(102);(105);(106);(22);(111);(157);(112);(113);(158);(159);(117);(118);(119);(122);(160);(123);(124);(126);(161);(127);(128);(130);(135);(162);(136);(137);(27);(138);(139);(140);(142);(143);(144);(146);(163)
6. Staff educational programs	34	(93);(150);(164);(99);(63);(100);(64);(154);(102);(103);(104);(84);(109);(22);(110);(157);(112);(114);(116);(119);(160);(123);(124);(126);(161);(127);(162);(136);(137);(138);(139);(141);(144);(45)
7. Staff cohorting	32	(90);(91);(150);(98);(63);(100);(152);(101);(64);(155);(103);(104);(106);(84);(148);(107);(78);(113);(158);(87);(121);(160);(75);(126);(161);(131);(133);(134);(135);(136);(138);(142)
8. Equipment cohorting/single-use equipment	21	(90);(150);(63);(64);(104);(105);(22);(121);(128);(131);(135);(146);(91);(92);(149);(114);(87);(124);(161);(101);(123)
9. Control of antibiotic use	17	(165);(96);(99);(103);(106);(84);(109);(78);(113);(114);(168);(121);(122);(137);(138);(139);(45)
10. Flagging of CRE patients	14	(92);(96);(98);(100);(153);(104);(107);(112);(118);(124);(125);(137);(140);(142)

[a]CRE, carbapenem-resistant Enterobacteriaceae.

showed a lower risk estimate only when studies in which carbapenemase-producing Enterobacteriaceae were described were included.

The most successful interventions to stop the spread of CRE were barrier/contact precautions, patient cohorting, and active surveillance. Our findings correspond to the

Table 6. Identified environmental sources and reservoirs for CRE[b]

Environmental source or reservoir	Studies
Sinks	(169);(159)[a];(170)[a];(118)[a];(130);(167)[a];(135)[a];(133);(171);(150)[a]
Patient bed (e.g., bedrail, mattress)	(126)[a];(161)[a];(171);(160)[a];(150)[a];(96)
Mechanical ventilation equipment	(165)[a];(161)[a];(172)[a];(135)[a];(160)[a]
Positive cultures from nurses (hands)	(144)[a];(145)[a];(150)[a];(96)
Endoscope	(115);(173)[a];(85)
Duodenoscope	(98)[a];(174)[a]
Urinary catheter	(166);(138)[a]
Monitor (e.g., vital signs, television)	(160)[a];(96)
Shower/shower equipment	(130);(171)
Table	(165)[a];(150)[a]
Ureteroscope	(175)[a]
Razor	(101)[a]
Incubator	(144)[a]
Radiant warmer	(145)[a]
Suction equipment	(171)
Wastewater drainage system	(138)[a]
Stethoscope	(138)[a]
Intravenous pole	(160)[a]
Infusion pump	(150)[a]
Janet syringe	(96)
Cabinet	(96)
Intravenous infusion counter apparatus	(96)
Enteral feeding formula	(96)

[a]The study proved the source or reservoir by molecular typing of carbapenem-resistant Enterobacteriaceae isolates.
[b]CRE, carbapenem-resistant Enterobacteriaceae.

guidelines presented by the Centers for Disease Control and Prevention (CDC), which mainly highlight active surveillance and contact precautions (180, 181). Surprisingly, antimicrobial stewardship was mentioned in only 17 out of 95 studies, although multiple antimicrobial classes were identified to be risk factors for CRE acquisition.

Only 27 out of 95 studies reported environmental sources or reservoirs for CRE within their hospitals (Table 6). This indicates that for many outbreaks the source or reservoir was not determined. Contaminated sinks were the most frequently described, and correspond to the reservoirs identified for other nosocomial pathogens, such as carbapenem-resistant *P. aeruginosa* (177) and ESBL producing *Klebsiella* spp. (176).

Strengths and limitations

A strength of our study was the inclusion of both Enterobacteriaceae that showed in vitro resistance to any carbapenem antibiotic and Enterobacteriaceae that were found to produce carbapenemase enzymes. This is important because carbapenemase-production does not always confer high-level carbapenem resistance and therefore leads to false-negative results when only phenotypic tests are used to identify carbapenem-resistant Enterobacteriaceae (182). This review included only two studies in which carbapenemase producing but carbapenem-sensitive and -resistant isolates were studied. However, the mechanism of resistance does influence transmission, and thus, epidemiology, as we showed different risk estimates, especially for the non-antibiotic-related risk factors, when each mechanism was analyzed. With the knowledge that we have up to now, we cannot explain this difference. As we included all kinds of mechanisms, this can also be seen as a limitation of the study.

The study also has some limitations; the first is the large heterogeneity of all studies included. Studies with different target populations for example neonates, adults, or transplant patients, were selected. In addition, different microbiological methods were used to identify the CRE isolates, different Enterobacteriaceae species were included, and different prevention strategies were installed. To limit the influence of the study heterogeneity, the random-effects model of DerSimonian and Laird (10) was implemented in the meta-analyses, and different subgroup analyses were performed.

Second, we included both studies reporting CRE colonization and studies reporting CRE infections in hospitalized patients. However, not all studies describing risk factors for CRE infection checked whether the patients were previously colonized with CRE or not. Likewise, they did not check whether patients from the control group were colonized with CRE before or during the infection. For these studies, we cannot rule out the possibility that their reported risk factors are not specific for CRE acquisition but are specific for progression to infection after CRE colonization.

Third, both publication bias indicators showed a significant result for several risk factors (9/13), indicating that publication bias was present. To limit publication bias, the studies that we included were not limited by language, date of publication, country of publication, carbapenem resistance mechanism, study design, or patient characteristics.

Conclusions and implications

This systematic review shows that not only antibiotic use but also many other risk factors are associated with CRE acquisition. The most significant risk estimate found in our meta-analyses was found for the risk factor medical devices, followed by carbapenem use. We identified risk factors related to the emergence/selection of CRE, but also risk factors related to the transmission of the CRE isolates. To prevent or to control hospital outbreaks, bundled interventions are needed. These interventions need to focus on

both antibiotic stewardship and reduction of the use of indwelling devices to reduce the spread of CRE within the hospital. Indwelling medical devices do present a very high risk for the acquisition of CRE but are also a risk for the acquisition of infections in general. Therefore, a very useful prevention measure is the active decrease in the rate of use (deimplementation) of medical devices.

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SUPPLEMENTAL MATERIAL

Supplement 1. PRISMA Checklist.

Supplement 2. Literature search strategy (list of search terms).

Supplement 3. Study Quality assessment using the STROBE guideline.

Supplement 4. Forest plots of additional meta-analyses of antibiotic exposure as risk factor and/or protective factor for the acquisition of carbapenem-resistant Enterobacteriaceae.

Supplement 5. Forest plots of additional meta-analyses of other risk factors and/or protective factors for the acquisition of carbapenem-resistant Enterobacteriaceae.

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Chapter 2.2

A systematic review and meta-analyses show that carbapenem use and medical devices are the leading risk factors for carbapenem-resistant *Pseudomonas aeruginosa*

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ABSTRACT

A systematic review and meta-analyses were performed to identify the risk factors associated with carbapenem-resistant *Pseudomonas aeruginosa* and to identify sources and reservoirs for the pathogen. A systematic search of PubMed and Embase databases from 1 January 1987 until 27 January 2012 identified 1,662 articles, 53 of which were included in a systematic review and 38 in a random-effects meta-analysis study. The use of carbapenem, use of fluoroquinolones, use of vancomycin, use of other antibiotics, having medical devices, intensive care unit (ICU) admission, having underlying diseases, patient characteristics, and length of hospital stay were significant risk factors in multivariate analyses. The meta-analyses showed that carbapenem use (odds ratio [OR] = 7.09; 95% confidence interval [CI] = 5.43 to 9.25) and medical devices (OR = 5.11; 95% CI = 3.55 to 7.37) generated the highest pooled estimates. Cumulative meta-analyses showed that the pooled estimate of carbapenem use was stable and that the pooled estimate of the risk factor “having medical devices” increased with time. We conclude that our results highlight the importance of antibiotic stewardship and the thoughtful use of medical devices in helping prevent outbreaks of carbapenem-resistant *P. aeruginosa*.

INTRODUCTION

Pseudomonas aeruginosa is one of the most common nosocomial pathogens (1). *P. aeruginosa* can cause infections in patients with serious underlying disorders, such as a suppressed immune system or cystic fibrosis (CF), or in patients in intensive care units (ICU)(2, 3). Further, infections with *P. aeruginosa* in such patients lead to increased morbidity and mortality (2-4).

P. aeruginosa is intrinsically resistant to various antibiotics and is capable of acquiring additional resistance by either chromosomal mutations or horizontal gene transfer (5). The most important mechanisms are loss or alteration of outer membrane porins and increased efflux pump activity (6-8). The emergence of multidrug-resistant (MDR) *P. aeruginosa* is a problem of global concern, and there are currently reports of hospital outbreaks of MDR *P. aeruginosa* from countries around the world, including The Netherlands (9-13). These outbreaks are frequently caused by *Pseudomonas aeruginosa* clones with metallo-β-lactamases, such as Verona integron-encoded metallo-β-lactamase (VIM) and imipenemase (IMP). Importantly, outbreaks may be large and sustained, despite the adoption of infection control measures (12, 14).

In 2006, a summary on this subject was published by Falagas and Kopterides, who published a systematic review of the problem (15). However, there have been many more published reports regarding nosocomial (MDR) *P. aeruginosa* since 2006. Therefore, in the current publication, a more extensive and up-to-date systematic review was performed, focusing on carbapenem resistance, non-CF patients and including conventional and cumulative meta-analyses. The aim of the analysis was to answer the following two questions. First, what are the risk factors for the presence of carbapenem-resistant *P. aeruginosa* among hospitalized patients? Second, what environmental sources and/or reservoirs were identified in these outbreaks? This knowledge will be useful for worldwide health care centers that are facing the threat of MDR *P. aeruginosa*, and will help in designing strategies to stop the emergence of spread of these MDR pathogens.

METHODS

The systematic review and meta-analyses presented in this publication include all of the items in the checklist detailed in the PRISMA guideline (16).

Study and data collection

Eligible articles were identified by searching PubMed (Medline) and Embase databases. Additional articles were identified by hand searching the reference lists of included reviews. Searches were performed for the period from 1 January 1987 until 27 January

2012. Search terms included "*Pseudomonas*" as a title word, in combination with the key-words "'resistant," "multidrug resistance," "VIM," "IMP," "metallo-beta-lactamase" or "MBL" and "risk factors," "determinants," "outbreak," "transmission," "nosocomial," "health care related," "health care associated," "epidemiology," or "source," including all possible ways of writing. The authors included peer-reviewed articles relating to carbapenem-resistant *P. aeruginosa*, that also described the risk factors associated with the presence of car-bapenem-resistant *P. aeruginosa* using a multivariate model and in which a nosocomial infection was described. We excluded studies relating to non-human infections, studies that only included patients with CF, reviews, commentaries, editorials, letters, and ab-stracts. We also excluded studies published before 1987, the year of the U.S. approval of imipenem (17). Environmental sources and reservoirs were searched for in both included and excluded studies. A study was excluded from the meta-analyses (i) if it reported only hazard ratios, (ii) if it reported only prevalence ratios or risk ratios, (iii) when confidence intervals were missing, and (iv) if it included only patients with *P. aeruginosa* bacteremia.

We extracted detailed information from the included studies. We based the classifica-tion of studies regarding the different study designs on the description of the methods in a particular study, not on the study design claimed to be used by the authors (*e.g.*, a reported retrospective cohort study can methodologically be a case-control study). We contacted the corresponding and/or first authors of 47 articles by e-mail in order to retrieve the full-text articles or to retrieve missing information.

Study quality

To assess the quality, risk of bias, and generalizability of the included studies a quality assessment was performed using the STROBE guidelines for included cross-sectional studies, as well as the Newcastle-Ottawa quality assessment scale for included case-control and cohort studies (18, 19). The quality of the studies was not considered an exclusion criterion.

Statistical analysis

We merged all reported risk factors with a reported odds ratio (OR) and 95% confidence interval (95% CI) into 10 different groups: group 1, carbapenem use; group 2, quinolone use; group 3, vancomycin use; group 4, other antibiotic use; group 5, medical devices; group 6, ICU admission; group 7, underlying diseases; group 8, patient characteristics; group 9, length of hospital stay; and group 10, other. We selected the 10 groups using the results of the systematic review. For each of the first nine groups, a meta-analysis was performed. That was not possible for the group 10 (other), as the risk factors were too diverse. An additional meta-analysis was performed for the risk factors quinolone use, vancomycin use, and other antibiotic use together. All meta-analyses were performed using StatsDirect Statistical Software (Altrinchem, United Kingdom). The risk factors

reported by the studies included in the analyses were diverse; therefore, a random effects model was fitted to the data based on the method of DerSimonian and Laird (20). A P value of <0.05 was considered statistically significant, and no correction was made for multiple testing. The risk of publication bias across the studies was assessed by the Egger and Begg-Mazumdar (Kendall's tau) indicators. Both bias indicators had to show a significant result before it was concluded that publication bias was present. Additionally, two cumulative meta-analyses were performed for the groups 1 (carbapenem use) and 2 (medical devices), as these two groups showed highly significant results using conventional meta-analyses. A random effects model, based on the method of DerSimonian and Laird, was also fitted to these cumulative meta-analysis (20).

RESULTS

Description of included studies

A total of 1,662 articles were identified when the search results of PubMed and Embase were combined (Figure 1). After applying exclusion criteria as described in Materials and Methods, 256 articles were read in their entirety (full-text) (Figure 1). The corresponding and/or first authors of 47 out of 256 articles were contacted by e-mail. Authors from 19 out of 47 articles responded to e-mail requests. Nine full-text articles were received by mail or e-mail, and from four articles missing information was retrieved. For two articles, the requested information was not available. Fifty-three studies were finally included in the analyses after exclusion of articles that did not meet our inclusion criteria as described in Materials and Methods (Figure 1). These studies represented 3,966 patient cases (ranging from 5 to 345 cases per publication) from 15 different countries (Tables 1 and 2). Eight of 53 of the studies included patients with bacteremia only and are shown in Table 2. All 53 studies had an observational study design and were written in English. Eight studies reported that a retrospective cohort study was performed, whereas conceptually they could be considered case-control studies. Five multicenter studies were also included. The percentage of male gender ranged from 38.6% to 84.0%. Patient age ranged from several days old (neonates) to very old, with 97 years as oldest.

Not all studies provided detailed information regarding the microbiological methods used. However, 23 of the 53 studies did describe the method used for the identification of *P. aeruginosa*, of which 10 studies used the Vitek system. Only 19 of the 53 studies described isolate genotyping, with 16 studies using pulsed-field gel electrophoresis (PFGE), 1 using multiple-locus variable-number tandem repeat analysis (MLVA), 1 using restriction fragment length polymorphism (RFLP), and 1 using repetitive-element-based PCR. The median number of cases, as included in the multivariate analyses in these 19 studies, was 30 (ranging from six to 204 cases). The median number of genetically

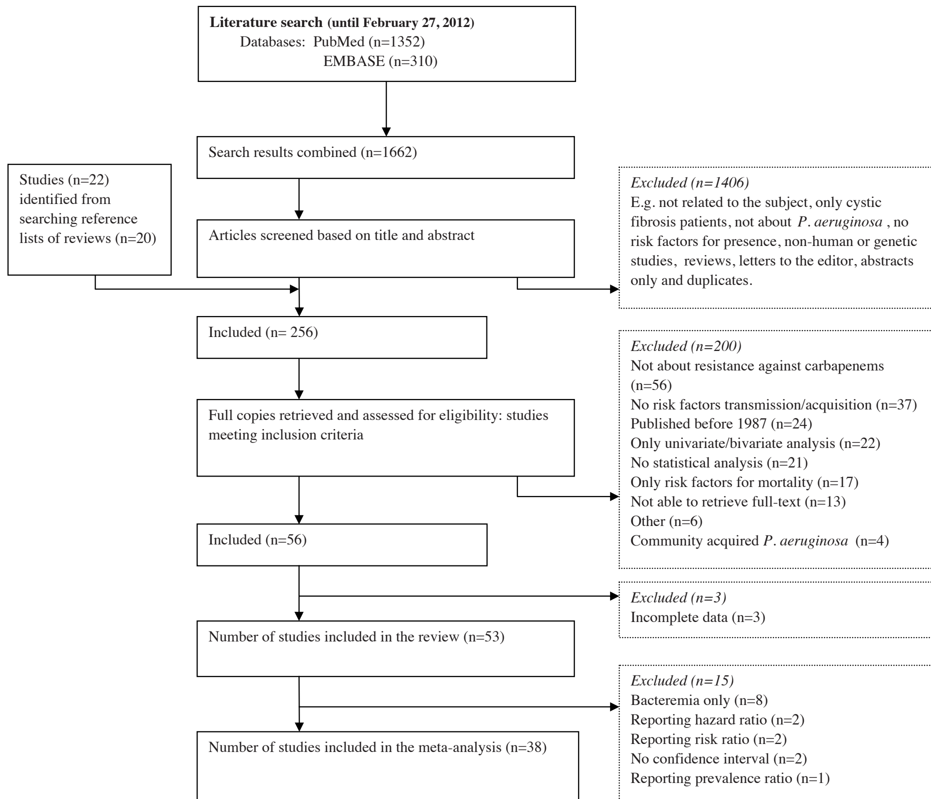


Figure 1. Flow diagram of study selection for the systematic review on carbapenem resistant *P. aeruginosa*

identical clusters identified was 2 (ranging from 1 to 8). The median size of the clusters described in these genotyping studies was 4 (ranging from 2 to 47). Seven of the 53 studies also identified the presence of blaVIM and blaIMP genes (using PCR amplification). The average number of cases in these seven studies was 32 (ranging from 5 to 47 cases).

The statistically significant risk factors calculated from the multivariate analyses, specifically the presence of carbapenem-resistant *P. aeruginosa* (Table 1), were extracted and merged into 10 different classes. The definitions of the risk factors from the different studies were not uniform.

When considering all statistically significant risk factors from the multivariate analyses of 45 studies (n) that had not included "only bacteremic patients," it was observed that the presence of medical devices was the most reported risk factor (n= 21) (Table 1). The risk factors extracted from the eight studies including only patients with bacteremia are shown in Table 2. Eight out of the 53 studies not only identified risk factors but also identified protective factors for presence of a carbapenem-resistant *P. aeruginosa*, including quinolone use, exclusive feeding by formula and duration of antibiotic treatment (Table 3).

Table 1. Sources and characteristics of included studies (n= 45) and risk factors for transmission and acquisition of carbapenem resistant *P. aeruginosa*, based on multivariate analyses[a]

Risk factor	No. of factors	Sources[b]	No. of cases	Case control studies	
			Range	No.	OR range
Carbapenem use	19	Harris, 2011(21); Lautenbach, 2010(22); Lepelletier, 2010(23); Cezario, 2009(24); Mueller, 2008(25); Onguru, 2008(26); Pena, 2007(27); Mentzelopoulos, 2007(28); Fortaleza, 2006(29); Ohmagari, 2005(30); Ozkurt, 2005(31); 2x Zavascki, 2005(32); Cao, 2004(33); Harris, 2002(34); Troillet, 1997(35); Carmeli, 1999(36); Lodise Jr, 2007(37);Montero, 2010(38)	5 -354	12	3.6 - 76.0
Quinolone use	11	van der Bij, 2011(12); Kohlenberg, 2010(39); Pena, 2009(40); Yang, 2009(41); Pena, 2007(27); Lautenbach, 2006(42);Zavascki, 2006(43); Nouer, 2005(44); Defez, 2004(45); Lodise Jr, 2007(37); Montero, 2010(38)	15 - 354	5	2.5 - 48.4
Vancomycin use	3	Harris, 2002(34); Fortaleza, 2006(29); Onguru, 2008(26)	75 - 120	3	1.8 - 2.9
Other antibiotic use	18	2x Furtado, 2010(46); Lepelletier, 2010(23); 2x Martinez, 2009(47); 2x Onguru, 2008(26); 2x Aloush, 2006(48); Fortaleza, 2006(29); Zavascki, 2006(43); Nouer, 2005(44); Ozkurt, 2005(31); Zavascki, 2005(32); Defez, 2004(45); 2x Harris, 2002(34); Richard, 1994(49)	15 - 120	9	2.2 - 43.7
Medical devices	21	Nagao, 2011(50); Park, 2011(51); Kohlenberg, 2010(39); 2x Cezario, 2009(24); Cortes, 2009(52); Fortaleza 2009(53);Martinez, 2009(47); Pena, 2009(40); Mueller, 2008(25); Onguru, 2008(26); Pereira, 2008(54); Zavascki, 2005(32); 2x Defez, 2004(45); Cao, 2004(33); 2x Dropulic, 1995(55); Talon, 1995(56); Thuong, 2003(57); Lodise Jr, 2007(37)	6 - 204	13	2.1 - 64.3
ICU admission	8	van der Bij, 2011(12); Lepelletier, 2010(23); Eagye, 2009(58); Furtado, 2009(59); Mueller, 2008(25); Aloush, 2006(48); Zavascki, 2006(43); Harris, 2002(34)	35 - 120	5	1.1 - 13.3
Underlying disease	12	Furtado, 2010(46); 3x Fortaleza 2009(53); Pena, 2007(27); 3x Zavascki, 2006(43); Fortaleza, 2006(29); Ohmagari,2005(30); Troillet, 1997(35); Talon, 1995(56)	17 - 260	6	1.0 - 25.0
Patient characteristics	19	Park, 2011(51); 2x Furtado, 2010(46); Lepelletier, 2010(23); 2x Eagye, 2009(58); Cezario, 2009(24); Aloush, 2006(48); Zavascki, 2005(32); Ohmagari, 2005(30); 2x Defez, 2004(45); Berthelot, 2001(60); Carmeli, 1999(2);2x Mammina, 2008(61); 3x Montero, 2010(38)	18 - 354	10	1.0 - 13.9
Length of hospital stay	13	Harris, 2011(21); Furtado, 2010(46); Lautenbach, 2010(22); Lepelletier, 2010(23); Yang, 2009(41); Pereira, 2008(54); Onguru, 2008(26); Ozkurt, 2005(31); Harris, 2002(34); Carmeli, 1999(2); 2x Montero, 2010(38); Arruda, 1999(62)	20 - 354	8	1.0 - 6.7
Other	18	van der Bij, 2011(12); Harris, 2011(21); Lautenbach, 2010(22); Furtado, 2010(46); Montero, 2010(38); Pena, 2009(40); 2x Aloush, 2006(48); Fortaleza, 2006(29); Zavascki, 2006(43); 2x Ozkurt, 2005(31); 2x Defez, 2004(45); Paramythiotou, 2004(63); Berthelot, 2001(60); Carmeli, 1999(36); Dropulic, 1995(55)	34 - 354	10	1.7 - 13.2

[a]From the initial 53 studies, those focused on only patients with bacteremia (n=8) were excluded. OR, odds ratio.
[b]Sources are identified by first author, year, and reference number. 2x or 3x, two or three different factors per reference.

Table 2. Summary of studies (n= 8) regarding *P. aeruginosa* bacteremia, reporting risk factors for transmission and acquisition of carbapenem-resistant *P. aeruginosa*, based on multivariate analyses[a]

						Risk factors					
Study[c]	Country	Study design	Hospital setting	No. of cases	Quality score[b]	For what	Factor	Risk estimate		95%CI	*P* value
Joo, 2011(64)	Korea	cc	mix	46	4	imp	Aminoglycoside use	OR	3.60	1.39 - 7.31	0.025
							Urinary catheter	OR	3.19	1.39 - 7.31	0.006
							Carbapenem use	OR	2.87	1.26 - 6.56	0.012
							Fluoroquinolone use	OR	2.54	1.08 - 5.96	0.033
Tumbarello, 2011(65)	Italy	cc	mix	106	6	mr	Central venous catheter	OR	17.99	6.45 - 50.09	<0.001
							Previous antibiotic therapy	OR	2.79	1.10 - 7.07	0.03
							Corticosteroid use	OR	2.73	1.06 - 7.00	0.03
Yang, 2011(66)	Korea	cc	pea	7	4	mr	Admission to ICU	OR	6.82	1.3 - 35.8	0.023
Johnson, 2009(67)	USA	rc	mix	113	7	mr	Hospital-acquired BSI	OR	2.41	1.39 - 4.18	0.002
							Previous transplantation	OR	2.38	1.51 - 3.76	<0.001
							Admission to ICU	OR	2.04	1.15 - 3.63	0.015
Tam, 2007(68)	USA	cc	mix	18	4	car	Additional week of hospitalization	OR	1.25	1.04 - 1.51	0.019
Falagas, 2006(69)	Greece	cc	mix	16	4	mr	Carbapenem use	OR	9.0	2.4 - 34.3	0.001
Kang, 2005(70)	Korea	rc	mix	28	6	imp	Carbapenem use	OR	40.96	8.92 - 188.3	<0.001
							Fluoroquinolone use	OR	5.60	1.64 - 19.11	0.006
							Invasive procedure within previous 72 hours	OR	4.51	1.56 - 13.04	0.005
El Amari, 2001(71)	Switzerland	cc	mix	81	4	mr	Previous monotherapy (incl. imipenem)	OR	2.5	1.3 - 4.8	0.006

[a]OR, odds ratio; CI, confidence interval; cc, case control; rc, retrospective cohort; mix, mixed; pea, peadiatric general; imp, imipenem; mr, multi-resistant including carbapenems; car, carbapenem; BSI; bloodstream infection. [b]Newcastle-Ottowa quality assessment scale.
[c]Studies are reported by first author, year, and reference number.

Possible sources and reservoirs

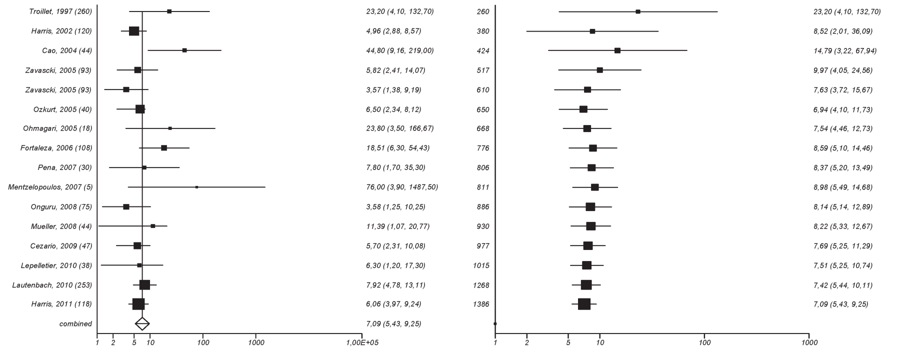
Several environmental sources and reservoirs were identified (Table 4). In some outbreaks, a single source could be identified (*e.g.*, a damaged bronchoscope or a contaminated automated urine collection machine), and the outbreak stopped after removing, repairing, or cleaning this source. However, often a reservoir was identified that was possibly not actually the main source of infection but rather a consequence of the presence of a colonized or infected patient that had led to contamination of the environment (*e.g.*, via sinks or mattresses).

Study quality

For all included studies (n= 53) a quality assessment was performed. Validation of case-control studies (n= 38) according to the Newcastle-Ottawa quality assessment scale, resulted in all studies scoring between 4 and 6 stars of a possible 10 (19). However, the validation of cohort studies (n= 12) according to the Newcastle-Ottawa quality assessment scale, resulted in scores between 6 and 7 stars of 13. The most important reasons for not awarding a star were (i) the use of hospital controls, (ii) the use of medical records, (iii) no information about follow-up of patients, and (iv) different matching criteria between studies. Validation of the two cross-sectional studies and the single study with an observational study design, all according to STROBE guidelines, resulted in scores of 15, 17 and 18 points of a total of 22, respectively (18). The main reasons not to award points in these analyses were due to the limited description of the statistical analysis in the methods and results sections of the articles.

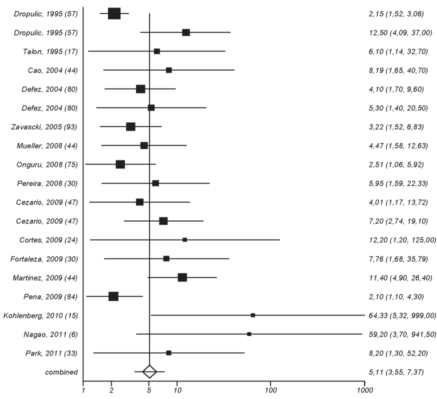
Nine meta-analyses

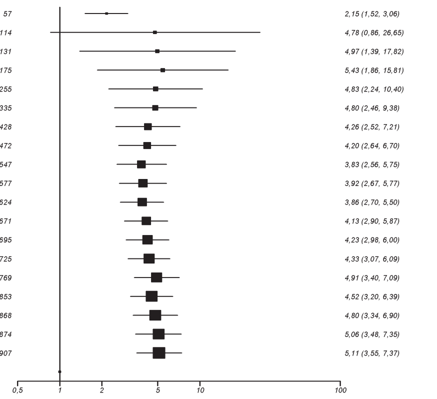
Thirty-eight of 53 studies were included in the 9 conventional meta-analyses, reporting 106 risk factors and 5 protective factors. Eight studies were excluded because only risk factors for *P. aeruginosa* bacteremia were reported. Five studies were excluded because they reported hazard ratios (n=2), risk ratios (n=2) and a prevalence ratio (n=1). Two studies were excluded because of missing confidence intervals. Thus, nine different meta-analyses were performed, plus an additional meta-analysis combining three risk factors (quinolone use, vancomycin use, and other antibiotic use). The results of the nine meta-analyses are shown in Table 5, and their forest plots are shown in Figure 2. When combining the risk factors quinolone use, vancomycin use and other antibiotic use, and performing an additional meta-analysis, the pooled odds ratio was 3.07 (95%CI= 2.27 to 4.15). Publication bias indicators showed significant results for the risk factors carbapenem use, medical devices, patient characteristics, and length of hospital stay (Table 5). For the additional meta-analysis, publication bias indicators showed no significant results. Carbapenem use (OR= 7.09, 95%CI= 5.43 to 9.25) and medical devices (OR= 5.11, 95%CI= 3.55 to 7.37) resulted in the highest pooled ORs in the meta-analyses. Therefore,



1. Conventional meta-analysis; source (number of case patients), odds ratio, 95% confidence interval.

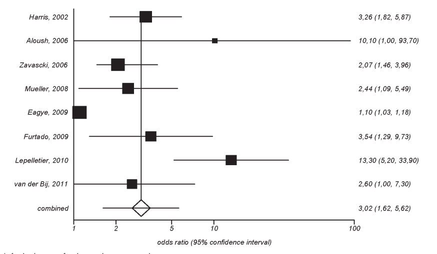
2. Cumulative meta-analysis; number of case patients, odds ratio, 95% confidence interval



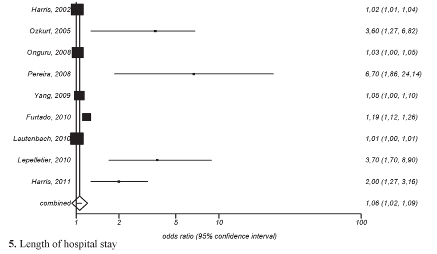


1. Conventional meta-analysis; source (number of case patients), odds ratio, 95% confidence interval.

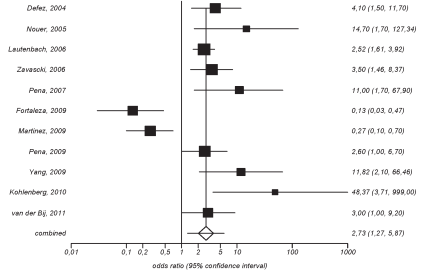
2. Cumulative meta-analysis; number of case patients, odds ratio, 95% confidence interval



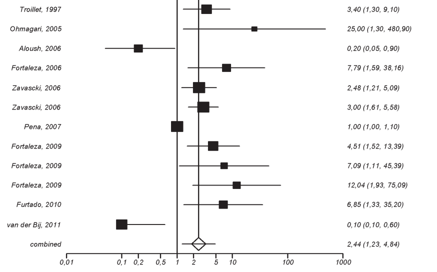
1. Admission to the intensive care unit



5. Length of hospital stay



2. Quinolone use



6. Underlying disease

Figure 2. (continued on next page)

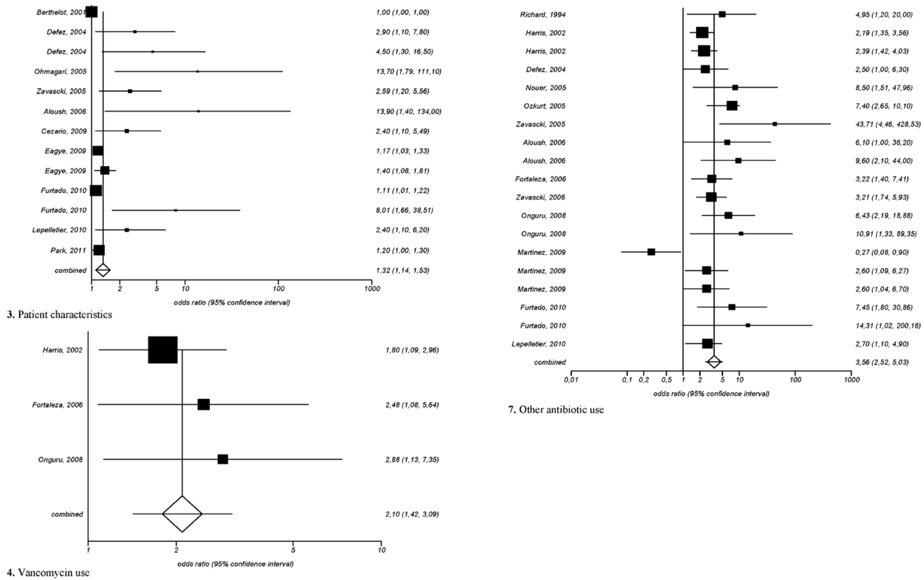


Figure 2. (a) Forest plots of conventional and cumulative meta-analyses of the risk factor carbapenem use in a random effects model, shown on a logarithmic scale. Plots: 1, conventional meta-analysis including the source given as first author and year of publication, number of case patients (in parentheses), odds ratio, and 95% confidence interval; 2, cumulative meta-analysis including number of case patients, odds ratio, and 95% confidence interval. (b) Forest plots of conventional and cumulative meta-analyses of the risk factor medical devices using a random effects model, shown on a logarithmic scale. Plots: 1, conventional meta-analysis including source and number of case patients as indicated for panel a, odds ratio, and 95% confidence interval; 2, cumulative meta-analysis including number of case patients, odds ratio, and 95% confidence interval. (c) Forest plots of individual and pooled odds ratios for seven different risk factors of transmission and acquisition of carbapenem resistant *P. aeruginosa*, using a random effects model, shown on a logarithmic scale.

cumulative meta-analyses were performed for these two risk factors. Results are shown in a forest plot (Figure 2a and 2b). For carbapenem use, all years showed statistically significant results. For the risk factor medical devices, the result was not significant when the estimate was updated the second time. When the estimate was updated for the third time, results became significant once more.

Even when excluding cohort and cross-sectional studies (n= 8) from the meta-analyses, our estimated results changed only slightly. The mean change was +0.2, ranging from -0.1 (risk factor length of hospital stay) to +1.31 (risk factor underlying diseases). All previous significant result calculations remained significant after removal of these eight studies.

Table 3. Summary of studies reporting protective factors for transmission and acquisition of carbapenem resistant *P. aeruginosa*, based on multivariate analyses.

		Risk factor results[b]				
Study[a]	**Country**	**Risk factor**	**Risk estimate**		**95%CI**	***P* value**
van der Bij, 2011(12)	The Netherlands	Cystic fibrosis as an underlying disease	OR	0.10	0.1 - 0.6	NR
Fortaleza, 2009(53)	Brazil	Quinolone use	OR	0.13	0.03 - 0.47	0.002
Martinez, 2009(47)	Spain	Quinolone use	OR	0.27	0.1 - 0.7	NR
Martinez, 2009(47)	Spain	Antipseudomonal cephalosporins use	OR	0.27	0.08 - 0.9	NR
Mammina, 2008(61)	Italy	Exclusive feeding by formula	HR	0.18	0.05 - 0.61	0.006
Mammina, 2008(61)	Italy	Length of stay >2 weeks	HR	0.10	0.00 - 0.11	0.011
Lodise Jr, 2007(37)	USA	Risk factor 1 + 2 + 3[c]	PR	0.60	0.4 - 0.9	0.02
Aloush, 2006(48)	Israel	Having a malignant disease	OR	0.20	0.05 - 0.9	0.03
Berthelot, 2001(60)	France	Duration of antibiotic treatment	OR	0.78	0.69 - 0.87	NR
Arruda, 1999(62)	Brazil	Number of antimicrobial drugs	OR	0.33	NR	0.006

[a]Studies are reported by first author, year, and reference number.
[b]OR, odds ratio; HR, hazard ratio; PR, prevalence ratio; CI, confidence interval; NR, not reported.
[c]Combination of risk factors: 1, prior receipt of mechanical ventilation for 11 days or more; 2, prior carbapenem exposure for 3 days or more; 3, prior fluoroquinolone exposure of 3 days or more.

