ANTIMICROBIAL USE AND RESISTANCE IN HOSPITALIZED PATIENTS

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ANTIMICROBIAL USE AND RESISTANCE IN HOSPITALIZED PATIENTS

Gebruik van antimicrobiële middelen en resistentie bij patiënten opgenomen in een ziekenhuis

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CHAPTER 1

General introduction

HISTORICAL PERSPECTIVE

A global rise of increasing antibiotic resistance, albeit with wide variations between countries and regions, is well documented in the literature (1). Antibiotic resistance is costly in both human and financial terms. Infection with a resistant bacterium increases the cost of health care, length of hospital stay, and mortality compared to infections with bacteria that are susceptible to common antibiotics (2-4).

Since the 1990s, concern about resistance has spread from medical specialists to health-care officials, politicians, and the public, with numerous agency and governmental reports (5). In 1997, a joint committee of the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) published the "Guidelines for the prevention of antibiotic resistance in hospitals (6). In the same year, the resistance problem was subject of a meeting of EU medical officers in Luxemburg and subsequently a EU conference on "The Microbial Threat" was organized in 1998 in Copenhagen. The results of this meeting were published in a report entitled "The Copenhagen Recommendations" (7). Accordingly, in 1999 the EU Health Council adopted a resolution concerning future Community action in terms of public health and antibiotic resistance (8). Also the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) recognized at that time the importance of launching strategies for the control of the resistance problem (9, 10).

Although the resistance problem in the Netherlands is relatively limited, antibiotic resistance is on the increase (11-13). The professional community responded in 1996 by starting the Dutch Working Party on Antibiotic Policy (Dutch acronym is SWAB), an initiative of the Dutch societies of Medical Microbiology, Infectious Diseases and of Hospital Pharmacists. The Advisory Council for Health Research (RGO) received a request for advice from the minister of Health, Welfare and Sport in 1999 concerning strategies to contain the antibiotic resistance problem. In 2000, the Council published their advice (14). This advice, and the decision made by the Minister of Health have been of great importance to the SWAB. Since 2001, the SWAB has been designated to co-ordinate the surveillance of antibiotic use and resistance in the Netherlands (15).

All the above mentioned reports vary in emphasis, but can be summarized as advocating development and implementation of systems for: 1) monitoring of antibiotic resistance; 2) monitoring of antibiotic use; 3) promoting good antibiotic practice; and 4) an effective infection control program to minimize the spread of resistance. In addition, it is recommended that the relationship between antibiotic use and resistance should be monitored and that research on different areas should be performed. Although, in aggregate the use of antibiotics is greatest in the community, much of the above mentioned activities are concentrated in hospitals where the density

of antibiotic use is much higher than in the community. Consequently, antibiotic resistance problems are greatest in hospitals. Cross colonization of resistant strains between hospitalized patients further aggravates the problem. The theme of this thesis is, therefore, antibiotic use and resistance in *hospitals*. This thesis focuses on monitoring of quantitative antibiotic use and on the epidemiology of resistant bacteria in the intestinal and oropharyngeal microflora of hospitalized patients.

SURVEILLANCE OF QUANTITATIVE ANTIBIOTIC USE IN HOSPITALS

Reliable data on the use of antibiotics in hospitals are essential for the interpretation of prescribing habits, the evaluation of compliance with clinical guidelines, and the linkage with antibiotic resistance data. Increasingly, antibiotic use is measured and compared between institutions, regions and countries (13, 16-18). It is therefore important that these data are collected, analyzed and presented in a standardized manner. Various methods are available to quantify antibiotic use. Table 1 summarizes different aspects that have to be defined before starting the collection of antibiotic use data. These factors will be discussed in more detail (19, 20).

Table 1. Key factors in designing a surveillance system for antibiotic use in hospitals

Select (classes of) antibiotics to include in the surveillance
Identify sources of valid and available data
Determine appropriate units of measurement (numerator and denominator)
Determine the frequency of data collection and reporting
Determine the detail and level of aggregation needed

Sources of data. Pharmacy purchase records are often used to estimate antibiotic consumption. Purchase records may be obtained from invoices and delivery documents. With purchase data it is possible to capture the total amount of antibiotics used in a specific institution. However, purchase data may overestimate the total use since they include antibiotics that are not be dispensed or administered to the patient. This might be the result of wastage during preparation or destruction of antibiotics that have exceeded their expiration date. Moreover, antibiotics issued from the hospital pharmacy to nursing homes and other institutions affiliated with hospitals should be excluded.

In some hospitals, antibiotic dispensing data are available. However, these data may overestimate antibiotic use since antibiotics that are dispensed to the patient may not be administered. Corrections have to be made for antibiotics that are send back from the hospital wards.

The most valid source data are to be found in patient administration records. This latter source is increasingly used now that electronic medical record systems are being introduced in health care.

Units of measurement. Various units of measurement have been used to express antibiotic consumption. These units of measurement are made up of a numerator and a denominator. As numerator, the number of grams, defined daily doses, packages, prescriptions, days on treatment or patients exposed can be used. The World Health Organization recommends to use the defined daily dose (DDD) for drug utilization studies (21). The DDD (expressed in grams) is the assumed average maintenance dose per day for a drug used for its main indication in a 70 kg adult. DDD may change over time. In 2005 for example, the DDD of parenteral administered amoxicillin with clavulanic acid has been changed from 1 to 3 grams. It is therefore important to always specify which ATC/DDD version has been used in the methodology section of studies. Antibiotic use data are also often presented in terms of financial expenditures (22, 23). Since hospitals negotiate different purchase prices, costs of antibiotics are poor yardsticks to compare antibiotic usage between hospitals and, even more so, between countries. However, data on costs of the individual antibiotics may be informative when comparing prescribing habits in different settings. Low prices may explain high usage, whereas high prices may reduce the use of specific drugs.

The WHO recommends the number of bed days for normalizing antibiotic use in hospitals. The number of bed days may be calculated by multiplying the number of admissions with the average length of stay or the number of beds multiplied by the average occupancy rate. The number of patient days may be obtained by subtracting the number of admissions from the number of bed days as the number of bed days over estimates actual treatment-days by including both the day of admission and the day of discharge. Other denominators that may be used to calculate rates of antibiotic use are the number of admissions or discharges and the number of (occupied) beds.

Frequency of data collection. According to the aim of the data collection, the frequency of data collection should be determined. In most national surveillance studies data are reported on a yearly basis (13, 16, 17). If one would like to study seasonal effects or to link the use data to resistance data, more informative timeframes are needed and data collected and analyzed on a monthly or quarterly basis are usually preferable (24).

Level of aggregation. While collecting data on antibiotic use, the antibiotics included in the data analyses should be defined clearly. The Anatomical Therapeutical Classification System (ATC) is the most commonly used classification system and is recommended by WHO (21). In the ATC system, antibiotics are divided into 14 main

categories according to the organ or system on which they act, and then according to their therapeutic, pharmacological, and chemical properties. It is a 5-level hierarchical code assigned to each chemical substance. Table 2 shows an example of this system. ATC-codes may also change over time. It is therefore important to specify the ATC/DDD version that was used in the methodology section of studies. Most studies on antibiotic use do refer the J01 group of the ATC-group. This group comprises all antibacterial agents for systemic use in humans, and represents the majority of antibiotics used in hospitals. Before starting to collect antibiotic use data, the aim of collecting antibiotic use data should be considered. If the aim is to monitor antibiotic use in the local hospital and to provide feedback to prescribers and to link the data with local resistance data, it seems best to analyze antibiotic use by discipline or unit. If one measures antibiotic use in order to compare or benchmark use with other hospitals at the regional or national level, data collection may be restricted to surveillance of use at the hospital level, or to distinguish between general wards and intensive care units.

Table 2. Example of ATC classification of ceftazidime[a]

J	General anti-infective agents for systemic use
J01	Antibacterial agents for systemic use
J01D	Other beta-lactam antibacterials
J01DD	Third-generation cephalosporins
J01DD02	Ceftazidime

[a] from the 2005 edition of the ATC/DDD system

In the past years, particular emphasis has been put on the above mentioned technical and logistic aspects regarding the collection of data on antibiotic use. According to the United States Centers for Disease Control and Prevention (CDC), epidemiological surveillance is defined as "the ongoing and systematic collection, analysis, and interpretation of health data in the process of describing and monitoring a health event. This information is used for planning, implementing, and evaluating public health interventions and programs (25). This definition implies that a surveillance program that is not used as an evaluation tool is useless and that surveillance programs should have an impact on the control and prevention of diseases under surveillance. Therefore, much more attention should be paid to the *interpretation* of antibiotic use data in order to give relevant feedback to physicians and policy makers.

EPIDEMIOLOGY OF COLONIZATION WITH ANTIBIOTIC RESISTANT BACTERIA IN PATIENTS DURING AND AFTER HOSPITALIZATION

Colonization versus infection

An important distinction in the epidemiology of antibiotic resistant bacteria should be made between infection and colonization. Colonization is the prolonged presence of a microorganism in or on a host, with growth and multiplication, but without any overt nor subclinical expression or deleterious effects on the host at the time the microorganism is isolated (26). Colonization is a normal process, an ecological event that occurs during and after birth until the normal flora is established. Thereafter, this commensal flora evolves dynamically, and changes over the lifetime of the host.

Infection, on the other hand, is characterized by damage to host tissues or systems that may or may not result in serious clinical illness, e.g. when antibiotic resistant bacteria contaminate wounds, the bloodstream or other sterile tissues and produce a systemic inflammatory response (26). The occurrence of hospital-acquired infections most often involves a three-step process: first, colonization of a patient's mucosa or skin with a potential pathogen; secondly, access of the pathogen to a site where it may invade tissues, often in association with a foreign body such as an intravascular catheter or an endotracheal tube; and third, an imbalance among the pathogen's virulence factors and the host's defense factors, which eventually results in the infection (27).

Factors that facilitate intestinal overgrowth and transmission of resistant bacteria

The prevalence of colonization with resistant bacteria within hospital settings is determined by admission and discharge rates of colonized and noncolonized patients, and on the likelihood that noncolonized patients acquire colonization with resistant strains (28). Among patients in health care settings, a variety of factors may facilitate intestinal colonization, overgrowth and subsequent transmission of resistant pathogens (Figure 1) (29).

The hands of health care workers are considered to be the major vectors of transmission of pathogens from patient to patient (29). Dissemination of pathogens from the patient's intestinal tract to environmental surfaces and patient's skin creates what has aptly been termed a "fecal veneer" in health care settings. Healthcare workers' and patients hands frequently become contaminated after contact with this veneer (30, 31). Fecal incontinence and diarrhea, as well as factors that reduce standards of hygiene contribute to the likelihood of fecal contamination (29).

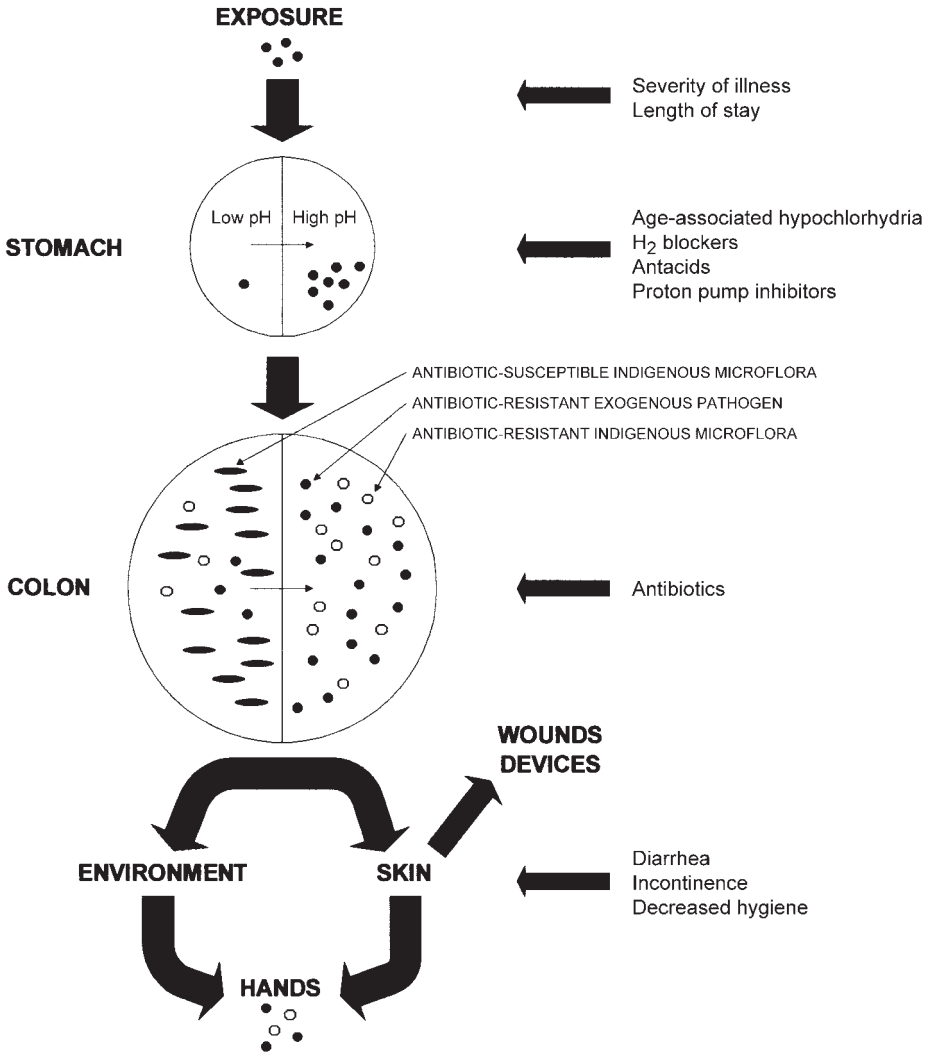

Figure 1. Factors that facilitate intestinal overgrowth and transmission of nosocomial pathogens. The left halves of the circles illustrate the presence of normal acidity in the stomach and intact indigenous microflora in the colon; the right halves illustrate the effects of increased stomach pH and antibiotic selective pressure in the colon (adapted from (29)).

