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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?

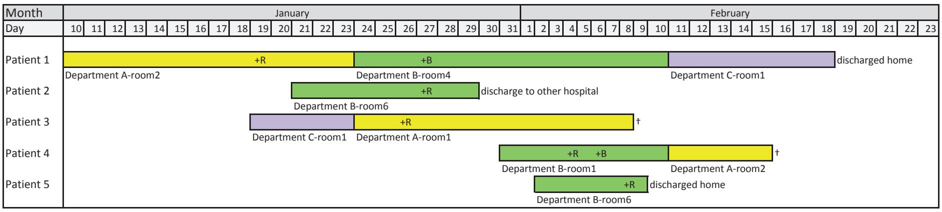

Figure 1. Epidemiological timeline of 5 individual patients. The different colors are different departments. +R; rectal swab positive for the specific microorganism, +B, blood culture positive for the specific microorganism.

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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