DISCUSSION

Summary of evidence

This systematic review identified the nine most significant and most reported risk factors for the presence of carbapenem-resistant *P. aeruginosa*, and summarized the sources and reservoirs of these bacteria within the hospital environment. The nine risk factors were in order of statistical significance, (i) carbapenem use, (ii) medical devices, (iii) other antibiotic use, (iv) ICU admission, (v) quinolone use, (vi) underlying diseases, (vii) vancomycin use, (viii) patient characteristics, and (ix) length of hospital stay. The risk factor carbapenem use showed the strongest pooled odds ratio in the meta-analysis (Table 5). However, the most frequently reported risk factor was medical devices, which showed the second strongest pooled odds ratio (Table 5). The cumulative meta-analyses (Figure 2a and 2b) of these two risk factors showed that the estimate of the risk factor carbapenem use was stable for studies published after 2005. Before 2005, only a few studies published were included, and therefore the estimate fluctuated per publication. However, after 2005, the worldwide use of carbapenem increased, mainly due to the appearance of endemic and epidemic multiresistant microorganisms, especially bacteria expressing extended-spectrum beta-lactamases in ICUs (where most of the studies included in this publication were performed) (100-103). The estimate of the risk factor

Table 4. Environmental sources and reservoirs identified when searching 1,662 + 22 studies for carbapenem resistant *P. aeruginosa*

Environmental source/reservoir	Reference(s)[a]
Automated urine analyzer	Hallin, 2012(72); Nagao, 2011*(50)
Urine vol-measuring device	Sekiguchi, 2007(73)
Air-conditioning system	Pinna, 2009(74)
Sinks	Kouda, 2011(75); Babu, 2011(76); Crivaro, 2009(77); Hota, 2009(78); Mayank, 2009(79); Crespo, 2004(80); Boutiba-Ben Boubaker, 2003(81); Bertrand, 2000(82); Bert, 1998(83); Griffith, 1989(84)
Scopes	Boutiba-Ben Boubaker, 2003(81); Bronchoscope: DiazGranados, 2009(85); Sorin, 2001(86); Panzig, 1999(87); ERCP scope: Fraser, 2004(88); Endoscope: Pitten, 2001(89)
Water tap	Mentzelopoulos, 2007*(28); Bukholm, 2002(90)
Trap water	Leung, 2008(91)
Tap water	Mayank, 2009(79); Pitten, 2001(89); Bert, 1998(83)
Sanitation related contamination	Kouda, 2011(75); Panzig, 1999(87); Verweij, 1997(92)
Contaminated patient room	Kouda, 2011(75); Cezario, 2009*(24); Mayank, 2009(79); Boutiba-Ben Boubaker, 2003(81); Landman, 2002(93)
Positive cultures from nurses	Crivaro, 2009(77); Mayank, 2009(79); Vilar-Compte, 2003(94); Bertrand, 2000(82); Zheng, 1990(95)
Bed pan sterilizer	Verweij, 1997(92)
Milk bank pasteurizer	Gras-Le Guen, 2003(96)
Bottle warmer	Gras-Le Guen, 2003(96)
Stethoscope	Crespo, 2004(80)
Mechanical ventilation related	Cezario, 2009*(24); Kikuchi, 2007(97); Landman, 2002(93)
Suction apparatus	Babu, 2011(76); Mentzelopoulos, 2007*(28); Bertrand, 2000(82)
Ice packs	Bertrand, 2000(82)
Mops	Babu, 2011(76)
O_2 bottles, O_2 tubing	Mayank, 2009(79)
Contaminated Cystoscopy room	Pena, 2003(98)
Contaminated urodynamic lab	Climo, 1997(99)

[a]References are reported by first author, year, and reference number. Studies followed by an asterisk were included in the systematic review.

medical devices decreased between 1995 and 2008, and increased from 2008 to 2011. We hypothesize that the estimate increased after 2008 due to an increase in the number of medical device days during this time period. The decrease in estimate from 1995 to 2008 can be explained by the relatively few studies included in the first part of the cumulative meta-analysis.

We also looked whether studies identified environmental sources and/or reservoirs, not only in included studies, but also in those excluded. Only 31 outbreaks reported environmental sources or reservoirs (Table 4). This implies that in most epidemics a source

Table 5. Conventional meta-analyses of the different risk factors for acquisition and transmission of carbapenem resistant *P. aeruginosa*[a]

Risk factor	No. of factors	Pooled OR (random effects)	95% CI	Range of OR in individual studies	Risk of publication bias			
					Egger	*P* value	Kendall's tau	*P* value
Carbapenem use	16	7.09	5.43-9.25	3.6-76.0	1.39	0.02	0.47	0.01
Medical devices	19	5.11	3.55-7.37	2.1-64.3	2.30	<0.001	0.49	0.003
Other antibiotic use	19	3.56	2.52-5.03	0.3-43.7	1.49	0.06	0.38	0.02
ICU admission	8	3.02	1.62-5.61	1.1-13.3	2.96	0.002	0.07	0.90
Quinolone use	11	2.73	1.27-5.87	0.1-48.4	0.89	0.56	0.45	0.06
Underlying disease	13	2.44	1.23-4.84	0.1-25.0	1.34	0.06	-0.05	0.77
Vancomycin use	3	2.10	1.42-3.09	1.8-2.9	NC	NC	NC	NC
Patient characteristics	13	1.46	1.22-1.75	1.0-13.9	2.02	<0.001	0.56	0.007
Length of hospital stay	9	1.06	1.02 - 1.09	1.0 - 6.7	3.05	0.0003	0.56	0.04

[a]OR, odds ratio; CI, confidence interval; NC= not calculated because there were too few strata

or reservoir is not identified, not reported, or not searched for. If carbapenem-resistant *P. aeruginosa* was identified in the innate environment, it was often unclear or not proven that the presumed reservoir was indeed the primary source of infection. In fact, sinks are most frequently reported and thought to be the main reservoir of carbapenem-resistant *P. aeruginosa* in hospitals (Table 4).

It was remarkable that in three of the studies included in the analyses, vancomycin use was identified as a risk factor for acquiring carbapenem-resistant *P. aeruginosa* (26, 29, 34). All three articles hypothesize that this may have been due to antibiotic selection pressure, with the reduction or elimination of competing Gram-positive bacteria post-antibiotic treatment having facilitated the colonization of the skin or gastrointestinal tract of patients with Gram-negative bacteria, including *P. aeruginosa*.

Limitations and strengths

The limitations of this study are mostly related to the heterogeneity of the studies included in the analyses. From our investigations, it was obvious that every reported outbreak generally involved different target populations, microbial sources, microbiological methods, active surveillance to find cases, and methods for identifying whether there was transmission or endogenous selection.

A limitation of the meta-analyses was the diverse models used by the different studies when performing multivariate regression analysis. Also, in almost all cases, the models used were not described. This problem is already known to be a major limitation of studies utilizing meta-analyses, as "confounders" can seriously alter the combined estimate. We know that the confounders that are adjusted for are different, whereas in meta-analysis we require them to be the same. However, from a clinical point of view

they have to be different, because every situation (selection or transmission), outbreak or level of endemicity is different. Even if we knew every specific model used, it would not solve the problem of heterogeneity. For all of these reasons, we used a random effects model.

The statistical results may also have been influenced by publication bias, and the Egger and Kendall's tau publication bias indicators showed significant results for several risk factors (Table 5). However, the authors tried to include as many studies as possible, despite differences in language or size of the outbreak. Nevertheless, a full-text article was not available for 20 studies, data were incomplete for two studies, and there may also be unpublished studies that we could not access. However, this number of studies is small relative to the number of studies included after title/abstract selection (n=256), so its influence on our results is likely to be limited.

We excluded studies including only patients with CF. These patients are chronically infected with *P. aeruginosa*, with strains acquired mostly in the community, and are a different patient population from the population of our interest (104).

Previously, a review by Falagas and Kopterides (2006) also identified risk factors associated with *P. aeruginosa* infection (15). Several of the current risk factors observed (Table 1) match the risk factors observed by Falagas and Kopterides. However, in contrast to the review by Falagas and Kopterides, the current study focuses on carbapenem resistance and includes only studies that analyzed data using a multivariate model. Also, almost one-half of the studies included in this publication were published after 2006. Finally, we also included all studies that indicated a source or reservoir of their *P. aeruginosa* outbreak, and we conducted conventional and cumulative meta-analyses, results that are not available in the review by Falagas and Kopterides.

Conclusions and implications

This systematic review shows that the risk factors for *P. aeruginosa* infection and transmission are diverse. However, the use of carbapenem antibiotics was the most significant risk estimate from this meta-analysis, which highlights the importance of antibiotic stewardship in controlling *P. aeruginosa* outbreaks. During an outbreak involving one or more (clonal) strains, the use of these antibiotics could be a risk factor for acquisition of that clonal strain(s), by making the patient more vulnerable to colonization or infection. Importantly, antibiotic use is a risk factor that can be influenced in order to reduce the chance of outbreaks occurring. Another risk factor is the use of medical devices and reduction of device days. The use of medical devices and the number of device days are also the most frequently reported risk factors resulting from our meta-analyses. The increased use of medical devices, and for longer periods of time, means that patients are becoming more vulnerable to acquiring MDR *P. aeruginosa* (105). On the other hand,

other important risk factors for outbreaks involving MDR *P. aeruginosa* such as patient characteristics, underlying diseases or ICU admission, cannot be easily influenced.

This systematic review also shows that it is difficult to identify the actual source of *P. aeruginosa* outbreaks. Therefore, basic infection prevention measures remain very important. For example, contact isolation of patients and strict compliance with hand hygiene measures remain the major steps necessary to stop further transmission of outbreak isolates. This is important whether or not an assumed or proven exogenous source is responsible for the outbreak.

We believe that it is important that prospective studies relating to outbreaks of carbapenem-resistant *P. aeruginosa* report on sources and reservoirs of infection, and that analysis of any data be performed using a multivariate statistical model. This information is extremely valuable with respect to planning future research and control measures for antibiotic-resistant *P. aeruginosa*. It is also very important for authors to genetically type strains associated with infection in order to identify clonal clusters of isolates. This data allows the infectious disease specialist to determine whether infection and spread are related to selection (risk factor carbapenem/antibiotic use) or transmission (*e.g.*, risk factor medical devices). The systematic review and meta-analysis published here shows the nine most important risk factors for the presence of carbapenem-resistant *P. aeruginosa* bacterial isolates among hospitalized patients. The identification of these risk factors is useful in controlling future outbreaks of by these organisms. In this case, risk factors such as antibiotic use and high numbers of device days have to be reduced or eliminated in order to help prevent the appearance and spread of carbapenem-resistant *P. aeruginosa*. In this study, carbapenem use was identified with the highest pooled odds ratio. Therefore, use of this class of antibiotics especially should be reduced.

Finally, it is important to decrease the use of antibiotics, especially the use of carbapenems, in order to help prevent resistant *P. aeruginosa* outbreaks. In addition, it is highly recommended that an infectious disease consultant with a broad view on the prevalence of MDR bacteria and knowledge of the most recent guidelines for antibiotic use in the hospital concerned be consulted. Indeed, studies have shown that consultation with an infectious disease consultant significantly increases the correct administration of microbiologically correct antibiotic therapy (106-108).

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Chapter 2.3

Clinical and molecular epidemiology of extended-spectrum beta-lactamase-producing *Klebsiella* spp.: a systematic review and meta-analyses

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ABSTRACT

Healthcare-related infections caused by extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella* spp. are of major concern. To control transmission, deep understanding of the transmission mechanisms is needed. This systematic review aimed to identify risk factors and sources, clonal relatedness using molecular techniques, and the most effective control strategies for ESBL-producing *Klebsiella* spp. A systematic search of PubMed, Embase, and Outbreak Database was performed. We identified 2,771 articles from November 25th, 1960 until April 7th, 2014 of which 148 were included in the systematic review and 23 in a random-effects meta-analysis study. The random-effects meta-analyses showed that underlying disease or condition (odds ratio [OR] = 6.25; 95% confidence interval [CI] = 2.85 to 13.66) generated the highest pooled estimate. ESBL-producing *Klebsiella* spp. were spread through person-to-person contact and via sources in the environment; we identified both monoclonal and polyclonal presence. Multi-faceted interventions are needed to prevent transmission of ESBL-producing *Klebsiella* spp.

INTRODUCTION

Healthcare-related infections (HRIs) are a major clinical problem worldwide. In 2011, the World Health Organization (WHO) reported that in a mixed patient population the pooled HRI-prevalence was 10.1% in low- and middle-income countries and 7.6% in high-income countries (1). Prolonged hospital stay, higher costs, increased antimicrobial resistance, and risk of potentially life-threatening conditions indicate the enormous burden of HRIs (2). Further, we are facing HRIs caused by multidrug-resistant gram-negative bacteria (MDR-GNB) without a parallel progression of the novel antibiotic classes (3).

Klebsiella spp. have been recognized as the most frequent cause of MDR-GNB outbreaks, particularly after the emergence of the extended-spectrum beta-lactamase (ESBL) enzymes (4, 5). As a result, infections in hospitalized patients with this ESBL-producing *Klebsiella* spp. have raised public concern due to the clinical outcomes and limited antibiotic options (6). Patients whose care requires devices, and patients who are identified with multiple antibiotic-resistant strains in the intensive care unit (ICU) are at highest risk to acquire an infection with an ESBL-producing *Klebsiella* spp. (7, 8). High discriminatory subtyping methods are beneficial to determine clonality of the outbreak strains with pulsed-field gel electrophoresis (PFGE) as the well-known 'gold standard' for molecular epidemiological studies and for current clinical use (9).

It requires deep understanding of all outbreaks to optimally control transmission of ESBL-producing *Klebsiella* spp. (10). Recent guidelines about the management of MDR-GNB underscore the need of well-managed and multi-faceted interventions (11). Therefore, it is necessary to investigate the transmission dynamics and the risk factors for hospital outbreaks. This systematic review aimed to answer the following four questions. First, what are the risk factors for the presence of ESBL-producing *Klebsiella* spp.? Second, what are the main sources and reservoirs for this microorganism? Third, how can we identify the transmission patterns and the clonal relatedness among isolates from patients who acquired ESBL-producing *Klebsiella* spp.? Fourth, what are the most effective control strategies for ESBL-producing *Klebsiella* spp.?

MATERIALS AND METHODS

This systematic review and meta-analysis followed the guidelines outlined in the PRISMA statement (supplement 1)(12).

Search strategy and selection criteria

We searched PubMed, Embase, and the Outbreak Database (until April 7th, 2014) to identify studies which examined the transmission of multidrug-resistant (MDR) *Klebsi-*

ella spp., identified potential risk factors, described modes of transmission, described laboratory methods used for the identification, and described the effective interventions to prevent transmission of MDR *Klebsiella* spp. with using the terms as applied in supplement 2. The search strategy was not limited by language, date of publication, country, study design, enzyme type, or patient characteristics. We excluded studies about: 1) pathogenesis, validation of molecular techniques, drug options, cost, 2) non-human studies, 3) studies only about carriers, health-care workers (HCWs), or family members, 4) studies only about environmental contamination, 5) case report with no statement on transmission, 6) non-hospital studies, 7) letters, editorials, communications, weekly reports, and reviews. However, we also searched the eligible citations of all relevant reviews. TCH initiated full searches and AFV independently repeated the search for a 5 percent subset of articles.

Data extraction

We first screened all articles based on titles and abstracts and then we subsequently assessed the articles in full text according to the inclusion and exclusion criteria. TCH initiated the screening and extracted the data with help of AFV and MCV. To retrieve articles that could not be found in full-text, we contacted first authors or corresponding authors of 80 publications. We also contacted the authors of 16 publications to obtain missing information about associated factors and cluster analyses. We defined the categories of MDR *Klebsiella* spp. as ESBL, possible ESBL and non-ESBL. We used the ESBL definition according to group 2b Bush criteria (13). We found several articles that showed resistance to cephalosporins before the term "ESBL" was established in 1989 (14). These studies were included as being 'ESBL'. Ultimately, we only focused on studies about ESBL-producing *Klebsiella* spp. within one hospital.

Data analyses

We included articles related to ESBL-producing *Klebsiella* spp. that described the factors associated with the presence or acquisition of ESBL-producing *Klebsiella* spp. using a multivariate model. We took into account studies that have suggested and proven the sources of ESBL-producing *Klebsiella* spp. using molecular typing techniques. However, we also included studies that suggested the potential reservoirs. In addition, we included studies about the associated factors for mortality related to ESBL-producing *Klebsiella* spp.

In order to assess clonal relatedness and transmission patterns of ESBL-producing *Klebsiella* spp., studies that only performed phenotypic typing methods were excluded and studies that did use molecular typing were included. We merged studies that used polymerase chain reaction (PCR)-based techniques for typing. We assessed the result of molecular typing methods and calculated the total number of identified patterns.

We defined a cluster as ≥ two similar patterns of ESBL-producing *Klebsiella* spp. isolates. Likewise, a unique isolate was defined as a single pattern. The term monoclonal presence referred to a single cluster and the term polyclonal presence referred to ≥ two clusters. We calculated the total number of patterns, the clusters including cluster sizes, and the single patterns. If the information was available, we performed the cluster analyses based on the number of patients, otherwise on the number of isolates. We also reviewed studies about infection control strategies and prevention programs. We assessed the standard interventions possibly combined with additional control strategies, and reported which were most successful strategies to stop transmission. We compiled data from two studies that were presented in four publications in the result section (15-18).

Statistical analysis

We combined all associated factors that reported an odds ratio (OR) and a 95% confidence interval (95% CI) into ten different categories: 1, medical devices (*e.g.*, mechanical ventilation, intra-vascular devices); 2, prior cephalosporin exposure; 3, prior quinolone exposure; 4, prior other antibiotic exposure; 5, prior antifungal exposure; 6, length of hospital stay; 7, patient characteristics (*e.g.*, age); 8, underlying disease or condition (*e.g.*, malignant disease); 9, medical procedures (*e.g.,* surgical intervention); 10, other (*e.g.*, exposure to the hands of HCWs). Studies reporting associated factors for mortality were excluded. Random-effects meta-analyses were performed for all categories except the "prior antifungal exposure", "medical procedures" which had less than three factors and "other" that comprised many various factors. Lytsy *et al.*, used three different models of multivariable analyses to find reliable estimates for the most important variables. However, we chose to only include model 1 in our meta-analyses (18). We applied the method of DerSimonian and Laird and meta-analyses were performed using StatsDirect statistical software (StatsDirect, Version 2.8.0, Altrincham, StatsDirect Ltd, 2013) (19). We considered P values <0.05 as statistically significant. Bias assessment plots were constructed to explore publication bias using the Egger and Begg-Mazumbdar (Kendall's tau) indicators (19, 20).

Study quality

The methodological quality of all studies in the random-effects meta-analyses was assessed using the strengthening the reporting of observational studies in epidemiology (STROBE) guidelines (Table A and Table B in supplement 3) or the Newcastle-Ottawa quality assessment scale (Table A and Table C in supplement 3), based on the study design (21, 22). Furthermore, the methodological quality of all molecular epidemiological studies in cluster analysis was assessed by the strengthening the reporting of molecular epidemiology for infectious disease (STROME-ID) guidelines (Table D in supplement 3) (23). However, the study quality was not considered as an exclusion criterion.

RESULTS

Description

We identified a total of 5,608 articles as potentially relevant when using our search strategy (Figure 1). Of these, 835 articles met the eligibility criteria and 25 articles were retrieved from citations of interesting reviews. We received 45 full-text articles from 80 authors. We got three further responses to the information requests that were sent to the authors of 17 articles. We ultimately included 148 articles in this systematic review (Figure 1). Five articles were written in Spanish, one article was written in French, one article was written in Turkish and 141 articles were written in English. The non-English articles were translated prior to data extraction. Most studies were conducted in Europe (39.9%; n= 59) particularly in France (n= 16), followed by Asia (18.9%; n= 28), North America (16.9%; n= 25), Africa (9.5%; n= 14), South America (10.8%; n= 16), multiple regions: Europe and Asia (3.4%; n= 5) and Australia (0.7%, n= 1) with 49 countries in total. All

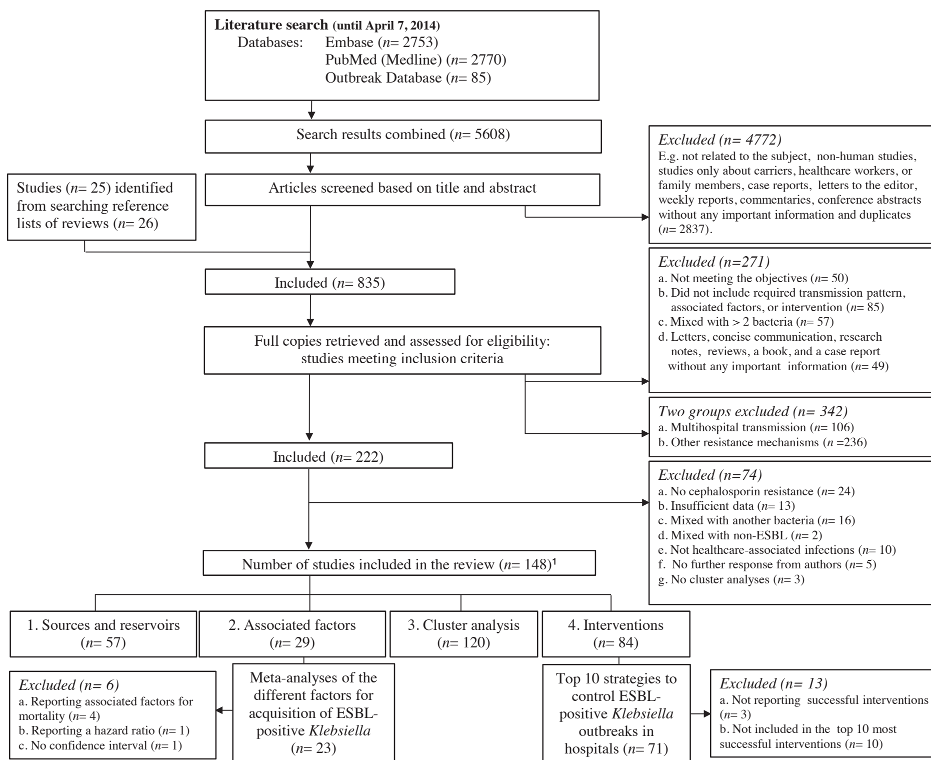


Figure 1. Flow diagram of study selection for the systematic review and random-effects meta-analyses on extended-spectrum beta-lactamase-producing *Klebsiella* spp. [1]Number of studies included in the review comprise the sources and reservoirs, associated factors, cluster analysis and successful interventions. [2]No response was obtained from the first and/or corresponding authors for the requested article.

studies were published between 1991 and 2014 with study periods of less than a month up to seven years. Thirty-one studies (20.9%) indicated the suspected or identified index case. The study population was predominated by adult patients (45.3%, n= 67), followed by neonates (33.1%; n= 49), pediatric patients (5.4%; n= 8), and mix groups including the studies located in hospitals but which did not mention the study population (16.2%; n= 24). Seventy-six percent of all studies took place at the ICU (n=113). These studies can be further divided in neonatal ICU (38.1%; n= 43), pediatric ICU (3.5%; n= 4), adult ICU (48.7%; n= 55) and mix ICU units (9.7%; n= 11). However, fifty-two studies were located both at ICU and non-ICU.

Associated factors

We identified 26 studies reporting associated risk factors with a statistically significant OR above one (Table 1) and seven studies reporting associated protective factors with a statistically significant OR below one (Table 2) for the presence of ESBL-producing *Klebsiella* spp. In addition, four studies identified the associated factors for bloodstream infections caused by ESBL-producing *K. pneumoniae* (Table 1). In general, prior antibiotic exposure was the most common associated factor in all studies. Four studies reported associated factors for mortality (24-27). It was published in these four studies that the presentation with septic shock had the highest odds ratio (205.99) (24).

Table 1. Associated risk factors for the presence of ESBL-producing *Klebsiella* spp. based on multivariate analyses.

Associated risk factor	No. of factors	RE	RE or RE range	No. of cases (range)	Studies
Underlying disease or condition	17	OR	1.04 – 60.60	26 - 292	(28); (29); (30); (31)(2x); (18)(6x); (32); (33)(4x); (34)
Other antibiotic exposure	15	OR	1.55 – 95.21	10-292	(35); (24)(a); (36); (30); (31); (37)(a); (38); (39); (40); (41)
	1	HR	4.60	206	(42)
Length of hospital stay	11	OR	1.05 – 12.60	18 - 80	(43); (29); (37)(a); (44); (38); (45); (18)(3x); (46)(a)
	1	HR	1.26	206	(42)
Medical devices	9	OR	2.11 – 5.23	18 – 292	(47)(2x); (24)(a); (36); (38); (45); (34)(3x)
Prior cephalosporin exposure	9	OR	4.51 – 7.60	17 – 88	(28); (36); (48)(a); (18)(2x); (32)(3x); (46)(a)
Other[b]	8	OR	1.66 – 9.30	18 – 94	(49); (43); (31); (38)(2x); (50); (32); (33)
Patient characteristics	3	OR	1.14 – 13.10	10 – 48	(35); (37)(a); (16)
	1	HR	1.57	206	(42)
Prior quinolone exposure	3	OR	2.86 – 25.37	30 - 78	(36); (41); (46)(a)
Medical procedures[b]	2	OR	9.34 – 10.35	52 - 60	(24)(a); (40)
Prior antifungal exposure[b]	2	OR	5.3 – 12	204	(30)(2x)

Abbreviations: RE, risk estimate, [2x], [3x], [4x] or [6x], two, three, four or six different factors per reference.
[a] Bloodstream infections.
[b] This category was not included in a random-effects meta-analysis study.

Table 2. Associated protective factors for the presence of ESBL-producing *Klebsiella* spp. based on multivariate analyses.

Associated protective factor	No. of factors	No. of patients	RE	RE or RE Range	Studies
Medical devices	2	206	HR	0.22 – 0.52	(42)
Prior penicillin+ β lactamase inhibitor exposure[a]	2	88	OR	0.16 – 0.27	(32)
Others[b]	2	27 – 292	OR	0.22 – 0.50	(39); (34)
Prior antibiotic exposure[a]	1	54	OR	0.003	(44)
Prior carbapenem exposure[a]	1	206	HR	0.22	(42)
Age[c]	1	47	OR	0.95	(36)
Prior cephalosporin exposure	1	204	OR	0.1	(30)

Abbreviations: RE, risk estimate; HR, hazard ratio; OR, odds ratio.
[a] This factor was classified in the category of prior antibiotic exposure for a random-effects meta-analysis study.
[b] This category was not included in a random-effects meta-analysis study.
[c] This factor was classified in the category of patient characteristics for a random-effects meta-analysis study.

Eight random-effects meta-analyses

Twenty-three studies were included in the seven random-effects meta-analyses, reporting 54 associated risk factors with a statistically significant OR above one and five associated protective factors with a statistically significant OR below one (Figure 1 and Table 3). The category of underlying disease or condition (OR = 6.25; 95% CI = 2.85 to 13.66) and prior cephalosporin exposure (OR = 4.65; 95% CI = 2.83 to 7.65) generated the highest pooled estimates (Figure 2). The publication bias indicators showed no significant results (Table 3).

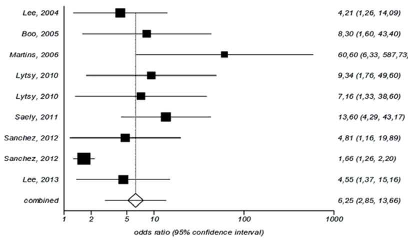
Sources and reservoirs

Fifty-seven studies identified environmental sources and/or surrounding reservoirs for ESBL-producing *Klebsiella* spp. (Table 4). Contaminated sinks were the most reported sources in the environment (13.8%; n= 4) whereas the patients were the main reservoirs (48.9%; n= 23), followed by the hands of HCWs (25.5%; n= 12). Interestingly, one study showed food as a transmission source for ESBL-producing *K. pneumoniae* (50).

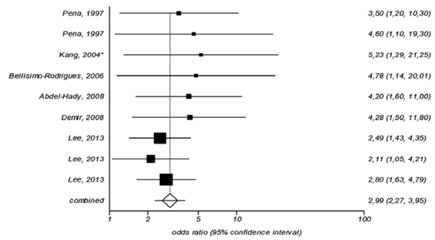
Cluster analyses

One hundred-twenty studies described the molecular methods used to type ESBL-producing *Klebsiella* spp. and the analyses of genetic similarity (Table 5). In particular for ESBL-producing *K. pneumoniae*, ninety-two studies used PFGE and 16 studies used PCR-based techniques which were predominated by enterobacterial repetitive intergenic consensus sequence – polymerase chain reaction (ERIC-PCR) in seven studies. Multilocus sequence typing (MLST) was performed in seven studies as well as random amplified polymorphic DNA (RAPD). Nineteen studies performed more than one mo-

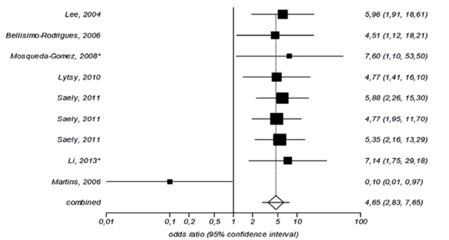
1. Underlying disease or condition



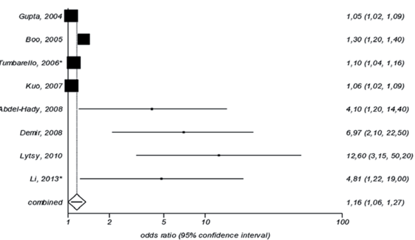
5. Medical devices



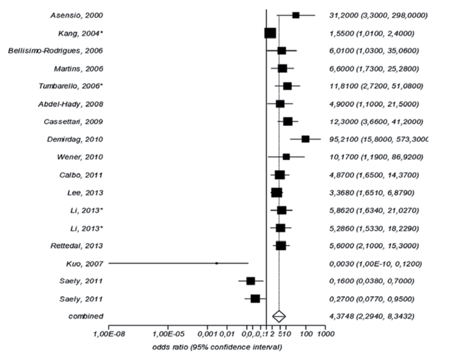
2. Prior cephalosporin exposure



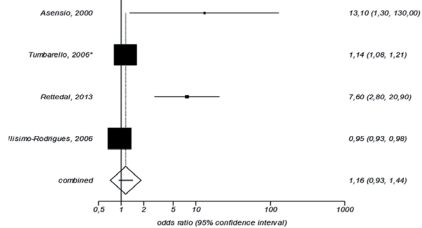
6. Length of hospital stay



3. Prior other antibiotic exposure



7. Patient characteristics



4. Prior quinolone exposure

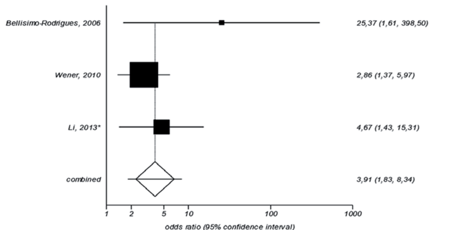


Figure 2. Forest plots of random-effects meta-analyses: individual and pooled odds ratios for associated risk factors and associated protective factors for presence of ESBL-producing *Klebsiella* spp. among patients in hospitals. *Bloodstream infections

Table 3. Random-effects meta-analyses of the different associated risk factors and associated protective factors for the presence of ESBL-producing *Klebsiella* spp. among patients in hospitals.

Associated factor	No. of factors[a]	Pooled OR	95% CI	Range of OR in individual studies	Risk of publication bias: Kendall's tau	*P* value	Egger	*P* value
Underlying disease or condition	9	6.25	2.85 – 13.66	1.66 – 60.60	0.39	0.18	2.61	< 0.01
Prior cephalosporin exposure	9	4.65	2.83 – 7.65	0.10 – 7.60	< 0.01	0.92	- 2.26	0.14
Prior other antibiotic exposure	17	4.38	2.29 – 8.34	0.003 – 95.21	0.16	0.39	1.51	0.16
Prior quinolone exposure	3	3.91	1.83 – 8.34	2.86 – 25.37	NC	NC	NC	NC
Medical devices	9	2.99	2.27 – 3.95	2.11 – 5.23	0.33	0.26	1.53	< 0.01
Length of hospital stay	8	1.16	1.06 – 1.27	1.05 – 12.60	0.55	0.08	3.25	< 0.01
Patient characteristics	4	1.16	0.93 – 1.44	0.95 – 13.10	< 0.01	0.75	4.11	0.18

Abbreviations: OR, odds ratio; CI, confidence interval; NC, not calculated because there were too few strata.
[a] Only studies included that reported 95% CI and *P* values

lecular method to analyze clusters. The average number of patients in the 120 studies was 22.5 (ranging from 1 to 295 patients). The median range of the number of patterns was 1 to 10 (median range of cluster-size medians from 1 to 15). In 111 studies that provided both number of clusters and the cluster size based on the number of patients, the median number of clusters was 2 ranging from 0 to 15 and the median of the cluster-size medians was 6 with a range of 0 to 81. In particular for *K. pneumoniae*, the median number of clusters was 2 and the median of cluster-size medians was 6. Further, the median number of clusters and the median of the cluster-size medians were 0 for *K. oxytoca*. Three surveillance studies reported an identical pattern indicating the clonality of identified ESBL-producing *Klebsiella* spp. strains in hospitals. In particular for the outbreak events, 43 studies showed monoclonal and 34 studies showed polyclonal presence of ESBL-producing *Klebsiella* spp. among patients within the hospital.

Effective interventions

The identification of ESBL-producing *Klebsiella* spp. transmission in hospitals should be followed by infection control strategies. However, not all studies provided detailed information regarding the interventions. We identified 84 studies that described the interventions during the study period. Twenty-eight studies reported the standard interventions that did not succeed. All but three studies described the additional and/or successful strategies to prevent the spread of ESBL-producing *Klebsiella* spp. in hospitals. Ultimately, we presented the top ten strategies to control ESBL-producing *Klebsiella* spp.

Table 4. Environmental sources and reservoirs for ESBL-producing *Klebsiella* spp., identified from $n = 57$ studies.

Reservoirs or Sources	No of studies	Studies
Innate Environment		
Bottles	1	(51)
Door handles, a siphon and a table	1	(52)(a)
Entire unit (Intensive Care Unit)	2	(53);(54)
Hospital kitchen-screened surfaces	1	(50)(a)
Incubator	2	(55)(a); (38)(a)
Liquid soap	2	(56)(a); (57)(a)
Mask	1	(58)(a)
Moist surfaces near sinks and faucets	1	(59)(a)
Roll boards in operating rooms	1	(60)(a)
Room surface	2	(55)(a); (38)(a)
Single use products		
A. Intravenous medication or solution (re-used repeatedly)	2	(61)(a); (62)(a)
B. Intravenous glucose preparation (multiple-dosed)	1	(51)
C. Oxygen saturation probes (re-used repeatedly)	1	(63)(a)
Sinks	4	(64)(a); (55)(a); (65)(a); (66)(a)
Suction pump located in the room of an infected patient	1	(67)(a)
Suction tube	1	(38)(a)
Thermometers	2	(63)(a); (68)
Two water reservoirs from humidifiers	1	(69)(a)
Ultrasonography coupling gel container of the emergency room	1	(70)(a)
Weighing scale machine for babies	1	(71)(a)
Human		
Transient Hand Carriage	1	(72)
A. Patients	23	(73); (74)(a); (64)(a); (75)(a); (76)(a); (49)(a); (77)(a); (78)(a); (79)(a); (80); (35)(a); (81)(a); (82)(a); (83)(a); (84)(a); (85)(a); (86)(a); (30)(a); (87); (88)(a); (89)(a); (90)(a); (91)(a)
B. Health Care Workers	4	(82)(a); (92)(a); (90)(a); (93)
Food handlers	1	(50)(a)
Handholding due to work overcharge	1	(94)
Hands	11	(95); (82); (68); (55)(a); (38)(a); (45)(a); (54); (96)(a); (67)(a); (57)(a); (97)
Artificial nails	1	(43)(a)
Onychomycosis	2	(98)(a); (99)&(39)(a)
C. Family (Mother to Child)		
Breast milk	2	(63)(a); (15)(a)
Peripartum colonization of neonates	1	(70)(a)

[a] This study proved the source or reservoir with use of a molecular typing technique.

Table 5. Summary of studies (n=120) reporting cluster analyses on identified ESBL-producing *Klebsiella* spp. using typing techniques.