Increased severity of disease and prolonged hospitalization are risk factors for acquisition of resistant pathogens since these factors result in increased opportunities for interaction with health care workers and contaminated surfaces or devices (29). Previous studies have shown that gram-negative bacteria are infrequently found in oropharyngeal cultures from normal subjects and that the prevalence of these bacteria

is strikingly increased among ill patients (32-34). Furthermore, the number of patients already colonized with resistant bacteria (= colonization pressure) in the hospital may be an important factor in determining chances of cross-colonization (35).

A low pH of the stomach reduces the number of ingested bacteria that enter the intestinal tract. Drugs that inhibit secretion of acid have been associated with an increased risk of colonization (36, 37). The association found between nasogastric tubes and/or enteral feeding may be explained by the fact that these interventions bypass or buffer the gastric acid barrier. Nasogastric tubes may also facilitate colonization of the oropharynx by bacteria that have the ability to adhere to plastic surfaces and form biofilms (38).

Gastric colonization has been assumed to be important in the pathogenesis of colonization and infection of the respiratory tract (39, 40). In critically ill patients, intragastric acidity may be reduced because of underlying illness, advanced age, or the administration of stress-ulcer prophylactic agents or enteral feeding. However, the importance of this gastropulmonary route of colonization has been questioned (41-43).

Colonization with resistant bacteria may remain undetectable within a largely susceptible microflora until, because of the selective growth advantage provided by antibiotics, bacterial outgrowth of the resistant pathogen occurs such that the detection limit of the culture method is exceeded. Poorly absorbed antibiotics can reach the colon in active form where they suppress susceptible bacteria and select pre-existing resistant bacteria. Also parenteral administered antibiotics that are secreted in the bile or from the intestinal mucosa may affect the normal intestinal microflora (44, 45). To what extent disturbances occur depends on the spectrum of the agent, the dose, the route of administration, pharmacokinetic and pharmacodynamic properties, and in-vivo inactivation of the antibiotic (45).

In addition to the selection of pre-existing resistant bacteria, resistance may also develop *de novo* in the intestinal tract. Susceptible bacteria may become resistant due to genetic mutations or through the induction or acquisition of resistance genes from other bacteria (46-48).

Reasons for assessing colonization with resistant bacteria

There are several reasons for assessing bacterial colonization with resistant strains (49). Bacterial colonization is an important step in the pathogenesis of infections. Many of the bacteria that comprise the gastro-intestinal microflora may cause infec-

tions (27, 50, 51). Oropharyngeal colonization with gram-negative bacteria plays a critical part in the pathogenesis of ventilator-associated pneumonia caused by gram-negative bacteria (41, 42, 52). The most frequent hospital infections caused by *Enterobacteriaceae* and *enterococcus* species are urinary tract infections, bloodstream infections, intra-abdominal infections, skin- and soft tissue infections and endocarditis (53, 54). Knowledge of the prevalence and degree of resistance in the fecal and oropharyngeal microflora on admission and during hospitalization may therefore contribute to an optimal choice of empirical therapy in the event of nosocomial infections (55). Bacterial colonization is of further interest since fecal bacteria might act as a reservoir for resistance determinants, plasmids, transposons and other moving genes (46-48). Infection rates often represent only the tip of an iceberg, whereas the true bacterial load is represented by colonization rates. The epidemiology of bacterial colonization is of interest as well, since the digestive tract is often the source from where resistant bacteria can spread and cause hospital epidemics.

Moreover, the dissemination of antibiotic resistant bacteria and resistance genes between hosts is not confined to a specific reservoir (Figure 2). After discharge from the hospital, patients may remain colonized with resistant bacteria acquired in the hospital and resistance may disseminate into the community, nursing homes or other institutes. Population based surveillance of colonization, with resistant *Enterobacteriaceae* and enterococci as indicator bacteria, appears to be a sound method to study the transfer of resistance between different reservoirs of resistance.

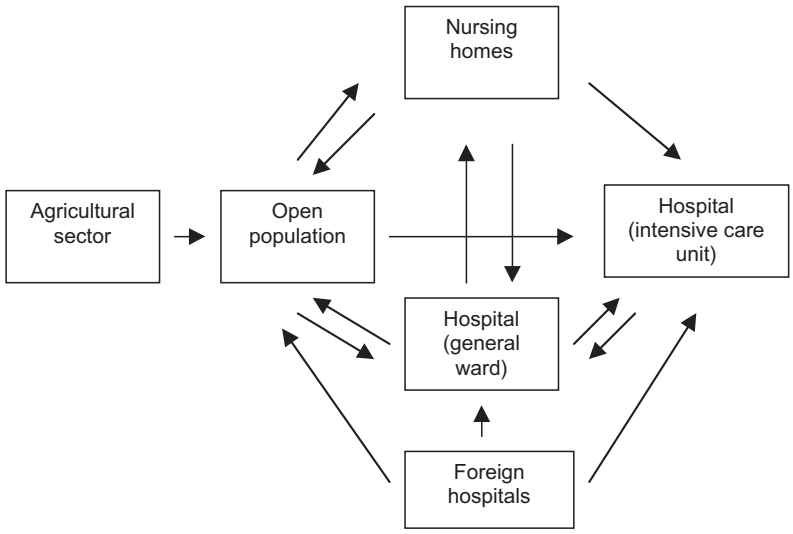

Figure 2. Interactions between different resistance compartments

AIM AND OUTLINE OF THE THESIS

The general aim of the studies in this thesis is to explore the current emergence of antibiotic resistance in hospitals. This aim is addressed in two research projects. The first project concerns the optimization of surveillance of quantitative antibiotic use in hospitals and the second project concerns the epidemiology of colonization with antibiotic resistant bacteria in patients during and after hospitalization.

The introductory chapter has illustrated that several authorities recommend to pay more attention to the monitoring of antibiotic use in hospitals, since resistance and use have been linked by a substantial amount of evidence. The question how best to measure and monitor antibiotic use is therefore further explored. In the past years, emphasis has been put in particular on technical and logistic aspects regarding the collection of data on antibiotic use. Now the following step is the interpretation of these data in order to give relevant feedback to physicians and policy makers. In **chapter 2** of this thesis, studies are described in which the interpretation of antibiotic use data is the main theme.

- **Chapter 2.1** focuses on the importance of units of measurement for a meaningful understanding of trends in antibiotic use data with regards to antibiotic resistance risks.
- In **chapter 2.2** we describe the surveillance of antibiotic use in the Netherlands in the period 1997-2002. Data are expressed in DDD per 100 bed days and in DDD per 100 admissions and hospital resource indicators are involved in the interpretation of the data.
- **Chapter 2.3** is devoted to the identification of determinants of antibiotic use in Dutch hospitals. Appropriate interventions based on surveillance data may help to contain the resistance problem. Therefore, insight is needed in hospital characteristics that predict quantitative antibiotic use and that may serve to identify hospitals with low or elevated levels of quantitative antibiotic use.

In **chapter 3** of the thesis, the epidemiology of colonization and resistance dynamics during and after hospitalization is assessed to identify risk factors for resistance emergence and to determine the relevance of transmission from the community into the hospitals, and vice versa. Colonization and resistance dynamics of *Enterobacteriaceae*, *P. aeruginosa* and *Enterococcus* species were assessed in the different studies.

- In **chapter 3.1** the impact of hospitalization on the prevalence of resistant *E. coli* in the intestinal flora of surgical patients of three Dutch university-affiliated hospitals is determined.
- **Chapter 3.2** describes the development of a method for the screening of the intestinal microflora for aerobic resistant gram-negative bacteria. This study was conducted in the course of a large epidemiological study (chapter 3.3 and 3.5).
- **Chapter 3.3** and **chapter 3.4** present the findings of studies to the colonization and resistance dynamics of aerobic gram-negative bacteria and *enterococcus* species in the intestinal and oropharyngeal microflora of patients admitted to intensive care units and general wards during and after hospitalization.
- The study described in **chapter 3.5** aims at investigating the risk factors for colonization with antibiotic resistant gram-negative bacteria during stay in the hospital.

In **chapter 4**, the main findings of the studies in this thesis are discussed and some methodological issues are considered that are relevant to several studies in these thesis. Finally, recommendations for future research are given.

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CHAPTER 2

Optimization of monitoring of quantitative antibiotic use in hospitalized patients

CHAPTER 2.1

An additional measure for quantifying antibiotic use in hospitals

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ABSTRACT

Objectives. The number of Defined Daily Doses (DDD) per 100 patient days is often used as an indicator for the selection pressure exerted by antibiotics in the hospital setting. However, this unit of measurement does not fully describe the selection pressure and is sensitive to changes in hospital resource indicators. Additional information is required to facilitate interpretation of this indicator. The number of DDD per 100 admissions could be a valuable additional tool. The aim of this study is to investigate the importance of units of measurement in quantifying antibiotic use data with regards to antibiotic resistance risks.

Methods. Trends in antibiotic use in acute care Dutch hospitals between 1997-2001 were studied. Antibiotic use was expressed in DDD per 100 patient days and in DDD per 100 admissions.

Results. From 1997 to 2001, total systemic antibiotic use significantly increased from 47.2 to 54.7 DDD per 100 patient days whereas expressed in DDD per 100 admissions it remained constant. Some individual antibiotics increases in DDD per 100 patient days were not accompanied by increases in DDD per 100 admissions and vice versa. The mean number of total DDD per hospital decreased (not significantly) between 1997 and 2001. The mean number of patient days, admissions and length of stay decreased significantly.

Conclusions. Knowledge of variation in resource indicators and additional expression of the data in DDD per 100 admissions is imperative for a meaningful understanding of observed trends in antibiotic use expressed in DDD per 100 patient days. Further research is needed to determine the correlation between different measures of antibiotic use and the level of antibiotic resistance.

INTRODUCTION

The increasing prevalence of antibiotic-resistant bacteria poses a major threat to the health of hospitalized patients (1). The relationship between emergence of resistance and antibiotic use and misuse is well recognized. It is evident that antibiotics affect not only the micro-organism and the individual patient, but also the population as a whole (2). At the hospital population level three factors are important with respect to the selection pressure exerted by antibiotics (3). First, the total amount of an antibiotic used in a particular geographical area (i.e. the entire hospital or a ward or unit) over a certain period of time. Secondly, the number of patients treated with the antibiotic (because they serve as the major "sources" of resistant bacteria). Thirdly, the density of these patients, i.e. the proportion of patients on antibiotics in the hospital. Together these factors represent the selection density in the hospital environment (3). As the selection density increases, the number of resistant strains in the hospital environment increases and the number of susceptible strains able to survive in this environment decreases (3). This may facilitate the spread of resistant bacteria and resistance genes. Antibiotics may also exert their selective pressure after treatment, as antibiotics may affect the microbial community as long as they remain intact and at growth inhibitory levels (3).

The World Health Organization (WHO) Collaborating Centre for Drug Statistics and Methodology recommends using the number of defined daily doses (DDD) per 100 patient days to quantify antibiotic use (4). The DDD is a technical unit of measurement and corresponds to the assumed average maintenance dose per day, for the main indication of the drug, in adults. The number of DDD per 100 patient days has been used as a proxy for the selection density and is an indicator for the selection pressure exerted by antibiotic use in the hospital setting. However, this measure does not fully describe the actual selection density, since it does not provide information on the number and proportion of patients actually exposed to antibiotics.

Over the last decade several national surveillance systems on antibiotic use and/or resistance have been set up (5-8). Critical assessment of the units of measurement used to quantify antibiotic use and discussions about the interpretation of these units are, however, rarely presented in the scientific literature (9-11). Most of the surveillance systems use the number of DDD per 100 patient days to compare consumption rates over time and between hospitals, geographical regions and countries. In our view, conclusions drawn from these surveillance systems should be interpreted with care. The number of DDD per 100 patient days does not fully address the selection density and is sensitive to changes in hospital resource indicators over time. Additional information is required to facilitate interpretation. The number of DDD per

100 admissions could be a valuable additional unit of measurement. The aim of this study is to investigate the importance of units of measurement in presenting antibiotic use data with regards to antibiotic resistance risks. We therefore compared and analyzed trends in the use of antibiotics in Dutch hospitals between 1997 and 2001 expressed in both DDD per 100 patient days and in DDD per 100 admissions.

METHODS

Population

Data on the use of antibiotics in acute care Dutch hospitals between 1997-2001 were collected by means of a questionnaire distributed to Dutch hospital pharmacies by the Working Party on Antibiotic Policy (SWAB) (for source data see NethMap 2003 on-line at www.swab.nl). Pharmacies were requested to report on the annual consumption of antibiotics for systemic use, as defined by group J01 of the Anatomical Therapeutic Chemical (ATC) Classification system for the classification of drugs. Outpatient use and dispensing of antibiotics to nursing homes were excluded. For each hospital the annual number of admissions and days spent in the hospital (bed days) were recorded. The number of bed days was calculated by multiplying the number of admissions with the average length of stay or the number of beds multiplied by the average occupancy rate; the choice between these methods was dependent on the preference of the individual hospital administrations.