Methods	No. of Strains: Median (Range)	No. of Patterns: Median (Range)[a]	No. of Clusters: Median (Range)	Cluster Size: Median of Medians (Range of Median)	Unique Isolates Median (Range)	No of Studies	Studies
Based on the number of patients							
A. K. pneumoniae							
PFGE	25 (2 - 235)	4 (1 - 55)	2 (0 - 15)	6 (0 - 81)	2 (0 - 45)	92	(72)(b,c); (100)(b,c); (101); (75)(e); (76); (70)(b,c); (102)(b,d,g); (49)(b,c); (78)(b,c); (79); (80)(b,c); (103)(b,d,e); (104)(b,d); (82)(b,c); (105); (56)(b,c); (106)(b,c,l,m); (107); (81)(b,c); (69)(b,c); (108)(b,c); (83)(b,c); (109)(b,d); (94)(b,d); (110)(b,c); (111)(b,c,f); (112); (42)(b,d); (113); (92)(b,c); (114)(b,d); (43)(b,c); (115)(g,k,l,m); (116); (98)(b,c); (68)(b,d); (84)(b,d); (85)(b,d); (115)(g,k,l,m); (116); (98)(b,c); (68)(b,d); (84)(b,d); (85)(b,d); (25)(m); (55)(e); (71)(b,c); (30); (117)(b,c); (118); (119); (120)(g); (45); (121); (88)(b,d); (17) and (18)(b,c,e,i); (122)(b,d); (48)(g); (123)(b,d); (124)(b,c); (89)(b,d,e,l,m); (125)(b,d); (126)(e); (27)(e); (90)(e,m); (127); (96)(b,c); (128); (40)(l,m); (129)(b,d); (130); (131); (132)(b,d); (67)(b,d); (50)(b,d); (133)(g); (134); (135)(b,c); (62)(b,c,m); (136)(b,d); (137); (138); (15) and (16) (b,d,i); (33)(b,c); (66)(b,c); (139)(b,c); (140); (141)(l); (142)(b,c); (143)(b,d); (144); (145)(b,c,e); (146)(b,d,g,k,l,m)
PCR	24 (4 -295)	4 (1 - 125)	1 (0 - 10)	5.5 (1 - 87)	2.5 (0 - 21)	16	(147)(b,d,e); (76); (148)(b,c); (149)(b,c); (110)(2x,b,c,n); (150)(b,c); (30); (71)(b,c); (151)(b,c,g); (152)(m); (153)(b,d); (91)(b,c); (93)(e,g); (154)(e); (155)(g); (143)(b,c)
RAPD	18 (8 - 40)	3 (1 - 17)	1 (1 - 4)	11 (7 - 19)	1 (0 - 15)	7	(77)(b,c); (35)(b,c); (63)(b,c); (104)(b,d); (108)(b,c); (92)(b,c); (156)(b,d)
MLST	21.5 (1 - 46)	2.5 (1 - 15)	2 (0 - 3)	2 (0 - 19)	1 (0 - 13)	7	(157); (127); (129)(h); (15) and (16)(b,d,i); (155)(g); (143)(b,d,l); (158)(b,f)
Ribotyping	18 (8 - 57)	5.5 (1 - 15)	2 (1 - 6)	6 (2 - 14)	2.5 (0 - 9)	6	(95)(b,d); (64)(b,c); (159)(b,d); (147)(b,d,e); (68)(b,d); (121)
ME-AFLP	8 (-)	1 (-)	1 (-)	8 (-)	0 (-)	1	(60)(b,c)
MLEE	19 (-)	11 (-)	1 (-)	9 (-)	10 (-)	1	(160)(b,c)
B. K. oxytoca							
PFGE	2 (1 - 101)	1 (1 - 27)	1 (0 - 3)	1 (0 - 8)	1 (0 - 24)	7	(104)(b,c); (115)(g); (118); (119); (122)(b,c); (132)(b); (65)(b,d,l)
RAPD	2 (-)	1 (-)	1 (-)	2 (-)	0 (-)	1	(104)(b,c)
Based on the number of clinical strains							
A. *K. pneumoniae*							

Table 5. Summary of studies (n=120) reporting cluster analyses on identified ESBL-producing *Klebsiella* spp. using typing techniques. (continued)

Methods	No. of Strains: Median (Range)	No. of Patterns: Median (Range)[a]	No. of Clusters: Median (Range)	Cluster Size: Median of Medians (Range of Median)	Unique Isolates Median (Range)	No of Studies	Studies
PFGE	24.5 (18 - 31)	2 (2 - 2)	2 (1 - 2)	11 (9 - 13)	0 (0)	3	(161)(b,c,m); (158)(b,f); (162)(b,d,f)
RAPD	37 (-)	28 (-)	6 (-)	2 (-)	22 (-)	1	(86)(f)
B. K. oxytoca							
PFGE	13 (3 - 23)	5 (1 - 9)	1 (0 - 2)	3.5 (0 - 7)	1 (1 - 1)	2	(59)(f); (158)(b,f)
Based on the number of patients or clinical strains + family and/or environmental strains							
PFGE	49 (30 - 49)	10 (2 - 25)	7 (2 - 7)	7 (2 - 15)	3 (0 - 18)	3	(156)(b,d); (163)(b,d,e); (52)(b,d,f)
PCR	39 (30 - 48)	9.5 (2 - 17)	3 (2 - 4)	15 (-)	6.5 (0 - 13)	2	(37)(j,l); (52)(b,d,f)
Raman spectroscopy	30 (-)	2 (-)	2 (-)	15 (-)	0 (-)	1	(52)(b,d,f)

Abbreviations: PFGE, pulsed-field gel electrophoresis; PCR, polymerase chain reaction (ERIC-PCR, enterobacterial repetitive intergenic consensus sequence; AP-PCR, arbitrarily primed-PCR; REP-PCR, repetitive sequence-based PCR; BOX-PCR; IRS-PCR, infrequent-restriction-site PCR; RFLP-PCR, restriction fragment length polymorphism); RAPD, random amplified polymorphic DNA; MLST, multi-locus sequence typing; ME-AFLP, multi-enzyme amplified fragment length polymorphism; MLEE, multilocus enzyme electrophoresis; (-), Only 1 study was reported; [2x], This study performed two rounds of ERIC-PCR on a different dataset.
[a]We assessed the result of molecular typing methods and reported the total number of identified patterns as number of patterns; [b] Outbreak; [c] Monoclonal; [d] Polyclonal; [e] The number of patients were not literally written; [f] Number of clinical isolates for cluster analysis due to available data or different time points of the isolates; [g] Different presentation of data between text and figures on papers, hence we used data from the figures (except Liu 1998, Lavigne 2004, Wu 2006 and Cassettari 2009); [h] MLST was only performed on the index patient; [i] Two publications used same isolates, hence, we combined the data; [j] This study was performed on 48 patient samples that consisted of 46 healthcare-related infections and 2 unidentified samples; [k] Data of number of patterns was not available; [l] Data of cluster size was not available; [m] Data of unique isolates was not available; [n] Data of unique isolates on the second round was not available.

Table 6. Top 10 strategies to control ESBL-producing *Klebsiella* spp. outbreaks in hospitals.

No.	Intervention	No. of studies	Studies
1	Reinforcement of hand hygiene	33	(95); (171); (72); (64); (77); (78); (172); (80); (148); (159); (35); (82); (56); (108); (109); (161); (94); (43)(a); (68); (84); (150); (51)(a); (55); (86)(a); (71); (30); (87); (54)(a); (67);(57)(a); (15); (142); (145)
2	Control of antibiotic use	24	(171); (49); (78); (148); (35); (56); (109); (161); (94); (28); (55); (71); (30); (37); (87); (45); (88)(a); (67); (57)(a); (91); (93); (158); (162); (145)
3	Strict hygienic practices	18	(64); (78); (82); (94); (59)(a); (84); (173); (86)(a); (88)(a); (124); (67); (134); (15); (33)(a); (141)(a); (142)(a); (16); (145)
4	a. Screening programs with an active microbiological surveillance	15	(35); (82); (63)(a); (84); (45); (88)(a); (124); (132); (67); (91); (15); (33)(a); (66)(a); (141)(a); (162)
	b. Cohorting of patients	15	(171); (72); (172); (35); (82); (150)(a); (55); (87); (88)(a); (54)(a); (124); (15); (141)(a); (162); (145)
5	Single-use equipment	14	(95); (70)(a); (80); (148); (159); (35); (109); (68); (84); (61); (67); (134); (62)(a); (15)
6	Barrier precautions	12	(100); (78); (82); (113); (150)(a); (60); (71); (30); (67); (134); (33)(a); (145)
7	Patient isolation	11	(64); (77); (148); (35); (82); (94); (87); (67); (52)(a); (15); (142)(a)
8	Good cooperation between department and infection control team	9	(35); (61); (55); (87); (88)(a); (124); (135); (142)(a); (145)
9	Personnel educational programs	8	(80); (82); (56); (61); (71); (87); (67); (162)
10	a. Removal of contaminated tools	7	(64); (70)(a); (59)(a); (60); (89)(a); (15); (66)(a)
	b. Temporary closure of contaminated rooms	7	(174)(a); (56); (63)(a); (84); (86)(a); (87); (16)
	c. Medical-equipment disinfections	7	(82); (69); (30); (175); (134); (62)(a); (15)

[a] The study added this additional intervention that was successful to control the events of ESBL-producing *Klebsiella* spp. in the hospital.

in hospitals from 71 studies (Table 6). Reinforcement of hand hygiene (46.5%) was the most successful intervention in all studies, followed by adequate compliance with the antibiotic control programs (33.8%). Removal of contaminated tools was also found in the list of top 10 strategies to control ESBL-producing *Klebsiella* spp. infections within the hospital.

DISCUSSION

Summary of evidence

This is the first systematic review and meta-analyses to identify the transmission pattern of ESBL-producing *Klebsiella* spp. among hospitalized patients worldwide. Our random-effects meta-analyses showed the underlying disease including malignancy or particular condition (OR = 6.25; 95% CI = 2.85 to 13.66) and prior cephalosporin exposure (OR = 4.65; 95% CI = 2.83 to 7.65) as the most significant associated factors for the presence of ESBL-producing *Klebsiella* spp. Consistent with our study, Cornejo-Juárez *et al.*, indicated the increased risk of ESBL-*Escherichia coli* bacteremia in patients with hematologic malignancies who had prior cephalosporins exposure (164). Our finding was also in line with a systematic review of ESBL-producing Enterobacteriaceae in Latin America that reported prior antibiotic exposure, in particular cephalosporins as the associated risk factor with a statistically significant OR above one for the acquisition of ESBL-producing *Klebsiella* spp. (165). Nevertheless, based on the study by Piroth *et al.*, antibiotic use can be an associated protective factor with a statistically significant OR below one (166). Moreover, our study reported the fact that infection with ESBL-producing *Klebsiella* spp. can cause fatal outcomes in association with other associated risk factors (167). Our study confirmed the importance of interaction between hospitalized patients, environmental sources, and surrounding reservoirs, in particular the HCWs. Obviously, the colonized and/or infected patients were the consistent reservoirs (Table 1). Further, it was clear that surrounding reservoirs and sources must be taken into consideration in order to end the vicious cycle of the spread of ESBL-producing *Klebsiella* spp. In general, likewise in case of Methicillin-resistant *Staphylococcus aureus*, HCWs who are colonized with ESBL-producing *Klebsiella* spp. play a pivotal role in the transmission through their hands (168). Even more, using artificial nails and having onychomycosis can be the transmission source for the presence of ESBL-producing *Klebsiella* spp. (39, 43).

Molecular typing

PFGE still is the gold standard for molecular typing, in particular to prove the transmission of ESBL-producing *Klebsiella* spp. among patients (9). However, it is useful to perform another molecular technique in order to confirm the result of the main diag-

nostic technique or transmission (52, 104). Furthermore, our findings showed that both monoclonal and polyclonal presence occur in outbreak events. This indicated that clonal dissemination plays a role in outbreaks (63). However, it is also important to consider the horizontal plasmid transfer among different bacterial species (52). Studies on plasmid fingerprinting showed the importance of plasmid genotyping to predict the mode of plasmid transmission among patients (76, 104).

Interventions

To stop the spread of ESBL-producing *Klebsiella* spp., reinforcement of hand hygiene and an antibiotic control program were the most successful interventions. Firstly, hand hygiene is a simple and low cost intervention to prevent the presence of ESBL-producing *Klebsiella* spp. The introduction of hand antiseptic will reduce the contamination of the hands with of ESBL-producing *Klebsiella* spp. (169). Recent guidelines also highlight hand hygiene as the top priority to prevent the transmission of nosocomial infections (11). Secondly, a meta-analysis study suggested cycling empirical antibiotic therapy to prevent antibiotic resistance (170). This adjustable cycling model confirmed the antibiotic control program as an important strategy for ESBL-producing *Klebsiella* spp. transmission.

Study quality

Most studies included in the meta-analyses and cluster analysis had a low study quality using the recommended guidelines. However, in particular for the studies that were assessed with STROME-ID guidelines, they had wide range of key objectives that did not focus solely on molecular epidemiology of ESBL-producing *Klebsiella* spp. Nonetheless, reporting the cases of ESBL-producing *Klebsiella* spp. according to STROME-ID guidelines can give clear information about the evidence to detect the transmission dynamics with molecular typing and helps in making the health-policy decision. In general, the studies that were included answered our research questions; however, our study indicated the importance to report structured articles that follow appropriate guidelines for a high study quality.

Strengths and limitations

The major strength of our study was the inclusion of a large number of hospitalized patients with ESBL-producing *Klebsiella* spp. In the meta-analyses, we identified several risk factors that were associated with the presence of ESBL-producing *Klebsiella* spp. We also summarized the most successful interventions to prevent the spread of ESBL-producing *Klebsiella* spp., which to our knowledge has never been done before. However, our study also has some limitations. Firstly, the heterogeneity of all included studies in combination with the diverse models used in all individual statistical analyses,

here combined in the meta-analyses. Therefore, we performed a random-effects model for the meta-analyses. Secondly, due to the different definitions of colonization and infection in the studies, we termed them as presence of ESBL-producing *Klebsiella* spp. As a consequence, reporting them separately was not possible. Thirdly, our study focused on clonal spread; hence, we did not include studies about plasmid transfers. Fourthly, some studies only performed molecular typing investigation on selected samples due to cost effectiveness. Therefore, most probably we have bias on the number of clusters. Fifthly, publication bias might have occurred since we have a broad range of articles from different objectives to comprehensively answer our research questions.

Conclusion

The presence of ESBL-producing *Klebsiella* spp., which results in increased morbidity and mortality, may occur by either direct spread from patient to patient or indirect transmission via surrounding reservoirs and sources in the environment. Obviously, molecular typing techniques can identify transmission of ESBL-producing *Klebsiella* spp. within the hospital. Prior antibiotic exposure holds a key role for the presence of ESBL-producing *Klebsiella* spp. particularly cephalosporin use. Multi-faceted interventions, including reinforcement of hand hygiene and control of antibiotic use, are necessary to prevent the spread of ESBL-producing *Klebsiella* spp. Further studies on plasmid transfer are needed to learn more about transmission of ESBL-producing *Klebsiella* spp. within a hospital. In addition, it is important to report studies in a more structured way that systematically follows suitable guidelines.

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SUPPLEMENTAL MATERIAL

Supplement 1. PRISMA 2009 Checklist

Supplement 2. List of search terms

Supplement 3. Study quality. Quality assessment scores of the 23 articles that were included in the random-effects meta-analysis study (Table A). Quality assessment scores of the 23 articles that were included in the random-effects meta-analysis study, using the STROBE guidelines (Table B). Quality assessment scores of the 15 case-control studies that were included in the random-effects meta-analysis study, using the Newcastle Ottawa Scale (Table C1). Quality assessment scores of the three cohort studies that were included in the random-effects meta-analysis study, using the Newcastle Ottawa Scale (Table C2). Quality assessment scores of 120 articles that were included in the cluster analysis study (Table D).

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Chapter 3

Healthcare-related pathogens: sources and transmission

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Chapter 3.1

VIM-positive *Pseudomonas aeruginosa* in a large tertiary care hospital: matched case-control studies and a network analysis

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ABSTRACT

Emergence of multidrug-resistant *Pseudomonas aeruginosa* is of global concern. We aimed to identify epidemiological relationships, the most common way of transmission, and risk factors for presence of Verona Integron-encoded Metallo-β-lactamase (VIM)-positive *P. aeruginosa* (VIM-PA). We conducted a network analysis and matched case-control studies (1:2:2). Controls were hospital-based and matched with cases for ward, day of admission (control group 1 and 2) and time between admission and the identification of VIM-PA (control group 1). The network was visualized using Cytoscape, and risk factors were determined using conditional logistic regression. Between August 2003 and April 2015, 144 case patients and 576 control patients were recruited. We identified 307 relationships in 114 out of these 144 patients, with most relationships (84.7%) identified at the same department <3 months after a previous case patient was discharged. In the multivariable model, having undergone ≥1 gastroscopy (odds ratio [OR] = 4.40, 95% confidence interval [CI] = 2.00 to 9.65 and OR = 2.47; 95% CI = 1.12 to 5.49), >10 day use of selective digestive tract decontamination (SDD) (OR = 2.97; 95% CI = 1.02 to 8.68 and OR = 4.61; 95% CI = 1.22 to 17.37), and use of quinolones (OR = 3.29; 95% CI = 1.34 to 8.10 and OR = 3.95; 95% CI = 1.13 to 13.83 and OR = 4.47; 95% CI = 1.75 to 11.43) were identified as risk factors when using both control groups. The network analysis indicated that the majority of transmissions occurred on the wards, but through unidentified and presumably persistent sources, which are most likely in the innate hospital environment. Previous use of certain antibiotic regimens made patients prone to VIM-PA carriage. Additionally, gastroscopy could be considered as a high-risk procedure in patients with risk factors. Our results add to the growing body of evidence that infection control measures targeting VIM-PA should be focused on reducing antibiotics and eliminating sources in the environment.

BACKGROUND

The emergence of multidrug-resistant strains of *Pseudomonas aeruginosa* (MDRPA) is of global concern (1, 2). Infections with this resistant microorganism lead to increased morbidity and mortality in patients; especially in specific patient groups, such as those in intensive care units (3-6). MDRPA hospital outbreaks are mostly caused by MDRPA which produce carbapenemases, with as most clinically significant the metallo-β-lactamases (MBL) (2). Currently, the Verona Integron-encoded MBL (VIM) is the most widespread MBL in *P. aeruginosa* (2, 7-9). Sources are often hard to eradicate because *P. aeruginosa* is known to form a biofilm in environmental niches which protects it from cleaning and disinfection actions (10, 11).

Since 2003, a VIM-positive clone of *P. aeruginosa* (VIM-PA) has emerged in our hospital and became entrenched causing multiple episodes of colonizations and infections in patients (9, 12). A systematic review published by our research group showed that the leading risk factors for acquiring MDRPA were carbapenem use and having medical devices (13). However, risk factors are likely to be outbreak specific because of different local circumstances and patient populations.

The aim of this study was first to identify epidemiological relationships between patients with a VIM-PA, and to identify the most common way of transmission. Second, we aimed to identify risk factors for presence of VIM-PA among colonized and/or infected patients with a case-control study. When a case-control study is used to understand an outbreak, it is often not clear what the best control group is. Both under- and overmatching may affect the results; in essence, the choice of the control determines the outcome. Therefore, our third aim was identifying the most appropriate control group.

METHODS

Ethics statement

Written approval to conduct this study was received from the medical ethics research committee of the Erasmus MC University Medical Centre (Erasmus MC), Rotterdam, the Netherlands (MEC-2015-240). This study is registered in the Dutch National Trial Register (NTR5145).

Setting

This retrospective study was conducted at the Erasmus MC in Rotterdam, the Netherlands, using data from August 2003 until April 2015. In this 1200-bed university hospital all medical specialties are available; organized into 48 departments. The Department of Adult Intensive Care (adult ICU) comprises of three high-level ICU wards, and each

ward has only single-patient rooms. At the ICU, patients expected to be on a mechanical ventilator for >48h or anticipated to be admitted to the ICU for >72h receive selective digestive tract decontamination (SDD). The SDD regimen is identical to the regimen used by de Smet *et al.*, including four days of cefotaxime intravenously (14). The total number of clinical admissions and clinical admission days from 2003 until 2015 are available in supplement 1.

Patient inclusion and microbiological analysis

Patients were included if identified with VIM-PA between 48 hours after admittance to and 48 hours after discharge from a department in the main Erasmus MC building. Patients were excluded if admitted only to the Erasmus MC Sophia Children's Hospital or only to the Erasmus MC Cancer Institute. These buildings are physically separated from the main building, and have their own employees. To our knowledge, there has been no cross-over of VIM-PA to and from these separate buildings. In addition, 22 patients that were involved in an outbreak resulting from a contaminated duodenoscope used for endoscopic retrograde cholangiopancreatography were excluded. The exact cause, source and transmission route were known and it was therefore investigated and reported separately (15).

Cultures taken for clinical diagnostic purposes were processed in the laboratory using standard microbiological methods. In case of suspected growth of carbapenemase-producing *P. aeruginosa* or MDRPA, an in-house polymerase chain reaction (PCR) for detection of blaVIM on LightCycler 480 (Roche Diagnostics, Almere, The Netherlands) was performed using previously reported primers (9, 12). For screening for VIM-PA, swabs were obtained from throat and rectum, cultured overnight at 35°C in a Tryptic Soy Broth with ceftazidime (2mg/L) and vancomycin (50mg/L), followed by our in-house PCR test on the broth. Positive PCR results were confirmed by subculturing the broth on a blood agar (BD Diagnostics, Breda, The Netherlands); *P. aeruginosa* growing on this agar plate was subjected to blaVIM PCR. Identification and susceptibility testing was performed using Vitek2 (bioMérieux, Marcy l'Etoile, France). Since January 2013, the MALDI-TOF (Bruker Daltonics, Bremen, Germany) was used for identification. Clonal relatedness of VIM-PA from clinical and screening cultures was determined using the DiversiLab system with the *Pseudomonas* kit (bioMérieux).

General infection prevention and control measures were installed after each case was identified (*e.g.* isolation). However, in 2011 these measures were intensified; at two adult intensive care units (ICUs), twice-weekly screening for VIM-PA (*i.e.* rectum and throat cultures) was implemented from October 2011. However, after April 2014, this was reduced to once a week. After August 2014, the weekly screening halted; however, was re-implemented in September 2014 because a new case of VIM-PA was identified from a clinical sample. Additionally, at the ICU rectum and throat cultures on VIM are taken

upon admittance and discharge of patients. At the neurological high care ward, screening took place once a week from August 2013 until January 2016.

Network analysis

Admission histories in time and department/room location of patients identified with VIM-PA were retrieved to define epidemiological relatedness. Each identified relation was classified in one out of four categories (Table 1). Then, the data were imported into Cytoscape v3.2.1 (http://www.cytoscape.org) and the network was visualized (16). It was analysed whether a patient only 'received', only 'transmitted', or 'received and transmitted' the VIM-PA following the definitions in Table 1. "Only received" indicated that a patient did not have epidemiological links to patients identified with a VIM-PA at a later time, "only transmitted" indicated that a patient did not have epidemiological links to patients identified with a VIM-PA earlier in time. "Received and transmitted" indicated that a patient had epidemiological relationships to patients identified with VIM-PA earlier in time and later in time.

Table 1. Definitions of epidemiological relatedness

	Definite[1]	Probable	Possible-I	Possible-II	Impossible
Same patient room	1	1	0	0	0
Same department	1	1	1	1	0
Same period	1	0[a]	1	0[a]	0[a]/0/1

0= no; 1= yes, 0[a]= <3 months after previous positive patient was discharged. [1]Definition definite was not possible at the intensive care units because only single patient rooms are present.

Case-control studies

The risk factor analysis was performed in individual matched retrospective case-control studies, using a 1:2:2 ratio, with hospital-based controls. All information was extracted from the electronic medical records. A list of all patient and treatment related variables collected for cases and controls is presented in supplement 2.

Control groups

Patients in control group 1 and 2 were matched for the following three characteristics: I: admitted to the same ward where the case supposedly acquired the VIM-PA (*i.e.* the ward where the patient was admitted 48 hours before the positive culture) (exact match), II: being admitted on the same date as the case (best match), III: having the same days of exposure as the case (*i.e.* the days between admittance and the date of the first positive culture with VIM-PA) (best match). If exact matching was not possible -with the exception of ward- exposure time was found to be the most imperative factor. Patients in control group 3 and 4 were matched for the following characteristics: I: admitted to

the same ward as the case (exact match), II: admittance on the same date as the case (best match). The control patient had to be free of colonization or infection with VIM-PA. This could be proven either by negative screening cultures or by the absence of clinical cultures with VIM-PA. A control patient could not serve as a control more than two times; within and between the four different control groups. Also, a case patient could never be selected as a control patient.

Statistical analyses

For continuous variables, means or medians were calculated. For categorical variables, percentages were calculated. The conditional logistic regression model was used in both univariate and multivariable analyses. Univariate analyses were conducted using the COXREG procedure in SPSS version 21 (IBM Cor*P*., Armonk, New York, USA). Characteristics with a P-value of <0.1 in univariate analysis were included in the multivariable analyses. Treatment variables could be included as 1) use yes/no, 2) use for 0/1-3/≥4 days or 3) use for 0/1-3/4-10/≥11 days. Selection for inclusion in the multivariable model of either category 1, 2 or 3 of a certain antibiotic was based on: 1) >5 patients in present in each group, 2) estimates of the different categories had to show a difference of at least 1 odds ratio (OR). Multivariable analyses were conducted using conditional logistic regression with dynamic ridge penalties in the R Project for statistical computing version 3.3.1 (Vienna, Austria). Subgroup analyses were performed for ward of acquisition of case patients and matched controls being ICU or being non-ICU, as well as for case patients in clonal clusters as indicated by the typing results. Additionally, analyses were performed between patients in control group 1&2 and 3&4. Results were presented as ORs with 95% confidence intervals (CI). P-values <0.05 were considered statistically significant. Graphs were created using GraphPad Prism Version 7.01 (GraphPad Software, Inc. CA, USA).

RESULTS

Included patients

Out of 166 patients identified with a VIM-PA between August 2003 and April 2015, eight children were excluded because they were admitted only to the Erasmus MC Sophia Children's hospital and one patient was excluded because admitted only to the Erasmus MC Cancer Institute. In addition, 13 patients were excluded because the VIM-PA was identified within 48 hours after admission. Ultimately, 144 patients were included in the network analysis and as case patients in the case-control study (supplement 1). Nineteen different wards of acquisition were identified, including three ICUs (*i.e.* two general adult ICUs and 1 thoracic ICU). The top 5 locations of acquisition were the two

general adult ICUs (87 patients, 60.4%), the gastro-intestinal surgical ward (10 patients; 6.9%), and the gastroenterology and hepatology ward (7 patients, 4.9%). Typing showed that the VIM-PA of 29 (20.1%) patients belonged to clonal cluster A, 105 (72.9%) to clonal cluster B, 7 (4.9%) did not belong to clonal cluster A or B, and it was not possible to type strains from 3 patients (2.1%).

Network analysis

From 2003 until 2015, we identified 307 relationships in 114 out of 144 patients (Table 1, Figure 1). Thirty out of 144 patients did not have relationships to other case patients (20.8%). When considering the definitions (Table 1) we identified nine probable relationships, 38 possible-I relationships and 260 possible-II relationships at 12 different departments. Most relationships (92%) were identified at the ICU. Twenty-five patients (17.4%) only 'received' the VIM-PA, 22 (15.3%) only 'transmitted' VIM-PA and 67 (46.5%) 'received and transmitted' VIM-PA.

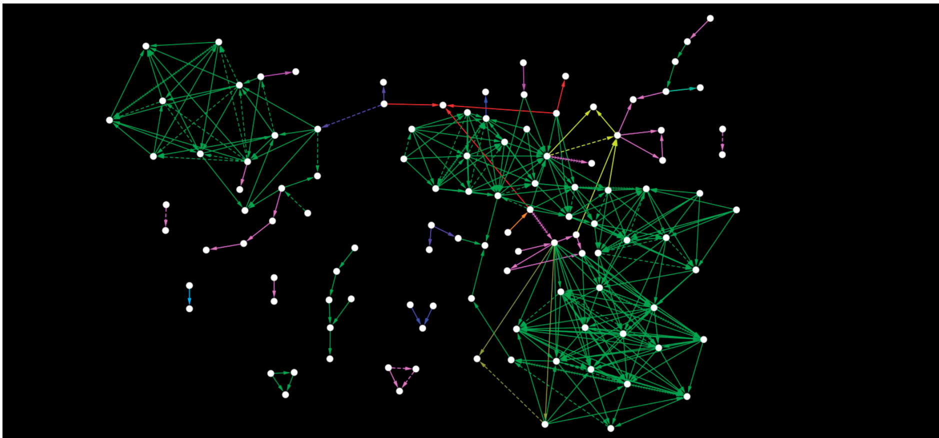

Figure 1. Network of 307 relationships in 114 out of 144 patients identified with VIM-PA. Thirty out of 144 patients did not have relationships to other case patients (20.8%). Edge colours represent different Erasmus MC departments; green represents the two adult ICU wards. Line shapes represent the different epidemiological relationships as described in Table 1: contiguous line= probable, dash line= possible A, solid line= possible B. The arrow shows the direction of the relationship.

Case-control studies

Matching

It was impossible to perfectly match all cases to four controls. Overall, perfect matching was achieved in 38.2% (range: 16.0%-66.0%). For cases, the median days from admission to acquisition of VIM-PA was 14 days (range: 1-114 days). In the control groups, the median error in days was 4, 4, -1 and -1 days respectively. Seventeen patients served 2 times as control patient between the 4 control groups (11.8%).

Risk factors for acquisition

Patient related clinical variables with crude odds ratios, 95% CI and P-values are presented in Table 2. Compared to control group 1&2 and control group 3&4, the median length of admission was significantly longer and the 1-year mortality rate was significantly higher in case patients (Table 2).

Treatment related variables with crude odds ratios, 95% CI and P-values are presented in Table 3 and in supplement 3. When comparing cases to control group 1&2, multivariable analysis revealed five risk factors; two patient related clinical risk factors, and three treatment related risk factors (Figure 2). The highest odds ratio was identified for having undergone ≥1 gastroscopy 6 months prior to the identification of VIM-PA (OR= 4.40, 95%CI= 2.00 to 9.65, P<0.001). When comparing cases to control group 3&4, one patient related clinical protective factor was identified (*i.e.* malignancies) and one risk factor (*i.e.* gastroscopy) (Figure 2). Also, four treatment related risk factors were identified; use of piperacillin/tazobactam, 1-3 day and >3 day use of quinolones and >10 day use of SDD (Figure 2). The highest odds ratio was identified for >10 day use of SDD (OR= 4.61, 95%CI= 1.22 to 17.37, P=0.024). In both case-control studies, previous use of quinolones, use of SDD for >10 days, and having undergone ≥1 gastroscopy 6 months prior to the identification of VIM-PA were identified as risk factors, and could therefore be considered robust risk factors.

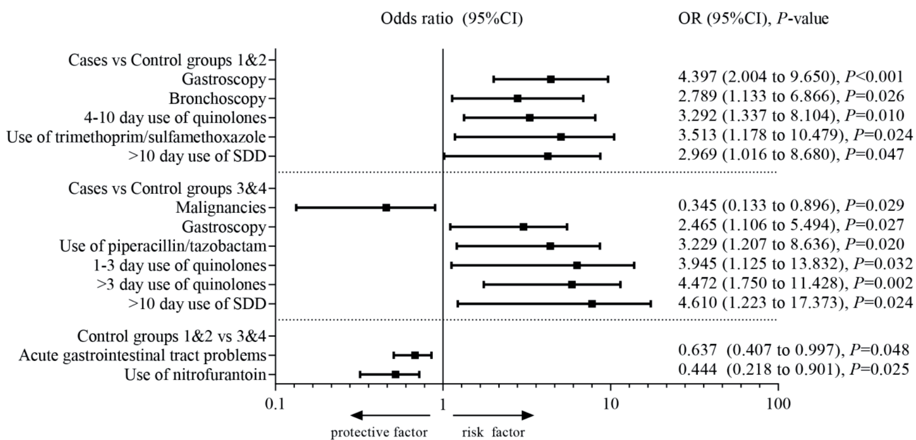

Figure 2. Risk factors and protective factors identified using multivariable analysis. Abbreviations: SDD= selective digestive tract decontamination, 95%CI= 95% confidence interval.

Univariate results of the subgroup analyses are presented in supplement 4 and 5. Multivariable results are displayed in Figure 3. There are differences in identified risk factors in the different subgroups. For example, for patients with DiversiLab type A endoscopies did not seem to play a role, whereas in type B they did. Also, at the ICU

Table 2. Patient related clinical variables, univariate analyses between cases and controls 1&2 and between cases and controls 3&4

Variables	Cases (n= 144)	Controls 1&2 (n= 288)	Crude OR (95% CI)	*P*-value	Controls 3&4 (n= 288)	Crude OR (95% CI)	*P*-value
Basic characteristics							
Age, years, median (range)	58.3 (17-82)	60.3 (17-92)	0.998 (0.986-0.1010)	0.723	61.1 (17-91)	0.995 (0.982-1.007)	0.407
Male gender (%)	83 (57.6)	174 (60.4)	0.886 (0.584-1.345)	0.570	167 (58.0)	0.985 (0.650-1.493)	0.944
28-day mortality (%)	41 (28.5)	69 (24.0)	1.283 (0.804-2.048)	0.296	40 (13.9)	**2.677 (1.577-4.544)**	**<0.001**
1-year mortality (%)	82 (56.9)	95 (33.0)	**2.680 (1.754-4.094)**	**<0.001**	63 (21.9)	**4.950 (3.072-7.976)**	**<0.001**
Transferred from another hospital (%)	50 (34.7)	60 (20.8)	**2.000 (1.279-3.128)**	**0.002**	50 (17.4)	**2.405 (1.530-3.782)**	**<0.001**
Median length of admission (range)	55 (3-338)	25 (1-258)	**1.021 (1.014-1.028)**	**<0.001**	12 (1-1102)	**1.021 (1.014-1.027)**	**<0.001**
Erasmus MC 1y before VIM-PA (%)	64 (44.4)	111 (38.5)	1.278 (0.851-1.920)	0.237	109 (37.8)	1.348 (0.881-2.064)	0.169
Erasmus MC ICU 1y before VIM-PA (%)	14 (9.7)	15 (5.2)	1.867 (0.901-3.867)	0.093	14 (4.9)	2.131 (0.976-4.652)	0.058
Surgery (%)	87 (60.4)	119 (41.3)	**2.165 (1.432-3.275)**	**<0.001**	121 (42.0)	**2.119 (1.399-3.209)**	**<0.001**
Underlying diseases							
Cystic fibrosis (%)	2* (1.4)	3 (1.0)	NA	NA	2 (0.7)	NA	NA
Chronic respiratory illness (%)	29 (20.1)	43 (14.9)	1.488 (0.861-2.571)	0.155	43 (14.9)	1.518 (0.868-2.655)	0.144
Chronic kidney failure (%)	5 (3.5)	3 (1.0)	3.33 (0.797-13.948)	0.099	11 (3.8)	0.903 (0.304-2.689)	0.855
Acute kidney failure; use of CVVH (%)	28 (19.4)	18 (6.3)	**3.471 (1.844-6.533)**	**<0.001**	10 (3.5)	**6.651 (3.022-14.638)**	**<0.001**
Chronic liver failure (%)	5 (3.5)	7 (2.4)	1.480 (0.442-4.952)	0.525	17 (5.9)	0.588 (0.217-1.594)	0.297
Acute liver failure (%)	0 (0)	6 (2.1)	NA	NA	3 (1.0)	NA	NA
Chronic problems of the gastrointestinal tract (%)	19 (13.2)	28 (9.7)	1.377 (0.758-2.502)	0.294	26 (9.0)	1.603 (0.822-3.126)	0.166
Acute problems of the gastrointestinal tract (%)	28 (19.4)	39 (13.5)	1.493 (0.894-2.494)	0.126	54 (18.8)	1.047 (0.627-1.750)	0.861
Auto-immune disease (%)	7 (4.9)	18 (6.3)	0.762 (0.308-1.886)	0.556	26 (9.0)	0.520 (0.221-1.223)	0.134
Human immunodeficiency virus (%)	0 (0)	2 (0.7)	NA	NA	4 (1.4)	NA	NA
Diabetes (%)	24 (16.7)	37 (12.8)	1.353 (0.776-2.360)	0.287	48 (16.7)	1.000 (0.579-1.726)	1.000
Solid organ transplant recipient (%)	20* (14.0)	20 (6.9)	**2.257 (1.139-4.473)**	**0.020**	30 (10.4)	1.463 (0.784-2.729)	0.232

Table 2. Patient related clinical variables, univariate analyses between cases and controls 1&2 and between cases and controls 3&4 (continued)

Variables	Cases (n= 144)	Controls 1&2 (n= 288)	Crude OR (95% CI)	*P*-value	Controls 3&4 (n= 288)	Crude OR (95% CI)	*P*-value
Stem cell/bone marrow transplant recipient (%)	7* (4.9)	6 (2.1)	3.562 (0.875-14.493)	0.076	1 (0.3)	NA	NA
Use of immunosuppressive agents (%)	55 (38.2)	71 (24.7)	**1.920 (1.236-2.985)**	**0.004**	55 (19.1)	**2.493 (1.602-3.879)**	**<0.001**
Malignancies (%)	32 (22.2)	76 (26.4)	0.777 (0.472-1.280)	0.322	92 (31.9)	**0.570 (0.347-0.936)**	**0.026**
Neutropenia, <500 cells/μL (%)	3** (2.7)	4** (2.1)	0.883 (0.159-4.889)**	0.886	7**(2.4)	0.667 (0.164-2.713)**	0.571
Endoscopies							
Colonoscopy (%)	7 (4.9)	10 (3.5)	1.400 (0.533-3.678)	0.495	12 (4.2)	1.202 (0.432-3.345)	0.724
Sigmoidoscopy (%)	7 (4.9)	5 (1.7)	2.800 (0.889-8.822)	0.079	8 (2.8)	1.750 (0.635-4.826)	0.280
Endoscopic ultrasound (%)	7 (4.9)	26 (9.0)	0.490 (0.202-1.189)	0.115	19 (6.6)	0.684 (0.260-1.802)	0.443
Gastroscopy (%)	75 (52.1)	50 (17.4)	**6.609 (3.803-11.485)**	**<0.001**	59 (20.5)	**4.529 (2.79-7.335)**	**<0.001**
ERCP (%)	17 (11.8)	15 (5.2)	**2.520 (1.190-5.336)**	**0.016**	10 (3.5)	**3.917 (1.678-9.143)**	**0.002**
Bronchoscopy (%)	41 (28.5)	34 (11.8)	**3.159 (1.841-5.422)**	**<0.001**	33 (11.5)	**3.030 (1.800-5.103)**	**<0.001**
Transoesophageal Echocardiography (TEE) (%)	12 (8.3)	13 (4.5)	1.846 (0.842-4.046)	0.126	13 (4.5)	1.901 (0.849-4.257)	0.118
Medical devices							
Mechanical ventilation (%)	129 (89.6)	210 (72.9)	**4.921 (2.284-10.600)**	**<0.001**	185 (64.2)	**8.211 (3.857-17.481)**	**<0.001**
Tracheostomy (%)	29 (20.1)	38 (13.2)	**1.801 (1.011-3.211)**	**0.046**	9 (3.1)	**7.883 (3.443-18.045)**	**<0.001**
Extracorporeal membrane oxygenation (%)	2 (1.4)	0 (0)	NA	NA	0 (0)	NA	NA
Central venous catheter (%)	103 (71.5)	124 (43.1)	**4.121 (2.477-6.854)**	**<0.001**	77 (26.7)	**9.040 (5.101-16.023)**	**<0.001**

Abbreviations: VIM-PA= Verona Integron-encoded Metallo-β-lactamase (VIM)-positive *Pseudomonas aeruginosa*, 95% CI= 95% confidence interval, y= year, ICU= intensive care unit, CVVH= Continuous Veno-Venous Hemofiltration, ERCP= Endoscopic Retrograde Cholangiopancreatography.

*1 case missing because no medical background was available.

** For 32 cases, 99 controls 1&2 and 108 controls 3&4 no information about neutrophils was available.

Table 3. Treatment related variables, univariate analyses between cases and controls 1&2 and between cases and controls 3&4

Variables^	Cases (n= 138#)	Controls 1&2 (n= 288)	Crude OR (95% CI)	*P*-value	Controls 3&4 (n= 288)	Crude OR (95% CI)	*P*-value
Antifungals (%)	82 (59.4)	79 (28.0)*	**4.863 (2.886-8.194)**	**<0.001**	50 (17.7)*	**5.890 (3.647-9.512)**	**<0.001**
Antivirals (%)	16 (11.6)	19 (6.7)*	1.913 (0.899-4.071)	0.092	17 (6.0)*	**2.053 (1.011-4.169)**	**0.047**
Aminoglycosides (%)	51 (37.0)	53 (18.8)	**2.988 (1.769-5.046)**	**<0.001**	24 (8.5)	**6.832 (3.692-12.643)**	**<0.001**
Amoxicillin/clavulanic acid (%)	39 (29.1)	48 (17.0)*	**2.014 (1.196-3.394)**	**0.009**	40 (14.2)*	**2.388 (1.428-3.992)**	**0.001**
Carbapenems (%)	58 (42.0)	58 (20.7)	**3.124 (1.898-5.142)**	**<0.001**	26 (9.2)	**6.762 (3.810-12.000)**	**<0.001**
Cephalosporins (%)	104 (77.6)	131 (46.5)	**3.942 (2.383-6.520)**	**<0.001**	107 (37.9)	**6.407 (3.692-11-121)**	**<0.001**
Colistin (%)	17 (12.7)	20 (7.1)*	1.896 (0.926-3.880)	0.080	12 (4.3)*	**4.282 (1.756-10.437)**	**0.001**
Macrolides (%)	60 (44.8)	64 (22.7)	**2.702 (1.696-4.304)**	**<0.001**	27 (9.6)	**6.881 (3.885-12.190)**	**<0.001**
Metronidazole (%)	48 (35.8)	48 (17.0)	**2.445 (1.534-3.898)**	**<0.001**	32 (11.3)*	**4.689 (2.630-8.360)**	**<0.001**
Nitrofurantoin (%)	17 (12.7)	12 (4.3)*	**3.486 (1.543-7.878)**	**0.003**	24 (8.5)	1.527 (0.767-3.039)	0.228
Penicillin (%)	29 (21.6)	49 (17.4)	1.226 (0.739-2.032)	0.431	31 (11.0)	**2.100 (1.199-3.678)**	**0.009**
Piperacillin/tazobactam (%)	49 (36.6)	50 (17.7)*	**2.774 (1.666-4.621)**	**<0.001**	32 (11.3)*	**4.572 (2.604-8.025)**	**<0.001**
Quinolones (%)	94 (70.1)	88 (31.2)	**6.087 (3.553-10.429)**	**<0.001**	53 (18.8)	**9.193 (5.284-15.996)**	**<0.001**
Trimethoprim/sulfamethoxazole (%)	32 (23.9)	23 (8.2)*	**3.606 (1.930-6.740)**	**<0.001**	19 (6.7)*	**3.834 (2.098-7.007)**	**<0.001**
Vancomycin (%)	74 (55.2)	51 (18.1)	**6.355 (3.652-11.057)**	**<0.001**	27 (9.6)	**9.847 (5.437-17.836)**	**<0.001**
Other antibiotics (%)	32 (23.9)	28 (9.9)*	**3.074 (1.680-5.627)**	**<0.001**	30 (10.6)*	**2.684 (1.512-4.763)**	**0.001**
Selective digestive tract decontamination (%)	92 (68.7)	109 (38.7)	**4.583 (2.641-7.952)**	**<0.001**	59 (20.9)	**10.864 (5.748-20.536)**	**<0.001**

Abbreviations: 95% CI= 95% confidence interval, #data on antibiotics of the first six cases was missing, bold= statistically significant, *P*-value <0.05. *= included in multi-variable analysis. ^for categorical data see supplement 3.

antibiotic use (trimethoprim/sulfamethoxazole) was identified as a risk factor in combination with having undergone ≥1 gastroscopy and bronchoscopy 6 months prior to the identification of VIM-PA, whereas at non-ICU wards it was a combination of having undergone ≥1 gastroscopy 6 months prior to the identification of VIM-PA and surgery or being admitted at the Erasmus MC before.

Univariate differences between control group 1&2 and 3&4 are presented in supplement 6. Multivariable analysis revealed only two differences between the control groups, regarding protection by acute gastrointestinal tract problems and use of nitrofurantoin (Figure 2).

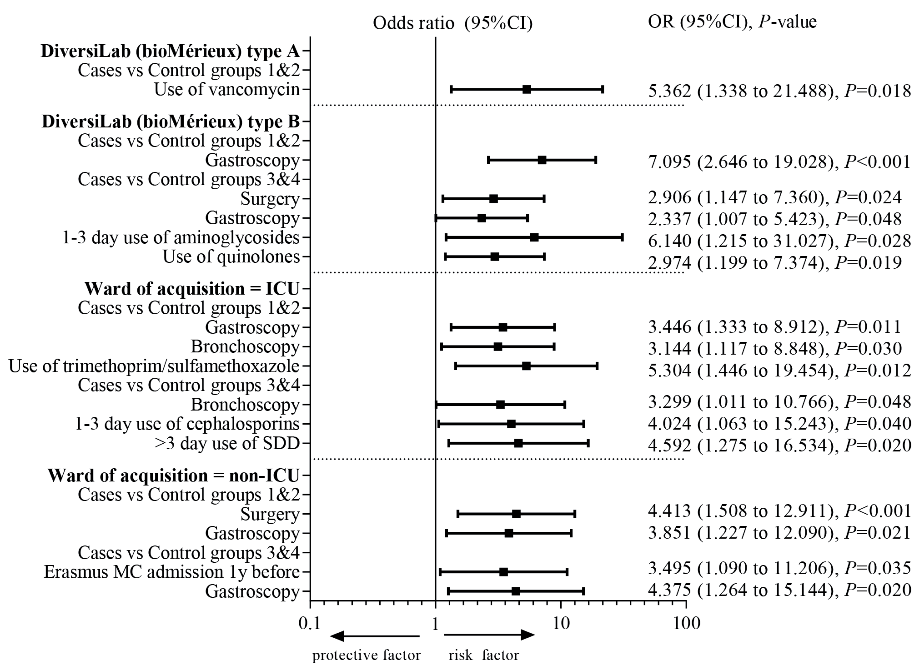

Figure 3. Risk factors identified using multivariable analysis of subgroups DiversiLab type A or type B and ward of acquisition of the case patient being the intensive care unit (ICU) or non-ICU wards. Abbreviations: SDD= selective digestive tract decontamination, 95%CI= 95% confidence interval, ICU= intensive care unit.

DISCUSSION

Our study aimed to identify epidemiological relationships, the most common way of transmission and risk factors for presence of VIM-PA. In the network analysis, we did not identify definite relationship and only nine probable relationships. Therefore, the same patient room, either sharing a patient room or being admitted at the same patient room within 3 months, is not the most likely source. However, there was a relation with the

same department. Surprisingly, the same admission period seems not to be important; most relationships were identified within 3 months after the previous positive patient was discharged. Thus, the majority of transmissions occurred on the wards in a wide time frame. Therefore, it must have occurred through unidentified sources, which may be either undetected patients or unidentified sources in the innate environment. Given the fact that patients at the ICUs and neurology high-care ward were frequently screened; we assume that undetected patients are not plausible. Our hypothesis is that persistent sources in the innate environment play an important role in the route of transmission of this pathogen. This is in agreement with current knowledge on the behaviour of this bacterium, as well as previous outbreak reports that identified the environment as source/reservoir (13, 17).

The case-control studies showed first that previous use of certain antibiotics were associated with an increased risk of acquisition of VIM-PA; especially the use of quinolones, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole should be avoided if possible. Second, gastroscopy and bronchoscopy were identified as risk factors (Figure 2). Third, the results of the two different case-control studies were largely in line with each other, with three common risk factors (*i.e.* previous use of quinolones, use of SDD for > 10 days, and having undergone ≥1 gastroscopy 6 months prior to the identification of VIM-PA) that could therefore be considered as robust. The assumption would be that certain antibiotics change the normal gut or throat flora in such a way that multidrug-resistant bacteria more easily attach to and colonize either the gut or throat. Nevertheless, multidrug-resistant microorganisms have to be offered to the patient, and this may occur through endoscopic procedures by contaminated endoscopes or using water from a contaminated source. Both the previous use of antibiotics and prior procedures with flexible endoscopes have been highlighted in previous studies as risk factors for acquisition of various multidrug microorganisms, including VIM-PA (18, 19).

The group of antibiotics that favours presence of VIM-PA (*i.e.* increases a patients' susceptibility to acquire VIM-PA) depends on the choice of the control group. Furthermore, we learned that although highly significant factors were obtained with one group of controls, these can disappear when other groups are compared as these groups differ in inclusion criteria or definition; the results highly depend on the choice of the control.

Limitations

Network analysis

Criteria for epidemiological relationships, especially relationships in time and space, are not clearly defined for outbreaks with multidrug-resistant bacteria. We developed criteria which are easy to apply; however, inherent to this is a simplification of the truth. We propose that these definitions would be modified or extended in case data from future studies warrants.

Case-control studies
First, this is a single-centre case-control study, which possibly hampers generalizability. Second, matching on ward of acquisition and length of stay prior to the positive culture might have caused additional matching on *e.g.* comorbidities and disease severity. However, we have done this deliberately. When comparing ICU to non-ICU patients, disease severity and possibly also comorbidities will be risk factors just because the groups are not similar. Third, misclassification of exposure could be present; not all control patients were cultured for VIM-PA, which could lead to VIM-PA carriers present in control grou*P.* However, this misclassification if present could only have led to an underestimation of the identified effects. Fourth, perfect matching was only achieved in 38%. This seems low; however, 100% perfect matching is not possible for large case-control studies including patients who have complicated medical histories and futures. Possibly, the percentage of perfect matching could be added as an item to the STROBE statement (20).

In one of the subgroup analyses, differences were identified between DiversiLab clonal cluster A and B (Figure 3). However, although widely applied, the DiversiLab system can be considered a limitation of the study, since available data regarding the DiversiLab system for *P. aeruginosa* are contradictory. A review by Brossier *et al.* on the performance of the DiversiLab system for *P. aeruginosa* concluded that the results should be interpreted with caution, and always in combination with epidemiological data, as was done in our study (21).

Conclusion

The network analysis indicated that the majority of transmissions occurred on the wards, but through unidentified and presumably persistent sources, which are most likely in the innate hospital environment. Previous use of certain antibiotic regimens made patients prone to VIM-PA carriage. Additionally, gastroscopy could be considered as a high-risk procedure in patients with risk factors.

Recommendation

If there is an outbreak with VIM-PA, we showed that first; the entire ward should be seen as reservoir and as contaminated. Therefore, cleaning and disinfection practices should be installed and possible sources should be eliminated. We also feel that it is especially important to search for unknown reservoirs in the environment. Second, use of particularly quinolones should be avoided because this could make a patient ‘prone’ for acquiring VIM-PA. Third, we showed that in an outbreak setting gastroscopy and bronchoscopy could be seen as high-risk procedures. Finally, a case-control study should be executed to identify outbreak specific risk factors. Because we showed that if you

change matching criteria outcomes do differ, it would be advisable to always include multiple definitions for control inclusion.

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SUPPLEMENTAL MATERIAL

Supplement 1. The number of clinical admissions and clinical admission days and the number of patients included in this study from 2003 until 2015.

Supplement 2. List of all variables extracted from electronic medical records of included case and control patients.

Supplement 3. Treatment related variables, categorical, univariate analysis. Variables with an asterisk (*) were selected for multivariable analysis.

Supplement 4. Subgroup analysis DiversiLab (bioMérieux) type A and type B, univariate analysis. Variables with an asterisk (*) were selected for multivariable analysis.

Supplement 5. Subgroup analysis ICU or non-ICU as ward of acquisition of VIM-PA, univariate analysis. Variables with an asterisk (*) were selected for multivariable analysis.

Supplement 6. Crude odds ratios; control group 1&2 versus control group 3&4. Variables with an asterisk (*) were selected for multivariable analysis.

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Chapter 3.2

High prevalence rate of digestive tract bacteria in duodenoscopes: a nationwide study

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ABSTRACT

Increasing numbers of outbreaks caused by contaminated duodenoscopes used for Endoscopic Retrograde Cholangiopancreatography (ERCP) procedures have been reported, some with fatal outcomes. We conducted a nationwide cross-sectional study to determine the prevalence of bacterial contamination of reprocessed duodenoscopes in The Netherlands. All 73 Dutch ERCP centers were invited to sample ≥2 duodenoscopes using centrally distributed kits according to uniform sampling methods, explained by video instructions. Depending on duodenoscope type, four to six sites were sampled and centrally cultured. Contamination was defined as (i) any microorganism with ≥20 colony forming units (CFU)/20 mL (AM20) and (ii) presence of microorganisms with gastrointestinal or oral origin, independent of CFU count (MGO). Sixty-seven out of 73 centers (92%) sampled 745 sites of 155 duodenoscopes. Ten different duodenoscope types from three distinct manufacturers were sampled including 69 (46%) Olympus TJFQ180V, 43 (29%) Olympus TJF-160VR, 11 (7%) Pentax ED34-i10T, 8 (5%) Pentax ED-3490TK and 5 (3%) Fujifilm ED-530XT8. Thirty-three (22%) duodenoscopes from 26 (39%) centers were contaminated (AM20). On 23 (15%) duodenoscopes MGO were detected, including *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumonia* and yeasts. For both definitions, contamination was not duodenoscope type dependent (P values: 0.20 and higher). In 39% of all Dutch ERCP centers, at least one AM20-contaminated patient-ready duodenoscope was identified. Fifteen per cent of the duodenoscopes harbored MGO, indicating residual organic material of previous patients, that is, failing of disinfection. These results suggest that the present reprocessing and process control procedures are not adequate and safe.

BACKGROUND

Very recently, an increasing number of infectious outbreaks involving multidrug-resistant organisms (MDRO) caused by contaminated duodenoscopes, used for Endoscopic Retrograde Cholangiopancreatography (ERCP) procedures, have been reported in both Europe and USA (1-5). These include outbreaks of infections with carbapenem-resistant Enterobacteriaceae, such as *Escherichia coli* and *Klebsiella pneumoniae* (1, 2), of which some have been associated with fatal outcomes (6). Post-ERCP infections typically range between 2% and 4% (7). It is not clear to what extent these infections are caused by the procedure itself (*i.e.* endogenous infections) or to what extent contaminated duodenoscopes are the source of infection (*i.e.* exogenous infections). For example, in one specific outbreak with a persistently contaminated duodenoscope, 14.4% of all patients who underwent an ERCP were found to be colonized or infected (8). Outbreaks can be traced by bacterial typing. Especially when MDRO strains are involved, detection is easier as laboratories usually store these resistant strains and (retrospective) typing can be performed. This raises the question whether outbreaks with duodenoscopes are a new and emerging problem or whether outbreaks are only detected more frequently because of increased awareness facilitated by recognizable MDRO in patients (2, 9).

During procedures in the gastrointestinal tract, all flexible endoscopes including duodenoscopes become heavily exposed to gastrointestinal flora (10). Therefore, flexible endoscopes are reprocessed after each procedure: a multistep process involving flushing, manual cleaning, automated cleaning, high-level disinfection and drying. Duodenoscopes are more difficult to reprocess compared with other flexible endoscopes (10). This is due to their complex design, which includes a side viewing tip, forceps elevator and elevator channel. Patient-ready duodenoscopes can be contaminated because of breaches in the reprocessing protocol, inadequate handling or because the current technique of reprocessing may be inadequate for the currently available duodenoscope design (11). Recent outbreaks have been documented to occur even when manufacturers' Instructions For Use (IFU) for reprocessing were followed to the letter (2, 5, 9).

In the Netherlands, as in many other parts of the world, process control is used. This means that reprocessing is considered to be adequate when it is performed according to the IFU and according to the standard handbook of the Dutch Steering Group for Flexible Endoscope Cleaning and Disinfection (SFERD) (12). This handbook is based on regulations applicable in the Netherlands as well as the guidelines of the European Society of Gastrointestinal Endoscopy (ESGE) (13). Despite international outbreaks and outbreaks in Dutch ERCP centers, both the IFU and SFERD do not include microbial surveillance after disinfection as a routine practice (14, 15). Recently, contamination of duodenoscopes has been assessed in several studies (16-18). Most studies were performed in a single university center, making it difficult to extrapolate their results and estimate

the true burden on a national level (17, 18). A study among 21 centers was conducted by Brandabur et al, showing contamination rates with a wide variability across centers (16). To date, no such study has been conducted in a nationwide setting using a uniform sampling and culture method as well as examining all possible contamination sites. Given the increase in the number of publications pertaining duodenoscope contamination and the potentially severe consequences for patients, there is an urgency to develop a more thorough understanding of the scale of the problem. Therefore, the aim of this study was to determine the prevalence of microbial contamination of patient-ready duodenoscopes in all ERCP centers in the Netherlands.

METHODS

Setting

We conducted a prospective nationwide cross-sectional study among all Dutch ERCP centers. In the Netherlands, over 16.000 ERCP procedures are performed in 73 ERCP centers yearly (19). All 73 Dutch ERCP centers were asked to sample at least two duodenoscopes at their own choosing and, if present, to include the newest Olympus TJF-Q180V (Olympus, Zoeterwoude, The Netherlands). Duodenoscopes were eligible for sampling if they were reprocessed and ready for patient use, for example, after high level disinfection or after drying in the storage cabinet. No data were recorded about the moment of sampling, surveillance methods or adherence to reprocessing or sampling protocols. No patient data were included in this study; therefore, there was no need for approval by the Medical Ethical Research Committee.

Sample collection

Sampling was performed independently by local staff of the included ERCP centers, using a centrally distributed sample collection kit, according to a strict and uniform sampling protocol (see supplementary files). This method was developed by a multidisciplinary team of reprocessing staff, medical device experts, infection control professionals, medical microbiologists and gastroenterologists based on the SFERD standard handbook (12). The sampling protocol was explained using 12 instruction videos available online (see online supplementary videos). As examples, the sampling and labelling procedure was shown in detail using one Olympus TJF-160VR and one Pentax ED34- i10T (Pentax, Dodewaard, The Netherlands) duodenoscope. Duodenoscopes were sampled while placed in the Automated Endoscope Reprocessor or on a sterile surface. Depending on the duodenoscopes type, four to six sites were sampled. The four sites present in all duodenoscope types were: (i) a flush of the biopsy channel, (ii) a flush of the suction channel, (iii) a swab from the forceps elevator and (iv) a single brush through the biopsy

and suction channel. Type-dependent samples were: (i) a swab of the removable protection cap and (ii) a flush of the elevator channel or air/water channel, if these channels were unsealed. Channels were flushed with sterile physiological saline solution of which at least 20 mL was collected at the distal tip in a sterile container. The flush fluid was aspirated with a sterile needle and injected in two 9.5 mL BD Vacutainers without additives (Becton Dickinson, Etten-Leur, The Netherlands). Forceps elevator and protection cap were sampled with ESwabs (Copan Italia S.p.A., Brescia, Italy). Type dependent, Olympus BW-412T or Pentax CS6021T single-use endoscope cleaning brushes were used to brush the biopsy and suction channel. Both ESwabs and the brush tip were transported in ESwab medium. Instructions were to swab first, second to flush the channels and finally to brush the channels. The decision to reprocess the endoscope after sampling was up to the respective centers and was not documented for the purpose of the current study. Samples were sent to the Erasmus MC department of Medical Microbiology and Infectious Diseases for culturing.

Culturing and interpretation

Samples were cultured on the day of receipt. Channel flushes were filtrated over a 0.45 μm filter of which the filtrate was forced on R2A agar. ESwabs and brush tips were vortexed in their ESwab medium of which 0.75 mL was poured on a blood agar. Samples were incubated at 35°C, examined for growth for 72 hours and read at 24 hours, 48 hours and 72 hours. Culture results were presented in colony forming units (CFU)/20 mL per microorganism. Results were sent to the respective ERCP centers without further interpretation: further action was up to the respective ERCP center and was not documented for the purpose of the current study. At the time of study conduct, Dutch guidelines for endoscopy centers stated that in case of contamination with a subset of indicator microorganisms with ≥20 CFU/20 mL or in case of persistent contamination, endoscopes should be quarantined and possible causes be investigated (12). Cultured microorganisms were categorized depending on their origin into gastrointestinal, oral, skin and waterborne flora. Contamination was defined according to two definitions: (i) microbial growth with ≥20 CFU/20 mL of any type of microorganism (AM20) as used by the ESGE guideline and Dutch SFERD handbook (12, 13), or (ii) presence of microbial growth (≥1 CFU/20 mL) of gastrointestinal and/or oral microorganisms (MGO).

Statistical analysis

Categorical data are presented in percentages. Mean (range) and median (IQR) are given for continuous and skewed data, respectively. The χ^2 test was used to compare categorical data and Student's t-test or Mann Whitney U-test was used to compare continuous data. Contamination rates of duodenoscope types and sample sites were compared according to a logistic regression model, using the SAS procedure GENMOD.

This model adjusted for the multiple samples of each unique duodenoscope, with each duodenoscope clustered within their respective ERCP center. Duodenoscope types were compared with the newest Olympus TJF-Q180V type as a reference and sample sites were compared with the flush of the biopsy channel. For both analyses, duodenoscope types or sample sites could be included if there was at least one contamination case and one non-contamination case. Analyses were performed using SAS V.9.4 (SAS, Cary, North Carolina, USA) and SPSS V.21.0 (IBM, Armonk, New York, USA).

RESULTS

Between June 2015 and March 2016, 67 out of 73 (92%) Dutch ERCP centers sampled 745 sites of 155 endoscopes. Five endoscopes were excluded: four duodenoscopes from one center whose samples were cultured in their own microbiology department and one gastroscope from another center as this type of endoscope does not have a forceps elevator, that is, no duodenoscope (Figure 1). Twenty-six samples from 17 duodenoscopes were excluded, as these sites did not correspond with the specified duodenoscope type. This resulted in an inclusion of 150 duodenoscopes with a total of 701 samples from 66 (92% of all centers) ERCP centers (Figure 1). The median time between local sampling

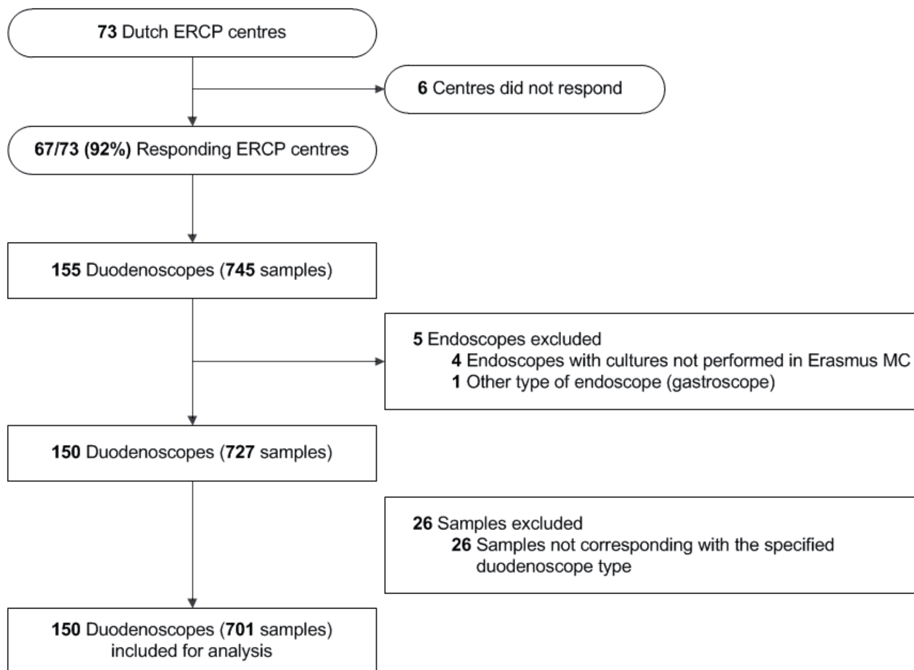

Figure 1. Flow diagram. ERCP, Endoscopic Retrograde Cholangiopancreatography.

and culturing in the Erasmus MC was 1 day (IQR 1–2). Table 1 provides an overview of the contamination prevalence per duodenoscope type and sample site for AM20 and MGO contamination definitions.

Table 1. Prevalence of AM20 and MGO contamination for duodenoscopes and sample sites.

Duodenoscope type	N	AM20		MGO	
		Contam.	Not contam.	Contam.	Not contam.
All duodenoscopes	150	33 (22%)	117 (78%)	23 (15%)	127 (85%)
Olympus TJF-Q180V	69	15 (22%)	54 (78%)	15 (22%)	54 (78%)
Olympus TJF-160VR	43	13 (30%)	30 (70%)	6 (14%)	37 (86%)
Olympus TJF-160R	8	1 (13%)	7 (87%)	0	8
Olympus TJF-140R	2	0	2	0	2
Olympus TJF-145	2	0	2	0	2
Pentax ED34-i10T	11	3 (27%)	8 (73%)	0	11
Pentax ED-3490TK	8	0	8	0	8
Pentax ED-3680TK	1	0	1	1 (100%)	0
Fujifilm ED-530XT8	5	0	5	0	5
Fujifilm ED-530XT	1	1 (100%)	0	1 (100%)	0
Sample site		**AM20**		**MGO**	
		Contam.	**Not contam.**	**Contam.**	**Not contam.**
All sample sites	701*	47 (7%)	654 (93%)	35 (5%)	666 (95%)
Biopsy channel	146	5 (3%)	141 (97%)	6 (4%)	140 (96%)
Suction channel	137	4 (3%)	133 (97%)	5 (4%)	132 (96%)
Forceps elevator	148	14 (10%)	134 (90%)	7 (5%)	141 (95%)
Brush	139	17 (12%)	122 (89%)	14 (10%)	125 (90%)
Protection cap	56	6 (11%)	50 (89%)	3 (5%)	53 (95%)
Elevator channel	53	0	53	0	53
Air/water channel	26	1 (5%)	21 (95%)	0	22

*Sampling of all possible sites would have yielded 745 samples: 44 (6%) sites were not sampled. This included 4/150 (3%) biopsy channel, 13/150 (9%) suction channel, 2/150 (1%) forceps elevator, 11/150 (7%) brush, 9/65 (13%) protection cap, 2/55 (4%) elevator channel and 3/25 (12%) air/water channel samples.
AM20, Microbial growth with ≥20CFU/20mL of any type of microorganism; MGO, Presence of any microbial growth of gastrointestinal or oral microorganisms; Contam., contaminated. Not contam., not contaminated.

Contamination according to the AM20 definition was found in 33 (22%) out of the 150 reprocessed and patient-ready duodenoscopes. Duodenoscopes were most often contaminated with skin flora (n=17; 11%) and to a lesser extent with waterborne flora (n=12; 8%), gastrointestinal flora (n=10; 7%) or oral flora (n=4; 3%). Contamination according to the MGO definition was found in 23 (15%) duodenoscopes. Table 2 shows all different microorganisms that were cultured, among others *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Table 2. Cultured microorganisms of 150 duodenoscopes.

Gastrointestinal flora independent of CFU count			**Oral flora independent of CFU count**		
	No. of duodenoscopes	**Quantity range**		**No. of duodenoscopes**	**Quantity range**
Yeasts	7	6 - 100 CFU	*Moraxella* spp.	4	1 CFU
Klebsiella pneumoniae	4	100 - >100 CFU	*Streptococcus salivarius*	4	1 – 15 CFU
Enterobacter cloacae	3	100 - >100 CFU	*Moraxella osloensis*	3	1 CFU – 100 CFU
Escherichia coli	2	50 and 100 CFU	*Streptococcus mitis*	2	30 and 50 CFU
Enterococcus faecalis	1	100 CFU	*Neisseria flavescens*	1	1 CFU
Enterococcus faecium	1	1 CFU	*Rothia* spp.	1	10 - 30 CFU
Klebsiella oxytoca	1	100 - >100 CFU	*Streptococcus mutans*	1	2 CFU
Pseudomonas aeruginosa	1	100 CFU	*Streptococcus orali*	1	5 CFU
Staphylococcus aureus	1	>100 CFU	*Streptococcus* spp.	1	10 CFU
Skin flora ≥20 CFU			**Water-borne flora ≥20 CFU**		
	No. of duodenoscopes	**Quantity range**		**No. of duodenoscopes**	**Quantity range**
Bacillus spp.	5	40 - 100 CFU	*Stenotrophomonas maltophilia*	3	100 - >100CFU
Micrococcus luteus	5	100 CFU	*Acinetobacter* spp.	2	80 and 100 CFU
Staphylococcus epidermidis	4	50 - 100 CFU	*Agrobacterium radiobacter*	2	20 and 100 CFU
Kocuria spp.	2	25 and 100 CFU	*Paraoccus yeeii*	2	30 and 100 CFU
Staphyloccocus hominis	2	25 and 100 CFU	*Achromobacter xylosoxida*	1	100 CFU
Staphyloccocus warneri	2	50 and 80 CFU	*Alternaria* spp.	1	>100 CFU
Kocuria rhizophila	1	>100 CFU	*Pseudomonas monteilii*	1	100 CFU
Micrococcus spp.	1	30 CFU	*Pseudomonas putida*	1	100 CFU
Staphylococcus auricularis	1	>100 CFU	*Sphingomonas paucimobilis*	1	100 CFU
Staphylococcus spp. (CNS)	1	60 CFU	*Rhizobium* spp. or *sphingobium* spp.	1	>100 CFU

CFU, colony forming units; CNS, coagulase – negative staphylococci.

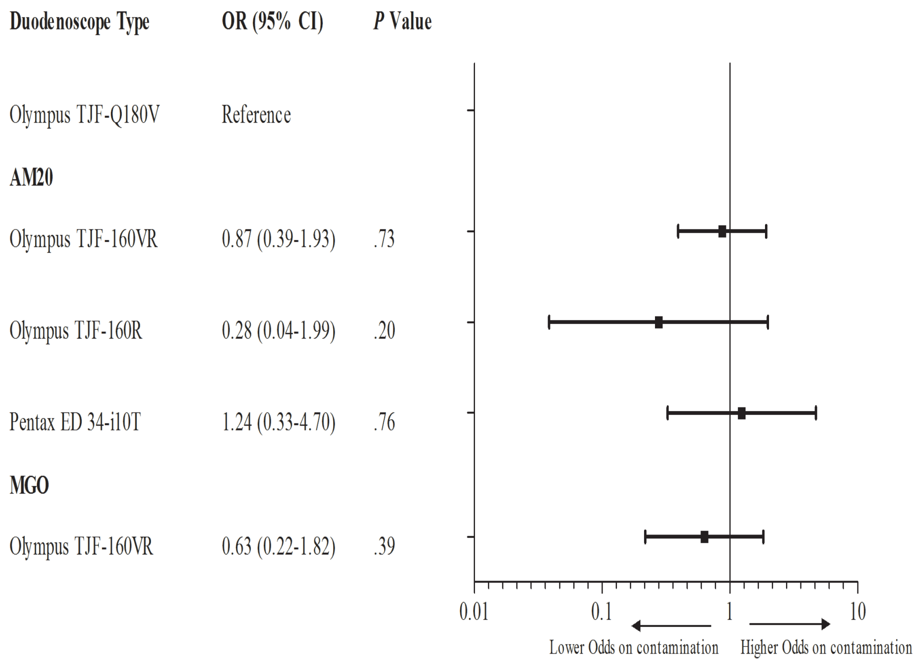

Figure 2. OR for each duodenoscope type on contamination. AM20, microbial growth with ≥20CFU/20 mL of any type of microorganism; CFU, colony forming units; MGO, presence of any microbial growth of gastrointestinal or oral microorganisms.

Ten different duodenoscope types from three distinct manufacturers (*i.e.* Olympus, Pentax and Fujifilm) were sampled. Contamination as defined by AM20 was identified in five different duodenoscope types and contamination as defined by MGO was identified in four different types. As shown in Figure 2, contamination for AM20 (four duodenoscope types included) as well as MGO (two duodenoscope types included) was shown not to be type-dependent (all P>0.05).

The AM20 contaminated duodenoscopes originated from 26 (39%) centers across the Netherlands. No difference (P=0.10) was shown in contamination prevalence between academic tertiary medical centers (n=3/8; 38%), specialized peripheral medical centers (n=13/23; 57%) or general peripheral medical centers (n=10/35; 29%). This was also the case for MGO-contaminated duodenoscopes originating from 19 (28%) centers. No difference was found (P=0.25) between academic tertiary medical centers (n=3/8; 38%), specialized peripheral medical centers (n=9/23; 39%) and general peripheral medical centers (n=7/35; 20%).

Microorganisms were cultured from 166 (24%) sample sites of 97 (65%) duodenoscopes. Additionally, 54 (8%) sample sites of 41 (27%) duodenoscopes contained two or

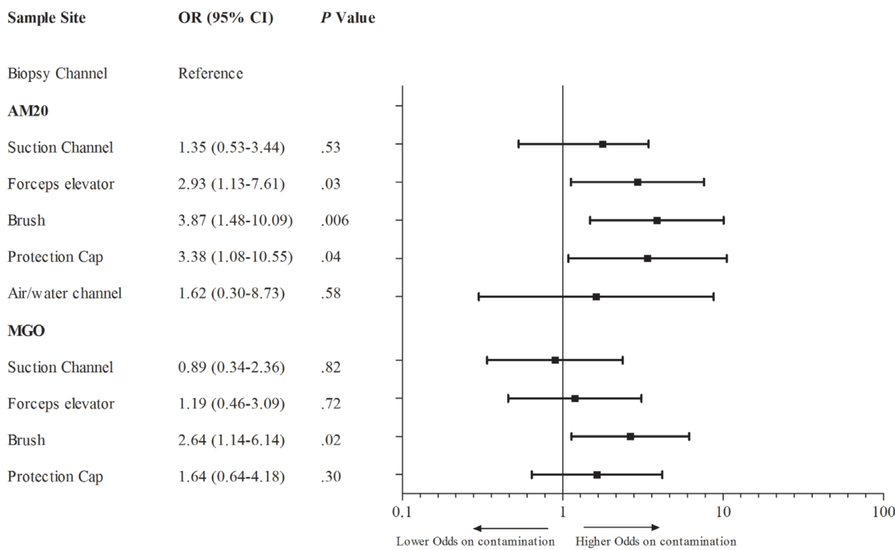

Figure 3. OR for each sample site on contamination. AM20, microbial growth with ≥20CFU/20 mL of any type of microorganism; CFU, colony forming units; MGO, presence of any microbial growth of gastrointestinal or oral microorganisms.

more microorganisms, in some cases up to five different microorganisms. As shown in table 1, all sample sites, except the flush of the elevator channel, were found positive for AM20 or MGO contamination. The flush of the biopsy channel was used as a reference to compare the contamination prevalence of all sample sites. Three sample sites had a higher probability of being contaminated (Figure 3). According to the AM20 definition, the swab of the elevator (OR 2.93, 95% CI 1.13 to 7.61; P=0.03) and the swab of the protection cap (3.38, 95% CI 1.08 to 10.55; P=0.04) were more often contaminated. The brush of the biopsy/suction channel was more often contaminated for both AM20 (OR 3.87, 95% CI 1.13 to 7.61; P=0.006) and MGO (OR 2.64, 95% CI 1.14 to 6.14; P=0.02) definitions.

DISCUSSION

In our nationwide prevalence study, we found that over one-fifth of sampled duodenoscopes were contaminated according to AM20 definition, with 39% of Dutch ERCP centers having at least one contaminated duodenoscope intended to be ready for patient use. Furthermore, MGO were cultured on 15% of the sampled duodenoscopes, indicating the presence of organic residue of previously treated patients. Our observations coincide with worldwide reported outbreaks indicating that exogenous transmission

of bacteria and associated infections and even viral infections related to contaminated duodenoscopes continue to threaten patients undergoing ERCP (1-3, 5, 20). Therefore, stringent measures are required to lower the number of contaminated duodenoscopes in order to minimize the risk of interpatient microbial transmission during ERCP and to prevent future outbreaks.

The prevalence of duodenoscope contamination in this study was in line with reports from several retrospective single tertiary center studies (18, 21, 22). Recent studies by Brandabur *et al.* and Ross *et al.* performing post procedure or everyday morning cultures reported remarkably lower contamination rates (16, 17). This could be explained by the fact that continuous feedback of microbial surveillance resulted in a raised alertness, resulting in lower contamination rates over time. In the centers included in the present study, it is not common practice to perform surveillance cultures, especially no daily or post procedure cultures, as Dutch guidelines do not demand these (12, 23). Other contributing factors could be differences in sampling and culture methods. For example, we used a more sensitive contamination cut-off and a longer incubating time than Brandabur *et al.* and Ross *et al* (16, 17). The present study was conducted in 2015–2016 after multiple MDRO-outbreaks were reported (inter)nationally, including reports of outbreaks in Dutch ERCP centers as early as 2009 and 2012 (14, 15). Despite current national awareness about the potential consequences of contamination, our results were concordant with a cross-sectional multicenter (n=37) Canadian study published in 2002 in which a contamination prevalence of 30% was reported using a contamination cut-off of 10 CFU/mL (24).

The most recent duodenoscope types introduced into the market have distinct design changes, including sealing of the elevator channel and a sealed protection cap, aimed at preventing contamination and the need for reprocessing at these locations. In 2012, an outbreak in our hospital was linked to the newest Olympus TJF-Q180V duodenoscope (5). After the outbreak, the duodenoscope was investigated by Olympus and an independent expert. One of the conclusions was that TJF-Q180V's specific design features hampered adequate cleaning and disinfection (15). To further investigate these matters, we asked participating centers to include the TJF-Q180V duodenoscope, if present. The current study shows that contamination for both AM20 and MGO were not restricted to certain duodenoscope types. This is in line with outbreaks that have been reported involving various duodenoscope types from all three manufacturers (6). Moreover, Brandabur *et al.* also reported that culture positivity was not affected by scope type (16). Despite differences in design, none of the available duodenoscope types seem excluded from the risk of contamination.