Analysis

The ATC/DDD classification from the World Health Organization (WHO), version 2002, was used to calculate the number of DDD of the various antibiotics (4). The number of patient days was obtained by subtracting the number of admissions from the number of bed days as the number of bed-days overestimates actual treatment days by including both the day of admission and the day of discharge. For the period 1997-2001 an overall pooled mean (i.e. weighted mean) was calculated for each year by aggregating data on antibiotic use, patient days and admissions from all hospitals. The use of antibiotics was expressed in DDD per 100 patient days and in DDD per 100 admissions. Trends in antibiotic use and hospital resource indicators were studied by a mixed model for repeated measurements with the hospitals as cofactor. *P* values < 5% were considered statistically significant. All statistical analyses were performed using SAS 8.2 (SAS Institute, Cary, NC, USA).

RESULTS

In 1997 the total systemic use of antibiotics in Dutch hospitals was 47.2 DDD per 100 patient days, and use significantly increased to 54.7 DDD per 100 patient days in 2001 ($p < 0.001$) (Table 1). However, total systemic use expressed as DDD per 100 admissions remained constant (Table 1). The mean number of total DDD per hospital decreased not significantly from 67176 to 59129 (-12%).

In addition, varying trends in antibiotic use were revealed by the two units of measurement for some subgroups of antibiotics and also for individual agents. For example, the use of ß-lactamase-sensitive penicillins, cephalosporins and macrolides increased significantly when expressed in DDD per 100 patient days, but not when expressed in DDD per 100 admissions; for penicillins with an extended spectrum and trimethoprim-sulphamethoxazole, a decrease was found when expressed in DDD per 100 admissions, but not per 100 patient days.

The use of penicillins in combination with ß-lactamase inhibitors, co-amoxiclav and piperacillin-tazobactam, increased significantly when expressed in DDD per 100 patient days. However, this increase was observed for piperacillin-tazobactam ($p = 0.003$) when only admissions were used as the criterion (data not shown).

The use of lincosamides and fluoroquinolones expressed in both DDD per 100 patient days and in DDD per 100 admissions increased significantly. This increased use was due to significant increases in the use of clindamycin ($p < 0.001$) and ciprofloxacin ($p < 0.001$), respectively (data not shown).

Between 1997 and 2001 changes in hospital resource indicators were observed. The mean number of patient days per hospital decreased significantly from 142339 to 108128 (-24%: $p < 0.001$) and the mean number of admissions significantly decreased from 17405 to 15677 (-10%, $p = 0.02$). The mean length of stay decreased significantly from 8.2 to 6.9 days (-16%, $p < 0.001$).

DISCUSSION

The manner in which antibiotic usage is expressed does matter. Proper expression of antibiotic use is needed for the interpretation of prescribing habits, the evaluation of compliance with clinical guidelines and the linkage with antibiotic resistance data. The DDD system provides a convenient tool for the quantification of antibiotic use and allows comparisons between different settings, regions, or even countries. Different units of measurement can be used as denominator, depending on the questions posed. If antibiotic resistance development is the issue then the measure of antibiotic use should be a reflection of the antibiotic selection pressure exerted.

Table 1. Use of antibiotics for systemic use (J01) in Dutch hospitals between 1997 and 2001 expressed in DDD per 100 patient days (DAY) and in DDD per 100 admissions (ADM)

	Year											
	1997		1998		1999		2000		2001		Trend 1997 - 2001	
Class of antibiotic (ATC group)	DAY	ADM	DAY	ADM	DAY	ADM	DAY	ADM	DAY	ADM	P value DAY	P value ADM
Tetracyclines (J01A)	1.6	13.4	1.6	13.2	1.7	12.8	1.6	12.2	1.6	11.2	0.996	0.514
Penicillins with extended spectrum (J01CA)	6.5	53.1	6.5	52.1	6.4	49.5	6.0	45.8	6.1	41.8	0.229	<0.001
Beta-lactamase-sensitive penicillins (J01CE)	1.2	9.4	1.0	8.4	1.1	8.2	1.1	8.5	1.4	9.4	0.003	0.0885
Beta-lactamase-resistant penicillins (J01CF)	4.1	33.6	3.8	30.4	3.9	30.0	4.4	33.8	4.3	30.0	0.110	0.241
Combinations of penicillins, incl. beta-lactamase-inhibitors (J01CR)	14.4	117.6	14.3	115.3	15.6	121.5	16.9	128.7	18.0	124.5	<0.001	0.290
Cephalosporins and related substances (J01DA)	5.1	41.9	5.5	44.4	5.6	43.3	5.9	44.6	6.1	42.3	<0.001	0.436
Carbapenems (J01DH)	0.43	3.5	0.38	3.0	0.33	2.5	0.44	3.3	0.35	2.4	0.398	0.722
Trimethoprim and derivatives (J01EA)	0.46	3.7	0.51	4.1	0.50	3.9	0.35	2.7	0.51	3.5	0.294	0.749
Combinations of sulfonamides and trimethoprim (J01EE)	2.6	21.1	2.6	20.6	2.5	19.1	2.4	17.9	2.3	15.6	0.062	<0.001
Macrolides (J01FA)	1.9	15.4	1.9	15.5	2.2	17.2	2.1	16.2	2.3	15.6	<0.001	0.265
Lincosamides (J01FF)	0.80	6.6	0.88	7.1	1.1	8.3	1.2	9.2	1.3	9.1	<0.001	<0.001
Aminoglycosides (J01GB)	2.0	16.0	2.1	16.9	2.0	15.8	2.2	16.6	2.0	14.0	0.214	0.766
Fluoroquinolones (J01MA)	4.0	32.7	4.4	35.3	5.0	38.9	4.9	37.2	5.5	38.0	<0.001	<0.001
Glycopeptides (J01XA)	0.42	3.4	0.42	3.4	0.44	3.4	0.51	3.9	0.46	3.2	<0.001	<0.001
Total antibiotics for systemic use (J01)	47.2	385.9	47.7	384.6	50.0	389.0	52.1	396.1	54.7	377.2	<0.001	0.838

At the population level the selection pressure is thought to depend on the volume of antibiotics used in a particular geographical area, the number of patients exposed and the proportion of patients treated with antibiotics (3). The denominator should thus preferably include information on all these factors.

In the present study, data on antibiotic use in Dutch hospitals between 1997 and 2001 were expressed using two different units of measurement, DDD per 100 patient days and DDD per 100 admissions. From our data it is evident that trends over time in DDD per 100 patient days did not always correlate with trends in DDD per 100 admissions. Differences in trends between the two units of measurement seem to be the result of changes in resource indicators over time. We measured a 24% decrease in the mean number of patient days per hospital. The mean number of admissions also decreased, but to a lesser extent (-10%). The mean length of stay decreased with 16%. The mean number of total DDD of antibiotics used also decreased (-12%). Taken these findings together we can easily understand the differences found when total use was expressed in DDD per 100 patient days (+16%) and in DDD per 100 admissions (-2%). Small discrepancies seem to be the result of the use of pooled and geometric means.

Without further information, an increase in DDD per 100 patient days might be interpreted as an actual increased use per patient. However, the number of DDD per 100 admissions remained constant. From our data we can only conclude that on average patients used the same number of DDD and were admitted to the hospital for a shorter period of time. This resulted in an intensification of antibiotic therapy per patient day.

An increase in the number of DDD per 100 patient days is often interpreted as worrisome with regards to the potential of antibiotic resistance development. However, in the Dutch situation, a constant use per patient combined with a significant decrease in the number of admissions are indicative for a lowering of the selection pressure exerted by antibiotic use over the years. Moreover, an intensification of antibiotic therapy per patient day suggests a shortening of duration of antibiotic treatment. Short duration of therapy may lead to less selection of resistant micro organisms (12).

It appears that the number of DDD per 100 patient days can only be used as a reliable and robust monitor of the selection density over time or between geographical areas when relevant hospital resource indicators remain constant. Furthermore, neither unit of measurement fully represents the selection density. Neither DDD per 100 patient days nor DDD per 100 admissions indicates the number of patients exposed or the proportion of patients on antibiotics. It is arguable that the selection density does not best represent selection pressure or predict resistance development in a given geographical setting. For example, the number of exposed individual

commensal microflora might best express selection pressure. However there is a lack of studies to determine the correlation between different measures of antibiotic use and the level of antibiotic resistance.

In conclusion, the data presented in this article showed that to understand trends in antibiotic use over time or between hospitals or countries, data should not only be presented in DDD per 100 patient days. Knowledge of variation in resource indicators and additional expression of the data in DDD per 100 admissions are imperative for a meaningful understanding of observed trends in antibiotic use expressed in DDD per 100 patient days. Further research is needed to determine the correlation between different measures of antibiotic use and the level of antibiotic resistance.

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CHAPTER 2.2

Changes in antibiotic use in Dutch hospitals over a 6-year period: 1997-2002

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ABSTRACT

Objectives. To analyze trends in antibiotic use in Dutch hospitals over the period 1997-2002.

Methods. Data on the use of antibiotics and hospital resource indicators were obtained by distributing a questionnaire to all Dutch hospital pharmacies. Antibiotic use was expressed as the number of defined daily doses (DDD) per 100 patient days and as DDD per 100 admissions.

Results. Between 1997 and 2002, mean length of stay decreased by 18%. The mean number of admissions remained almost constant. Total antibiotic use significantly increased by 24%, from 47.2 in 1997 to 58.5 DDD per 100 patient days in 2002 ($p <$ 0.001), whereas expressed as DDD per 100 admissions it remained constant. Antibiotic use varied largely between the hospitals. Moreover, the mean number of DDD per hospital of amoxicillin with clavulanic acid, clarithromycin, cefazolin, clindamycin and ciprofloxacin increased with 16%, 38%, 39%, 50% and 52%, respectively. Total antibiotic use was higher in university hospitals than in general hospitals.

Conclusions. Between 1997 and 2002 patients hospitalized in the Netherlands did not receive more antibiotics but, since they remained in the hospital for fewer days, the number of DDD per 100 patient days increased. For macrolides, lincosamides and fluoroquinolones increases in both DDD per 100 patient days and in DDD per 100 admissions were observed. It is arguable whether these trends result in an increase in selection pressure towards resistance in the hospitals. Continuous surveillance of antibiotic use and resistance is warranted to maintain efficacy and safety of antibiotic treatment.

INTRODUCTION

The increasing prevalence of antibiotic resistant micro-organisms poses a major threat to the health of hospitalized patients (1, 2). Its relationship with antibiotic use and misuse is well recognized. Specific criteria for appropriate use of antibiotics in order to avoid resistance should therefore be developed (3). Quantitative and qualitative data on the use of antibiotics in hospitals are needed to evaluate strategies that are implemented to contain antimicrobial resistance. Obviously, resistance rates also need to be measured.

In Sweden, Denmark and the Netherlands yearly reports are issued in which resistance rates and antibiotic use data are reported (4-6). In the Netherlands, Janknegt et al. collected data on the use of antibiotics in Dutch hospitals during the period 1991-1996 (7). In 1996 the Working Party on Antibiotic Policy (acronym is SWAB; www.swab.nl) was founded by the Dutch Society for Medical Microbiology (NVMM), the Society for Infectious Diseases (VIZ) and the Dutch Association of Hospital Pharmacists (NVZA). Main activities of the SWAB are development of guidelines and educational programs to promote appropriate use of antibiotics and the surveillance of antibiotic use and resistance. These activities are supported with a structural grant by the Ministry of Health, Welfare and Sport of the Netherlands. In 2000 SWAB's working group on the use of antimicrobial agents started with the collection of national data on antibiotic use in hospitals. These data are presented in NethMap, the annual report of the SWAB (6).

In a recent editorial of this journal it was stated that physicians would not directly benefit from these national reports in their daily practice, but that these reports may help to increase their general awareness of the problem of antibiotic resistance (8). Furthermore these reports may provide a knowledge base for policy decisions, guidelines and research strategies.

The aim of this study was therefore to analyze and report on antibiotic use in Dutch hospitals between 1997 and 2002.

METHODS

Population

All Dutch hospitals, 94 general hospitals and 8 university hospitals, were approached to participate in the national surveillance system of the SWAB. Specialized hospitals, such as psychiatric and orthopedic hospitals as well as rehabilitation centers were excluded. Data on the use of antibiotics in acute care Dutch hospitals between 1997 and 2002 were collected by means of a questionnaire distributed to all Dutch hospital pharmacies by the SWAB. Data from inpatient wards as well as day care wards

had to be included, whereas outpatient use and dispensing to nursing homes had to be excluded from the data report.

Antibiotic use

Pharmacies were requested to report on the annual consumption of antibiotics for systemic use, group J01 of the Anatomical Chemical Classification (ATC) system. The use of different (sub) classes of antibiotics was expressed as defined daily doses (DDD) per one hundred patient days and per one hundred admissions (9).

The ATC/DDD classification from the World Health Organization (WHO), version 2002, was used to calculate the number of DDD of the various antibiotics. The DDD is defined as the assumed average maintenance dose per day for a drug used for its main indication in an adult (10).

Hospital resource data

For each hospital the annual number of admissions and days spent in the hospital (bed days) were recorded. The number of patient days was obtained by subtracting the number of admissions from the number of bed days as the number of bed days over estimates actual treatment days by including both the day of admission and the day of discharge. The mean length of stay was calculated by dividing the mean number of patient days by the mean number of admissions.