The differences in the type of cultured flora can give an indication where in the reprocessing process the duodenoscopes were contaminated. Several guidelines that advocate active microbiological surveillance give guidance on how to interpret culture

results (13, 25). In this study, a substantial number of duodenoscopes were contaminated with skin and waterborne flora. Contamination with skin flora is thought to arise from handling and therefore could potentially easily be reduced by improved handling during reprocessing and transport. However, the presence of skin flora could be due to contamination during sampling. We cannot rule out this cause as sampling on site was not audited. Dutch centers have to use filtered water for reprocessing facilities and process control involves quarterly microbiological control of the rinse water (12). In our view, persistent contamination with waterborne flora demands a thorough investigation as it can be caused by several factors, including contamination of the water supply, inadequate filtering of the water supply and inadequate drying of the endoscope during storage. Contamination with MGO indicates inadequate reprocessing as originating from the gastrointestinal tract. This type of contamination could be due to a breach in the reprocessing procedure or because the reprocessing procedure cannot be adequately performed due to reprocessing, endoscopic or procedure specific risk factors. Currently, we are working on a Dutch guideline in which actions following positive cultures will be described extensively. The guideline will be submitted for international publication in the near future. Differences in Automated Endoscope Reprocessors, endoscope hang time and different reprocessing methods do not seem to affect contamination rates (16, 26, 27). Beside the complex design of the duodenoscope (5, 6, 28), endoscope age has also been suggested as a risk factor (5, 18, 26), with Brandabur *et al.* proposing the number of procedures as a better indicator for endoscope usage (16). Contamination does not seem to be confined to duodenoscopes: single-center studies show that coloscopes and gastroscopes can have similar contamination rates (18, 22). However, compared with duodenoscopes, other gastrointestinal endoscopes are far less the reason of recent reported outbreaks (7). We hypothesize that this could be due to differences between types of procedures as ERCP procedures tend to be more invasive, entering sterile body cavities and could have a more compromised patient population. The latter defines the more serious and therefore detectable clinical outcome of transmission of microorganisms by ERCP compared with other gastrointestinal endoscopes.

In the present study, the brush, the forceps elevator and the protection cap had the highest probability of detection of contamination. The forceps elevator is a site known to be prone to persistent contamination (2, 5, 16, 17). The brush is also noted as a site that can harbor the involved microorganism during an outbreak (17). Borescope channel inspections of gastroscopes and coloscopes performed by Ofstead *et al.* revealed that all reprocessed endoscope channels contained fluid, discoloration and debris (29). This underlines that the biopsy channel is subject to heavy wear and tear: devices are introduced frequently, causing soiling of the channel which adds to the risk of contamination (30). Remarkably, in the present study, the elevator channel was not contaminated in any duodenoscope and the air/water channel in only one duodenoscope. Sampling of

these specific channels is often not performed during surveillance and often not even in the case of an outbreak (17).

In current guidelines and studies, there is no international consensus on a uniform sampling and culturing method, although several differences could potentially affect culture outcomes. The location and the number of sample sites differ greatly: in some instances, a channel brush (18, 31) or swab of the forceps elevator (12, 24) is omitted. When the channel brush or the forceps elevator would not be cultured in the present series, 19% (6/32) or 9% (3/32), respectively, of the AM20 contaminated duodenoscopes would have been missed. Some studies and guidelines advocate a different order of sampling, such as retrograde sampling or the flush-brush-flush method, as it might have a higher sensitivity (14, 25, 31, 32). The cleaning brush that is used for sampling could disrupt present biofilms and affect subsequent samples. However, in this study, the brush sample was performed last. A sample flush with a neutralizer instead of saline solution can prevent false negative outcomes due to the biocidal activity of residual disinfectants (33, 34) and is advocated by the French guideline and several French studies (18, 21, 33, 35). The toxicity of the neutralizers might also cause false negatives (36), and theoretically the endoscope should not contain any residual disinfectant after a successful reprocessing cycle. Other guidelines including the Dutch guideline, according to which our sampling protocol was designed, do not require a neutralizer based on current evidence (12, 13, 25, 31). However, if a neutralizer effectively prevents false negative outcomes, the contamination rates in this study could be even higher. A longer incubation time is associated with a higher culture positivity rate. Saliou *et al.* state that endoscope samples should be incubated for at least 1 week. In their study, after 48 hours only 55.5% of the final number of contaminated endoscopes were found positive (18). Some studies and guidelines use an incubation time of 48 hours (16, 17, 25, 31). In this study, we have chosen for a 72 hours period: the microorganisms of concern would be detected and the study results could by compared with the centers' previous microbiological surveillance results. Also, the choice of growing media for incubation of flush samples can affect the culture positivity rate. R2A agar, as used in this study, has a high sensitivity, especially for slower growing microorganisms (37, 38). To be able to compare test results and omit false negative test results, standardized and uniform instructions for sampling, culturing and interpretation of culture results should be devised which, based on results in this study, should include a channel brush and a swab of the forceps elevator as these sites pose the highest risk of contamination.

To the best of our best knowledge, this is the first study assessing contamination of duodenoscopes nationwide. Another strength of our study is that we cultured all samples in one microbiology laboratory using a standardized protocol. Finally, because of the extensive sampling method we were able to analyses all possible contamination sites. This study has some limitations. This study could only be conducted nationwide as

a cross-sectional study without follow-up samples of the duodenoscopes: improvement of contamination rates or persistent contamination was not assessed. Furthermore, sampling was conducted independently by local staff. Although we provided strict sampling protocols with clear video instructions on how the culture procedure should be performed, we were not able to check for adherence to the sampling protocol. Also the conditions in which the endoscopes were sampled (*i.e.* just disinfected or after drying with or without alcohol flush or positive air flow) were not recorded. Potential differences in culture outcomes between sampling post-disinfection or post drying, differences in drying times or other storage or reprocessing parameters could not be assessed. However, all assessed duodenoscopes were ready for use in patients and should not be contaminated, regardless of the moment of reprocessing. We hypothesize that the effect of these factors on the presence of especially gastrointestinal and or oral flora is rather small, as we see this as a failure of the reprocessing process. Last, a small amount of sites were not sampled, which could cause underestimation of the total number of contaminated duodenoscopes.

The observed nationwide high prevalence of contamination of patient-ready duodenoscopes is a clear indication that the current combination of reprocessing and process control is not sufficient. All participating hospitals are dedicated endoscopy centers following the national guideline that underlines process control. This includes reprocessing exactly according to the manufacturer's instructions and extensive yearly audits (12, 23). As adherence to reprocessing protocols was not observed, this study shows real-life outcomes of patient-ready duodenoscopes with little bias. Regardless of whether the precise cause of contamination was a breach in the reprocessing process or the complex duodenoscope design, process control was not able to identify and prevent such large-scale inadequate reprocessing. This calls for concerted action by all parties involved, that is,: manufacturers, regulatory bodies, government agencies, gastroenterologists and medical microbiologists. Nowadays, ERCP has evolved into a minimally invasive interventional procedure having replaced more invasive and complicated surgical procedures. It is an essential procedure practiced all over the world with over 650 000 procedures performed in USA annually (39). During revision of the market clearance of the Olympus TJF-Q180V duodenoscope, the U.S. Food and Drug Administration (FDA) stated that a decrease in ERCP capacity would be unacceptable (40). However, contaminated duodenoscopes put patients at risk of developing clinically relevant infections by transmission of microorganisms. In 2015, the FDA issued a warning that some parts of duodenoscopes may be extremely difficult to access and adequate cleaning of all areas may not be possible (28). Since then additional measures have been suggested (11), including alternative reprocessing methods or implementation of microbial surveillance as proposed by Centers for Disease Control and Prevention (10, 31). Eventually, radical changes in the design of duodenoscopes should ensure thorough cleaning and

disinfection. However, development and market introduction of such newly designed duodenoscopes will require substantial time. A complicating factor is that standardized procedures to test duodenoscopes in their ability to be adequately cleaned and disinfected are not available. Therefore, on the short term, we should not solely rely on process control as there is no scientific proof that this serves as a reliable proxy for safe and clean duodenoscopes. Uniform guidelines and instructions for microbial surveillance should be developed. Also, an international registry for contaminated scopes should be instituted in order to truly estimate the scale of the problem and track its impact and revolution over time.

To conclude, this nationwide cross-sectional study shows high prevalence rates of contamination of duodenoscopes in Dutch ERCP centers. The recent reports on infections due to contaminated endoscopes will probably be due to involvement and alertness on highly resistant microorganisms, but also the more and more complex designs of endoscopes can play a role in this emergence. Additional preventive measures including microbial surveillance strategies are needed to reduce the number of contaminated duodenoscopes.

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SUPPLEMENTAL MATERIAL

Supplement 1: Sampling protocol Olympus ERCP duodenoscope
Supplement 2: Sampling protocol Pentax ERCP duodenoscope

ONLINE ONLY SUPPLEMENTARY VIDEOS:

Video 1. Instruction video Olympus TJF-160VR: swab forceps elevator
Video 2. Instruction video Olympus TJF-160VR: swab protection cap
Video 3. Instruction video Olympus TJF-160VR: flush elevator wire channel
Video 4. Instruction video Olympus TJF-160VR: flush suction channel
Video 5. Instruction video Olympus TJF-160VR: flush biopsy channel
Video 6. Instruction video Olympus TJF-160VR: brush biopsy/suction channel
Video 7. Instruction video Pentax ED34-i10T: swab forceps elevator
Video 8. Instruction video Pentax ED34-i10T: swab protection cap
Video 9. Instruction video Pentax ED34-i10T: flush suction channel
Video 10. Instruction video Pentax ED34-i10T: flush air/water channel
Video 11. Instruction video Pentax ED34-i10T: flush elevator wire channel
Video 12. Instruction video Pentax ED34-i10T: brush biopsy/suction channel

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Chapter 3.3

An outbreak of *Clostridium difficile* infections due to new PCR ribotype 826: epidemiologic and microbiologic analyses

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ABSTRACT

The aim of this study was to investigate an unusual outbreak of five patients with a total of eight episodes of a *Clostridium difficile* infection on a gastrointestinal surgical ward of a Dutch tertiary-care university-affiliated hospital. Clinical case investigations and laboratory analyses were performed. Laboratory analyses included PCR ribotyping, multiple-locus variable-number tandem repeat analysis typing, toxin typing, antimicrobial susceptibility testing and whole genome sequencing. The outbreak was associated with recurrent and severe disease in two out of five patients. All episodes were due to a unique ribotype that was not recognized in the collection of an international network of reference laboratories and was assigned PCR ribotype 826. PCR ribotype 826 is a toxin A-, toxin B- and binary toxin- positive ribotype which according to molecular typing belongs to clade 5 and resembles the so called hypervirulent ribotype 078. The presence of a clonal outbreak was confirmed by whole genome sequencing, yet the source of this newly identified ribotype remained unclear. This newly identified *C. difficile* PCR ribotype 826 is part of clade 5 and might also have increased virulence. The recognition of this outbreak highlights the need of ongoing *C. difficile* infection surveillance to monitor new circulating ribotypes with assumed increased virulence.

INTRODUCTION

We identified an outbreak of eight episodes of *Clostridium difficile* infection (CDI) in five patients within a 4-month period (1 December 2015-31 March 2016). The outbreak occurred on a gastrointestinal surgical ward of a Dutch tertiary-care hospital. In this case series, we describe the clinical characteristics of affected patients and microbiologic investigations that were performed on the identified strain.

METHODS

The case series was conducted at a gastrointestinal surgical ward of the Erasmus University Medical Center in Rotterdam, the Netherlands. The Erasmus MC participates in the national sentinel CDI surveillance program and therefore sends all samples from hospitalized CDI patients to the national Reference Laboratory for PCR ribotyping (I.K. Sanders *et al.*, paper presented at the 25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID 2015), abstract P0793, 2015) (1). In case of an outbreak (defined as more than two isolates of the same type detected less than 7 days apart in one hospital either with onset of symptoms on the same ward, or accompanied by an increased CDI monthly incidence within the hospital; http://www.rivm.nl/Documenten_en_publicaties/Algemeen_Actueel/Uitgaven/Infectieziekten/CDiffNL/Tenth_Annual_Report_of_the_National_Reference_Laboratory_for_*Clostridium_difficile*_and_results_of_the_sentinel_surveillance), additional analyses can be performed by the Reference Laboratory. These include multiple-locus variable-number tandem repeat analysis (2), PCRs for toxin genes (3), PCRs for clade-specific makers (4), antimicrobial susceptibility screening tests (Etest) and whole genome sequencing (5).

Patient information and medical history from all CDI cases during this outbreak were collected from the electronical medical records. Defined daily doses for all antibiotics used up to three months before development of CDI and Charlson comorbidity scores were calculated (6). CDI was classified as severe if one or more of the following conditions were present (attributable to CDI): fever (temperature of 38.5^{0}C or higher), rigors, hemodynamic instability, ileus, peritonitis, mental status changes, admission to ICU, end organ failure, leukocytosis (>15 x 109), leukopenia (<2 x 109), hypoalbuminemia (<30g/L), >1.5-fold increase in creatinine level above baseline, serum lactate >2.2mmol/L, pseudomembranous colitis, colonic wall thickening, pericolonic fat stranding or ascites. All other cases were classified as mild CDI (7, 8).

Written approval to conduct the case series was received from the medical ethics research committee of Erasmus University Medical Center, Rotterdam, the Netherlands (MEC-2015-306).

RESULTS

The CDI incidence rate on the gastrointestinal surgical ward was 3.3 per 10,000 patient-days (July 2009 to November 2015) and increased to 19.8 per 10,000 patient-days (December 2015-March 2016). In total, six patients with CDI were diagnosed, five of whom had the same PCR ribotype.

The index case of this outbreak (patient A) was an 83-year-old man who underwent pancreaticoduodenectomy to treat a carcinoma of the common bile duct 1 month earlier. In December 2015, during a readmission that was due to infected ascites, he developed diarrhoeal symptoms and was diagnosed with hospital-acquired CDI. Within 1 week after the start of his symptoms, two other patients (patients B and C) on the same ward were diagnosed with hospital-acquired CDI. All three patients were treated with a 7- to 11-day oral course of metronidazole and discharged.

In January 2016, a fourth hospital-acquired CDI case (patient D) on the ward was noticed. In February 2016, patient A was readmitted because of a CDI recurrence, and a fifth case (patient E) was reported. Patient A was readmitted once more to treat a second recurrence in February, and patient D was also diagnosed with a CDI recurrence in March. In total, four of eight CDI episodes (in two patients) were classified as severe CDI. None of the patients was admitted to the intensive care unit because of CDI, and no CDI-related mortality (within 30 days) occurred. All patients had received antibiotic therapy before acquiring CDI, and total defined daily doses of antibiotics administered before the onset of CDI ranged from 21 to 63 (median, 26.9). Four out of five patients had received therapy with proton pump inhibitors before the CDI diagnosis. The median Charlson comorbidity score was 2 (range 0 to 8).

In accordance with local guidelines, all patients who had or who were suspected to have CDI were placed in a single room and were not allowed to use shared sanitation. Medical personnel wore protective disposable gowns and gloves when entering the room, and handwashing with soap and water was endorsed. Isolation precautions were discontinued 48 hours after resolution of diarrhoeal symptoms. In reaction to this CDI outbreak, additional infection prevention measures were implemented on the ward during certain time periods (Figure 1).These additional infection prevention measures included cleaning and disinfection using 1000 ppm chlorine of the following items: automatic bedpan washer (daily), toilet chairs (after each use), utility room and sanitation (once or twice a day) and all patients rooms of half the department (once, after recognition of the fifth case). Additionally, the metal bedpans were replaced by cardboard single-use bedpans. Moreover, after the fifth case was diagnosed, 56 environmental swabs were taken on two different sampling days: 19 and 24 February. Samples were taken from the following sites: sink, water tap, grip of cabinet, alarm system, dustbin, chairs/tables and bed curtains of a room that had been occupied by a CDI patient

(before final cleaning); the same items in a clean room (after cleaning and disinfection with 1000 ppm); and toilet, shower chair, sink, shower curtain, sack of laundry and towel dispenser of a shared bathroom (after cleaning and disinfection). Environmental swabs were inoculated in *Clostridium difficile* enrichment-modified broth (*C. difficile* enrichment broth; Mediaproducts, Groningen, The Netherlands) for 1 week and subcultured on CLO plates (*C. difficile* agar; bioMérieux, Marcy l'Étoile, France). No antibiotic restriction policy was implemented during this outbreak.

Stool samples of all five patients tested positive for toxin B and binary toxin genes in the Xpert *C. difficile* (Cepheid, Sunnyvale, CA, USA); however, the TcdCΔ117 deletion specific for ribotype 027 was not identified. Investigations at the reference laboratory demonstrated the presence of TcdA and confirmed the presence TcdB and the binary toxin genes. In addition, a 39 bp deletion in TcdC was detected.

All five isolates and one isolate obtained from an environmental culture (taken from the sack of laundry in the shared bathroom after cleaning and disinfection) displayed the same PCR ribotyping profile. The profile was not recognized in the Dutch Reference Library (which is able to recognize 221 different PCR ribotypes), but it most resembled the profile of ribotypes 078, 126 and 066 most (all belonging to clade 5) (Figure 2a). A data set of sized fragments obtained by capillary gel-based electrophoresis PCR ribotyping (1) was sent as FSA file to international *C. difficile* reference laboratories (including the Leeds collection encompassing more than 800 PCR ribotypes , the WEBRIBO system, the US Centers for Disease Control and Prevention database and databases from Sweden, Portugal, Belgium and Canada), but no match was found. The new strain was assigned as ribotype 826 by the Leeds ribotyping reference network. PCR analysis of a clade 5-specific DNA marker (4) revealed that all ribotype 826 isolates were positive for the marker, confirming that ribotype 826 is part of clade 5.

According to Clinical and Laboratory Standards Institute (CLSI) breakpoints, all isolates were susceptible for erythromycin (minimum inhibitory concentration (MIC) <2 mg/L), clindamycin (MIC <2 mg/L), metronidazole (MIC <2 mg/L) and vancomycin (MIC <2 mg/L), but resistant to ciprofloxacin (MIC >32 mg/L) and moxifloxacin (MIC >32 mg/L) (9).

The isolates were 100% identical with no summed tandem repeat differences, thereby confirming a clonal complex according to multiple-locus variable-number tandem repeat analysis.

In addition, whole genome sequencing was performed (Figure 2b). To provide phylogenetic context, reference strains 078, 126/078, 045, 033 and 066 and four patient samples from confirmed strain 078 cases were included. In total, 1678 single nucleotide polymorphisms (SNPs) were identified within this sample selection, which is the expected variation between different ribotypes of one clade. Within the outbreak isolates, only two SNPs were identified (there was one SNP difference between the isolate from

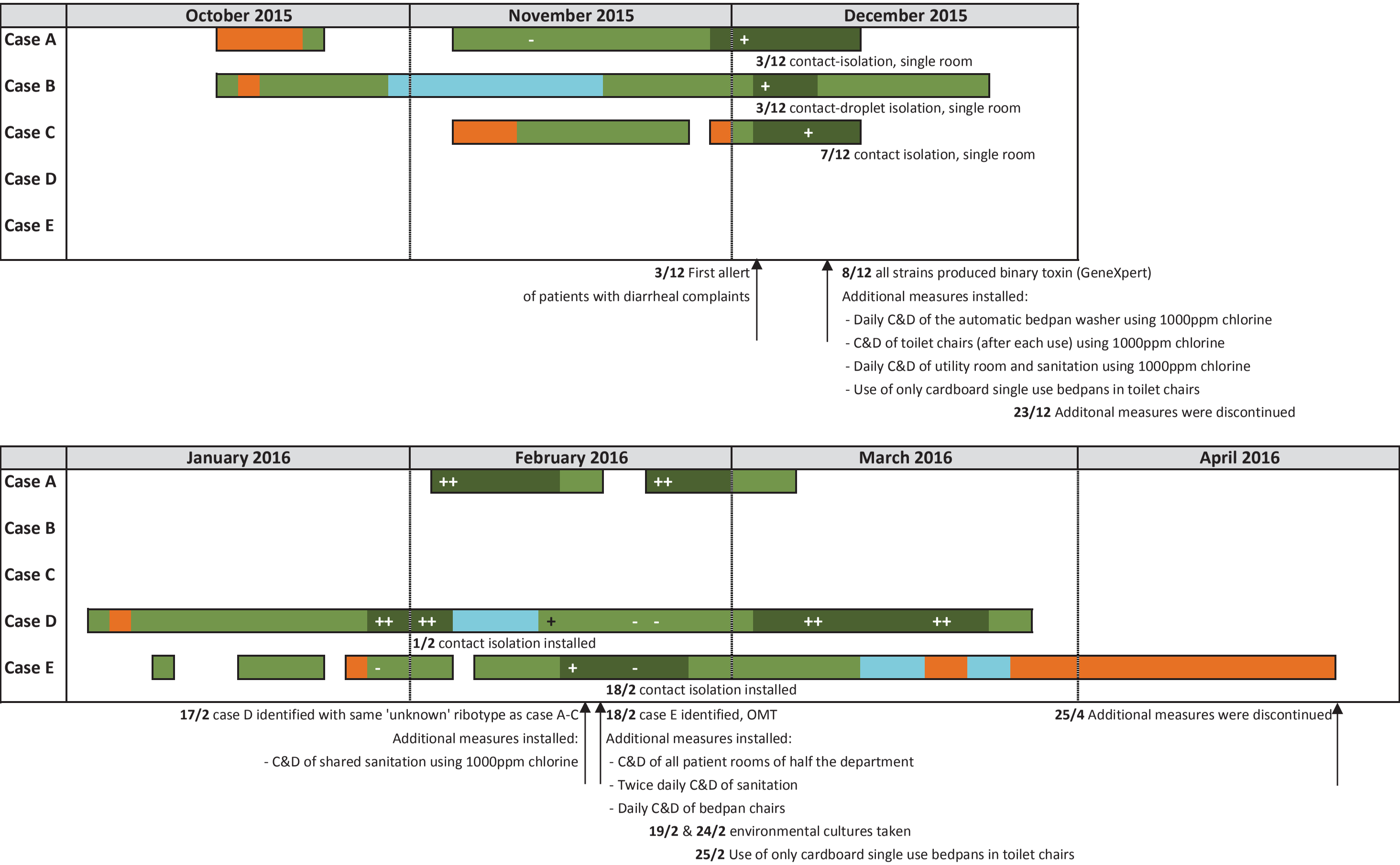

Figure 1.

Figure 1. Epidemic curve of five patients infected with *Clostridium difficile* caused by PCR ribotype 826. Green, outbreak, non-ICU ward; orange, other non-ICU ward; blue, ICU; dark green, diarrhoeal episode; white +, positive culture for *C. difficile* and mild *C. difficile* infection; white ++, positive culture for *C. difficile* and severe *C. difficile* infection; black +, positive C. *difficile* culture without diarrhoea; white -, negative culture for *C. difficile*. C&D, cleaning and disinfection; ICU, intensive care unit; OMT, outbreak management team.

the recurrence in patient A compared to the initial patient A isolate, and one SNP difference between the patient D /patient E isolates and the initial patient A isolate). Clonality of these cluster isolates was thus confirmed by whole genome sequencing, as the commonly used cutoffs for classifying isolates as clonal is zero to two SNPs (5).

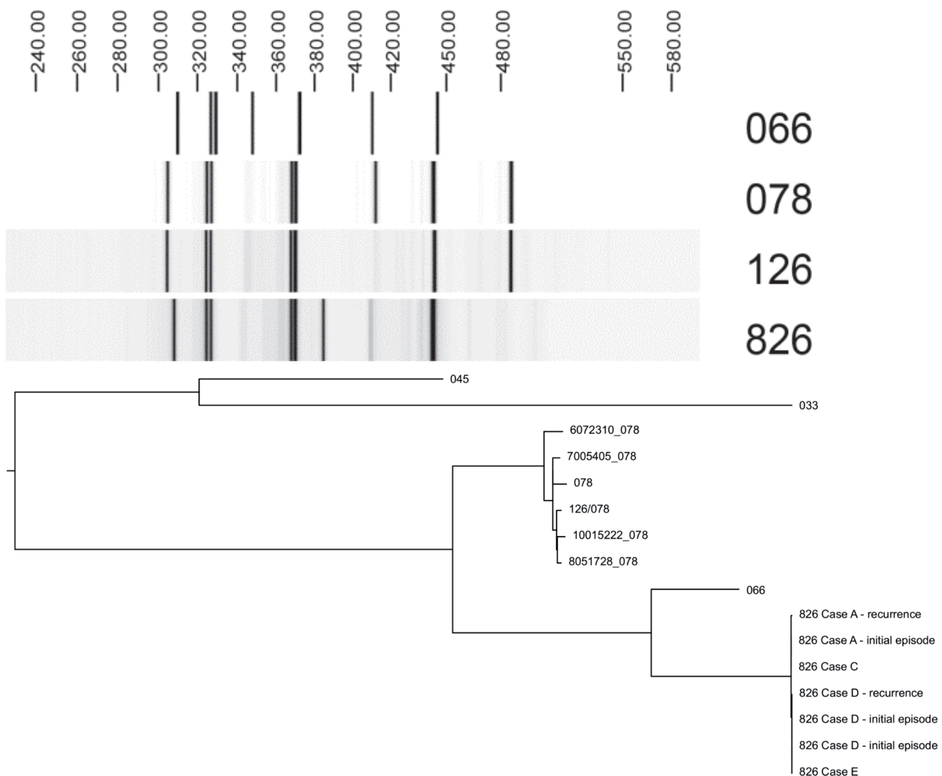

Figure 2. (a) PCR ribotyping patterns for ribotype 066, 078, 126 and 826. Upper row indicates fragment sizes. (b) Phylogenetic tree of ribotype 826 outbreak isolates and related ribotypes. 078, reference ribotype 078 strain; 066, reference ribotype 066 strain; 045, reference ribotype 045 strain; 126078, reference ribotype 126/078 strain 7005405_078/10015222_078; 8051728_078, 6072310_078; clinical patient CDI samples with confirmed ribotype 078; 4_826, sample from patient A (recurrent episode); 3_826, sample from patient A (initial episode); 6_826, sample from patient C; 1_826, sample from patient D (recurrent episode); 2_826, sample from patient D (initial episode); 8_826, sample from patient D (initial episode, repeat sample); 5_826, sample from patient E. Isolate from patient B could not be sequenced.

DISCUSSION

The occurrence of this CDI outbreak was uncommon, as it occurred on a ward where transmission of *C. difficile* was rare, as proven by sentinel CDI surveillance. Also, two of five patients had recurrent disease and were severely affected. Cases were due to the newly identified ribotype 826. Additional investigations showed that ribotype 826 belongs to clade 5 with a characteristic clade 5-specific DNA marker and a 39 bp deletion in TcdC. Whole genome sequencing revealed that ribotype 826 resembles ribotype 078 quite well. CDI cases due to clade 5 ribotypes have been reported to be associated with the highest 14-day mortality (10). We therefore assume that this new ribotype also has increased virulence, thus explaining the occurrence of this outbreak.

Whole genome sequencing results demonstrated clonality, thereby confirming transmission, but unanswered questions remain, including the source of this ribotype and how transmission occurred. The index patient could have introduced this ribotype into the ward, although no unusual profession, recent travel or other remarkable expositions were reported. Alternatively, an undetected asymptomatic carrier might have introduced the ribotype and spread it to other patients. Transmission could have occurred via shared items, as contamination was demonstrated in one of the environmental cultures, but unfortunately environmental swabs were only taken after the last patient was detected. The outbreak ceased with the implementation of additional infection prevention measures, suggesting that these cleaning and disinfection measures were effective, probably together with a raised awareness among the healthcare workers.

Because most PCR ribotypes of clade 5 are also found in animals, it is tempting to speculate that the newly recognized ribotype 826 derives from animals. The lack of this PCR ribotype in the databases of human collections supports this hypothesis. Unfortunately, reference laboratories for animal-associated *C. difficile* infections are not available that could be used to match our isolates. To our knowledge, no additional 826 isolates have been detected since this outbreak.

This outbreak indicates that new *C. difficile* ribotypes with increased virulence still emerge at unexpected locations and without a clear source. Given the increased virulence and still unknown source of this newly identified ribotype, ongoing CDI surveillance remains essential, and other institutions should now be aware of ribotype 826.

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Chapter 4

Healthcare-related pathogens: detection of transmission

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Chapter 4.1

Instant typing is essential to detect transmission of extended-spectrum beta-lactamase-producing *Klebsiella* species

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ABSTRACT

Infections with multidrug-resistant (MDR) microorganisms are an increasing threat to hospitalized patients. Although rapid typing of MDR microorganisms is required to apply targeted prevention measures, technical barriers often prevent this. We aimed to assess whether extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella* species are transmitted between patients and whether routine, rapid typing is needed. For 43 months, the clonality of all ESBL-producing *Klebsiella* isolates from patients admitted to Erasmus MC University Medical Center in Rotterdam, the Netherlands was assessed with Raman spectroscopy. A cluster was defined as n ≥2 patients who had identical isolates. Primary patients were the first patients in each cluster. Secondary patients were those identified with an isolate clonally related to the isolate of the primary patient. Isolates from 132 patients were analyzed. We identified 17 clusters, with 17 primary and 56 secondary patients. Fifty-nine patients had a unique isolate. Patients (n=15) in four out of the 17 clusters were epidemiologically related. Ten of these 15 patients developed an infection. Clonal outbreaks of ESBL-producing *Klebsiella* species were detected in our hospital. Theoretically, after Raman spectroscopy had detected a cluster of n ≥2, six infections in secondary patients could have been prevented. These findings demonstrate that spread of ESBL-producing *Klebsiella* species occurs, even in a non-outbreak setting, and underscore the need for routine rapid typing of these MDR bacteria.

INTRODUCTION

Infections with multidrug-resistant (MDR) microorganisms are an increasing threat to hospitalized patients, leading to high morbidity and mortality because of ineffective antibiotic treatment (1, 2). In general, carriage of antimicrobial resistant organisms occurs de novo by induction and selection during therapy (endogenous sources) or by transmission of already resistant organisms (exogenous sources). In healthcare settings transmission occurs either direct – patient to patient – or indirect via surrounding reservoirs or sources in the environment (3). Exogenous infections can be prevented using measures aiming at preventing transmission. Nevertheless, Gram-negative bacteria producing beta-lactamase enzymes such as extended-spectrum beta-lactamase (ESBL) or carbapenemases are currently of major concern. These resistant bacteria are a major cause of healthcare-related infections, especially in patients with a prolonged hospital stay (4).

Although the spread of ESBL-producing *Klebsiella* spp. has not yet been elucidated, current data indicate that they are mainly polyclonal with some small clusters in the hospital (5, 6). However, if these small clusters go unnoticed and/or appropriate infection control measures are not taken, they may result in large hospital-wide outbreaks (7). Because the prevalence of ESBL-producing *Klebsiella* spp. is increasing worldwide, hospitals must remain vigilant (4, 8). It should however be noted that the prevalence differs among patient groups, clinical and geographic settings (4, 8).

Rapid typing of MDR microorganisms can be of great support to demonstrate spread of related microorganisms. As a result targeted infection prevention measures are to be applied to stop transmission. Although MDR bacteria can be easily detected in a routine setting using proper indicator antibiotics combined with confirmation assays, rapid typing of these isolates is often not routinely performed. This is mainly due to technical barriers: most typing techniques are time consuming and laborious and therefore difficult to implement in routine diagnostics. Raman spectroscopy is a rapid technology that is used for whole organism fingerprinting and is applied in microbiological laboratories. This technique provides highly information-rich spectra which are required for maximum discriminatory power to distinguish unrelated microorganisms – which is required in outbreak management (9-11).

In this study, we applied this rapid typing technique to all ESBL-producing *Klebsiella* spp. that were identified in our hospital in the Netherlands for 43 months, in order to answer the following questions: first, are ESBL-producing *Klebsiella* spp. transmitted between patients? Second, is routine, rapid typing needed in an apparently non-outbreak setting?

MATERIALS AND METHODS

Ethics statement

Screening was performed as part of the infection control strategy using non-invasive sampling. The microbiological and epidemiological analyses were in first instance performed to develop new strategies for infection control. Also, according to the Dutch regulation for research with human subjects, neither medical nor ethical approval was required to conduct the study since the data were retrospectively recorded. However, we received approval from the medical ethics research committee of the Erasmus University Medical Center (Erasmus MC) in Rotterdam, the Netherlands to conduct this study (MEC-2011-085). The existing data from the electronic medical records could not be recorded anonymously as the patient name is always present. Before data analyses however, the opt-out list available at our department was consulted and patient names were removed from the dataset by A.F. Voor in 't holt. The authors had no direct interaction with the patients and no new patient data were collected during the study period.

Population

This study was conducted at the Erasmus MC in Rotterdam, the Netherlands. This is a 1,320-bed university hospital, with 97 beds in the adult or pediatric intensive care unit (ICU) (Erasmus MC, 2012). All medical specialties are available. Table 1 presents the number of hospital admissions from 1 January 2010 until 1 August 2013, the number of patients identified with *Klebsiella* spp. - susceptible and ESBL-producing isolates - and the number of patients who were included in the current study.

Table 1. Study characteristics.

	2010	2011	2012	2013[a]
No. of hospital admissions	40,626	41,773	41,001	21.893
No. of clinical admission days	292.209	288.799	299.736	n.a.
Klebsiella pneumoniae[b]	774	677	619	320
ESBL-producing *K. pneumoniae*[c]	54 (40)	47 (10)	62 (48)	34 (25)
ESBL-rate per 1000 hospital admissions	1,33	1,13	1,51	1,55
Klebsiella oxytoca[b]	370	338	351	161
ESBL-producing *K. oxytoca*[c]	7 (0)	5 (3)	15 (5)	3 (1)
ESBL-rate per 1000 hospital admissions	0,17	0,12	0,37	0,14

Abbreviations: n.a., not available, no., number
[a]1 January until 1 August 2013
[b]One per patient
[c]Between brackets: number of patients included in current study

Study Design and data collection

We included patients of all departments with a microbiologically confirmed ESBL-producing *K. pneumoniae* or *K. oxytoca* between 1 January 2010 and 1 August 2013. After detection, all patients were immediately managed in contact isolation and placed in single-occupancy rooms. Contact isolation included the use of gloves and gowns, and disinfection of hands and wrists with hand alcohol when entering and leaving the room. No active surveillance and/or contact investigation were carried out. Isolates were cultured from clinical samples, either because of 1) assumed infection or 2) surveillance purposes in the ICU and hematology departments - patients receiving selective digestive tract decontamination are routinely tested for the presence of (resistant) Gram-negative bacteria twice weekly. This study consisted of two study periods. During study period I, 1 January 2010 until 1 September 2012, isolates were collected and typed retrospectively. Also, clinical data were collected retrospectively. However, carbapenemase-producing isolates were immediately typed after detection at that time. During study period II (1 September 2012 until 1 August 2013), typing with Raman spectroscopy was performed immediately after detection and clinical data were collected prospectively. In this report, data from both study periods were combined. Preventive measures were equal in both periods.

For each first isolate, we recorded patient data and bacteriological data, which were obtained from electronic patient records. Age of the patient was defined as age at day of detection of the first ESBL-producing *Klebsiella* isolate. Mortality was defined as death from any cause within one year after the day of detection of the first ESBL-producing *Klebsiella* isolate (12).

To identify healthcare-related infections and the specific type of infection, we used the criteria published by the Centers for Disease Control and Prevention (CDC) (13). We investigated microbiological data and medical records of included patients to distinguish colonization from infection.

Microbiological analysis

Cultures were performed at the diagnostic laboratory of the department of Medical Microbiology and Infectious Diseases (Erasmus MC, Rotterdam, The Netherlands). In study period I, bacteria were taken from frozen stock cultures kept at -80^{0}C, and were subsequently analyzed with Raman spectroscopy. In study period II, bacteria were typed with Raman spectroscopy immediately after the initial culture.

Identification and susceptibility testing for *K. pneumoniae* and *K. oxytoca* were performed using VITEK 2 (bioMérieux, Lyon, France), and results were interpreted according to the EUCAST clinical breakpoints. ESBL confirmation was performed with either the combination disk-diffusion test (Rosco Diagnostica, Taastrup, Denmark) or the Etest (bioMérieux, Lyon, France). *K. oxytoca* isolates were regarded as ESBL-producing when

resistant to ceftazidime and demonstrating synergy between ceftazidime and clavulanic acid. *K. oxytoca* isolates were regarded as hyperproducers of K1 (KOXY) chromosomal beta-lactamase if they showed resistance to cefuroxime, piperacillin-tazobactam and aztreonam, borderline resistance to cefotaxime and cefepime, but remained susceptible to ceftazidime (14). Presence of carbapenemases was confirmed using real-time PCR for blaIMP, blaKPC, blaNDM, blaOXA-48, and blaVIM genes (15).

Clonal relatedness

Clonal relatedness was investigated with Raman spectroscopy using SpectraCellRA analysis (SCRA). Cultures, sample preparation, and SCRA measurements were performed according to the operators manual (version 1.7) (16). Analyses and calculations were performed as described previously (17). A cluster was defined as n ≥2 patients who had identical isolates as indicated with Raman spectroscopic analysis. A distinction was made between primary and secondary patients, and patients with a unique isolate. A primary patient was defined as the first patient in time in a cluster. Secondary patients were all subsequent patients who had a proven clonal relationship with the primary patient. Unique was defined as patients with a non-cluster isolate. When analyzing, data from primary patients and from patients with a unique isolate were combined since they were both the first patient in time with a certain type. The only difference was that primary patients generated secondary patients, and patients with a unique isolate did not.