Statistical analysis

Regarding the period 1997-2002 an overall pooled mean (i.e. weighted mean) was calculated for each year by aggregating data on antibiotic use and patient days of all hospitals. Drug utilization was compared between hospitals and over time by a mixed model for repeated measurements. The response variables applied were the number of DDD per 100 patient days and the number of DDD per 100 admissions. P values < 0.05 were considered statistically significant. All statistical analyses were performed by SAS 8.2 (SAS Institute, Cary, NC, USA)

RESULTS

Hospital resource indicators

Between 1997 and 2002 a decrease in the mean length of stay was found in both the total cohort of hospitals and the subgroups of university and general hospitals (Table 1). The mean number of admissions remained almost constant. As the mean

number of patient days is calculated by multiplying the mean number of admissions with the mean length of stay, a decrease was also found in the mean number of patient days.

Hospital use

The number of hospitals that issued data on antibiotic use varied from 49 (48%) in 1997 to 59 (58%) in 2002. The reasons given for not participating were other priorities (56%), not able to generate data on antibiotic use (25%) or no interest (19%).

In 1997 total systemic use in hospitals was 47.2 DDD per 100 patient days and significantly increased by 24% to 58.5 DDD per 100 patient days in 2002 ($p < 0.001$) (Table 2). However, total systemic use expressed as DDD per 100 admissions remained almost constant at 385.9 in 1997 and 391.6 in 2002 ($p = 0.866$) (Table 3). The mean number of total DDD per hospital did not change between 1997 and 2002 (67176 and 66714 DDD in 1997 and 2002 respectively).

Regarding trends in antibiotic use over the years five main categories can be distinguished:

1. For macrolides, lincosamides and fluoroquinolones we found a significant increase over the years for both units of measurement;
2. for amphenicols and monobactams a significant decrease in both units of measurement was found;
3. for tetracyclines, ß-lactamase-resistant penicillins, carbapenems, trimethoprim and derivatives, intermediate-acting sulfonamides, aminoglycosides and imidazole derivatives a constant use in both units of measurement was found;
4. for total systemic use, combinations of penicillins including ß-lactamase inhibitors, ß-lactamase-sensitive penicillins, cephalosporins and glycopeptides a significant increase in DDD per 100 patient days and a constant use in DDD per 100 admissions has been

Table 1. Resource indicators of Dutch hospitals, 1997-2002

	Hospitals		Admissions			Patient days			Length of stay		
	1997 (n)	2002 (n)	1997 (mean)	2002 (mean)	% change 1997-2002	1997 (mean)	2002 (mean)	% change 1997-2002	1997 (mean)	2002 (mean)	% change 1997-2002
All hospitals	49	59	17405	17038	-2.1	142339	114038	-19.9	8.2	6.7	-18.3
University hospitals	8	7	25670	24441	-4.8	226264	191374	-15.4	8.8	7.8	-11.4
General hospitals	41	52	15793	16041	+1.6	125963	103628	-17.7	8.0	6.5	-18.9

Table 2. Antibiotic use in Dutch hospitals (DDD per 100 patient days), 1997-2002

ATC code	Antimicrobial group	Relevant example antibiotic(s)	DDD per 100 patient days			Average change per year (%)	Trend 1997-2002 (*P* value[a])
			1997	2002	Absolute change 1997-2002		
J01AA	Tetracyclines	Doyxcycline	1.6	1.6	0.00	0.071	0.933
J01BA	Amphenicols	Chloramphenicol	0.017	0.0039	0.00	-62.1[b]	0.007
J01CA	Penicillins with extended spectrum	Amoxicillin	6.5	6.2	-0.34	-1.1	0.212
J01CE	Beta-lactamase-sensitive penicillins	Benzylpenicillin	1.2	1.2	0.082	1.4	0.004
J01CF	Beta-lactamase-resistant penicillins	Flucloxacillin	4.1	4.5	0.36	1.7	0.116
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	Amoxicillin with clavulanic acid, piperacillin with tazobactam	14.4	20.6	6.2	7.4	<0.001
J01DA	Cephalosporins and related substances	Cefazolin, cefuroxim, ceftazidim	5.1	6.3	1.1	4.0	<0.001
J01DF	Monobactams	Aztreonam	0.011	0.0021	-0.009	-27.7[b]	0.018
J01DH	Carbapenems	Imipenem, meropenem	0.43	0.46	0.034	1.6	0.246
J01EA	Trimethoprim and derivatives	Trimethoprim	0.46	0.48	0.021	0.90	0.353
J01EC	Intermediate-acting sulfonamides	Sulfadiazine	0.061	0.00013	-0.061	-70.8[b]	0.229
J01EE	Combinations of sulfonamides and trimethoprim	Sulfamethoxazole with trimethoprim	2.6	2.4	-0.22	-1.7	0.0715
J01FA	Macrolides	Clarithromycin	1.9	2.7	0.77	7.1	<0.001
J01FF	Lincosamides	Clindamycin	0.80	1.5	0.67	12.9	<0.001
J01GB	Aminoglycosides	Gentamycin, tobramycin	2.0	2.1	0.13	1.3	0.334
J01MA	Fluoroquinolones	Ciprofloxacin	4.0	5.7	1.7	7.3	<0.001
J01MB	Other quinolones	Pipemidic acid	0.030	0.077	0.046	20.4[b]	-[c]
J01XA	Glycopeptides	Vancomycin	0.42	0.51	0.092	4.1	<0.001
J01XD	Imidazole derivatives	Metronidazole	1.2	1.4	0.26	4.1	0.622
J01XE	Nitrofuran derivatives	Nitrofurantoin	0.21	0.52	0.31	20.4[b]	-[c]
J01	Antibiotics for systemic use (total)		47.2	58.5	11.3	4.4	<0.001

[a] *P* value < 0.05 = statistically significant

[b] due to the low absolute use of these antibiotics the average change per year is of little practical importance

[c] not able to calculate ***P*** value due to small number of observations

Table 3. Antibiotic use in Dutch hospitals (DDD per 100 admissions), 1997-2002

ATC code	Antimicrobial group	Relevant example antibiotic(s)	DDD per 100 admissions			Average change per year (%)	Trend 1997-2002 (*P* value)[a]
			1997	2002	Absolute change 1997-2002		
J01AA	Tetracyclines	Doyxcycline	13.4	11.0	-2.4	-3.9	0.482
J01BA	Amphenicols	Chloramphenicol	0.14	0.03	-0.1	-28.1[b]	0.001
J01CA	Penicillins with extended spectrum	Amoxicillin	53.1	40.1	-13.0	-5.4	<0.001
J01CE	Beta-lactamase-sensitive penicillins	Benzylpenicillin	9.4	8.0	-1.4	-3.2	0.080
J01CF	Beta-lactamase-resistant penicillins	Flucloxacillin	33.6	28.9	-4.7	-2.9	0.265
J01CR	Combinations of penicillins, incl. beta-lactamase-inhibitors	Amoxicillin with clavulanic acid, piperacillin with tazobactam	117.6	135.5	17.9	2.9	0.159
J01DA	Cephalosporins and related substances	Cefazolin, cefuroxim, ceftazidim	41.9	41.8	-0.1	-0.05	0.415
J01DF	Monobactams	Aztreonam	0.09	0.01	-0.07	-30.5[b]	0.007
J01DH	Carbapenems	Imipenem, meropenem	3.5	3.1	-0.4	-2.4	0.754
J01EA	Trimethoprim and derivatives	Trimethoprim	3.7	3.2	-0.5	-3.1	0.902
J01EC	Intermediate-acting sulfonamides	Sulfadiazine	0.5	0.00087	-0.5	-71.9[b]	0.268
J01EE	Combinations of sulfonamides and trimethoprim	Sulfamethoxazole with trimethoprim	21.1	15.9	-5.3	-5.6	<0.001
J01FA	Macrolides	Clarithromycin	15.4	17.8	2.4	2.9	0.012
J01FF	Lincosamides	Clindamycin	6.6	9.8	3.3	8.5	<0.001
J01GB	Aminoglycosides	Tobramycin	16.0	13.9	-2.0	-2.7	0.458
J01MA	Fluoroquinolones	Ciprofloxacin	32.7	38.0	5.3	3.1	<0.001
J01MB	Other quinolones	Pipemidic acid	0.25	0.51	0.3	15.7[b]	-[c]
J01XA	Glycopeptides	Vancomycin	3.4	3.4	0.0	-0.01	0.026
J01XD	Imidazole derivatives	Metronidazole	9.6	9.5	-0.01	-0.01	0.458
J01XE	Nitrofuran derivatives	Nitrofurantoin	1.7	3.5	1.8	15.7[b]	-[c]
J01	Antibiotics for systemic use (total)		385.9	391.6	5.6	0.3	0.866

[a] *P* value < 0.05 = statistically significant

[b] due to the low absolute use of these antibiotics the average change per year is of little practical importance

[c] not able to calculate *P* value due to small number of observations

observed;

5. for penicillins with extended spectrum and combinations of sulfonamides and trimethoprim we found a constant use when expressed in DDD per 100 patient days and a significant decrease in the number of DDD per 100 admissions.

The proportion of all penicillins combined represented 55% of total systemic use in both 1997 and 2002. In an in-depth study of the individual antibiotics we found that the mean number of DDD per hospital of amoxicillin-clavulanic acid, clarithromycin, cefazolin, clindamycin and ciprofloxacin increased with 16%, 38%, 39%, 50% and 52%, respectively.

In university hospitals, total systemic antibiotic use increased significantly by 16.5% (from 57.6 in 1997 to 67.1 DDD per 100 patient days in 2002 (p = 0.002)), whereas in general hospitals total use increased significantly by 29.4% (from 43.6 in 1997 to 56.4 DDD per 100 patient days in 2002 ($p < 0.001$)). However, total systemic antibiotic use expressed as DDD per 100 admissions in university hospitals remained almost constant at 507.4 in 1997 and 525.2 in 2002. In general hospitals no increase was found when use was expressed as DDD per 100 admissions as well: 347.4 in 1997 and 364.2 in 2002. In university hospitals the mean number of DDD per hospital decreased by 1.5%, whereas in general hospitals an increase of 6.5% has been observed.

Moreover, a large variation in quantitative antibiotic use was found between the participating hospitals, in particular in general hospitals (Figure 1).

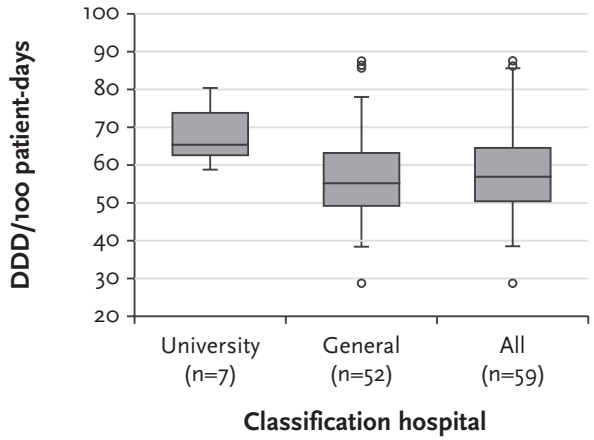

Figure 1. Variance in total use of antibiotics for systemic use (J01) in Dutch hospitals, 2002: university versus general hospitals.

DISCUSSION

Our data showed a decrease in the mean length of stay during the study period and a more or less constant mean number of admissions. These trends in hospital resource indicators are consistent with the demographics of all hospitals as registered by Statistics Netherlands (http://www.cbs.nl). In addition, we found that trends over time in DDD per 100 patient days did not consistently correlate with trends in DDD per 100 admissions.

In the present study total antibiotic use significantly increased by 24%, from 47.2 in 1997 to 58.5 DDD per 100 patient days in 2002. The total number of DDD and admissions remained almost constant between 1997 and 2002. However, length of stay decreased significantly during this period. This means that on average patients used the same number of DDD in a shorter period of time, which might be interpreted in different ways. Firstly, no changes in treatment policies occurred since most patients were already treated with antibiotics during the first days of hospitalization. Due to intensification of general care length of stay decreased. Another explanation might be that antibiotic courses are completed at home with antibiotics supplied by the hospital.

Between 1991 and 1996 total antibiotic use in Dutch hospitals increased by 14% from 37.2 to 42.5 DDD per 100 patient days in 1996 (7). This might also be the result of a decreasing length of stay over the years (12%) rather than an increase in DDD per admission. The first results of an European surveillance program demonstrated that the Nordic countries and the Netherlands all show a low total antibiotic use compared with other European countries (11).

In both university and general hospitals we found a constant use in DDD per 100 admissions and an increase in DDD per 100 patient days as well. Total systemic antibiotic use was notably higher in university hospitals than in general hospitals. This might be explained by the admission of patients with more complex infections or undergoing complex surgery and transplantation's requiring prophylaxis (12).

In the total cohort of hospitals the mean number of DDD per hospital of amoxicillin-clavulanic acid, clarithromycin, cefazolin, clindamycin and ciprofloxacin increased with 16%, 38%, 39%, 50% and 52%, respectively. As the number of admissions remained almost constant over the years this means an increase in the consumption of these antibiotics per admission. The increase in the use of cefazolin, an agent that is merely used for surgical prophylaxis may be explained by the publication of a national guideline on surgical antibiotic prophylaxis in 2000. This guideline strongly recommends the use of cefazolin for surgical prophylaxis. In our cohort of hospitals the percentage of hospitals using cefazolin increased from 37% in 1997 to 69% in 2002 (P = 0.001). It is not clear why the use of the other antibiotics is increasing.

Audits on antibiotic prescribing practices at the individual patient level are needed to clarify the increasing use of these antibiotics.