Spatial analysis

To investigate if patients identified in Raman spectroscopic clusters were epidemiologically related, four different definitions of epidemiological relatedness were created from highest to lowest likelihood (Table 2).

Table 2. Definitions of epidemiological relatedness.

	Definitions			
	Definite	Probable	Possible	Impossible
Same patient room	yes	yes	no	no
Same department	yes	yes	yes	no
Same period	yes	maybe[a]	maybe[b]	maybe[c]

[a]Same patient room within 3 months after primary patient has left.
[b]Same department within 3 months after primary patient has left.
[c]Same period but not the same department or patient room.

Statistical analysis

We calculated the transmission index (TI) to analyze the transmission dynamics using two different formulas. Firstly, it was calculated as the number of secondary patients divided by the number of primary patients and the number of patients with a unique isolate - with the results of Raman spectroscopy. Secondly, as the number of secondary patients who were epidemiologically related to the primary patient (definitions 'definite' and 'probable' combined, Table 2) divided by the number of primary patients and the number of patients with a unique isolate. Basic patient characteristics (*e.g.* age, gender, death from any cause within one year after positive culture) were analyzed as percentages and means using Microsoft Excel 2010.

RESULTS

Identification and characteristics of included patients

For the period from 1 January 2010 until 1 August 2013 we included 132 patients with an ESBL-producing *Klebsiella* isolate (Table 3, Figure 1). ESBL-producing *K. pneumoniae* was cultured in 123 patients and ESBL-producing *K. oxytoca* was cultured in nine patients (Table 3). Ninety isolates were obtained from clinical samples, and 42 isolates were obtained from surveillance cultures. In total, 17 clusters were identified with Raman spectroscopy, comprising 73 patients (cluster size ranging from two to ten patients), and 59 patients were identified with a unique isolate. Among the 73 patients with a cluster isolate, we identified 17 primary patients, and 56 secondary patients (Table 3). Eighty-six out of 132 patients (65.2%) developed an infection with an ESBL-producing *Klebsiella* spp. (Table 3). Fifty-one out of 17 primary plus 59 unique patients (67.1%) and 35 out of 56 secondary patients (62.5%) developed or had an infection with the ESBL-producing *Klebsiella* spp.

Eight patients were identified with an ESBL-producing *K. pneumoniae* isolate that was also resistant to imipenem and/or meropenem and three patients with an ESBL-producing *K. pneumoniae* isolate intermediately susceptible to imipenem and meropenem. The blaKPC gene was detected in six out of these 11 patients, the blaOXA-48 gene was demonstrated in isolates of three patients and the blaNDM gene was present in isolates of two patients. According to Raman spectroscopic analyses, seven out of these 11 patients had a unique isolate. In cluster 15 (n = 2), both patients had a KPC-producing isolate. In cluster ten (n = 10), two patients had an NDM-producing isolate.

Clinical epidemiology

From all 132 patients with an ESBL-producing *Klebsiella* spp. isolate, 130 were residents of the Netherlands. The median age of these 132 patients was 57.4; 58.3 for primary

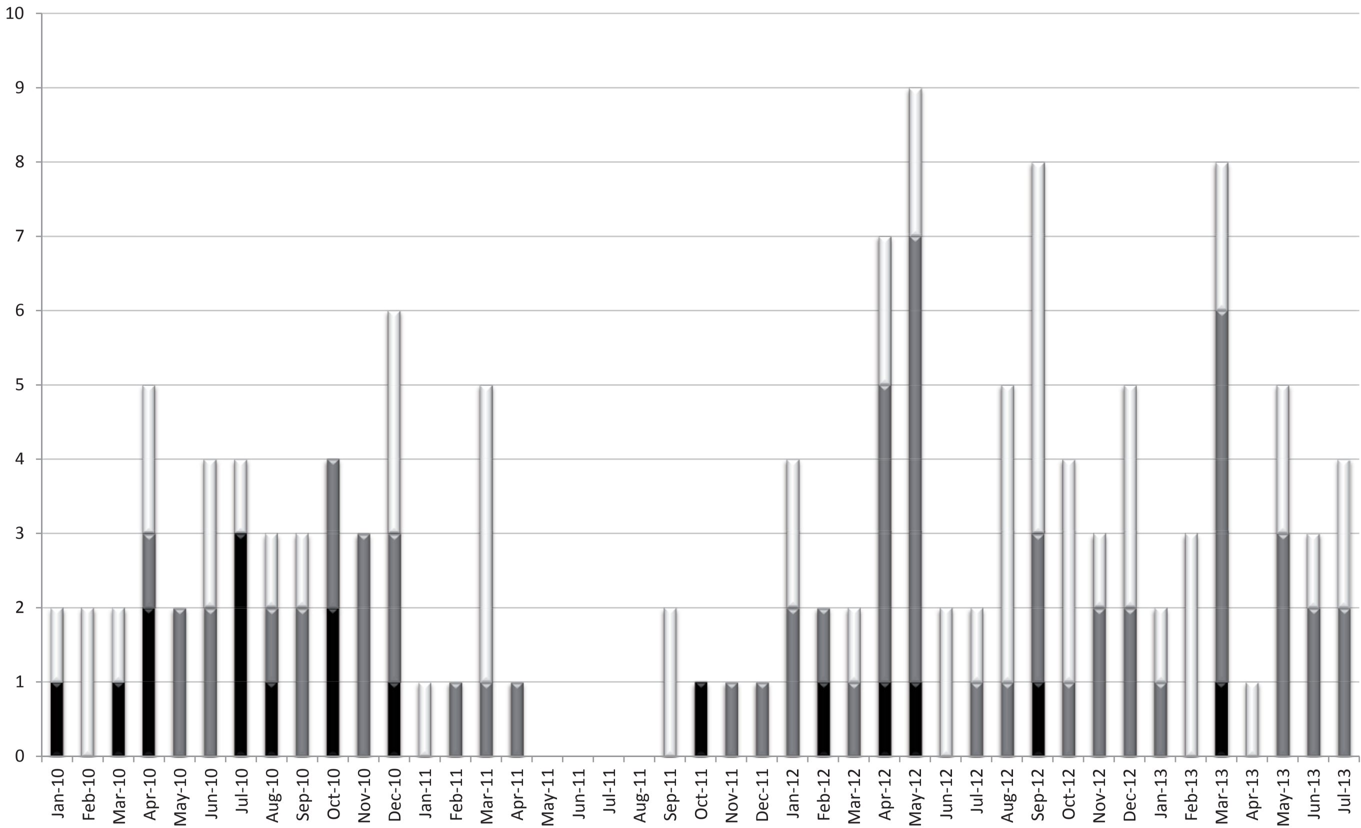

Figure 1. Distribution of patients identified with an ESBL-producing *Klebsiella* spp. (*n*= 132), January 2010 until August 2013. Black= primary patients (*n*= 17); the first patient in time in a cluster, identified with Raman spectroscopy. Grey= secondary patients (*n*= 56); all subsequent patients who had a proven clonal relationship with the primary patient. Light grey= patients with a unique isolate (*n*= 59).

Table 3. Characteristics of clusters and unique isolates as defined with Raman spectroscopy, January 2010 until August 2013.

	Total no. of patients	No. of primary patients[a]		No. of secondary patients	
		Infection	Colonization	Infection	Colonization
Total	132	51	25	35	21
K. pneumoniae	123	50	22	33	18
K. oxytoca	9	1	3	2	3
Cluster 1	2	0	1	1	0
Cluster 2	4	1	0	0	3
Cluster 3	9	1	0	5	3
Cluster 4	10	0	1	8	1
Cluster 5	5	0	1	4	0
Cluster 6	5	0	1	2	2
Cluster 7	3	1	0	1	1
Cluster 8	3	0	1	1	1
Cluster 9	2	0	1	1	0
Cluster 10	10	1	0	5	4
Cluster 11	2	0	1	1	0
Cluster 12	2	0	1	1	0
Cluster 13	5	1	0	2	2
Cluster 14	3	1	0	2	0
Cluster 15	2	1	0	0	1
Cluster 16	3	1	0	1	1
Cluster 17	3	0	1	0	2
Unique isolates	59	43	16	n.a.	n.a.

Abbreviations: n.a., not applicable, no., number.
[a]Including patients with a unique isolate

patients plus patients with a unique isolate (n = 17 + n = 59) and 54.8 for secondary patients (n = 56). Two clusters (cluster 6 and 11) consisted of newborns only. The overall mortality rate one year after detection of the bacteria was 17.4% (n = 23), consisting of three primary and nine secondary patients, and 11 patients with a unique isolate. Overall male percentage was 62.1%; 61.8% in primary patients plus patients with a unique isolate and 62.5% in secondary patients. Twenty-six patients (19.7%) were organ transplant recipients; three primary and eight secondary patients, and 15 patients with a unique isolate. The majority of patients received a kidney allograft (n = 22). Basic patient characteristics are displayed in table 4.

The most frequent specimen containing ESBL-producing *Klebsiella* spp. cultures for all patients was urine (45.5%), followed by rectum samples (21.2%). In primary patients plus patients with a unique isolate, 73.0% of patients who had a positive urine sample devel-

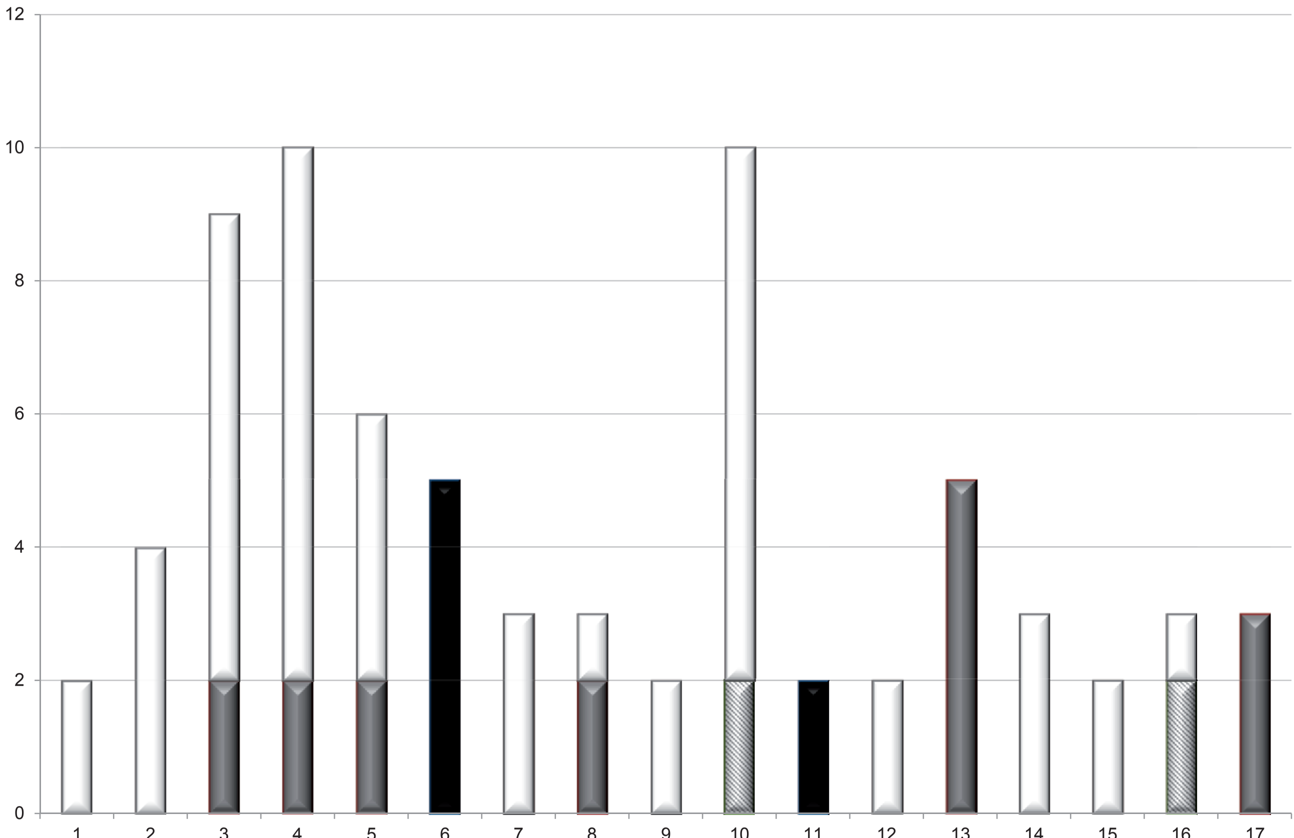

Figure 2. Epidemiological relatedness of patients in the 17 clusters. Y-axis: number of patients, x-axis: cluster number. Black= definite, grey= probable, wide downward diagonal lines= possible, white= impossible.

Table 4. Clinical characteristics of patients infected or colonized with ESBL-producing *Klebsiella* spp. and clinical characteristics of primary and secondary patients.

	No. of patients (%)		No. of patients (%)	
Variables	Infection (*n* = 86)	Colonization (*n* = 46)	Primary[b] (*n* = 76)	Secondary (*n* = 56)
Gender, male	61 (70.9)	21 (45.7)	47 (61.8)	35 (62.5)
Age, mean years (SD)	53.4 (24.0)	42.9 (29.7)	52.3 (23.4)	46.3 (30.1)
COPD	6 (7.0)	1 (2.2)	5 (6.6)	2 (3.6)
1-year mortality[a]	18 (20.9)	5 (10.9)	14 (18.4)	9 (16.1)
Organ transplantation	17 (19.8)	9 (19.6)	18 (23.7)	8 (14.3)
No. of primary patients[b]	51 (59.3)	25 (54.3)	n.a.	n.a.
No. of patients who had an infection	n.a.	n.a.	51 (67.1)	35 (62.5)

Abbreviations: COPD, chronic obstructive pulmonary disease, SD, standard deviation, no., number, n.a., not applicable.
[a]Death from any cause within one year after the first positive culture.
[b]Including patients with a unique isolate

Table 5. The number of infected and colonized patients in the eight clusters identified with Raman spectroscopy with a definite or probable epidemiological relationship.

	Definite			Probable		
	Infection			Infection		
Cluster no.	Prim	Sec	Colonization	Prim	Sec	Colonization
Cluster 3	0	0	0	1	1	0
Cluster 4	0	0	0	0	1	1
Cluster 5	0	0	0	0	2	0
Cluster 6	0	2	3	0	0	0
Cluster 8	0	0	0	0	1	1
Cluster 11	0	1	1	0	0	0
Cluster 13	0	0	0	1	2	2
Cluster 17	0	0	0	0	0	3

Abbreviations: no., number; prim, primary patients including patients with a unique isolate; sec, secondary patients. Epidemiological relatedness is presented as number of patients.

oped or had an infection, compared with 47.8% of secondary patients (P value 0.049). Twenty-three percent of primary patients who had a positive rectum sample developed or had an infection, compared with 53.3% of secondary patients (P value 0.102).

Spatial analysis

From the 132 patients, 95 were admitted to the hospital to 36 different departments. Thirty-seven patients were not admitted when the ESBL-producing *Klebsiella* isolate was

detected. Also, 31 patients (23.5%) were admitted to either the ICU or the hematology department.

Patients in cluster 6 (n = 5) and 11 (n = 2) were, according to our definitions, definitely related as patients were related in time and place (Figure 2). Cluster 6 and 11 both consisted of newborns infected (n =3) or colonized (n = 4) with *K. oxytoca* bacteria (Table 5). Patients in cluster 13 (n = 5) and 17 (n = 3) were probably related. In six clusters (cluster 3, 4, 5, 8, 10 and 16) a more diverse picture of epidemiological relatedness was found (Table 5, Figure 2). Patients in cluster one (n = 2), two (n = 4), seven (n = 3), nine (n = 2), 12 (n = 4), 14 (n = 3) and 15 (n = 2) could not be related to each other in time and place: transmission in the hospital was impossible according to the information in the medical records of the patients and our definitions (Figure 2).

When using the results of Raman spectroscopy, the transmission index was 0.74 (56 divided by 17 plus 59; definition 1). When dividing epidemiologically related secondary patients in clusters by all primary patients, the transmission index declined to 0.30 (23 divided by 17 plus 59; definition 2).

DISCUSSION

Summary of evidence

There are many ways of preventing antibiotic resistance, and especially the spread of antibiotic-resistant bacteria can be prevented in many different ways. A powerful policy is to prevent transmission of these resistant bacteria from patient to patient, the success of which depends on the speed and accuracy of the typing method used for early identification of related clusters. Rapid and accurate typing allows targeted infection control measures to be applied immediately, thereby preventing morbidity and mortality among patients and reducing hospital costs (18).

In the present study, we performed an in-depth analysis of the epidemiology of ESBL-producing *Klebsiella* spp. in a large university hospital using clinical samples and screening cultures over a 43-month period. Our results demonstrate that clonal outbreaks with ESBL-producing *Klebsiella* spp., as confirmed epidemiologically and by Raman spectroscopy, definitely occurred in our hospital and should have been prevented. In theory, as soon as clonality had been demonstrated using Raman spectroscopy (n ≥2), six out of ten infections in seven different clusters could have been prevented by implementing immediate additional infection control measures. These additional infection control measures include immediate disinfection of the patient rooms and sanitation involved and a contact investigation among roommates. The contact investigation is performed from the first admission date of the primary patient until the date of contact isolation or discharge of the last secondary patient. The possible (NDM) and impossible (KPC)

transmissions of carbapenemase-producing *Klebsiella* spp. in cluster 10 and cluster 15 were noted with typing at the time of detection. Additional screening of contact patients was performed, which did not reveal other colonized patients. Previous reports on ESBL-producing *Klebsiella* spp. have shown similar results, although some studies have identified large clusters (19). However, these studies often did not investigate whether patients in clusters - identified by molecular analysis - were also epidemiologically related (19).

Due to the retrospective nature of our analysis, our results represent merely the tip of the iceberg. We hypothesize that if patients had been routinely screened for MDR bacteria upon admission, during admission, upon discharge and/or after the finding of a primary case, we would have found more and larger clusters in which patients were epidemiologically related. The fact that we were not able to define relatedness in all secondary cases was presumably due in part to unidentified carriers (missed cases) and transmission. A possible additional theoretical explanation of secondary cases is community spread of these type of bacteria, as carriage in the community has been reported in the literature and is of rising concern. However, in the Netherlands spread in the community has not been reported so far (20).

The transmission index was 0.74. When we used epidemiological relatedness as a criterion for secondary patients, the transmission index decreased to 0.30. However, the R0 could not be calculated as each patient identified with an ESBL-producing *Klebsiella* spp. isolate was nursed in contact isolation and placed in a single-occupancy room immediately after detection. At that moment it was not clear whether this patient was a primary or a secondary patient. These prompt measures may have kept the transmission index low.

Limitations

Our study has a number of limitations. First, patients were not routinely cultured for carrying multi-resistant Enterobacteriaceae at admission or discharge during the study periods in our hospital and we were not able to include all the carriers identified in our hospital (Table 1). The presence of unidentified carriers therefore cannot be ruled out, and we may have underestimated the number of affected patients. Second, the surveillance cultures in our dataset were only obtained from ICU and hematology patients while the remaining cultures were obtained from clinical samples. Third, in seven of the 17 clusters identified using Raman spectroscopy no epidemiological relatedness between patients was found. This may be due to low numbers of cultures – thus missing cases – and/or to our definition of epidemiological relatedness that may have been too strict (Table 2). Since contamination of the environment as a source needs to be taken into consideration when studying transmission, we chose a 3-month window during which we still considered transmission to be possible (Table 2) (21, 22). Unfortunately,

neither nationwide guidelines nor even the CDC provide a definition of epidemiological relatedness. Finally, we did not include the identification of the specific ESBL genes (*e.g.* blaCTX-M, blaSHV, blaTEM) and plasmid types in our analysis of the 132 isolates. Therefore, transmission between different strains of plasmids carrying ESBL genes cannot be ruled out.

Conclusion and clinical implication

In summary, as transmission occurred during the entire study period and throughout the entire hospital, there is an urgent need for instant typing to detect and subsequently prevent the spread of ESBL-producing *Klebsiella* spp. We conclude that typing should continuously be performed, also in an apparent non-outbreak situation. In order to relate those patients who have clonally clustered isolates, it is important to actively screen contact patients for carriage of ESBL-producing *Klebsiella* spp. These recommendations are similar to those given in previous investigations and in the recent Dutch MDRO guideline (19, 23-26). A rapid typing method that is easy to implement and that can be used for the early identification of clusters is Raman spectroscopy. Through the implementation of immediate infection-prevention strategies, those small outbreaks identified early on can be stopped and further transmission can be prevented. However, further research is needed to investigate the presence of ESBL-producing *Klebsiella* spp. and their transmission dynamics in other hospital settings. This will give further insight in the transmission magnitude of *Klebsiella* spp. in the hospital.

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Chapter 4.2

Detection of healthcare-related extended-spectrum beta-lactamase-producing *Escherichia coli* transmission events using combined genetic and phenotypic epidemiology

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ABSTRACT

Since the year 2000 there has been a sharp increase in the prevalence of healthcare-related infections caused by extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*. However, the high community prevalence of ESBL-producing *E. coli* isolates means that many *E. coli* typing techniques may not be suitable for detecting *E. coli* transmission events. Therefore, we investigated if High-throughput MultiLocus Sequence Typing (HiMLST) and/or Raman spectroscopy were suitable techniques for detecting recent *E. coli* transmission events. This study was conducted from January until December 2010 at Erasmus University Medical Center, Rotterdam, the Netherlands. Isolates were typed using HiMLST and Raman spectroscopy. A genetic cluster was defined as two or more patients carrying identical isolates. We used predefined definitions for epidemiological relatedness to assess healthcare-related transmission. We included 194 patients; strains of 112 patients were typed using HiMLST and strains of 194 patients were typed using Raman spectroscopy. Raman spectroscopy identified 16 clusters while HiMLST identified 10 clusters. However, no healthcare-related transmission events were detected. When combining data from both typing techniques, we identified eight clusters (n=34 patients), as well as 78 patients with a non-cluster isolate. However, we could not detect any healthcare-related transmission in these 8 clusters. Although clusters were genetically detected using HiMLST and Raman spectroscopy, no definite epidemiological relationships could be demonstrated which makes the possibility of healthcare-related transmission events highly unlikely. Our results suggest that typing of ESBL-producing *E. coli* using HiMLST and/or Raman spectroscopy is not helpful in detecting *E. coli* healthcare-related transmission events.

INTRODUCTION

For many years, the spread of microorganisms expressing extended-spectrum beta-lactamase (ESBL) genes was limited to those circulating in hospitals, with most isolates being *Klebsiella pneumoniae* (1). Since the year 2000 however, there has been a sharp increase in the prevalence of ESBL-producing microorganisms worldwide, whereby *Escherichia coli* has replaced *K. pneumoniae* as the major carrier of ESBL encoding genes (2-6). Worldwide, ESBL-producing Enterobacteriaceae carriage rates in the community range from <10% in Europe, to >50% in Southeast Asia, with *E. coli* as the predominant colonizing species (7, 8). In addition, current research in the Netherlands shows that 5 to 7% of people in the Dutch community carry ESBL-producing *E. coli* isolates, with ST 131 being the most dominant sequence type (9-13). *E. coli* is the most common agent associated with infections of the urinary tract and bloodstream infections arising from these urinary tract infections. Importantly, an increase in the number of antibiotic resistant bacteria in the community results in an increase in the number of antibiotic resistant bacteria in patients admitted to hospitals. The monitoring and prevention of healthcare-related infections requires the early detection and differentiation of antibiotic resistant bacteria in order to identify possible sources of transmission. This then allows suitable action to be taken in order to prevent future transmission events and infections (14).

Resistant isolates can be routinely compared to detect hospital transmission using molecular typing methods - with Pulsed-field gel electrophoresis (PFGE) as the gold standard technique. Although this technique has a good discriminatory power, it is too laborious to detect clones on a routine basis. Therefore, investigations need to be performed to determine whether high-throughput techniques such as High-Throughput Multilocus Sequence Typing (HiMLST) and Raman spectroscopy are suitable techniques to be implemented in a routine setting. HiMLST is a genotyping technique which classifies isolates based on the sequence variations of seven housekeeping genes. While conventional MLST uses classical Sanger sequencing, HiMLST employs next-generation sequencing (NGS) to generate MLST sequence data using a high –throughput protocol (15). Raman spectroscopy is an easy to use and rapid technique which measures phenotypic expression profile differences between bacteria (16). This publication investigated whether either of these techniques, or a combination of both, could be used to differentiate between recent ESBL-producing *E. coli* transmission events in hospitals by comparing the output of the techniques with patients' hospital admission history.

METHODS

Ethics statement

Written approval to conduct the study was received from the medical ethics research committee of the Erasmus MC University Medical Center (Erasmus MC), Rotterdam, the Netherlands (MEC-2011-085). As per conclusion of our medical ethics research committee, written informed consent from patients for acquiring data from clinical records was not needed. The existing data from the electronic medical records could not be recorded anonymously as the patient name is always visible when collecting data. Before data analyses however, the opt-out list available at our department was consulted and patients were excluded when applicable. Also before analyses, patient names were removed from the dataset by A.F. Voor in 't holt and A.A. Wattel. The authors had no direct interaction with the patients during the study period and no new patient data were collected for this study.

Design and setting

For this retrospective study patients were included from January until December 2010 at Erasmus MC, Rotterdam, the Netherlands. In this university hospital all medical specialties are available. In 2010, 40,626 patients were admitted resulting in 292,209 admission days.

Bacterial collection and patient data

ESBL-producing *E. coli* isolates (according to the Clinical and Laboratory Standards Institute (CLSI) criteria), were obtained from the bacterial biobanks of the Department of Medical Microbiology and Infectious Diseases (MMIZ) at Erasmus MC (frozen stocks stored at -80^{0}C) (17). Only the first *E. coli* isolate per patient was included in the study. All isolates were obtained from clinical samples sent to MMIZ for analysis due to: 1) an assumed infection, or 2) for surveillance purposes in the intensive care unit (ICU) and hematology departments of Erasmus MC. Patients admitted to ICU and hematology departments routinely receive selective digestive tract decontamination and are tested for the presence of (antibiotic resistant) Gram-negative bacteria twice weekly. If (antibiotic resistant) Gram-negative bacteria are detected, affected patients are immediately moved to 'contact' or 'contact-droplet' isolation in single-occupancy rooms. Healthcare workers wear gloves and gowns during patient care. No extra active surveillance and/or contact investigation was performed for this study.

Patient data (*e.g.* age, gender, and region of residence), hospital admission data (*e.g.* period, department, and patient room) and bacteriological data were obtained from electronic patient records. The age of the patient was defined as his/her age on the day of detection of the first ESBL-producing *E. coli* isolate. Mortality was defined as death

from any cause within 28 days after the day of detection of the first ESBL-producing *E. coli* isolate. Twenty-eight-day mortality data was obtained from electronic patient records.

High-throughput multilocus sequence typing

Due to availability and costs, a random selection of isolates were subjected to the standardized MultiLocus Sequence Typing (MLST) scheme for *E. coli* as reported by Wirth *et al.*, 2006, using the High-Throughput MLST (HiMLST) strategy (15, 18). PCR primers were modified to reduce amplicon sizes, conserving the intact cores, and extended with universal tails to make the isolates suitable for HiMLST use. The HiMLST primer sequences used in this publication are shown in Table 1. Advantages of using the HiMLST technique in comparison to conventional MLST is that it allows genotyping of large numbers of isolates, requires less labour per isolate, and lowers overall costs (15). MLST types were generated using BioNumerics v6.6 software152 (Applied Math NV, Sint-Martens-Latem, Belgium), and by reference to the MLST database hosted at the University of Warwick (http://mlst.warwick.ac.uk/mlst/mlst/dbs/Ecoli).

Table 1. High-throughput MultiLocus Sequence Typing primer sequences used in this publication.

Gene	Forward primer	Reverse primer
adk	5′ - gacactatagattctgcttggcgctccggg - 3′	5′ - cactatagggccgtcaactttcgcgtattt - 3′
fumc	5′ - gacactatagggtatttagtccagtac - 3′	5′ - cactatagggatttaggcttgttgtctg - 3′
gyrb	5′ - gacactatagataactcctataaagtgtc - 3′	5′ - cactatagggaatgttgttggtaaagcag - 3′
icd	5′ - gacactatagccagccatgctgaaagtg - 3′	5′ - cactatagggcaccagagtcacagagtc - 3′
mdh	5′ - gacactatagtgcacgaaccagagacag - 3′	5′ - cactatagggatgtcgttcttatctctgc - 3′
pura	5′ - gacactatagcatgtccgctgatccttg - 3′	5′ - cactatagggcggtcgggaacggacctgc - 3′
reca	5′ - gacactatagacctttgtagctgtaccacg - 3′	5′ - cactatagggagcgtgaaggtaaaacctgtg - 3′

Raman spectroscopy

Phenotypic relatedness was investigated via Raman spectroscopy using a SpectraCellRA (SCRA) apparatus (RiverD International B.V., Rotterdam, The Netherlands). Raman spectroscopy is a label-free, optical technology based on the inelastic scattering of light by molecules (19). The Raman spectrum displays molecule-specific changes in wavelength – so-called spectroscopic fingerprints (19). These fingerprints reflect the overall molecular composition of a sample (19). The advantages of Raman spectroscopy are that this is an easy-to-use rapid technique. However, one disadvantage is that it is not currently a widely used technique. Cultures, sample preparation and SCRA measurements were performed according to the operator manual (version 1.7) (16). Raman spectroscopy analyses and calculations were performed as described previously (19).

Epidemiological relatedness

We defined a 'cluster' as two or more patients carrying identical isolates as indicated by HiMLST and/or Raman spectroscopic analysis. Within clusters, a 'primary patient' was defined as the first patient in time who was positive for an ESBL-producing *E. coli* isolate, while 'secondary patients' were all subsequent patients who were positive for an ESBL-producing *E. coli* isolate that was genetically or phenotypically related to the primary patient as identified by HiMLST and/or Raman spectroscopy. Non-cluster (unique) patients were defined as primary patients. The 'transmission index' was calculated for genotypically and phenotypically identical ESBL-producing *E. coli* isolates and was calculated as the number of secondary patients divided by the number of primary patients. To be able to distinguish community acquisition from healthcare-related transmission, we defined 'healthcare-related transmission' as ESBL-producing *E. coli* identified in a sample taken between 48 hours after admission and within 48 hours after discharge.

Likelihood of healthcare-related transmission

To determine the likelihood of epidemiological relatedness of isolates within clusters we defined 4 groups based on the likelihood of healthcare-related transmission: 1) 'definite', 2) 'probable', 3) 'possible', and 4) 'impossible' (Table 2). Patients were 'definitely related' if patients shared the same patient room within the same admission period. If patients shared the same patient room but did not have the same admission period, and if the second patient was admitted within two months after the first patient was discharged, then patients were defined as 'probably related'. 'Possibly related' patients only shared the same department during the same admission period. Alternatively, any second patient who was admitted within two months after their first patient was discharged was also defined as 'possibly related'. Patients 'impossibly related' were patients related neither in place nor in time. When using these definitions we assumed 1) that patients were not mobile outside their original ward and 2) that people who were mobile between rooms and wards did not transmit the microorganism.

Models of transmission

We compared different categories using 4 different models in order to determine the likelihood of healthcare-related transmission events (Table 2). In 'model 1', we combined hospital admission data of patients with isolates within the same cluster according to HiMLST. In 'model 2', we combined hospital admission data of patients with isolates within the same cluster according to Raman spectroscopy. In 'model 3' we combined 2 months of hospital admission data from patients with isolates within the same cluster and further sub-divided HiMLST clusters by adding data from Raman spectroscopy. 'Model 4' was identical to model 3, but the patient data collected was extended to 3 months.

Table 2. Definitions of likelihood of epidemiological relatedness.

	Definition						
	Definite	Probable	Possible		Impossible		
Same patient room	1	1	0	0	0	0	0
Same department	1	1	1	1	0	1	0
Same period	1	0[a]	1	0[a]	1	0	0

Abbreviations: 0= no; 1= yes
[a]Not the same period but same patient room (probable) or department (possible) within 2 months after primary patient was discharged.

Statistical Analysis

A non-parametric test was performed using an independent samples median test in order to compare median days of stay in the hospital before the detection of an ESBL-producing *E. coli* between patients in clusters and non-cluster patients. A P value of <0.05 was considered statistically significant and the analysis was performed using IBM SPSS version 21 (SPSS Inc., Chicago, IL, USA).

RESULTS

Selection of isolates and patients

In 2010, *E. coli* isolates were identified in 2,933 patients at Erasmus MC, including 204 patients (7.0%) with an ESBL-producing *E. coli*. The ESBL-rate per 1,000 hospital admissions was calculated as being 0.50. One hundred and ninety-eight ESBL-producing *E. coli* isolates from 194 patients were stored in MMIZ biobanks at Erasmus MC and were available for this study. We included 1 isolate per patient, except for 2 patients who were identified carrying 2 different ESBL-producing *E. coli* isolates. This difference was determined by differences in their antibiotic resistance profiles. In total, we included 196 isolates - of which 33 belonged to ST 131 - representing 194 patients in the current study (Figure 1).

Model 1

The first model included 113 randomly selected isolates (from 112 patients) typed using HiMLST (Figures 1 and 2). We identified 10 clusters (n = 65 isolates from 64 patients) with cluster size ranging from 2 to 33 patients - the largest cluster being ST 131, and 48 primary patients carrying a non-cluster, unique isolate ESBL (Table 3, Figure 2). After applying the definitions described in Table 2, we identified 3 possible healthcare-related transmission events within the cluster representing ST 131 (Table 3). All other patients were impossible to relate to each other with respect to time and place. For patients in

the 10 clusters, 14 isolates (21.5%) were considered as healthcare-related transmission (Table 3) and the remaining isolates considered as community acquired. Of the 2 patients previously described carrying 2 different ESBL-producing *E. coli* isolates, the isolates of a single patient were typed using HiMLST. Results showed that these isolates had the same sequence type (ST 1137). This was the only patient present with this sequence type.

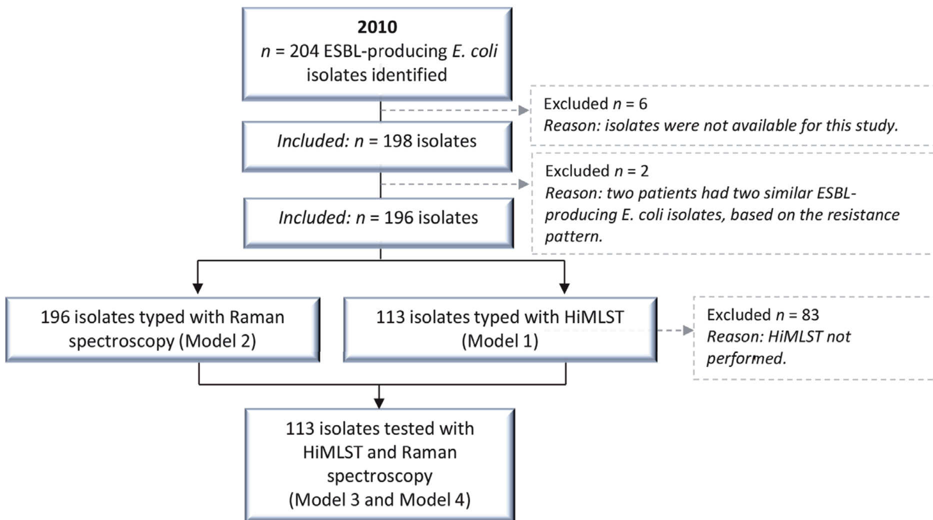

Figure 1. Flow diagram of the selection of isolates and patients identified with an ESBL-producing *E. coli* in 2010. The 113 isolates typed with HiMLST were a random selection out of the study total of 196 isolates. Abbreviations: HiMLST; High-throughput MultiLocus Sequence Typing, ESBL; extended-spectrum beta-lactamase.

Model 2

The second model of this study included 196 isolates (from 194 patients) typed using Raman spectroscopy. We identified 16 clusters (n = 101 isolates), with cluster sizes ranging from 2 to 26 patients, and 95 patients with a non-cluster isolate (Table 4). When applying the definitions as described in Table 2, only 1 patient was possibly related to another patient (Table 4). All other patients were impossible to relate with respect to time and place. From the patients in the 16 clusters, 33.7% (34 patients) were considered as originating from healthcare-related transmission events, and the remaining 66.3% considered as not originating from healthcare-related transmission events. For the 2 patients previously described with 2 different ESBL-producing *E. coli* isolates, both isolates from both patients showed different Raman spectroscopy cluster numbers.

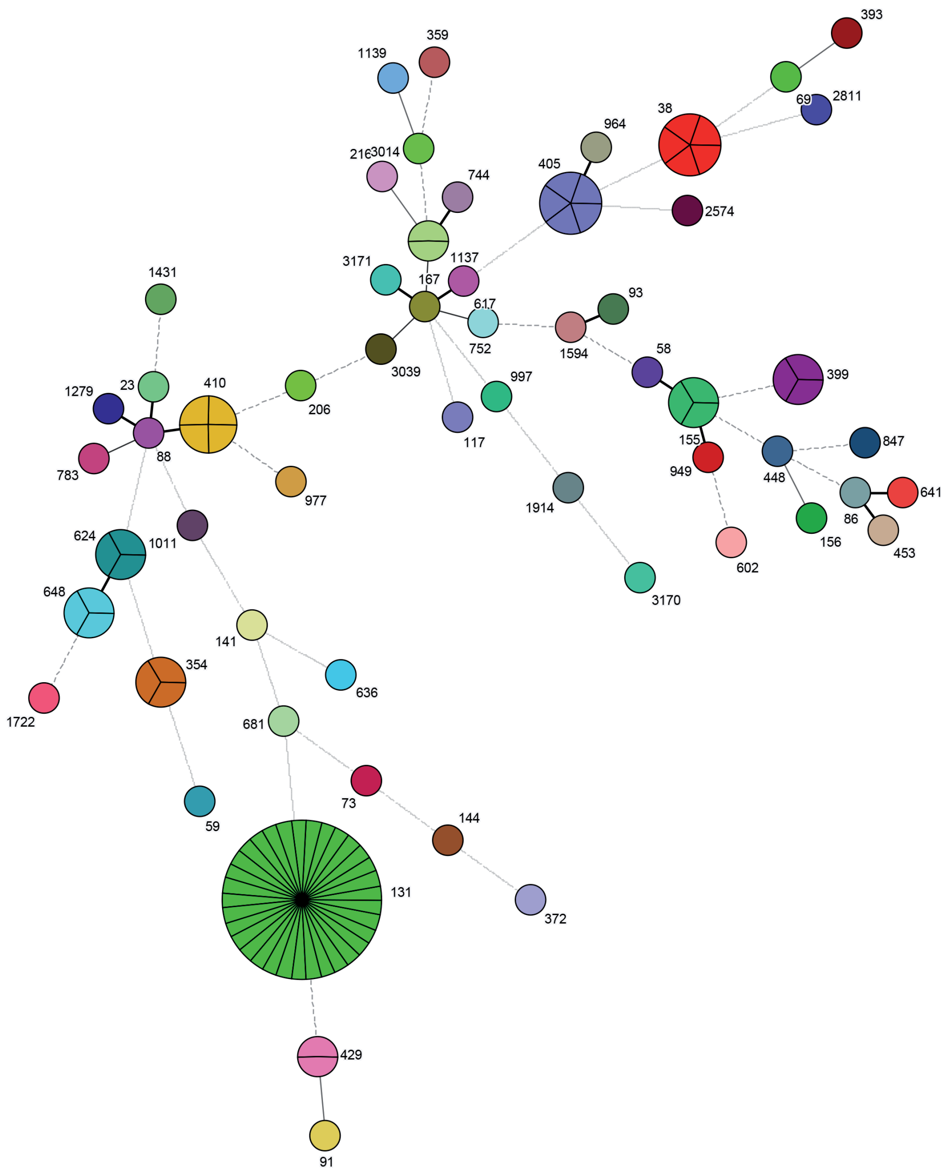

Figure 2. Representative genotypic ESBL-producing *E. coli* clusters observed using High-throughput multilocus sequence typing (HiMLST). The different colors represent different sequence types. 1 allele difference= thick solid line; 2 allele differences= medium solid line; 3 allele differences= thin solid line; 4 allele differences= dashed line; >4 allele differences= dotted line.

Table 3. Epidemiological relatedness of ESBL-producing *E. coli* isolates typed using HiMLST only.

Cluster no.	Sequence type	No. of patients	Healthcare-related[a]	Model 1			
				Definite	Probable	Possible	Impossible
1	38	5	3	0	0	0	5
2	131	33	3	0	0	3[b]	30
3	155	3	0	0	0	0	3
4	354	3	1	0	0	0	3
5	399	3	2	0	0	0	3
6	405	5	3	0	0	0	5
7	410	4	0	0	0	0	4
8	429	2	1	0	0	0	2
9	624	3	0	0	0	0	3
10	648	3	1	0	0	0	3
Total	n.a.	64	14	0	0	3	61

Abbreviations: HiMLST, High-throughput MultiLocus Sequence Typing; n.a., not applicable
[a]Positive isolate identified between 48 hours after admission and within 48 hours after discharge.
[b]Of which one isolate was considered as healthcare-related

Table 4. Epidemiological relatedness of ESBL-producing *E. coli* isolates typed using Raman spectroscopy only.

Cluster no.	No. of patients	Healthcare-related[a]	Model 2			
			Definite	Probable	Possible	Impossible
1	3	0	0	0	0	3
2	9	8	0	0	0	9
3	3	0	0	0	0	3
4	3	1	0	0	0	3
5	2	2	0	0	1	1
6	2	1	0	0	0	2
7	2	1	0	0	0	2
8	2	1	0	0	0	2
9	22	6	0	0	0	22
10	26	5	0	0	0	26
11	4	2	0	0	0	4
12	2	0	0	0	0	2
13	5	5	0	0	0	5
14	12	2	0	0	0	12
15	2	0	0	0	0	2
16	2	0	0	0	0	2
Total	101	34	0	0	1	100

[a]Positive sample identified between 48 hours after admission and within 48 hours after discharge.

Table 5. Characteristics of patients (n= 112) and isolates (n= 113) typed using HiMLST in combination with Raman spectroscopy.

	No. of patients	Median age[a] (range)	Male (%)	Crude Mortality (%)[b]	No. of isolates	Sample sites of isolates			
						Blood (%)	Urine (%)	Rectum/ throat (%)	Other (%)[c]
Total	112	53.2 (0-93)	51 (45.5)	9 (8.0)	113	7 (6.2)	73 (64.6)	17 (15.0)	16 (14.2)
Cluster 1	4	51.8 (41-80)	1 (25)	1 (25)	4	0 (0)	4 (100)	0 (0)	0 (0)
Cluster 2	15	60.0 (2-86)	11 (73.3)	0 (0)	15	1 (6.7)	10 (66.7)	2 (13.3)	2 (13.3)
Cluster 3	3	45.7 (27-70)	1 (33.3)	0 (0)	3	0 (0)	2 (66.7)	0 (0)	1 (33.3)
Cluster 4	2	63.8 (62-66)	1 (50)	0 (0)	2	0 (0)	2 (100)	0 (0)	0 (0)
Cluster 5	2	68.3 (56-80)	1 (50)	0 (0)	2	0 (0)	1 (50)	0 (0)	1 (50)
Cluster 6	2	55.0 (39-71)	1 (50)	0 (0)	2	0 (0)	0 (0)	2 (100)	0 (0)
Cluster 7	3	86.4 (57-88)	3 (100)	1 (33.3)	3	0 (0)	1 (33.3)	2 (66.7)	0 (0)
Cluster 8	3	66.3 (47-69)	1 (33.3)	0 (0)	3	0 (0)	3 (100)	0 (0)	0 (0)
Non-cluster isolates	78	55.3 (0-93)	31 (39.7)	7 (9.0)	79	6 (7.6)	50 (63.3)	11 (13.9)	12 (15.2)

Abbreviations: HiMLST, High-throughput MultiLocus Sequence Typing.

[a]Age at day of detection of ESBL-producing *E. coli*

[b]Death from any cause within 28 days after first positive culture

[c]Including: pus, wound fluid, peritoneal cavity fluid

Table 6. Epidemiological relatedness of ESBL-producing *E. coli* isolates typed using HiMLST in combination with Raman spectroscopy.

Cluster no.	No. of patients	Healthcare-related[a]	ST 131(%)	Model 3				Model 4			
				Definite	Probable	Possible	Impossible	Definite	Probable	Possible	Impossible
1	4	1	4 (100)	0	0	0	4	0	0	0	4
2	15	4	15 (100)	0	0	0	15	0	0	0	15
3	3	2	3 (100)	0	0	0	3	0	0	0	3
4	2	0	2 (100)	0	0	0	2	0	0	0	2
5	2	0	0 (0)	0	0	0	2	0	0	0	2
6	2	2	0 (0)	0	0	0	2	0	0	0	2
7	3	3	0 (0)	0	0	0	3	0	0	1	2
8	3	0	0 (0)	0	0	0	3	0	0	0	3
Total	34	12	24 (70.6)	0	0	0	34	0	0	1	33

Abbreviations: HiMLST, High-throughput MultiLocus Sequence Typing.

[a]Positive sample identified between 48 hours after admission and within 48 hours after discharge.

Model 3

The third model of this study included 113 isolates (from 112 patients) typed using both HiMLST and Raman spectroscopy. The median age of the 112 patients was 53.2 years (ranging from zero to 93) and 51 (45.5%) were male. The predominant sample site of the 113 isolates was urine (64.6%), followed by rectum and throat samples (15.0%; Table 5). Overall, 41.1% of patients lived in Rotterdam, the Netherlands. Data on city of residence was not available for 2 patients and only one patient lived abroad in Aruba (autonomy within the Kingdom of the Netherlands).

We identified 8 clusters when combining the results of Raman spectroscopy and HiMLST, - with cluster sizes ranging from 2 to 15 isolates (n= 34 patients, 30.4%), and 79 non-cluster isolates (78 patients, 69.6%; Table 5). This resulted in 86 primary patients (76.8%) and 26 secondary patients (23.2%), and a transmission index of 0.30. Of the 79 non-cluster isolates only 36 isolates (32.1%) were unique isolates according to both typing techniques, 11 isolates (9.8%) were part of a cluster according to Raman spectroscopy but not according to HiMLST, and 21 isolates (18.8%) were part of a cluster consistent with HiMLST but not consistent with Raman spectroscopy. In cluster 1 to 4, 100% of isolates belonged to ST 131 (Table 6), in cluster 5 to 8, no ST 131 isolates were identified.

Thirty-eight patients (33.9%) were detected with an ESBL-producing *E. coli* between 48 hours after admission and within 48 hours after discharge and were therefore considered as healthcare-related transmission events. The median length of stay in the hospital of these patients before detection was 11.5 days (ranging from three to 150 days). Of patients in clusters (n= 12), the median length of stay in the hospital before detection was 16.0 days (ranging from 3 to 49 days) and in patients with a non-cluster isolate (n= 26) the median length of stay in the hospital before detection was 11 days (ranging from 3 to 150 days) (P value 0.727). In total, 22 patients were identified with an ESBL-producing *E. coli* before admission, or within 48 hours after admission, and 52 patients had been discharged from hospital, or were outpatients, when the first positive culture was identified. These 74 patients were considered as having a community acquired ESBL-producing *E. coli*, though these 74 patients (66.1%) could still be a potential source of transmission events to other patients. After applying the definitions as described in Table 2, all patients were impossible to relate with respect to time and place (Table 6).

Model 4

In model 4, we included 113 isolates (from 112 patients) that were typed using both HiMLST and Raman spectroscopy. This was identical to model 3, but patient data was collected over a 3 month period. Model 4 clusters and sizes were similar to those found in the previous word using model 3. After applying the definitions as described in Table

2, only 1 patient was possibly related to another patient. All other patients were impossible to relate in time and place (Table 6).

DISCUSSION

In this study, genetically and phenotypically defined clusters of ESBL-producing *E. coli* were identified using HiMLST and Raman spectroscopy, but no epidemiological relationships could be found between patients assigned to various epidemiological clusters of ESBL-producing *E. coli*. The most prevalent sequence type was ST 131 (33/113; 29.2%), which was expected since it is the most predominant sequence type circulating in the community in both The Netherlands and worldwide (20). It was interesting that after sub-grouping the ST 131 isolates with Raman spectroscopy, 24/33 of these isolates could be subdivided into 4 different clusters, and 9 were considered as non-cluster (unique) isolates (model 3). However, despite this extra level of clustering, epidemiological relationships between these isolates and patients could still not be identified.

In outbreak settings, newer typing techniques such as whole genome sequencing (WGS) are proving to be helpful in healthcare- related transmission events settings, and are able to distinguish outbreak from non-outbreak bacterial strains (21, 22). However, this technique still needs threshold analyses for defining recent transmissions. Also, currently, the WGS technique is not generally available for use in routine patient settings due to the fact that it is a complex, laborious, time-consuming and expensive technique.

In this publication, clinical and molecular epidemiology (both genetic and phenotypic) data have been combined in an attempt to detect healthcare-related transmission events in a non-outbreak setting. In the Netherlands, recently introduced guidelines for multidrug-resistant microorganisms stated that all ESBL-producing *E. coli* in Dutch hospitals should be typed in order to better detect and manage healthcare-related transmission events (23). However, the exact definition of a 'healthcare-related transmission event' is not defined, and there are no defined typing techniques that are currently recommended for use. Additionally, it is not clear how any results obtained should actually be interpreted. Therefore, in this publication, the authors developed their own definitions for 'healthcare-related transmission event' and 'likelihood of healthcare-related transmission' (Table 2). In a systematic review, Kramer *et al.* determined how long *E. coli* can survive on inanimate surfaces, which differed from 1.5 hours up to 16 months (24). As this range is not practical, we selected the reference that was most applicable to the hospital setting when considering environmental contamination in our definitions of epidemiological relatedness (Table 2). However, Neely at al. found that most *E. coli* isolates had died in the environment only after 36 days (25). Therefore, we extended our time frame up to 2 months and incorporated this within the definitions 'probable' and

'possible' in Table 2. In case we still missed important links we extended the time frame used in model 3 from 2 to 3 months and used this time period in model 4 (Table 2). However, 33.9% of the 112 patients were considered as culture positive for ESBL-producing *E. coli* via a healthcare-related transmission event, but no healthcare-related transmission event was identified using our 'likelihood' definition. The question therefore arises if some of the 112 patients should actually be considered as 'community acquired', since there could still be some form of endogenous selection because of antibiotic use. This however is a subject for future research.

Limitations

This study has some limitations. Firstly, the routine surveillance cultures in our dataset were obtained only from the adult ICU (3 different departments; 10 patients), children's ICU (2 different departments; 2 patients) and hematology (1 department; 3 patients) while the remaining cultures were obtained from clinical samples. Therefore, the presence of unidentified ESBL-producing *E. coli* carrier patients cannot be ruled out. Also, the number of affected patients may have been underestimated, which would mean that the lack of epidemiological relatedness in this study could be a consequence of missing data. Secondly, we did not include the characterization of the specific ESBL genes (*e.g.* blaCTX-M, blaSHV, blaTEM) and plasmids in our analysis. It is known however that the IncFII plasmids harbor the CTX-M-15 enzyme, and that CTX-M-15 is mostly carried by the most prevalent ESBL-producing *E. coli* strain ST 131, which is also most prevalent in our study (26, 27). Finally, the possibility of ESBL antibiotic resistance gene transmission between other members of the Enterobacteriaceae and our *E. coli* isolates was not investigated.

Though this publication suggests that determining ESBL-producing *E. coli* transmission events is difficult using currently available, and high throughput, typing technologies the spread of antibiotic resistant organisms within healthcare settings remains a serious problem. For example, the isolation of hospitalized patients with ESBL-producing *E. coli* is a nationwide policy in the Netherlands, and more studies are required in order to determine if, and when, contact isolation is required or no longer indicated. Interestingly, Tschudin-Sutter *et al.* showed that the rate of spread of ESBL-producing *E. coli* to roommates in hospitals was low and suggested discontinuing contact isolation of infected or colonized patients. However, these authors only included 93 patients in a study period of almost 12 years (June 1999 through April 2011) (8). In any case, transmission prevention measures including antibiotic stewardship, cleaning and disinfection, barrier precautions and hand hygiene, should ideally be implemented in all healthcare settings (28, 29). For example, Lautenbach *et al.* identified prior antibiotic usage as the only independent risk factor for acquiring an infection with ESBL-producing *E. coli* (28). The fact that ESBL-producing *E. coli* transmission events are difficult to detect, means that

the correct training of healthcare personnel in infection control procedures is extremely relevant. This in order to reduce the likelihood of transmission events occurring at all.

Conclusion and clinical implication

ESBL-producing *E. coli* healthcare-related transmission events could not be successfully determined even when using predefined epidemiological definitions and both genotypic and phenotypic typing techniques (HiMLST and Raman spectroscopy). Even though the majority of isolates belonged to ST 131, no epidemiological relatedness was identified between patients carrying ST 131 *E. coli* strains. We therefore conclude that only the general use and development of more sensitive typing techniques (*e.g.* whole genome sequencing), coupled to increased throughput, will generate useful data for identifying ESBL-producing *E. coli* transmission events in healthcare environments. At the clinical level, the implementation of WGS should ideally be coupled to the screening of all patients at admission to hospitals as previously suggested (30).

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Chapter 5

Summarizing discussion and future perspectives

SUMMARIZING DISCUSSION

In this thesis, epidemiological studies were conducted in order to understand, describe, and evaluate the dynamics between patients and microorganisms in a hospital environment. We focused on risk factors, transmission, and detection of transmission.

Healthcare-related pathogens: risk factors

By performing systematic reviews with meta-analyses, we identified the leading risk factors, the leading protective factors, important environmental sources and reservoirs, and effective infection prevention strategies for carbapenem-resistant Enterobacteriaceae (CRE) (**Chapter 2.1**) (1), carbapenem-resistant *Pseudomonas aeruginosa* (**Chapter 2.2**) (2), and extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella* species (**Chapter 2.3**) (3); all highly-resistant microorganisms (HRMO). Figure 1 shows a summary of all patient-related and environmental factors identified in chapters 2.1, 2.2 and 2.3 for acquiring and preventing acquisition of these HRMO.

We identified that the most reported environmental source for CRE, carbapenem-resistant *P. aeruginosa*, and ESBL-producing *Klebsiella* species was the sink (Figure 1) (1-3), although in many individual studies a source was not described, not identified, or not searched for. The sink flora is, next to the waterborne flora, determined by the flora of admitted patients and by its use, after which a biofilm forms (4). Unfortunately, there are no studies about design and materials in preventing contamination of sinks with bacteria. In other words, the best sink to use in a hospital environment has not been engineered yet. Transmission of microorganisms via de sink occurs through splashing of water, or if materials used for a patient are put in or near the sink (5). Since the sink plays such an important role in transmission of HRMO, it is questionable whether a sink in a hospital environment is necessary. A study conducted by Hopman *et al.* in the Netherlands and a study conducted by Shaw *et al.* in Spain concluded that removal of sinks and water-free patient care significantly reduced colonization with Gram-negative bacteria in patients (6, 7). However, as Hopman *et al.* state in the limitation section of the article, removal of sinks could interfere with the transmission of *Clostridium difficile* spores, norovirus, and several other non-enveloped viruses, because they are resistant to hand alcohol. In these cases, after having contact, hands must be washed. Additionally, if your hands are soiled (*e.g.* with blood) or small surgical procedures are performed in for example a room on an intensive care unit (ICU), there must be a facility to wash your hands. **We conclude** and recommend that a sink must be seen as an important but dangerous object in a patient room. Sinks should be considered dirty and an important source for transmission of bacteria. Healthcare workers (HCW) and patients must become aware of this risk and act accordingly.

We identified that the second most identified source was transmission by contaminated hands of healthcare workers (HCW). Meanwhile, the low compliance rate to hand hygiene by HCW is still a concern (8). Unfortunately, interventions to increase compliance are of varying success (9, 10). Factors that impact compliance to hand hygiene by HCW are: (i) motivational factors (*e.g.* social influences, acuity of patient care, self-protection), and (ii), perceptions of the work environment (*e.g.* resources, knowledge, organizational culture) (11). A study conducted in the Netherlands introduced a multicomponent intervention program in 10 hospitals. Hand hygiene compliance increased from 42.9% to 51.4%, a significant increase (12). Connected to that, as most effective infection prevention strategy reported for ESBL-producing *Klebsiella* species, and the fourth most reported effective strategy to control CRE was improving adherence to hand hygiene (Figure 1). Therefore, **we conclude** that efforts to improve hand hygiene compliance are still important and must be and/or remain a top priority in every hospital.

The most reported effective infection prevention strategy for control of CRE were barrier and contact precautions when identifying a patient with CRE, and the second most reported infection prevention strategy was transfer of identified patients to a

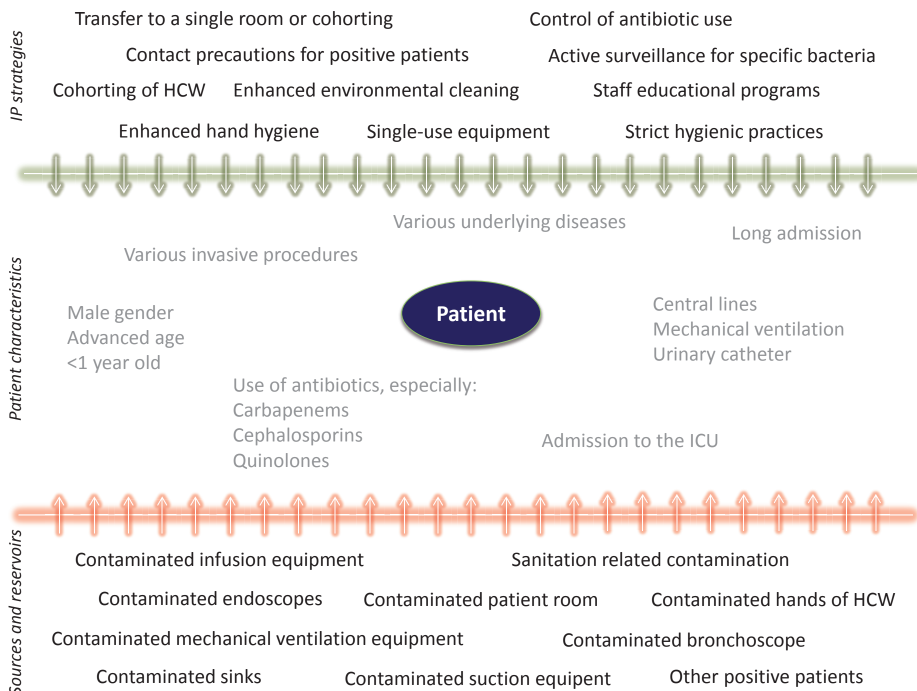

Figure 1. Infection prevention strategies, sources/reservoirs and patient-related risk factors for acquisition of highly-resistant microorganisms identified in chapters 2.1, 2.2 and 2.3.
Abbreviations: ICU; intensive care unit, HCW; healthcare workers, IP; infection prevention.

single-occupancy room, or cohorting of patients with the same microorganism (Figure 1). Concerning single-occupancy rooms, there is increasing evidence showing a relationship between hospital room design and infection control. A systematic review by Taylor *et al.* showed that there is moderately high evidence that single-occupancy rooms are an intervention for infection control, and a systematic review by Stiller *et al.* showed that single-occupancy rooms are beneficial for infection control (13, 14). Additionally, the 2018 USA guidelines for design and construction of hospitals and outpatient facilities states: "*The maximum number of beds per room in a medical/surgical patient care unit shall be one unless the necessity of a two-bed arrangement has been demonstrated* (15)." All other types of patient care units (*e.g.* oncology, intermediate care) mentioned in this guideline refer to this section (*i.e.* patient rooms shall comply with requirements of medical/surgical patient care unit – patient room) (15). Unfortunately, in the Netherlands no such guideline is available. Therefore, it is difficult for the Dutch infection control departments to demand only single-occupancy rooms. There are also other advantages of single-occupancy rooms; it was shown to improve patients' recovery because of increased privacy and increased patient support, to decrease length of hospital stay, to reduce patients' stress, to cause less medication errors and to increase doctor-nurse-family communication (16-19). However, opponents claim (i) that single-occupancy rooms are more expensive, (ii) that it may cause social isolation, (iii) that it affects the layout of the hospital (*e.g.* walking distances) which reduces quality of care, and (iv) that there is not enough evidence that proves that single-occupancy rooms reduce healthcare-related infections (16-19). The reason that only a few studies are performed on this topic is that it is difficult to perform RCTs with single-occupancy rooms as intervention. **We conclude** based on the results of chapter 2.1 that contact precautions need to be installed for patients identified with CRE, and that a single room is preferred above multi-occupancy rooms.

The risk factor we identified as having a high pooled odds ratio (OR) in all three systematic reviews in this thesis was use of antibiotics (Figure 1) (1-3). Additionally, as described by Paño Pardo *et al.* antibiotic use is likely the primary determinant for persistent CRE carriage (20). This urgently calls for reducing use, and also optimizing appropriate use of antibiotics (*i.e.* antibiotic stewardship) (21). A Cochrane systematic review by Davey *et al.* concluded that there is high-certainty evidence that interventions are effective in increasing compliance with antibiotic policy and reducing duration of antibiotic treatment (22). They also identified that less use of antibiotics did not increase mortality, but reduced length of hospital stay (22). However, as described by Parsonage *et al.*, there are numerous ethical problems to be considered when reducing use of antibiotics (23). In short, authors discussed if you can deny helpful and even potentially lifesaving antibiotics to a patient because there is a potential lack of therapies for that patient in the future (23). Antibiotics are a risk factor for acquiring HRMO because broad-spectrum

antibiotics (*e.g.* carbapenems, cephalosporins and fluoroquinolones) influence the normal gut flora and effectively kill or suppress the susceptible microorganisms, thereby enabling resistant bacteria to emerge or adhere, survive, and proliferate (*i.e.* antibiotic selective pressure). **We conclude** in all three chapters that antibiotic use is associated with acquisition of HRMO, and use needs to be reduced as much as possible. Also, we describe that since many risk factors are identified (Figure 1), bundled interventions are needed, and these should include antibiotic stewardship.

Healthcare-related pathogens: sources and transmission

Transmission is defined as the process, the mechanisms and the determinants by which an infectious agent or an infectious disease is spread from a source or reservoir to another person or across communities and countries (24). Transmission can be (i) direct (*e.g.* from person to person), or (ii) indirect (*e.g.* vehicle borne, vector borne or airborne) (24). All hospitals deal with outbreaks every now and then, and our tertiary hospital is no exception; as LeBourdais stated in 1974: "*Hospitals are bacterial collectors and distributers.*" In **chapters 2.1-2.3**, we evaluated outbreaks and transmission of microorganisms in our tertiary hospital (*i.e.* for two specific microorganisms) and nationwide (*i.e.* for a specific route of transmission).

Chapter 3.1 showed that when dealing with a large hospital-wide outbreak by Verona-integron-encoded metallo-beta-lactamase (VIM)-positive *P. aeruginosa*, the entire ward should be seen as reservoir and as contaminated, and unidentified persistent sources in the innate environment play an important role in transmission dynamics (25). This means that surveillance and cleaning of the environment is of utmost importance. In 2017, the World Health Organization (WHO) published a guideline for the prevention and control of CRE, carbapenem-resistant *Acinetobacter baumannii* and carbapenem-resistant *P. aeruginosa* in healthcare facilities (26). Although a low quality of evidence, the panel recommended that environmental surveillance cultures for these microorganisms may be considered when epidemiologically indicated (26). Also, because environmental contamination is associated with increased rates of patient colonization and infection. We identified quinolone use, use of the selective digestive tract decontamination regimen (SDD) and having undergone a gastroscopy as robust risk factors (25). A risk factor was defined as "robust" when identified using two different groups of control patients. Regarding SDD, Sánchez-Ramírez *et al.* concluded that SDD was effective in an ICU setting with a high level of resistance and subsequent high level of clinical infections (27). Several Dutch studies concluded the same in ICU settings with low levels of resistance (28-31). Additionally, SDD appeared to be cost-effective, and there was no relation between the use of SDD and the development of resistance in microorganisms in patients in the ICU (32-35). We hypothesize that the reason why we identified SDD as a robust risk factor, despite the antibiotic colistin being present in

SDD, could be because (i) the sites of the patient's body where VIM-positive *P. aeruginosa* primarily adhered was not in the gastrointestinal tract so could not be reached by SDD, (ii) the SDD could not reach VIM-positive *P. aeruginosa* in the gut because of a paralytic ileus, or (iii) topical application of colistin is not sufficient enough. Because SDD does kill other bacteria present in the gut, VIM-positive *P. aeruginosa* may adhere and proliferate. This is supported by James Hurley, who concluded that the incidence of ventilator-associated pneumonia caused by *P. aeruginosa* stayed similar in patients who did and who did not receive SDD (36). Regarding having undergone a gastroscopy; complex endoscopes have a relatively high chance of inadequate reprocessing compared to non-complex endoscopes, such as gastroscopes. However, outbreaks with as source the gastroscope have been reported, and to date no study was performed to determine the prevalence of microbial contamination of patient-ready gastroscopes, or critically assessed the gastroscope reprocessing procedures (37-39). **We conclude** that when studying microorganisms using a case-control study design, investigators need to consider using different control groups, and reason in the method section of the article why certain control groups were chosen. Additionally, use of quinolones and SDD can make patients prone to carriage of VIM-positive *P. aeruginosa*, and gastroscopy could be considered as a high-risk procedure in patients with risk factors.

In **Chapter 3.2**, we performed a nationwide cross-sectional study to assess the contamination rate of patient-ready duodenoscopes, after we proved and published in 2015 that these were a source for VIM-positive *P. aeruginosa* and the cause of an outbreak of this bacterium (40). Our study showed that 22% (33 duodenoscopes) of patient-ready duodenoscopes from 67 hospitals in the Netherlands were contaminated with ≥20 colony forming units (CFU), and 15% (23 duodenoscopes) were contaminated with gastrointestinal or oral bacteria, independent of CFU count (41). We also showed that this was not dependent on the duodenoscope manufacturer (*e.g.* Olympus, Pentax or Fujifilm), or duodenoscope type (41). This means that our study showed that patients are at risk. Since duodenoscopes are causally linked to outbreaks of CRE, and are difficult to clean and disinfect, it is a thin line between benefiting patients by the ERCP procedure and doing harm (41, 42). The different sampling conditions of the endoscopes and adherence to the strict sampling protocol could not be checked in our study, which is a limitation. **To conclude**, this study showed high prevalence rates of contaminated patient-ready duodenoscopes. This means that the current reprocessing and process control installed or the design of the endoscopes is not sufficient. This calls for further research and uniform guidelines and instructions, since they are currently lacking.

Third, **chapter 3.3** shows an outbreak of *C. difficile*; a microorganism first identified in 1935, causing infection, with as symptoms severe diarrhea and colitis (43). This outbreak was caused by a hypervirulent not-before published new *C. difficile* clone, and involved 5 patients (44). By constructing an epidemiological curve and by conducting molecu-

lar analyses transmission was confirmed. However, an environmental source was not identified. *C. difficile* can be hospital-acquired as well as community-acquired (45-47). Previously identified and known sources include (i) *C. difficile* colonized persons (*i.e.* in the absence of symptoms), as described by Crobach *et al.* in 2018: the most important unexplained reservoir for *C. difficile* transmission (48), (ii) animals, from wild animals to pets (49), and (iii), the environment, including water, plants and soil (49). With such a variety of sources successful infection prevention and control is difficult. In 2011, the burden of *C. difficile* infection (CDI) in the United States was estimated at almost 500,000 infections, and 29,000 deaths (47). In Europe, during a point prevalence survey during 2011-2012, 48% of all gastrointestinal infections registered were due to *C. difficile*, and most common in Hungary and UK-Wales (50). **We conclude** that new *C. difficile* ribotypes still emerge without a clear source. Therefore, ongoing surveillance as currently installed in the Netherlands needs to continue (51). In this way, outbreaks can be detected in an early phase and measures can be installed.

Overall, the conclusions from the studies presented in these three chapters show that bacteria are most often transmitted from an unidentified patient to the environment, object, or hands of HCW, to the next patient. Bacteria can remain on inanimate surfaces for months and therefore the hospital environment acts as a continuous source (52). Finally, outbreaks with HRMO happen, and close monitoring of high-risk environments and of high-risk patients is important to control transmission in an early phase.

Healthcare-related pathogens: detection of transmission

To detect local hospital outbreaks typing techniques are necessary. Therefore, we studied the then novel method Raman spectroscopy, a phenotypic typing method, in order to conclude whether or not to implement this technique in the diagnostic laboratory of our tertiary-care hospital. As described by Eberhardt *et al.*, advantages for diagnostic laboratories when using Raman spectroscopy are (i), minimal sample preparation, (ii), high specificity, (iii), label free, no dyes and toxic waste products, and (iv), non-destructive, non-invasive (wavelength and power dependent) (53). Disadvantages are (i) auto fluorescence (sample dependent), (ii) low sensitivity, (iii) long measurement times if weak Raman signal, and (iv) sophisticated data analysis are often necessary (53).

In **Chapter 4.1**, we evaluated Raman spectroscopy using SpectraCell*RA* analysis (RiverD International B.V., Rotterdam, The Netherlands) for a period of 43 months (54). We applied this typing method retrospectively and included patients identified with ESBL-producing *Klebsiella pneumoniae* or ESBL-producing *Klebsiella oxytoca*, and used it prospectively, also for patients identified with an ESBL-producing *K. pneumoniae* or ESBL-producing *K. oxytoca*. We could detect clonal outbreaks which were epidemiologically plausible, which could have possibly been prevented if instant prospective typing had been implemented in our hospital. However, we think that our results are the tip of

the iceberg, because we did not install routine admission screenings, and therefore have presumably missed a lot of unidentified carriers. For this study, we also developed definitions of epidemiological relatedness, and conducted a spatial analysis to combine this with the Raman data. The definitions were not only applicable to *Klebsiella* species, but we also applied them to ESBL-producing *Escherichia coli* (**chapter 4.2**), and VIM-positive *P. aeruginosa* (**chapter 3.1**) transmission events (25, 55). Our definitions could have been too strict. Therefore, it is possible that we misclassified or missed epidemiological relations that were present.

In **Chapter 4.2**, we studied if High-Throughput Multilocus Sequence Typing (HiMLST) and Raman spectroscopy could detect ESBL-producing *E. coli* healthcare-related transmission events, using four different models (55). We concluded that we did identify genetically and phenotypically defined clusters, however; patients in all four models were not epidemiologically related. This can be explained by (i), missing links (*i.e.* unidentified carriers), and/or (ii), the high community carriage rates of ESBL-producing *E. coli* sequence type (ST) 131. To solve this problem, we concluded that more sensitive typing techniques (*e.g.* whole genome sequencing or wgMLST) and admission screening are needed.

We conclude that epidemiological plausible clusters for ESBL-producing *Klebsiella* species; however, this could not be concluded for ESBL-producing *E. coli*. Additionally, epidemiological relatedness is often described in publications; however, definitions are almost never published in such a way that they are directly applicable to your own research. With our table of epidemiological relatedness, applied to three different scenarios, we hope to contribute to epidemiological research on transmission of bacteria. Raman spectroscopy had advantages and disadvantages. Eventually, Raman spectroscopy was not implemented as a routine typing technique in our hospital. This was mainly due to the software instabilities and the sudden unavailability of the method at that time.

FUTURE PERSPECTIVES

Healthcare-related pathogens: risk factors

The literature studies in chapters 2.1, 2.2, and 2.3 may serve as a basis and provide knowledge for observational or experimental research on HRMO. We feel that especially **(i)**, future research should not only focus on developing new antibiotics, but also on novel therapeutic strategies since resistance to all biologic antimicrobials will ultimately develop (23). **(ii)** Future studies should reconsider the design of the hospital sink, including the faucet, faucet aerator, sink tap, grating, plughole, and siphon because of biofilm formation of bacteria in these areas. **(iii)** More research is needed about single-

patient rooms and the effect on acquisition of HRMO. On May 18, 2018, the Erasmus MC University Medical Center, Rotterdam, the Netherlands (Erasmus MC), moved to a new building, with only single-occupancy rooms. This event provided a unique opportunity to perform such a study. Hundreds of patients that were housed in multiple-occupancy rooms in the old building, and hundreds of patients that are housed in the new building were and continue to be included. From these patients, admission and discharge cultures (perianal swab) were and continue to be performed. Additionally, the hospital environment was and continuous to be thoroughly sampled in both buildings. Hopefully, this study will add to the existing knowledge about changes in environment and in patients when moving from multiple-occupancy rooms to only single-occupancy rooms. **(iv)** It is not known whether there is a relationship between HRMO infection prevention policy and the prevalence of HRMO. Therefore, future studies should assess the cost-effectiveness of infection prevention strategies to prevent transmission and acquisition of HRMO and compare policies in settings with a high prevalence of HRMO to settings with a low prevalence of HRMO.

Healthcare-related pathogens: sources and transmission

P. aeruginosa can survive in environments with low and high availability of nutrients, can grow at temperatures between 10^0C and 42^0C, and forms biofilms (56). Therefore, this microorganism is difficult to remove from the environment. **(i)** The most optimal cleaning agent for *P. aeruginosa* environmental cleaning protocols has not yet been defined. This can be investigated by future studies. **(ii)** We identified use of SDD as a risk factor for acquiring VIM-positive *P. aeruginosa*; in future research a subgroup analysis of all patients with SDD could be performed to unravel any unidentified important patient characteristics. Additionally, future studies should determine if SDD can be optimized in settings were this microorganism is present. **(iii)** Since gastroscopy was identified as a high-risk procedure, future studies should investigate the prevalence of bacterial contamination of gastroscopes, and should assess the gastroscope reprocessing process.

Regarding contamination of duodenoscopes, future studies should **(i)** investigate the effect of other confounding factors, such as age and number of procedures of the duodenoscope and the time component (*i.e.* considering the warnings by for example the U.S. Food and Drug Administration and newly developed guidelines) on contamination levels of duodenoscopes. **(ii)** Since the reprocessing of duodenoscopes is not optimal, future studies should investigate different and novel cleaning and drying methods. **(iii)** A cross-sectional study design is useful when wanting to know a prevalence; however, follow-up data of duodenoscopes is needed to study persistence of colonization and to study the effects of interventions on contamination rates. This can be investigated by future studies.

Finally, our newly identified ribotype 826 *C. difficile* was not identified in databases of human collections, and animal collections are lacking. Therefore, we strongly support the development of a global database or national reference laboratory for animal-associated *C. difficile* infections can be realized.

Healthcare-related pathogens: detection of transmission

Considering the different typing methods in combination with epidemiological relatedness, it is **(i)** important that there are as less as unidentified carriers as possible. Therefore, future studies about transmission dynamics should assess in a setting with admission cultures of all patients if molecular relationships can be explained epidemiologically. **(ii)** In chapter 4.2, we did not find any epidemiological relationships between patients with phenotypically identical ESBL-producing *E. coli*. If admission cultures are installed, the different relationships should be studied again for this microorganism. Since literature on the effect and necessity of infection prevention measures for patients identified with ESBL-producing *E. coli* is contradictory, these data could be helpful. **(iii)** In chapters 4.1 and 4.2, we studied ESBL-producing *K. pneumoniae*, ESBL-producing *K. oxytoca* and ESBL-producing *E. coli*; future studies should investigate other HRMO. **(iv)** Transmission dynamics of HRMO should be studied in different settings; for example university compared to non-academic hospitals, or hospitals in countries with a high HRMO prevalence compared to hospitals in countries with a low HRMO prevalence. Additionally, network analyses should be performed to further understand transmission dynamics. To fully understand transmission dynamics, the network analyses should not only include movements of patients in one hospital, but also movements of patients between different hospitals. Furthermore, not only movements in and between hospitals should be included, but also for example admissions to nursing homes and rehabilitation clinics. Finally, **(v)** future studies should keep evaluating novel promising typing methods, as speed and accuracy can always be improved, and are important in fast, efficient and effective infection prevention and control.