We distinguished five categories concerning trends in antibiotic use over the years. With regard to resistance development it appears that an increase in both the number of DDD per 100 patient days and the number of DDD per 100 admissions (category 1) is worrisome and that no significant change or a significant decrease in both units of measurement (category 2, 3 and 5) are not worrisome. The trend in category 4 is less easy to interpret. An increase in the number of DDD per 100 patient days may be interpreted as an increase in the selection pressure towards resistance. However, this is arguable since the number of admissions and the total number of DDD remained almost constant over the years. Moreover, an intensification of antibiotic therapy suggests a shortening of duration of antibiotic treatment. Short duration of therapy may lead to less selection of resistant microorganisms (13, 14).

In the present study some methodological problems were encountered. Firstly, one possible source of bias was the variety of methods used by the different Dutch hospital pharmacies to quantify their antibiotic use. The majority of hospitals delivered data based on hospital purchases, only a few hospitals provided actual dispensing data. Ideally, one would prefer actual administration data as a source to measure antibiotic use in hospitals, with every dose actually administered to a patient electronically.

Secondly, we aimed to provide census data, covering at least 90% of the acute care hospital population in the Netherlands. The overall response to the inquiry was however 58%. In contrast with for example Denmark, the Dutch government does not make it compulsory for hospitals to deliver their data on the use of antibiotics (15). Consequently aiming at 90% coverage will be unrealistic. Since the variance in antibiotic use is very large between the hospitals, a representative selection of hospitals is only possible when insight is obtained in the determinants of hospital antibiotic use.

Another possible source of bias may be that as a result of earlier discharge of the less sick patients, patient days may increasingly originate from sicker patients who more often require antibiotic treatment. However, this is not likely, as the total number of DDD remained constant.

In this survey, data was collected by a questionnaire and processed manually, which is a relatively slow process. In the near future the SWAB wishes to start a national project in order to collect data on hospital drug use in a central data warehouse. This will facilitate the collection of data and the conversion to DDD per 100 patient days.

Data on the use of antibiotics at hospital level might be too crude for identifying subtle trends in antibiotic use of specific patient populations. Therefore, monitoring

antibiotic use patterns by specific populations within the hospital (e.g. intensive care and general ward patients; surgical and non-surgical patients) is warranted. In this way substantial changes can be demonstrated that would be overlooked if hospital-wide data is aggregated into national trends.

In conclusion, patients hospitalized in the Netherlands did not receive more antibiotics but, since they remained in the hospital for fewer days, the number of DDD per 100 patient days increased. It is arguable whether this results in an increase in selection pressure towards resistance in the hospitals, since the total number of DDD remained almost constant over the years. For macrolides, lincosamides and fluoroquinolones increases in both DDD per 100 patient days and DDD per 100 admissions were observed between 1997 and 2002. This might be worrisome since this trend is more likely to be associated with an increase in the selection pressure. Further research is needed to determine the relationship between antibiotic use, selection pressure and the emergence of resistance. To maintain efficacy and safety of antibiotic treatment, continuous surveillance of antibiotic use and resistance is necessary.

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CHAPTER 2.3

Determinants of quantitative antibiotic use in hospitals

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ABSTRACT

Objectives. Surveillance of hospital antibiotic use at the national level serves as an early warning system for the emergence of resistance. Appropriate interventions based on these data may help to contain the resistance problem. Insight is therefore needed into hospital characteristics that predict quantitative antibiotic use. These characteristics may serve to identify hospitals with low or elevated levels of quantitative antibiotic use. The aim of this study is to assess determinants of antibiotic use in 43 Dutch hospitals.

Methods. A multivariate backward stepwise linear regression model was performed with total systemic use of antibiotics as outcome variable and institutional, population and care providers' characteristics as potential explanatory variables.

Results. Total systemic use in 43 Dutch hospitals varied largely (median = 45.6, range 26.1 - 65.9 DDD per 100 patient days). In the multivariate analysis the number of pharmacists, microbiologists and infectious disease physicians per 100,000 patient days were significant determinants of quantitative antibiotic use (R^2 = 0.40).

Conclusions. Our study indicates that 40% of the variation in quantitative antibiotic use between hospitals can be explained by the relative number of hospital pharmacists, medical microbiologists and infectious disease physicians. This result is understandable considering that the relative number of such specialists in a hospital strongly correlates with the hospital's case mix of patients regarding infectious diseases. It is desirable to extend studies like this to further delineate the determinants that explain variability in antibiotic use.

INTRODUCTION

Surveillance of hospital antibiotic use at the national level serves as an early warning system for the emergence of resistance (1, 2). Appropriate interventions based on these data may help to contain the resistance problem. An important tool to encourage participation in such a surveillance program is to give feedback to the individual hospitals on the level of antibiotic use in their own hospital. It is however complex to provide the individual hospitals with appropriate information. The national surveillance system may report data on total systemic use of antibiotics in the individual hospital and compare this with the median use in the national cohort of participating hospitals. If the use of the individual hospital is elevated in comparison with the median value of all participating hospitals, this might be an indicator for further analysis. However, the individual hospital may still debate that it is a referral hospital or that it has many intensive care or haematology patients. Since it is not known which hospital dependent characteristics are associated with quantitative use of antibiotics it is difficult to comment on these arguments. Insight is therefore needed into hospital characteristics that predict quantitative antibiotic use. This may help to identify hospitals with low or elevated levels of quantitative antibiotic use and that are potential candidates for in depth studies to the quality of antibiotic prescribing. The aim of this study is therefore to assess determinants of antibiotic use in Dutch hospitals.

METHODS

1. Dependent variable: quantitative use of antibiotics

Data on the use of antibiotics (group J01 of the Anatomical Therapeutic Chemical (ATC) Classification system) in acute care Dutch hospitals in 2001, was collected by means of a questionnaire distributed to Dutch hospital pharmacies by the Working Party on Antibiotic Policy (acronym is SWAB). The ATC/DDD classification from the World Health Organization (WHO), version 2005, was used to calculate the number of defined daily doses (DDD) of the various antibiotics (3). Use of antibiotics was expressed in DDD per 100 patient days. The number of bed days and admissions were obtained from the Institute for Health care Information and Consultancy (Prismant). The number of patient days was calculated by subtracting the number of admissions from the number of bed days as the number of bed days over estimates actual treatment-days by including both the day of admission and the day of discharge.

2. Independent variables: hospital characteristics

Hospital characteristics consisted of institutional, population and care provider characteristics. If not specifically mentioned these data were derived from the year 2001 questionnaire that was sent to all Dutch hospitals by the Dutch Federation of University Medical Centers (NFU) and the Dutch Federation of Hospitals (NVZ). Enumerators were defined for all quantitative variables to make them relative to the size of the hospital.

Institutional characteristics. Hospitals were categorised in general, regional referral hospitals and university hospitals. Geographic location was encoded by the Nomenclature of Territorial Units for Statistics (NUTS) (4). The North (NL1) and East (NL2) provinces were grouped together to obtain sufficient hospitals per category. Teaching status for medical specialists was also included as an institutional characteristic.

Population characteristics. These characteristics directly or indirectly reflect the case-mix (i.e. the patients with or at risk for an infectious disease) of the hospital.

These included the number of haematologists (data obtained from the National Society of Internal Medicine Physicians (NIV)) and intensive care physicians, the number of inpatient surgical procedures, the number of inpatient bed days for the departments of internal medicine, pulmonology, surgery and urology, the number of transplantations, the number of dialyses, the ratio of the number of bed days of surgical and non-surgical departments, the number of treatments in daycare, the number of outpatient chemotherapy treatments, the proportion of the discharged population > 45 years of age and the mean length of stay.

A specific casemix-indicator was also included. This indicator was developed by Prismant to assess the differences in health care needs (in costs) of patients between the different hospitals.

Care provider characteristics. The number of hospital pharmacists, medical microbiologists and infectious disease specialists (data obtained from the National Society of Internal Medicine Physicians (NIV) were included. We also developed an indicator for the intensity of medical care provided by internal medicine physicians, pulmonologists, surgeons and urologists to their respective patients. The number of these physicians were weighted to their respective bed days.

3. Statistics

Descriptive analyses were performed to assess distribution and frequency of the dependent and independent variables. A multivariate backward stepwise linear regression model was constructed with total systemic use of antibiotics as the dependent outcome variable and all the institutional, population and care providers' charac-

teristics as the independent explanatory variables. Only explanatory variables with bivariate correlation with *P* values < 0.15 were included in the stepwise regression model. Two-sided *P* values of < 0.05 were considered to be statistically significant. The data were analyzed by using SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois).

RESULTS

Hospital characteristics and antibiotic use. In 2001, a total of 49 hospitals contributed data on the use of antibiotics. This sample represents about 50% of the total number of patient days in The Netherlands in 2001. Of 43 out of these 49 hospitals, data were available on hospital characteristics. These hospitals were used in the analysis of determinants of quantitative antibiotic use. Table 1 outlines the institutional, population and care providers' characteristics of the 43 participating hospitals. Median total systemic use in the total cohort of hospitals was 45.6 (range 26.1 – 65.9) DDD per 100 patient days (for source data see NethMap 2005) (5).

Characteristics associated with quantitative antibiotic use. Eleven hospital characteristics were identified as significant bivariate determinants of quantitative antibiotic use (Table 2). In the multivariate analysis the number of hospital pharmacists, medical microbiologists and infectious disease physicians per 100,000 patient days were significant determinants of quantitative antibiotic use in 43 Dutch hospitals. If the number of these medical specialists increases with one per 100,000 patient days, total antibiotic use increases with 2.2, 2.4 and 2.8 DDD per 100 patient days, respectively. These variables explained 40% of the variation in antibiotic use.

DISCUSSION

The number of hospital pharmacists, medical microbiologists and infectious disease physicians per 100,000 patient days determine the quantity of antibiotic use in a hospital. From many studies it is known that multidisciplinary teams including specialists from the departments of microbiology, infectious diseases and hospital pharmacy has been successful in improving the quality of antibiotic drug use (6, 7). Policy makers should foresee that, when inappropriate prescribing is due to under-treatment, quality improvement may result in an increase in total use of antibiotics and costs (8). This may thus explain the association that we found between the number of hospital pharmacists, medical microbiologists and infectious disease physicians per 100,000 patient days and an increased use of antibiotics.

Table 1. Institutional, population and care provider characteristics of 43 Dutch hospitals

Hospital characteristics	
Institutional characteristics	*n* (%)
Hospital category	
University hospital	5 (11.6)
Regional referral hospital	8 (18.6)
General hospital	30 (69.8)
Geographical situation	
North/East	10 (23.3)
West	22 (51.2)
South	11 (25.6)
Teaching status present	26 (60.5)
No. of beds, mean (SD)	522 (244)
Population characteristics	mean (SD)
No. of haematologists / 100,000 PD[a]	1.8 (2.2)
FTE[b] intensive care physicians / 100,000 PD	1.1 (1.6)
No. of surgical procedures / 100 PD	15.7 (4.4)
% Bed days internal medicine / total bed days	16.7 (3.5)
% Bed days pulmonology / total bed days	7.0 (2.5)
% Bed days surgery / total bed days	18.6 (3.2)
% Bed days urology / total bed days	8.1 (2.7)
No. of transplantations / 100 PD	0.2 (0.5)
No. of dialyses/ 100 PD[c]	10.0 (10.9)
Ratio bed days surgical and non-surgical departments	1.1 (0.2)
No. of treatments in daycare / 100 PD	9.2 (2.3)
No. of outpatient chemotherapy treatments / 100 PD	2.0 (1.1)
% Discharged population > 45 years of age[c]	57.3 (5.1)
Mean length of stay (days)	7.9 (0.9)
Casemix-indicator[d]	94.6 (11.2)
Care provider characteristics	mean (SD)
FTE hospital pharmacists / 100,000 PD	2.8 (1.4)
FTE medical microbiologists / 100,000 PD	1.6 (1.5)
FTE infectious disease physicians / 100,000 PD	0.5 (1.3)
FTE internal medicine physicians / 100,000 internal medicine bed days	61.0 (45.5)
FTE surgeons / 100,000 surgical bed days	45.9 (25.6)
FTE pulmonologists / 100,000 pulmonology bed days	41.1 (20.8)
FTE urologists / 100,000 urology bed days	81.4 (34.1)

[a] PD = patient days

[b] FTE = full-time equivalent

[c] No data available from university hospitals

[d] Data only available from 30 general and regional referral hospitals

In addition, we postulate that the relative number of such specialists in a hospital strongly correlates with the hospital's case mix of patients regarding infectious diseases and that variables currently not covered confound our results. We assessed many indirect population characteristics in order to include a meaningful indicator of the patient case-mix in our prediction model. The choice of these characteristics was deliberately limited to those that were freely available from public sources and from the yearly questionnaire of the Dutch Federation of University Medical Centres

Table 2. Bivariate and multivariate determinants of quantitative antibiotic use in 43 Dutch hospitals, 2001

Determinants	Bivariate analysis		Multivariate analysis[a]	
	F	P value	Non-standardized Betacoefficient	95% CI
Institutional characteristics				
Hospital category	0.004	6.219		
Population characteristics				
No. of haematologists / 100,000 PD[b]	0.002	10.436		
No. of transplantations / 100 PD	0.006	8.444		
Casemix-indicator[c]	0.052	4.099		
Care provider characteristics				
FTE[d] hospital pharmacists / 100,000 PD	0.054	3.957	2.168	0.171 - 4.165
FTE medical microbiologists / 100,000 PD	0.002	11.042	2.398	0.257 - 4.539
FTE infectious disease physicians / 100,000 PD	0.001	12.103	2.812	0.452 - 5.172
FTE internal medicine physicians / 100,000 internal medicine bed days	0.001	11.929		
FTE surgeons / 100,000 surgical bed days	0.003	9.900		
FTE pulmonologists / 100,000 pulmonology department bed days	0.032	4.913		
FTE urologists / 100,000 urology department bed days	0.103	2.776		

[a] R Squared = 0.395

[b] PD = patient days

[c] Data only available from 30 general and regional referral hospitals

[d] FTE = full-time equivalent

(NFU) and the Dutch Federation of Hospitals (NVZ). Potential determinants that require yearly collection of data from the individual hospitals, or patients, appears to be less ideal with this purpose in mind. Nevertheless, despite the limited number of explanatory variables our model explains 40% of antibiotic in Dutch hospitals.

We are aware of only two similar studies. Blix *et al.* found that size, type and geographical situation do not influence the level of antibiotic use in Norwegian hospitals (9). In an unpublished study, significant predictors of total antibiotic use in 32 US hospitals included the number of infections, inpatient surgeries and bacteremias per 1000 discharges, and the illness severity class and geographic location. This model explained 72% of total antibiotic use (10). The latter study shows that inclusion of more disease related variables in the model explains a higher proportion of the variability in antibiotic use. Other variables that might explain the variability in use might be those related to local antibiotic policies including the presence of guidelines, restricted lists of antibiotics and presence of antibiotic committees. Moreover, data on local resistance levels were not included in our analysis. Elevated resistance levels may result in higher dosage regimens, an increased duration of treatment or prescription of combination of antibiotics. Therefore resistance levels may also correlate with quantitative antibiotic use in hospitals.

In conclusion, it is desirable to extend studies like this to further delineate the determinants of variability in antibiotic use in acute care hospitals. This would enable us to give appropriate feedback to the individual hospitals on their use of antibiotics.

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We thank the pharmacists of the participating hospitals for providing data on antibiotic use. The Dutch Federation of University Medical Centers (NFU) and the Dutch Federation of Hospitals (NVZ) are acknowledged for providing data from the year 2001 questionnaires. W. Hoogen Stoevenbeld (Prismant) is thanked for his assistance in collection of the data. This study was supported with a structural grant by the Ministry of Health, Welfare and Sport to the Working Party on Antibiotic Policy (SWAB), nr. 366950.01.

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CHAPTER 3

Epidemiology of colonization with antibiotic resistant bacteria in patients during and after hospitalization

CHAPTER 3.1

Hospitalisation, a risk factor for antibiotic-resistant *E. coli* in the community?

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ABSTRACT

Objectives. The impact of hospitalization on the prevalence of resistant *Escherichia coli* in the intestinal flora of patients admitted to the surgical wards of three Dutch university- affiliated hospitals was analyzed prospectively.

Methods. Fecal samples were obtained on admission to the hospital, at the time of discharge, and 1 and 6 months after discharge. All samples were examined for resistance to nine antibiotic agents.

Results. For the total patient population, no significant differences in the prevalence of resistance were observed at the different sampling intervals, except for a significant decrease in cefazolin resistance between the time of discharge and 6 months after discharge (10% to 3%, $p < 0.05$). This decrease was mainly observed in patients from the university hospital Maastricht (azM), in which a significant decrease from 17% to 6% was detected ($p < 0.05$). Moreover, despite dissimilarities in patient characteristics and the marked variations in antibiotic use, no significant differences in the prevalence of antibiotic resistance were observed between the three hospitals, except for the overall higher prevalence of cefazolin resistant *E. coli* in azM patients ($p < 0.05$).

Conclusions. In this study, hospitalization did not seem to have any substantial effect on the prevalence of antibiotic resistant *E. coli* at the different time intervals. However as our study population consisted of surgical patients with a relatively moderate antibiotic use, and the prevalence of antibiotic resistance was only analyzed for fecal *E. coli*, further investigation should be encouraged, as the understanding of the interaction between different resistance reservoirs is important for directing future intervention studies.

INTRODUCTION

Many studies have shown that hospitalization can lead to an increase in antibiotic resistance in pathogenic bacteria (1,2). The effect of hospitalization on antibiotic resistance of commensal intestinal bacteria, like *Escherichia coli*, has received less attention. Resistant commensal bacteria acquired in the hospital may disseminate in the community through discharged patients.

In this study, the impact of hospitalization on the prevalence of resistance in *E. coli* of the intestinal flora of surgical patients of three university affiliated-hospitals in The Netherlands was analyzed prospectively. The prevalence of resistance was determined on admission, at time of discharge and 1 and 6 months after discharge. *E. coli* was used as an indicator organism, as this species is one of the dominant facultative aerobically growing bacteria of the intestinal flora.

METHODS

Patient population

From 1999 to 2001, patients admitted to the participating surgical wards of the university hospitals of Groningen (azG), Maastricht (azM), and Rotterdam (azR), The Netherlands, were asked to participate in the present study. Upon inclusion, patients were requested to collect a fecal sample at the following time intervals: within 24 hours after admission to the hospital, at time of discharge, and 1 and 6 months after discharge. Data on antibiotic use and hospitalization in the 3 months before hospital admission were recorded. At the time of discharge, the reason for surgical admission, the length of stay and the antibiotics prescribed were registered for each patient. Patients were not eligible for the study if they were referred from a nursing home or from another hospital ward. Patients were withdrawn from the analysis if no fecal samples were obtained on admission, at time of discharge or one month after discharge, if they stayed for < 2 days in the hospital, or if they were referred to an intensive care unit for > 24 hours after surgery. Approval of the Medical Ethical Committees was obtained before the start of the study. Only patients who had given their informed consent were included in the study.

Sample processing

The prevalence of antibiotic resistance was determined, as described previously (3). In short, after thawing, the samples were further diluted (10^{-2} - 10^{-4}) and 0.04 mL of these dilutions were spread over Eosine-Methylene-Blue (EMB) agar plates (Oxoid

CM 69, Basingstoke, UK) with and without antibiotics, using a spiral plater (Salm and Kipp, Utrecht, The Netherlands). In the present study, the minimum detection level for *E. coli* was 10^3 colonies per gram feces. The antibiotic concentrations used (see Table 2) in the agar plates were based on NCCLS guidelines and modified (where appropriate) to make comparison with previous studies possible (4, 5).

Analysis

The prevalence of antibiotic resistance (%) was defined as the number of fecal samples with resistant *E. coli* divided by the total number of samples with detectable *E. coli* and multiplied by 100. The prevalence of resistance against amoxicillin, cefazolin, ciprofloxacin, co-amoxiclav, gentamicin, nalidixic acid, nitrofurantoin, oxytetracycline, and trimethoprim was determined. The consumption of antibiotics during hospitalization in the three patient populations was expressed as the number of defined daily dosages (DDD) per 100 bed-days. Statistical comparisons were performed using the chi-square test or Fisher's exact test. P values < 0.05 were considered significant.

RESULTS

Patient populations

The overall response of participation in the study was ± 30%. The reason that patients did not want to participate was mainly because they were physically unable or had psychological reasons. Of the 400 patients, 268 were enrolled in the study. Fecal samples were collected on admission, at time of discharge, and 1 month after discharge (azG = 83, azM = 94, azR = 91), and for 221 patients a fecal sample was also collected 6 months after discharge (azG = 59, azM = 80, azR = 82).

There was no marked variation between the three patient populations, for mean age (azG = 51, azM = 58, azR = 63), use of antibiotics (azG = 25%, azM = 30%, azR = 18%) and hospitalization (azG = 27%, azM = 26%, azR = 32%) in the three months prior to admission. Almost half of the azM patients were admitted for gastrointestinal surgery (47%), whereas azR patients were admitted most often for vascular surgery (49%) and azG patients for oncology related surgery (52%). The majority of patients underwent surgery during hospitalization (azG = 99%, azM = 86%, azR = 98%). The median length of stay for the populations of azG, azR, azM was 6, 8, and 10 days, respectively.

Table 1 presents the consumption of antibiotics for systemic use during hospitalization. The percentages of patients receiving antibiotics in the patient popula-

tions of azM, azR and azG were 93%, 62%, and 34%, whereas the percentages of patients receiving antimicrobial prophylaxis were 78%, 49% and 34%, respectively. In the azM population, co-amoxiclav was used for antimicrobial prophylaxis, and 41% also received co-amoxiclav for therapy. For the azR patients the antibiotics used for therapy were diverse, whereas cefazolin with or without metronidazole was exclusively used for prophylaxis. The azG patients were most often prescribed ciprofloxacin for therapy (18%) and cefuroxime with or without metronidazole was used for prophylaxis (Table 1).

Table 1. Consumption of antibiotics during hospitalization in the three patient populations

	Antibiotic use in DDD per 100 bed days[a] (no. of patients prescribed a specific antibiotic)		
Antibiotic agent	azM	azR	azG
Aminopenicillins	1.4 (2)	0.1 (1)	-
Flucloxacillin	6.0 (3)	-	-
Co-amoxiclav	94 (81)	21.3 (8)	14.8 (4)
Cefazolin	0.2 (1)	2.0 (44)	1.5 (11)
Cefuroxime	-	4.0 (8)	-
Ceftazidime	1.1 (1)	-	-
Trimethoprim	0.5 (1)	-	-
Co-trimoxazole	0.3 (1)	1.6 (3)	0.8 (2)
Erythromycin	-	2.2 (2)	-
Clindamycin	2.7 (3)	4.4 (3)	-
Aminoglycosides	4.5 (6)	-	2.1 (3)
Ciprofloxacin	1.3 (4)	5.7 (2)	13.7 (17)
Metronidazole	0.8 (1)	3.3 (15)	1.1 (4)
Nitrofurantoin	0.4 (1)	-	-
Total	113	45	34

[a] from the 2002 edition of the ATC/DDD system

Antibiotic resistance

Table 2 presents the prevalence of antibiotic resistance for the samples with detectable *E. coli* at the different time intervals. No significant differences in the prevalence of resistance were found between admission, time of discharge, and 1 and 6 months after discharge, both for the total population and for the separate hospitals. One exception was the significant decrease in the prevalence of cefazolin-resistant *E. coli* from the azM patients between discharge (17%) and 6 months thereafter (6%). In addition, a similar decrease was found for the total patient population ($p < 0.05$). The azM patients showed a significantly higher prevalence of cefazolin resistance on admission, at time of discharge and 1 month after discharge compared with the azG patients and also at time of discharge for the azR patients ($p < 0.05$) (Table 2).

Table 2. The prevalence (%) of antibiotic resistance per hospital determined for samples with detectable *E. coli* [a]

				After discharge	
		Admission	Discharge	1 month	6 months
No. of fecal samples with detectable	azG	50	53	63	40
E. coli	azM	76	75	74	63
	azR	57	71	70	70
	total	183	199	207	173
Amoxicillin (25 mg/L)	azG	28	30	39	35
	azM	38	36	34	40
	azR	32	41	39	34
	total	28	36	38	36
Cefazolin (32 mg/L)	azG	0[b]	0[b]	2[b]	0
	azM	9	17	12	6[c]
	azR	4	4[b]	7	3
	total	5	10	7	3[c]
Ciprofloxacin (4 mg/L)	azG	6	6	2	3
	azM	3	4	4	2
	azR	2	3	4	4
	total	3	4	3	4
Gentamicin (16 mg/L)	azG	2	4	0	0
	azM	1	0	0	2
	azR	2	4	2	3
	total	2	3	1	2
Nalidixic acid (32 mg/L)	azG	4	6	3	8
	azM	5	7	6	2
	azR	5	6	4	4
	total	5	6	4	4
Oxytetracycline (25 mg/L)	azG	20	25	25	35
	azM	30	33	36	35
	azR	25	32	34	26
	total	26	31	32	31
Trimethoprim (8 mg/L)	azG	16	17	21	20
	azM	22	23	18	21
	azR	12	24	26	24
	total	17	22	21	22

[a] No resistance was found for nitrofurantoin (50 mg/L) and co-amoxiclav (32 mg/L)

[b] Significant difference compared with azM within a time interval ($p < 0.05$)

[c] Significant difference between discharge and 6 months after discharge within a hospital ($p < 0.05$)

DISCUSSION

Despite the dissimilarities in patient characteristics and the variations in quantities and types of antibiotics used during hospitalization, no significant differences in the prevalence of antibiotic resistance were observed between the three hospitals, except for cefazolin resistance. Even when the overall prevalence of antibiotic resistance remains stable at the different time intervals, the colonization density of

resistant bacteria in the commensal flora could increase. When resistant bacteria become part of the dominant flora, this enhances the chance of dissemination into the environment and to other hosts. However, in the present study, the prevalence of predominant resistant *E. coli* (degree of >50%) found in the patients' fecal samples was also found stable at the different time intervals (data not shown, $p > 0.05$).

Selection pressure by antibiotics is considered the most important factor in the emergence of antibiotic resistance. The azM patients were mostly exposed to antibiotics. This high use of antibiotics can be explained by the fact that the majority of azM patients underwent (clean-) contaminated surgery, which because of higher infection risk is an indication for antimicrobial prophylaxis (6), whereas the majority of azR and azG patients underwent 'clean' surgery (75% and 85% respectively). However, the higher selection pressure in azM patients due to the use of co-amoxiclav, did not seem to have any effect on the observed prevalence of co-amoxiclav or amoxicillin resistance. The patients that used antibiotics during hospitalization showed a significant higher prevalence of cefazolin resistance at time of discharge. One might speculate that the high use of co-amoxiclav induced the increase in cefazolin resistance observed at time of discharge. The use of cefazolin for prophylaxis in the azR did not seem to effect the prevalence of cefazolin resistance.

On surgical wards, antibiotic use is relatively low compared to other wards like hematology or intensive care units. In addition, the average length of stay in these wards is usually longer and patients are often severely immunocompromised, which facilitates dissemination of and colonization by antibiotic-resistant bacteria. Studying antibiotic resistance in these hospital populations would have lead perhaps to a more pronounced effect on the prevalence of antibiotic resistance.