FINAL NOTES

Epidemiology is the study of the occurrence and distribution of health-related events, states, and processes in specified populations, including the study of the determinants influencing such processes, and the application of this knowledge to control relevant health problems (24). Study includes: (i) surveillance, (ii) observation, (iii) screening, (iv) hypothesis testing, (v) analytic research, (vi) experiments, and (vii) prediction (24). In this thesis, epidemiological studies were conducted in order to understand, describe, and evaluate the dynamics between patients and microorganisms in a hospital environment.

Study designs

Back in 1904, summarizing and pooling of data in review articles was introduced (57). Currently, systematic reviews are well established studies to summarize all available evidence about a specific subject. Because of the possible high impact of results of systematic reviews on decision making in healthcare, the quality of reporting of meta-analyses (QUOROM) statement was introduced in 1996 (officially published in 1999) (58, 59). This statement aimed at enhancing quality of systematic reviews of randomized controlled trials (RCTs) (58). In 2009, the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement was introduced as an update of the QUOROM statement (www.prisma-statement.org) (60). Currently, in 2018, an update of the 2009 statement is under development. In addition, in 2018, the PRISMA statement checklist is mandatory to add as supplemental material when submitting a systematic review to over 180 journals. Hence, we also need to consider the limitations of systematic reviews. A first limitation that needs to be considered is the between study heterogeneity. This means that there are underlying differences among the included studies. This may be caused by (i) the selection of patients, (ii) differences in study setting, (iii) patient characteristics, (iv) measurements, and/or (v) methodological study differences (61). One can reason if heterogeneity between studies is present or not, or use the I^2 statistic (61, 62). The I^2 statistic is easily available, easy to apply, and easy interpretations are available (62). However, the I^2 statistic is often used inappropriately (63). Only the statistic itself can give wrong information, as for example a meta-analysis with an I^2 value of 80% can correspond with less variance than a meta-analysis with an I^2 value of 20%. Therefore, it should always be used together with the forest plot. However, still when using a forest plot, the I^2 statistic only shows you the extent to which confidence intervals from the different studies overlap with each other, nothing about the actual study to study dispersion in effects (63). In chapters 2.1, 2.2 and 2.3, heterogeneity between studies was present when using the I^2 statistic in combination with the forest plot. Therefore, in all these three studies, a random effects model was fitted in all meta-analyses. A random effects model allows for differences from study to study, and is therefore a good statistical option if heterogeneity between studies is present (64). A second limitation of systematic reviews is publication bias. Publication bias means that the research that is available differs in its results from the results of all the research that has been done (65). Often, studies with a non-significant result or negative results are not published. Publication bias was also present in almost all meta-analyses present in this thesis, as indicated by funnel plots (*i.e.* visual inspection) or by bias indicators by Egger *et al*, and Begg and Mazumdar (66, 67). It is possible to correct for publication bias by imputing the apparent missing values in funnel plots; however, this is not always preferable.

From chapter 3.1 we learned that the approach of using two different groups as controls showed that results you obtain from case-control studies highly depend on

the choice of the control patients. As described by Feinstein and Horwitz in 1983, the different choices of control patients suggest either that (i) the perfect control group does not exist, or (ii) that just no specific standards have been established in order to decide which controls to use (68). Additionally, described by Grimes and Schulz in 2005, poor choice of controls can lead to both wrong results and possible medical harm (69).

With a cross-sectional study as used in chapter 3.2, data is collected at a given point in time. Making causal inferences is not possible with this type of study, unless the exposure, in this case contamination, is stable over time (70). Kraemer *et al.* states that limitations of cross-sectional studies are acknowledged, however understated (71). To assess the validity of observational studies, including cross-sectional studies, a Cochrane systematic review by Anglemyer *et al.* included reviews, and investigated healthcare-outcomes assessed with observational study designs compared with those assessed by RCTs (72). They concluded that when conducting a review on a specific topic, on average there is little difference between results obtained from RCTs and results obtained from observational studies (72). However, unfortunately a direct comparison between cross-sectional studies and RCTs was not possible, because of lack of identified studies (72).

The future of hospital epidemiology

An epidemiologist is a professional who strives to study and control the factors that influence the occurrence of disease or other health-related conditions and events in defined populations and societies, has an expertise in population thinking and epidemiological methods, and is knowledgeable about public health and causal inferences in health (24). A healthcare epidemiologist should have knowledge about (i) disease exposure and transmission, (ii) an understanding of measures of incidence and prevalence, and (iii) basic knowledge of microbiology, bacteriology, virology and mycology (73). As described by Bryant *et al*, having at least one dedicated full-time healthcare epidemiologist, next to infection preventionists and clinical microbiologists, is a requirement for an effective infection prevention and control/healthcare epidemiology program in a healthcare institution (74). In the future, I hope we can accomplish this in every Dutch hospital; both academic and non-academic.

In this thesis, we described three topics, all about healthcare-related pathogens; (i) risk factors, (ii) sources and transmission, and (iii) detection of transmission. In my opinion, the three topics in this thesis are connected. We need to learn from other outbreaks and studies about infection prevention and microorganisms, we need to learn from our own outbreaks and gain deep understanding how and why it happened, and we need to constantly evaluate existing and new typing methods to quickly learn if isolates from patients are related or not. There are also four different levels within the three topics (Figure 2): (i) healthcare at individual hospital level, (ii) healthcare at regional level, (iii) healthcare at national level, and (iv) healthcare at international level.

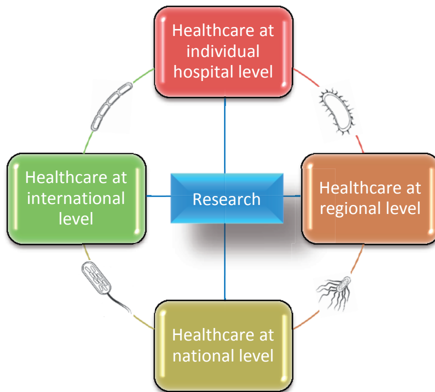

Figure 2. Connections of hospitals concerning risk factors, sources and transmission, and detection of transmission of microorganisms.

These levels are also connected. Concerning the individual hospital level, data collection and data connection can happen without any problems. However, when collecting and connecting regional, national or international patient data in order to epidemiologically interpret the results of molecular genotypic or phenotypic typing, problems of data ownership and patient privacy arise, especially when a website-based database is used. Therefore, agreements between institutes need to be signed and the patient privacy must be adequately protected. In the future, I hope we can develop first national guidelines, and then followed by international guidelines about data ownership and patient privacy. National and international collaborations are in my opinion the key. Overall, research is the connecting factor, which needs to be executed at each level, about every topic possible. Share information about infection prevention, share data, share research. In this way we can combat antimicrobial resistance and combat spread more effectively and efficiently, because transmission happens, and often through unidentified sources and/or reservoirs.

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Chapter 6

Nederlandse samenvatting

Dit proefschrift bestaat uit literatuuronderzoeken en observationele studies over zorggerelateerde ziekteverwekkers. Deze studies zijn onderverdeeld in de volgende drie onderwerpen: (i) het identificeren en beschrijven van risicofactoren voor het verkrijgen van zorggerelateerde ziekteverwekkers, (ii) bronnen en overdracht van zorggerelateerde ziekteverwekkers, en (iii) het detecteren van overdracht van zorggerelateerde ziekteverwekkers. Het doel van deze studies was het optimaliseren en het veiliger maken van zorg voor patiënten opgenomen in het Erasmus MC Universitair Medisch Centrum in Rotterdam (Erasmus MC). Ook geven de uitkomsten van de studies inzichten welke van nut zijn voor andere zorginstellingen, wereldwijd.

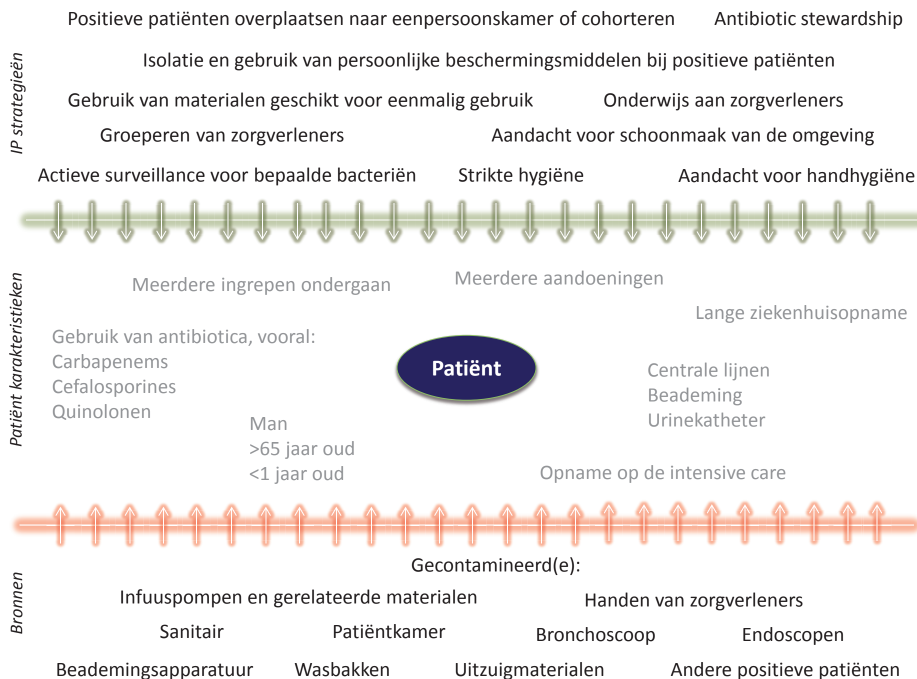
ZORGGERELATEERDE ZIEKTEVERWEKKERS: RISICOFACTOREN

Het eerste deel van dit proefschrift bestaat uit drie systematische literatuuronderzoeken. Een systematisch literatuuronderzoek heeft als doel alle beschikbare wetenschappelijke literatuur over een specifiek onderwerp samen te vatten, kwalitatief en/of kwantitatief. Het samenvoegen van resultaten van de verschillende studies kan ook door middel van een statistische methode; dit wordt ook wel een meta-analyse genoemd. Het doel van de literatuuronderzoeken in dit proefschrift was het identificeren van (i) de belangrijkste risicofactoren, (ii) de belangrijkste beschermende factoren, (iii) veel voorkomende bronnen in de ziekenhuisomgeving, en (iv) de meest effectieve infectiepreventie maatregelen voor het voorkomen van overdracht van verschillende zorggerelateerde ziekteverwekkers, ofwel micro-organismen. In **hoofdstuk 2.1** beschreven we dit voor carbapenem-resistente Enterobacteriaceae (CRE). Enterobacteriaceae zijn Gram-negatieve bacteriën; de bekendste genera binnen de familie van de Enterobacteriaceae zijn: *Escherichia*, *Klebsiella*, *Proteus*, *Enterobacter*, *Morganella*, *Salmonella* en *Serratia*. In **hoofdstuk 2.2** beschreven we carbapenem-resistente *Pseudomonas aeruginosa*, en in **hoofdstuk 2.3** extended-spectrum beta-lactamase (ESBL)-producerende *Klebsiella* species. Het enzym ESBL zorgt voor resistentie tegen belangrijke groepen van antibiotica, namelijk de penicillines en cefalosporines. Al deze micro-organismen worden ook wel bijzonder resistente micro-organismen (BRMO) genoemd. Ze zijn bijzonder resistent omdat ze resistent zijn tegen eerste keus antibiotica of tegen meerdere groepen antibiotica. De behandeling van infecties door BRMO is daardoor vaker complex, en er moet uitgeweken worden naar soorten antibiotica die men liever niet gebruikt vanwege bijvoorbeeld hoge toxiciteit. Ook worden patiënten bij wie deze bacteriën zijn geïdentificeerd in isolatie verpleegd om overdracht van de bacterie naar andere patiënten te voorkomen. Afhankelijk van het micro-organisme betekent dit een eenpersoonskamer (soms zelfs met sluiskamer), en zowel zorgverleners als bezoek dragen een schort en handschoenen, eventueel uitgebreid met een mondkapje en een muts. Het is belangrijk

verspreiding van BRMO binnen zorginstellingen te voorkomen omdat (i) het hebben van een infectie met een BRMO adequate behandeling bemoeilijkt of zelfs onmogelijk maakt, (ii) patiënten langer opgenomen liggen in isolatie en (iii) patiënten mogelijk een slechtere prognose hebben door het hebben van een BRMO. Hierbij is een samenvatting van kennis van uitbraken wereldwijd, en bevindingen en gedachtegangen van collega onderzoekers in publicaties cruciaal. In figuur 1 staan de belangrijkste bevindingen van hoofdstuk 2.1, hoofdstuk 2.2 en hoofdstuk 2.3 weergegeven.

De factor die in alle drie de studies een hoog risico op overdracht met BRMO gaf was het gebruik van diverse antibiotica. Dit laat zien dat zorgverleners zich moeten inzetten om antibiotica alleen te gebruiken als het daadwerkelijk nodig is.

De omgevingsbron welke het meest werd gerapporteerd voor alle onderzochte BRMO was de wasbak. Vanuit de wasbak kunnen de bacteriën via spatten terecht komen op voorwerpen rondom de wasbak. Via die voorwerpen kunnen ze dan vervolgens bij de patiënt komen. Dit pleit mogelijk voor het verwijderen van wasbakken in de patiënt omgeving. Echter, in verschillende situaties blijft een wasbak nodig: (i) bij patiënten geïdentificeerd met een micro-organisme dat resistent is tegen handalcohol, (ii) als



Figuur 1. De belangrijkste risicofactoren, veel voorkomende bronnen in de omgeving, en de meest effectieve infectiepreventie (IP) maatregelen voor overdracht en het voorkomen van overdracht van verschillende bijzonder resistente micro-organismen.

handen van zorgverleners visueel bevuild zijn met bijvoorbeeld braaksel, en (iii) bij het uitvoeren van kleine chirurgische ingrepen op de patiëntkamer. De meest gerapporteerde effectieve infectie preventie strategie voor CRE was het gebruik van persoonlijke beschermingsmiddelen en het instellen van isolatie. Voor ESBL-producerende *Klebsiella* was dit extra aandacht besteden aan handhygiëne.

ZORGGERELATEERDE ZIEKTEVERWEKKERS: BRONNEN EN OVERDRACHT

Overdracht, ofwel transmissie van micro-organismen wordt vaak gedefinieerd als het proces, het mechanisme en factoren waardoor een micro-organisme of ziekte verspreid vanuit een reservoir of omgevingsbron naar een persoon. Overdracht kan worden onderverdeeld in twee categorieën: (i) directe transmissie, van patiënt naar patiënt, of (ii) indirecte transmissie, van patiënt naar patiënt via de omgeving (*e.g.* via een voorwerp of via de lucht). In de volgende drie hoofdstukken beschrijven en evalueren wij overdracht van micro-organismen in het Erasmus MC (*i.e.* voor twee specifieke micro-organismen) en landelijk (*i.e.* voor een specifieke transmissie route),

Hoofdstuk 3.1 beschrijft een grote uitbraak veroorzaakt door Verona Integron-encoded Metallo-β-lactamase (VIM) –positieve *P. aeruginosa*. Dit micro-organisme is resistent tegen carbapenem antibiotica (*e.g.* imipenem en meropenem). Wij onderzochten onderliggende risicofactoren voor het oplopen van deze bacterie door middel van een patiënt-controle onderzoek. In een patiënt-controle onderzoek worden patiënten met de bacterie (cases) vergeleken met patiënten die vergelijkbaar zijn met de cases, maar zonder de bacterie. Omdat VIM-positieve *P. aeruginosa* niet vaak voorkomt, is dit efficiënter dan prospectief een grote groep patiënten volgen. In deze studie includeerden wij 144 patiënten met de bacterie en 576 controles. Door middel van een multivariaat statistisch model identificeerden wij de volgende risicofactoren: (i) het hebben ondergaan van een gastroscopie binnen 6 maanden voor identificatie van de bacterie, (ii) het gebruik van selectieve darm decontaminatie (SDD), antibiotica voorschreven op de intensive care, en (iii) het gebruik van chinolonen. Ook hebben wij een netwerkanalyse uitgevoerd, waarin wij vaststelden dat overdracht van de bacterie niet via directe transmissie plaatsvindt (*i.e.* van patiënt naar patiënt), maar indirect, via de omgeving. Wij concludeerden dat deze studie laat zien dat het gebruik van antibiotica zo mogelijk verminderd moet worden, een gastroscopie kan worden gezien als een hoog-risico procedure bij patiënten bij wie andere risicofactoren (*e.g.* antibiotica gebruik) ook aanwezig zijn, en er aandacht moet worden besteed aan het opsporen en elimineren van bronnen in de omgeving aangezien de netwerkanalyse liet zien dat overdracht van VIM-positieve *P. aeruginosa* vooral via de omgeving plaatsvindt.

Hoofdstuk 3.2 beschrijft een landelijke studie naar contaminatie van duodenoscopen gebruikt voor endoscopische retrograde cholangio- en pancreaticografie (ERCP). Een ECRP wordt ingezet om de galwegen en alvleesklier te onderzoeken, en wordt in Nederland ongeveer 17.000 keer per jaar uitgevoerd in 73 verschillende centra. Tijdens de ERCP procedure kunnen duodenoscopen gecontamineerd raken met darmflora. Deze flora bestaat voornamelijk uit bacteriën. Als duodenoscopen na elke procedure inadequaat gereinigd en gedesinfecteerd worden, kunnen patiënten besmet raken met de darmflora van de vorige patiënt(en). Een duodenoscoop is erg complex want heeft onder andere een zijwaarts gerichte tip, een liftmechanisme, en een liftkanaal. Hierdoor is een duodenoscoop lastiger te reinigen en desinfecteren dan andere endoscopen, bijvoorbeeld een gastroscoop of een coloscoop. Wereldwijd zijn er uitbraken met BRMO geïdentificeerd en gerapporteerd waarbij de duodenoscoop de bron was. Ook in het Erasmus MC is er een uitbraak gerapporteerd door een gecontamineerde duodenoscoop, waarbij 22 patiënten betrokken waren (Verfaillie *et al.*, Endoscopy 2015; 47(6): 493-502). Door deze wereldwijde uitbraken met als bron een duodenoscoop en het besef dat duodenoscopen niet adequaat gereinigd en gedesinfecteerd kunnen worden, vonden wij het belangrijk vast te stellen hoeveel duodenoscopen daadwerkelijk zijn gecontamineerd, en waaruit de flora bestaat. Met de huidige studie onderzochten wij hoeveel duodenoscopen in Nederland gecontamineerd zijn en of het uitmaakt van welke fabrikant (*i.e.* Olympus, Pentax of Fujifilm) de scoop afkomstig is. Zevenenzestig van de 73 (91.8%) Nederlandse ERCP-centra hebben aan onze studie meegedaan, en 155 duodenoscopen zijn geïncludeerd. Drieëndertig duodenoscopen (22%) waren gecontamineerd, waarvan 23 (15%) met darmflora. Het maakte niet uit van welke fabrikant de duodenoscoop was, contaminatie was evenredig verdeeld over alle 3. De resultaten van deze studie laten zien dat de huidige reinigings- en desinfectie procedures niet adequaat en veilig zijn, waarvoor een oplossing nodig is. Een mogelijke oplossing zou het aanpassen van het design van de duodenoscoop zijn, zodanig dat adequate reiniging en desinfectie wel mogelijk is. Helaas is dit geen korte termijn oplossing aangezien het ontwerpen en introduceren van een nieuwe duodenoscoop een langdurig traject is.

Hoofdstuk 3.3 beschrijft een uitbraak veroorzaakt door *Clostridium difficile*. *C. difficile* bevindt zich in de darmen, en kan ernstige diarree en darmontstekingen veroorzaken. De *C. difficile* welke deze uitbraak veroorzaakte was hypervirulent, en het bleek bovendien te gaan om een nieuw type. Bij de uitbraak waren 5 patiënten betrokken. Door het combineren van epidemiologische gegevens en moleculaire typeringsuitslagen kon transmissie worden vastgesteld; maar helaas werd geen bron in de omgeving ontdekt. De ontdekking en bevestiging van het plaatsvinden van deze uitbraak laat zien dat (landelijke) surveillance van dit micro-organisme nodig is; dit om nieuwe types met een mogelijke verhoogde virulentie tijdig op te sporen.

ZORGGERELATEERDE ZIEKTEVERWEKKERS: HET DETECTEREN VAN OVERDRACHT

Omdat het voorkomen van BRMO steeds meer toeneemt en daardoor ook uitbraken in ziekenhuizen door BRMO neemt de behoefte aan goede typeertechnieken toe. Moleculaire typeertechnieken helpen om patiënten geïdentificeerd met dezelfde bacterie te differentiëren in verschillende clusters, en zijn daardoor belangrijke tools in infectiepreventie. Er bestaan veel verschillende typeertechnieken, elk met voor- en nadelen. De keuze welke typeertechniek te gebruiken hangt af van veel factoren, bijvoorbeeld: (i) welk micro-organisme moet getypeerd worden, (ii) is er haast bij of niet, (iii) hoeveel patiënten zijn er betrokken over een periode van hoeveel maanden/jaren, (iv) wat kost de typeertechniek en (v) welke technieken en kennis over de technieken is er beschikbaar binnen een instelling. In **hoofdstuk 4.1** en **4.2** hebben we de toen nieuwe, snelle, veelbelovende fenotypische typeertechniek Raman spectroscopy, welke gebruik maakt van SpectraCellRA analysis (RiverD International B.V., Rotterdam), bestudeerd met als doel gegevens te verkrijgen om te bepalen of deze techniek in de routine diagnostiek van het Erasmus MC geïmplementeerd zou moeten worden.

In **hoofdstuk 4.1** hebben we over een periode van 43 maanden 132 patiënten geïdentificeerd met ESBL-producerende *Klebsiella pneumoniae* en *Klebsiella oxytoca* geincludeerd, en isolaten getypeerd met behulp van Raman spectroscopy. In deze studie ontwikkelden wij ook definities om te bepalen of patiënten epidemiologisch gerelateerd waren; gerelateerd in plaats en tijd (Tabel 1). Wij hebben deze definities ontwikkeld omdat de definities die beschikbaar zijn in wetenschappelijke literatuur of bij instanties zoals de Centers for Disease Control and prevention (CDC) niet specifiek genoeg zijn. Dit omdat de definitie "gerelateerd in plaats en tijd" niets zegt over of dit alleen kamergenoten moeten zijn of ook afdelingsgenoten, en of alleen patiënten opgenomen ten tijde van de opname van de case meegerekend moeten worden of ook patiënten die een paar dagen eerder op dezelfde kamer lagen. De definities ontwikkeld tijdens het uitvoeren van deze studie hebben wij ook toegepast in hoofdstuk 4.2 en in hoofdstuk 3.1. Door middel van de typeertechniek konden we vaststellen dat 73 van de 132 pati-

Tabel 1. Definities om te bepalen of patiënten epidemiologisch gerelateerd zijn.

	Definitief gerelateerd[1]	Waarschijnlijk gerelateerd	Mogelijk gerelateerd-I	Mogelijk gerelateerd-II	Onmogelijk gerelateerd
Dezelfde patiëntkamer	1	1	0	0	0
Dezelfde afdeling	1	1	1	1	0
Dezelfde tijdsperiode	1	0[a]	1	0[a]	0[a]/0/1

0= nee; 1= ja, 0[a]= <3 maanden nadat de vorige positieve patiënt was ontslagen. [1]De definitie 'definitief gerelateerd' was niet mogelijk op de intensive care, dit omdat daar alleen maar eenpersoonskamers aanwezig waren.

enten verdeeld waren over 17 clusters en 59 patiënten een uniek isolaat hadden. Door het toepassen van de definities in tabel 1 bleken patiënten in 2 clusters definitief aan elkaar gerelateerd te zijn, in 6 clusters was het een mix van waarschijnlijk, mogelijk en onmogelijk en in 7 clusters waren patiënten epidemiologisch onmogelijk aan elkaar gerelateerd. Alle eerste patiënten in de tijd in een cluster noemden wij primaire patiënten, in dit geval waren dit er dus 17. Alle opvolgende patiënten in de tijd in clusters noemden wij secundaire patiënten in ons geval waren dit 56 patiënten. Bij identificatie van een cluster (2 patiënten of meer) zouden extra infectiepreventie maatregelen kunnen worden ingezet waardoor verspreiding van de bacterie naar meer secundaire patiënten voorkomen zou kunnen worden. Deze maatregelen zouden grondige reiniging en desinfectie van patiëntkamers en sanitair, en het uitbreiden van een contactonderzoek kunnen inhouden. In theorie, bij het routinematig inzetten van Raman spectroscopy zou het dus mogelijk zijn om kolonisaties en infecties bij patiënten te voorkomen. Deze studie laat zien dat transmissie plaatsvindt, ook in een schijnbaar niet-uitbraak situatie. Wij concluderen dat het routinematig typeren van BRMO nodig is.

Door het hoge percentage van dragers van 1 specifieke ESBL-producerende *Escherichia coli* (*i.e.* sequence type 131) buiten het ziekenhuis is het lastig om door middel van typeertechnieken verschillende clusters te ontdekken. Dit is de reden waarom wij in **hoofdstuk 4.2** hebben onderzocht of de combinatie van typeertechnieken High-throughput MultiLocus Sequence Typing (HiMLST) en Raman spectroscopy meer inzicht zou geven in recente overdracht van ESBL-producerende *E. coli* in het Erasmus MC. Overdracht werd gedefinieerd volgens de definities in tabel 1. Wij includeerden 194 patiënten met een ESBL-producerende *E. coli*. Alleen gebruik makend van Raman spectroscopy resulteerde in 16 clusters, en alleen gebruik makend van HiMLST resulteerde in 10 clusters. Maar, patiënten binnen zowel de Raman spectroscopy als de HiMLST clusters waren niet epidemiologisch gerelateerd. Een combinatie van beide technieken leverde 8 clusters op, maar ook in deze 8 clusters waren patiënten niet epidemiologisch gerelateerd. Wij concludeerden dat ondanks dat er clusters werden geïdentificeerd door de verschillende typeertechnieken, of een combinatie van beiden, geen epidemiologische relaties werden gevonden. Wij concludeerden dat in het Erasmus MC het routinematig typeren van ESBL-producerende *E. coli* niet zinvol is met zowel HiMLST als Raman spectroscopy.

Raman spectroscopy had voordelen en nadelen en is tijdelijk geïmplementeerd geweest in de routine diagnostiek van het Erasmus MC voor specifieke resistente bacteriën. De techniek werd na een aantal jaren niet meer geleverd en wordt daarom nu niet meer gebruikt.

TOEKOMST

De in dit proefschrift beschreven studies geven aanleiding tot vervolgonderzoek. De 3 studies in hoofdstuk 2 over het onderwerp **identificeren en beschrijven van risicofactoren voor het verkrijgen van zorggerelateerde ziekteverwekkers** laten zien dat vervolgonderzoek moet worden gedaan naar: (i) het ontwikkelen van nieuwe antibiotica en het ontwikkelen van nieuwe therapeutische strategieën, (ii) het ontwikkelen van een wasbak speciaal voor gebruik in het ziekenhuis, (iii) het effect van eenpersoonskamers op overdracht van BRMO, en (iv) de kosteneffectiviteit van alle preventiestrategieën op dit moment geïmplementeerd in ziekenhuizen.

De drie studies in hoofdstuk 3 over het onderwerp **bronnen en overdracht van zorggerelateerde ziekteverwekkers** laten zien dat wat betreft VIM-positieve *P. aeruginosa* meer onderzoek moet worden gedaan naar: (i) het beste middel om de omgeving mee schoon te maken en te desinfecteren, (ii) het gebruik van SDD in combinatie met het voorkomen van VIM-positieve *P. aeruginosa*, en (iii) de prevalentie van contaminatie van gastroscopen. Wat betreft gecontamineerde duodenoscopen kan vervolgonderzoek worden gedaan naar: (i) het effect van factoren welke niet zijn meegenomen in de huidige studie, zoals het tijdscomponent en leeftijd van de duodenoscoop (*i.e.* in jaren oud en in het aantal en soort uitgevoerde procedures), (ii) het ontwikkelen van nieuwe reiniging en desinfectie methodes, en (iii) het uitvoeren van een prospectief longitudinale studie in plaats van een cross-sectioneel onderzoek. Het onderzoek naar *C. difficile* laat zien dat de database op dit moment beschikbaar in Nederland met alle verschillende types geïdentificeerd in Nederland kan helpen om snel het probleem vast te kunnen stellen. Maar, een dergelijke database van *C. difficile* geïdentificeerd in dieren ontbreekt. We hopen dat een dergelijke database in de toekomst wel beschikbaar zal zijn.

De 2 studies in hoofdstuk 4 over het onderwerp **het detecteren van overdracht van zorggerelateerde ziekteverwekkers** laten zien dat het belangrijk is om nieuwe typeermethodes te blijven evalueren; dit omdat het snel identificeren van een uitbraak essentieel is voor effectieve en efficiënte infectiepreventie. Ook kan naar de volgende onderwerpen vervolgonderzoek gedaan worden: (i) omdat het belangrijk is om zo veel mogelijk patiënten met het micro-organisme waarin je geïnteresseerd bent te identificeren, zou deze studie herhaald kunnen worden in een setting waar opnamekweken bij patiënten worden afgenomen. Hierbij wordt de kans op het ontbreken van schakels tussen patiënten kleiner. (ii) Wij hebben nu alleen ESBL-positieve *K. pneumoniae*, ESBL-positieve *K. oxytoca* en ESBL-positieve *E. coli* geëvalueerd. In toekomstige studies zouden ook andere BRMO onderzocht kunnen worden. (iii) Om overdracht van micro-organismen tussen patiënten nog beter te begrijpen, zou het zinvol zijn niet alleen te kijken naar patiëntbewegingen binnen 1 ziekenhuis, maar ook te kijken naar overplaatsingen naar bijvoorbeeld andere ziekenhuizen, verpleeghuizen en revalidatieklinieken.

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Chapter 7

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DANKWOORD

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CURRICULUM VITAE

Anne Fenny Voor in 't holt was born on September 2nd, 1989 in Nunspeet, the Netherlands. After finishing her secondary education at the Lambert Franckens College in Elburg in 2006, she started studying nursing at Windesheim University of Applied Sciences in Zwolle. After her final internship at the North Karelia Central Hospital in Joensuu, Finland, she also entered the pre-master program Health Sciences at the VU University in Amsterdam. In 2010, she obtained her bachelor degree in nursing, and finished the pre-master. After the pre-master program, she started a MSc program in Health Sciences and specialized in infectious diseases and public health. For her MSc internship she wrote a literature review about multidrug-resistant tuberculosis and streptomycin resistance at the Institute for Global Health and Development, Academic Medical Center, Amsterdam. After finishing her MSc degree in 2011, she started a second MSc program in clinical epidemiology at the Erasmus University in Rotterdam. During her final internship at the department of Medical Microbiology and Infectious Diseases (MMIZ) at the Erasmus MC she wrote a systematic review and meta-analysis about carbapenem-resistant *Pseudomonas aeruginosa*. After obtaining her second MSc degree in 2012, she started working at the MMIZ. First she was involved in the RADERMO study, about cost-effectiveness of Raman spectroscopy, and after that she worked as an epidemiologist of the Unit infection prevention. At the same time, she started her PhD under the supervision of Prof.dr. Margreet Vos and Dr. Juliëtte Severin of which the results are presented in this thesis. Anne currently continues her research about epidemiology of healthcare-related pathogens in combination with working as an epidemiologist at the Unit infection prevention of the MMIZ. Anne is married to Roderick Kluvers, and together they have a daughter; Emma (2013), and a son; Diederick (2017).

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11. Verfaillie CJ, Bruno MJ, **Voor in ‘t holt AF**, Buijs JG, Poley JW, Loeve AJ, Severin JA, Abel LF, Smit BJ, de Goeij I, Vos MC. Withdrawal of a novel-design duodenoscope ends outbreak of a VIM-2-producing *Pseudomonas aeruginosa*. *Endoscopy*. **2015**. 47(6), 493-502. PubMed PMID: 25826278.
12. **Voor in ‘t holt AF**, Severin JA, Lesaffre EM, Vos MC. A systematic review and meta-analyses show that carbapenem use and medical devices are the leading risk factors for carbapenem-resistant *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*. **2014**. 58(5), 2626-37. PubMed PMID: 24550343.

* These authors contributed equally to the work

PHD PORTFOLIO

Name: Anne F. Voor in 't holt
Erasmus MC department: Medical Microbiology and Infectious Diseases
Promotor: Prof.dr. Margreet C. Vos
Copromotor: Dr. Juliëtte A. Severin

PhD training	**Year**
National and international conferences and meetings	
- Annual *C. difficile* meeting, Leiden, The Netherlands	2016, 2017
- 26th ECCMID, Amsterdam, The Netherlands	2016
- 25th ECCMID, Copenhagen, Denmark	2015
- PhD day, Erasmus MC, Rotterdam, the Netherlands	2013-2014
- 23rd ECCMID, Berlin, Germany	2013
- MMIZ research day	2012, 2017
- Weekly 1 hour journal club and research meetings MMIZ	2012-2018
Presentations as first author	
- Oral presentation, regional IP meeting, Rotterdam, The Netherlands	2015, 2017
- 2 paper poster presentations 27th ECCMID, Vienna, Switzerland	2017
- Oral presentation annual *C. difficile* meeting, Leiden, The Netherlands	2016
- Paper poster presentation 26th ECCMID, Amsterdam, The Netherlands	2016
- Six-minute oral presentation 25th ECCMID, Copenhagen, Denmark	2015
- Paper poster presentation 25th ECCMID, Copenhagen, Denmark	2015
- Oral presentation MMIZ research meeting, Rotterdam, the Netherlands	2014-2018
- 2 paper poster presentations 23rd ECCMID, Berlin, Germany	2013
- Oral presentation MMIZ research day, Rotterdam, the Netherlands	2012, 2017
Presentations as a co-author	
- Paper poster presentation, Digestive Disease Week, Washington, USA	2018
- Oral presentation and paper poster presentation, 28th ECCMID, Madrid, Spain	2018
- Oral presentation, Digestive Disease Days, Veldhoven, the Netherlands	2017
- Paper poster presentation, UEGW, Barcelona, Spain	2017
- Paper poster presentation, ICPIC, Geneva, Switzerland	2017
- Oral presentation, 27th ECCMID, Vienna, Switzerland	2017
- Oral presentation, Digestive Disease Week, San Diego, USA	2016
- Paper poster presentation 26th ECCMID, Amsterdam, The Netherlands	2016
- Oral presentation 26th ECCMID, Amsterdam, The Netherlands	2016
- Oral presentation, NVGE, Dutch gastroenterology and hepatology congress	2016
- Paper poster presentation 17th ICI Diseases Hyderabad, India	2016

- Paper poster presentation ICAAC, Washington DC, USA — 2014

Supervision of students

University of applied sciences

- Supervision of a third-year Human Technology student — 2017-2018
- Supervision of 2 final-year Biology and Medical Laboratory Research students — 2014-2016
- Supervision of 2 final-year nursing students — 2013

University

- Supervision of 2 third-year medicine students — 2015-2016
- Supervision of a MSc student Health Sciences — 2015
- Supervision of 3 MSc students Infection and Immunity — 2014, 2017-2018

Courses

- The R project for statistical computing, MolMed, 1.8 ETCS — 2018
- Writing successful grant proposals, MolMed, 0.5 ECTS — 2015
- Research Integrity, Erasmus MC, 0.3 ECTS — 2014
- Biomedical English Writing and Communication, Erasmus MC, 3 ECTS — 2014
- Basic and advanced course on Microsoft Access, MolMed, 0.7 ECTS — 2014
- Advanced course on Microsoft Excel, MolMed, 0.4 ECTS — 2014

Seminars and master classes

- Medical Ethical Research Committee; European Clinical Trial Regulation — 2017
- Accessing deceased patient records in the Erasmus MC — 2016
- PREZIES workshop, national institute for public health and the environment — 2015, 2016
- Research Impact and Relevance: How to publish a world-class paper — 2014
- How to write a competitive proposal for Horizon 2020 — 2014

Other activities

- Review of a IJID, PLOS ONE, and ARIC research article — 2018
- Review of a Lancet Infectious Diseases, CMI and ARIC research article — 2017
- Review of a JHI, ARIC and CID research article — 2016
- Review of an EMI and ICHE research article — 2015
- Review of a PLOS ONE research article — 2014

Travel grants

- Vereniging Trustfonds Erasmus Universiteit Rotterdam — 2013, 2015

Research grants

- Weber Hospital Systems b.v.
  - Reduction of microorganisms in the Validated Disinfection Systems (VDS) automatic bed washer — 2018
  - Co-applicant
- Pentax Medical — 2018

- REDUCE Study – evaluation of the new Pentax ED34-i10T Video with Duodenoscope detachable distal-end cap.
- Co-applicant

- Erasmus MC 2017
- MOVE Study – do new single-occupancy rooms offer a microbial safer environment to patients compared to old multiple-occupancy rooms?
- Co-applicant

- 3M 2016
- DETECT Study - Duodenoscopes: Efficacy of ATP Tests Compared to visual inspection.
- Co-applicant

- Ministry of Health, Welfare and Sport 2016
- PROCESS Study - Prevalence of contamination of ERCP Endoscopes in the Netherlands.
- Co-applicant

- Erasmus MC Efficiency Research 'Doelmatigheid' 2015
- PRICE Study - Cost-effectiveness of the Erasmus MC infection prevention policy for highly-resistant microorganisms.
- Co-applicant