In this study, hospitalization did not seem to have any substantial effect on the prevalence of antibiotic resistant *E. coli* at the different time intervals. However, as our study population consisted of surgical patients with a relatively moderate antibiotic use, and the prevalence of antibiotic resistance was only analyzed for fecal *E. coli*, further investigation should be encouraged, as the understanding of the interaction between different resistance reservoirs is important for directing future intervention studies.

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CHAPTER 3.2

Comparative evaluation of three chromogenic agars for detection and rapid identification of aerobic gram-negative bacteria in the normal intestinal microflora

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ABSTRACT

Objectives. To compare three different chromogenic agars and MacConkey agar for the detection of aerobic gram-negative bacteria in the normal intestinal microflora and to assess the accuracy of the chromogenic agars for the direct identification of *Escherichia coli*.

Methods. A total of 164 Gram-negative clinical isolates (*E. coli*, *Proteus*, *Klebsiella*, *Enterobacter*, *Morganella* and *Pseudomonas* species) and 30 stool specimens were inoculated in parallel on four media: Chromagar *E. coli* / Coliform, Chromogenic urinary tract infection UTI medium, CHROMagar Orientation and MacConkey agar. All colonies that differed by color and/or morphology were selected for further identification by VITEK 1 and/or API 20E from each medium.

Results. On *E. coli* / Coliform agar five out of 32 (16%) *E. coli* strains failed to produce the color as described by the manufacturer. No remarkable discrepancies were found for the other clinical isolates. There was no significant difference in detection rate (DR) of aerobic gram-negative bacteria in stool specimens between the different chromogenic agars and MacConkey agar. The overall DR was about 84%, and varied from 100% for monomicrobial specimens to 33% for polymicrobial specimens. The positive predictive values (PPV) for the direct identification of *E. coli* on Chromagar *E. coli* / Coliform, Chromogenic UTI medium and CHROMagar Orientation were 1.00, 0.93 and 0.93, respectively. The negative predictive values (NPV) were 0.53, 0.68 and 0.69, respectively.

Conclusions. Chromogenic UTI medium and CHROMagar Orientation are the preferred media because of the higher NPV. The high PPV of these agars allows accurate and rapid identification of *E. coli*.

INTRODUCTION

MacConkey agar is the most widely used medium in the microbiological laboratory for the isolation and differentiation of *Enterobacteriaceae*. This medium differentiates aerobic gram-negative bacteria by detecting lactose utilization with a neutral red indicator. As a result of the absence of any differential genus-specific indicator property in the MacConkey agar, mixed gram-negative cultures are not always detected. Several chromogenic agars, which have been available for some years, allow the presumptive identification of aerobic gram-negative bacteria on the basis of colony morphology and distinctive color patterns [1-6]. By means of chromogenic substrates incorporated into the agar, chromogenic agar plates reveal genus- or species-specific enzyme activities of microorganisms. Thus, these media may allow better discrimination of the different species in mixed cultures, resulting in greater sensitivity of detection compared to MacConkey agar.

The present study was conducted in the course of a large epidemiological study of antibiotic resistance among bacteria present in stool specimens of hospitalized patients. The purpose of this study was to compare three different chromogenic agars and MacConkey agar for the detection of aerobic gram-negative bacteria in the normal intestinal microflora. As *E. coli* is the dominant aerobic gram-negative species in the gut, the accuracy of the chromogenic agars for the direct identification of *E. coli* was assessed.

MATERIALS AND METHODS

Culture media

Three chromogenic culture media (Chromagar *E. coli* / Coliform (Oxoid, Basingstoke, UK), Chromogenic UTI medium (Oxoid, Basingstoke, UK) and CHROMagar Orientation (Becton Dickinson, Heidelberg, Germany) were compared. Chromagar *E. coli* / Coliform and Chromogenic UTI medium were prepared from the dehydrated media according to the manufacturers' instructions. CHROMagar Orientation was received as ready-made agar plates. MacConkey agar (Becton Dickinson) served as the reference medium.

Determination of colony color

The identification of microorganisms is based on chromogenic substrates incorporated into the media to detect certain bacterial enzymes. The respective organisms react with the chromogenic agents in such a way that the colonies take on a char-

acteristic color and colony morphology. Chromogenic *E. coli* / Coliform medium is supplemented with two different chromogenic agents: one allows for the detection of ß-glucuronidase and releases a blue dye, whereas the other chromogen detects the presence of ß-galactosidase and releases a pink to red dye. Chromogenic UTI medium and CHROMagar Orientation are also supplemented with two chromogenic agents which are cleaved by the enzymes ß-galactosidase (pink to red dye) and ß-glucosidase (blue to blue-green dye). Additionally, tryptophan deaminase, an enzyme characteristically found in the Proteus-Morganella-Providencia (PMP) group of organisms is detected on the latter two media by the production of a brownish, diffuse pigment that stains the medium around the respective colonies.

Bacterial strains

A total of 164 isolates (*E. coli* (n=32), *Klebsiella* spp (n=28), *Enterobacter* spp (n=27), *M. morganii* (n=26), *Proteus* spp (n=21) and *P. aeruginosa* (n=30)) were screened for their colony colors on the three chromogenic agars. These clinical isolates, obtained from blood cultures from hospitalized patients, were identified according to local standard operating procedures and stored at –80°C. After subculturing on Colombia blood agar, the isolates were inoculated in parallel on the three chromogenic media by the same technician and incubated aerobically at 35 ± 2°C. The color of the isolates was determined after 18-24 hours and compared with the color catalog of the manufacturers.

Stool specimens

Thirty stool specimens were used for the evaluation of the three chromogenic media. The stool specimens were collected from surgical patients on admission to the hospital. The stool specimens were inoculated in parallel on the three chromogenic media and on MacConkey agar. All four plates were inoculated by the same technician, with 50 µL of 10^{-2} and 10^{-4} dilutions of the stool specimens in physiological saline. The plates were incubated aerobically at 35 ± 2°C and examined after 18-24 hours for the colony characteristics. Dilutions with the highest variety of different colonies were included in the final analyses. From each medium, all colonies that differed by color and/or morphology were selected for further identification. A description of colony appearance was recorded. At least three colonies were selected from the MacConkey medium. When no differences in color and/or morphology were observed these three colonies were randomly selected. After microscopic examination (Gram-stain) all colonies were identified by VITEK 1 (bioMérieux, Marcy l'Etoile, France) or API 20E system (bioMérieux). The accuracy of the presumptive identifica-

tion of *E. coli* on the basis of colony color is described in terms of positive- and negative predictive values.

RESULTS

Bacterial strains

Table 1 demonstrates the color reactions for the 164 clinical isolates on the three different chromogenic agars. On *E. coli* / Coliform agar five out of 32 *E.coli* strains failed to produce a purple color. These five isolates produced colorless colonies and were ß-glucuronidase negative as tested with the API 20E system. On both the Chromogenic UTI and CHROMagar Orientation medium, the *Proteus vulgaris* strains produced blue colonies, except for two strains that did not grow or yield colorless/beige colored colonies. These two strains also did not grow on *E. coli* / Coliform agar. Compared to the *E. coli* / Coliform medium, the Chromogenic UTI and CHROMagar Orientation have the advantage of inhibiting swarming of *Proteus* spp. No major discrepancies with the color catalogues of the manufacturers were found for the other clinical isolates.

Table 1. Evaluation of color reactions of 164 clinical gram-negative isolates on three chromogenic media

	Number of isolates with colors as described[a] / total numbers of isolates[b]		
Micro-organism	Chromagar *E. coli* / Coliform	Chromogenic UTI medium	CHROMagar Orientation
Enterobacter aerogenes	13 / 13	13 / 13	13 / 13
Enterobacter cloacae	13 / 14	14 / 14	14 / 14
Escherichia coli	27 / 32	32 / 32	32 / 32
Klebsiella pneumoniae	28 / 28	28 / 28	28 / 28
Morganella morganii	26 / 26	26 / 26	26 / 26
Proteus mirabilis	13 / 13	13 / 13	12 / 13
Proteus vulgaris	6 / 8	6 / 8	6 / 8
Pseudomonas aeruginosa	30 / 30	30 / 30	30 / 30
Total	156 / 164 (95%)	162 / 164 (99%)	163 / 164 (99%)

[a] The colors as described by the manufacturers of Chromagar E.coli/Coliform, Chromogenic UTI medium and CHROMagar Orientation, respectively, were as follows: *Enterobacter* spp, pink/blue/blue; *E. coli*, purple/pink/pink; *K. pneumoniae*, pink/blue/blue; *M. morganii*, colorless/brown/brown; *Proteus mirabilis,* colorless/brown/brown; *Proteus vulgaris,* colorless/blue/blue*; P. aeruginosa,* straw/fluoresce/fluoresce.

[b] After 18-24h of incubation at 37°C.

Stool specimens

The total number of gram-positive and negative strains isolated from the Chromagar *E. coli* / Coliform, the Chromogenic UTI medium, the CHROMagar Orientation and

the MacConkey agar were 84, 83, 91 and 93, respectively. After identification tests were performed for all isolates, the total number of different gram-negative micro-organisms was determined for each of the 30 stool specimens. One specimen yielded no growth of gram-negative microorganisms on all four media and 29 specimens yielded growth on at least one of the four media. Fifteen stool specimens yielded pure cultures of gram-negative bacteria and 14 mixed cultures.

Table 2 shows the distribution of gram-negative micro-organisms. *E. coli* was the dominant isolate. A total of 47 gram-negative micro-organisms were isolated on at least one medium. Two stool cultures yielded *Acinetobacter* spp. For one specimen this microorganism was only detected on MacConkey; from the other specimen the species were detected both on the MacConkey agar and the Chromagar *E. coli* / Coliform. No significant differences in detection rate (DR) of *Enterobacteriaceae* in stool specimens were found between chromogenic agars and MacConkey agar. The overall DR was 84% (Table 2), and varied from 100% for monomicrobial specimens to 33% for polymicrobial specimens (Table 3).

A total of 25 gram-positive microorganisms were isolated on at least one medium. The DR of gram-positive microorganisms on the Chromagar *E. coli* / Coliform, the Chromogenic UTI medium, the CHROMagar Orientation and the MacConkey agar were 48%, 64%, 76% and 44%, respectively.

Table 2. Distribution of gram-negative bacteria recovered from 30 stool specimens on different media

	Detected on any medium[a]	Chromagar *E. coli* / Coliform[b]	Chromogenic UTI medium[b]	CHROMagar Orientation[b]	MacConkey agar[b]
Acinetobacter spp	2	1	-	-	2
Citrobacter spp	4	4	1	3	2
Escherichia coli	27	26	27	27	26
Enterobacter spp	3	1	2	1	3
Hafnia alvei	2	-	1	-	1
Klebsiella spp	7	6	6	6	5
Morganella morganii	1	1	1	-	-
Serratia spp	1	1	1	1	1
Total	47	40	39	38	40
Detection rate		85%	83%	81%	85%

[a] Total number of strains detected on at least one of the four media

[b] No significant difference (Chi-square; *P* value = 0.4)

Presumptive identification of *E. coli* on the basis of colony color

After 18-24 hours incubation, it was easy to discriminate between gram-positive cocci and all other microorganisms on the basis of morphology alone. Gram-positive cocci grew as very small colonies and were excluded from the analysis of the accuracy of the identification of *E. coli* based on colony color. The remaining number

Table 3. Detection rates of aerobic gram-negative bacteria in mono- and polymicrobial stool specimens

	Number of stool specimens (detection rate)		
	1 micro-organism (*n* = 15)	2 micro-organisms (*n* = 8)	3 micro-organisms (*n* = 6)
Chromagar *E. coli* / Coliform	15 (100%)	4 (50%)	1 (17%)
Chromogenic UTI medium	15 (100%)	3 (38%)	1 (17%)
CHROMagar Orientation	15 (100%)	2 (25%)	2 (33%)
MacConkey agar	15 (100%)	4 (50%)	1 (17%)

Table 4. Accuracy of presumptive identification of *E. coli* on the basis of colony color

	Number of *E. coli* strains		
	Chromagar *E. coli* / Coliform	Chromogenic UTI medium	CHROMagar Orientation
True positive strains[a]	26	26	26
False-negative strains	15	6	8
White colonies	4	3	6
Pink colonies	11	-	-
Transparant colonies	-	1	2
Blue colonies	-	2	-
False-positive strains	-	2	2
Citrobacter freundii	-	1	-
Hafnia alvei	-	1	-
Gram-positive rod	-	-	2
True negative strains	17	13	18
Positive predictive value	1	0.93	0.93
Negative predictive value	0.53	0.68	0.69

[a] The colors as described by the manufacturers' of Chromagar *E. coli* / Coliform, Chromogenic UTI medium and CHROMagar Orientation, respectively, were purple, pink and pink.

of microorganisms isolated from Chromagar *E. coli* / Coliform, Chromogenic UTI medium and CHROMagar Orientation were 58, 47 and 54, respectively. The accuracy of the three chromogenic media to identify *E. coli* by means of purple or pink colonies is reported in Table 4. On the *E. coli* / Coliform medium 15 of the 41 isolated *E.coli* strains did not produce a purple color. On the Chromogenic UTI medium and CHROMagar Orientation the false-negative rates were six out of 32 strains and eight out of 34 strains, respectively. The false positive rates were very low for the three chromogenic media. The positive predictive values (PPV) for the direct identification of *E. coli* on Chromagar *E. coli* / Coliform, UTI and Orientation were 1, 0.93 and 0.93, respectively. The negative predictive values (NPV) were 0.53, 0.68 and 0.69, respectively.

DISCUSSION

Infections caused by gram-negative bacteria continue to be a major problem for hospitalized patients. According to the data from the National Nosocomial Infections Surveillance System, gram-negative bacteria are the leading cause of nosocomial infections in the United States [7]. The increase in antimicrobial resistance of these microorganisms demands effective surveillance programs. Screening for resistant bacteria in the fecal flora seems relevant for two reasons. Firstly, the gastrointestinal tract may be the reservoir of bacteria that cause clinical infections [8]; secondly, fecal bacteria, whether they cause infections or not, might act as a reservoir of mobile resistance genes [9].

Different methodologies have been used for the screening of stool specimens for antimicrobial resistant strains. Österblad et al. compared two frequently applied methods: 1) the replica plating method, i.e. the comparison of growth on antibiotic-free and antibiotic containing plates and 2) the selection of five colonies with different appearances from the MacConkey medium which are subsequently tested for resistance by an agar dilution method [10]. The rate of resistance detection by these two methods did not differ statistically for any of the antibiotics tested. In most studies, fecal isolates were only classified on the basis of colony morphology and Gram stain [11, 12]. Other studies focused only on *E. coli* as indicator bacterium of the intestinal flora [13, 14]. As the prevalence of resistance varies between species, it is essential to discriminate between the different gram-negative bacteria [15].

In the present study three chromogenic agars were compared with the MacConkey agar for the detection of aerobic gram-negative fecal cultures. No significant differences in overall DR of *Enterobacteriaceae* in stool specimens were found between chromogenic agars and MacConkey agar. To our knowledge DR of *Enterobacteriaceae* in stool specimens are not known from other studies using chromogenic media. The DR corresponded well however to the rates of urinary tract pathogens described in previous studies using these media [1, 2]. Hengstler et al. did not find any differences in recovery of gram-negative bacteria from urine specimens between the two chromogenic media and the MacConkey agar [2]. In another study five chromogenic media were compared for the detection of urinary tract pathogens. The DR in monomicrobial specimens varied from 94 to 100% (361 samples), in polymicrobial specimens from 90 to 96% (94 samples) [1]. In our study on stool specimens the DR for monomicrobial specimens was 100% and for polymicrobial specimens only 17-50% (Table 3). Although the sample size in our pilot study was small it appears that in these polymicrobial samples the dominant bacteria were always detected on MacConkey agar and on the three chromogenic media. Microorganisms that were not detected on either one of the four media were often present at very low colony

counts. Therefore, chromogenic media and the MacConkey agar are equally accurate when the objective is to study the dominant microorganisms in the fecal flora.

Although no difference in detection rate was found, the easy recognition of simultaneous growth of multiple organisms on colony color can be advantageous as can be explained by the following example. In a study of antimicrobial resistance using the "5 colony picking method" [10], one can define an algorithm for the selection of colonies from the agar. If, for example, 80 pink colonies and 20 blue colonies grow on a chromogenic agar, the proposed algorithm would result in selecting 4 pink and 1 blue colony for the antimicrobial resistance testing. This method leads to a proportional sampling of the dominant flora that is not possible with MacConkey agar.

In previous studies on antibiotic resistance about 60% of the isolated gram-negative bacteria from stool specimens were identified as *E.coli [4]* . Accurate identification of *E.coli* on colony color may considerably reduce time and costs of fecal screening programs. In our study Chromagar *E. coli* / Coliform showed a high rate of false negative strains; 15/41 (37%) strains did not react with the chromogenic substrate. From other studies it is reported that about 95-98% of *E.coli* isolates produce ß-glucuronidase [16, 17]. Carricajo and Hengstler found percentages from 84-91% [1, 2]. Chromogenic media supplemented with a chromogenic agent that interacts with ß-glucuronidase, as in Chromagar *E.coli* / Coliform, are therefore not accurate for rapid identification of *E. coli* owing to the low sensitivity. However, the high sensitivity of the other media combined with a high PPV often leads to a rapid and correct detection of *E.coli*.

Standard inocula for antimicrobial susceptibility testing are usually prepared by subculturing colonies from the chromogenic media and MacConkey agar to blood-based media or Mueller-Hinton agar. Samra et al. compared the accuracy of antimicrobial susceptibility testing by picking isolates from CHROMagar Orientation with the technique of picking isolates from MacConkey agar [5]. The antimicrobial susceptibilities of these isolates were determined by the disk diffusion technique according to the National Committee for Clinical Laboratory Standards (NCCLS). The numbers of susceptible isolates were identical. These investigators did not observe significant differences between the numbers of intermediate and resistant gram-negative isolates. From the study of Samra et al. we conclude that the chromogenic substances do not interfere with the susceptibility test results.

In summary, chromogenic agars are reliable media for the detection of aerobic gram-negative bacteria in the normal intestinal microflora. The easier recognition of different colonies on these media in particular, is a major advantage. The Chromogenic UTI medium and the CHROMagar Orientation are the preferred media because of higher negative predictive values. The high positive predictive values of these chromogenic agars allow accurate and rapid identification of *E. coli*.

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CHAPTER 3.3

Colonization and resistance dynamics of gram-negative bacteria in patients during and after hospitalization

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ABSTRACT

The colonization and resistance dynamics of aerobic gram-negative bacteria in the intestinal and oropharyngeal microflora of patients admitted to intensive care units (ICU) and general wards were investigated during and after hospitalization. A total of 3316 specimens were obtained on admission, once weekly during hospitalization, at discharge from the ICU, at discharge from the hospital and one and three months after discharge from the hospital. Five colonies per specimen were selected for identification and susceptibility testing. In both patient populations, the gram-negative colonization rates in oropharyngeal specimens increased during hospitalization and did not decrease in the three months after discharge. In rectal specimens, colonization rates decreased during hospitalization and increased after discharge. There was a change in species distribution among the dominant microflora during hospitalization. *Klebsiella* spp, *Enterobacter* spp, *Serratia marcescens* and *Pseudomonas aeruginosa* were isolated more often, whereas the frequency of *Eschericia coli* declined. The percentage of ICU patients colonized with ampicillin- and/or cephalothin-resistant fecal *E. coli* was significantly increased at discharge from the hospital and did not change in the three months after discharge. The emergence of multidrug resistance was observed for *E. coli* during patient stays in the ICU. Resistance frequencies in *E. coli* significantly increased with the length of stay in the ICU. For the general ward population, no significant changes in resistance frequencies were found during hospitalization. From a population perspective, the risk of dissemination of resistant gram-negative bacteria into the community through hospitalized patients appears to be low in general ward patients but is noticeably higher among ICU patients.

INTRODUCTION

Hospitals are considered particularly important for the containment of antimicrobial resistance. The combination of seriously ill patients, intensive use of antibiotics and cross-colonization has resulted in nosocomial infections with highly resistant bacterial pathogens (1-3). Infections caused by antibiotic resistant microorganisms are associated with higher mortality and morbidity rates and higher costs than are antibiotic-sensitive bacterial infections (4-6). The prevalence of resistance for nearly all important micro-organism/antibiotic combinations is generally higher among isolates from patients hospitalized on intensive care units (ICU) than that among non-ICU inpatients (7-9). *Enterobacteriaceae* and *Pseudomonas aeruginosa* have emerged as major causes of nosocomial infections and account for approximately 30% and 5%, respectively, of all bloodstream infections (10, 11). Antibiotic resistance surveillance programs have demonstrated an increase in resistance among these gram-negative pathogens (9-11).

Bacterial colonization is often a first step in the pathogenesis of nosocomial infections (12). Therefore, the choice of empirical antibiotic therapy depends, at least partly, on the colonization and resistance dynamics of the normal microflora.

Different mechanisms may lead to colonization of hospitalized patient with resistant strains (13, 14). First, these strains may enter the hospital upon the admission of patients already colonized with resistant strains. Secondly, during hospitalization, susceptible bacteria may develop resistance due to genetic mutations or through transfer of resistance genes. Thirdly, resistance may emerge through induction of genes that are already present in susceptible bacterial subpopulations. A lack of hygiene and infection control may facilitate the spread of these resistant bacteria from patient to patient (12).

After discharge, patients may remain colonized with resistant bacteria acquired in the hospital, and these may subsequently spread into the community. Whether resistant strains will survive and replicate depends largely on the selection pressure exerted by antibiotics. In the late 1960s and 1970s, numerous colonization studies were performed to clarify the epidemiology of nosocomial infections with resistant bacteria (15-23). These studies may now be of limited value due to major changes in antibiotic and patient characteristics. Other colonization studies in hospitalized patients have been based on single cultures from individual patients, without longitudinal follow-up during and after hospitalization (24-26). Moreover, isolates were often not identified up to the species level and were described as coliforms or aerobic gram-negative bacteria (16, 19, 24, 25). In these studies, an observed increase in resistance in *Enterobacteriaceae* may in fact have been the result of a shift in species distribution (26).

We therefore decided to design a prospective observational study with the aim to accurately assess the epidemiology of aerobic gram-negative colonization and

antibiotic resistance in the oropharyngeal and intestinal microflora of hospitalized patients from admission up to three months after discharge.

METHODS

Patients and study design

Setting. Erasmus MC is a 1,200-bed university, referral hospital in Rotterdam, The Netherlands. The study was conducted at two ICU's (surgical and neurosurgical) and five general wards including the departments of internal medicine, pulmonology, neurosurgery, urology and gastro-enterology. Patients were enrolled into the study between November 2000 and July 2003. The study was approved by the Medical Ethics Committee and informed consent was provided before participation in the study.

Inclusion and exclusion criteria. Patients were eligible for the study if they met all of the following criteria: ≥18 years of age; the length of stay was expected to be ≥ 5 days, and informed consent was given by the patient or his/her representative. The exclusion criteria for patients on general wards were as follows: preceding admission to an ICU or another general ward during the same hospitalization, the presence of an ileostoma, and a diagnosis of human immunodeficiency virus infection, tuberculosis or cystic fibrosis. The same criteria were applicable for ICU patients except that preceding admission to a general ward was allowed.

Collection of specimens. Rectal swabs (or stool specimens) and oropharyngeal swabs were obtained within 48 hours of admission, once a week if the length of stay was more than 7 days, at discharge from the ICU (ICU-patients only), and at discharge from the hospital. At discharge from the hospital and 1 and 3 months after discharge, patients were contacted by mail to send in a stool specimen and an oropharyngeal swab. A reminder was sent to non-responders 1 week later. Persistent non-responders were contacted again at subsequent times, 1 and 3 months after discharge.

Data collection. The following data were collected: age; gender; reason for admission; severity of acute illness on admission, graded according to the simplified acute physiologic score (SAPS) for ICU patients (27); co-morbidity on admission according to the definitions of the Dutch National Intensive Care Evaluation (http://www.stichting-nice.nl); residential status 48 hours before admission; hospitalization history in the 3 months prior to admission; and the consumption of antibiotics during hospitalization. Community pharmacies were asked for information about the use of antibiotics in the three months prior to and after hospitalization. Patients provided written informed consent for the acquisition of these medication lists.

Antibiotic use during hospitalization was expressed as the number of Defined Daily Doses (DDD) per 100 bed days whereas the use before and after hospitaliza-

tion was expressed as the number of DDDs per 100 inhabitant days. We used the DDDs of antibiotics for systemic use listed in the Anatomical Therapeutic Chemical (ATC) Classification System 2003 (28).

Microbiological methods

Isolation of gram-negative bacteria. All patient specimens were collected and analyzed at the microbiological laboratory of the Erasmus MC. Stool specimens were collected in plastic containers, and oropharyngeal and rectal swabs were transported in Amies transport medium. Stool specimens were diluted 1:10 in physiological saline containing 20% glycerol and stored at –20°C. After thawing, 10^{-2} and 10^{-4} dilutions in physiological saline were inoculated on chromogenic plates (Chromagar Orientation (Becton Dickenson, Heidelberg, Germany).

Swabs were diluted in 1 ml of Stuart transport medium and stored at –80°C until assayed. After thawing the samples were diluted further. Fifty microliters of the undiluted suspension and 50 µl of a 10^{-1} dilution in physiological saline were inoculated onto chromogenic plates. The plates were incubated aerobically at 37°C and examined after 18-24 hours for growth and colony characteristics. The dilution with a countable number of colonies (≤100) was used for the selection of colonies. If both dilutions yielded countable numbers of colonies, the dilution with the highest variety of different colonies was chosen. If no difference existed between the two dilutions, the lowest dilution was chosen. Five colonies were selected for further identification. An algorithm was developed for the selection of these five colonies. This method led to proportional sampling of the microorganisms present at concentrations above the detection limit; e.g., if 60 pink colonies and 40 blue colonies grew on the chromogenic agar, then the proposed algorithm resulted in selecting three pink and two blue colonies. When no differences in color and/or morphology were observed, five colonies were randomly selected.

Identification. In a previous study, we concluded that Chromagar Orientation allows the accurate identification of *E. coli* by colony color (29). Pink colonies were therefore directly identified as *E. coli*. The identification of other colonies was done with the VITEK 2 system (bioMerieux, Marcy l'Etoile, France) with proprietary data management software (version: VT2-R02.03) by using ID-GNI cards after subculturing on Colombia blood agar.

Determination of susceptibility. Minimal inhibitory concentrations (MICs) were determined by the VITEK 2 system using AST-N010/020 cards. The breakpoints of the National Committee for Clinical Laboratory Standards (NCCLS) were applied (30). The Advanced Expert System of the VITEK 2 system (version: AES.R02.00N) was

used for all readings and interpretations of susceptibility results. *E. coli* (ATCC 25922 and ATCC 35218) and *P. aeruginosa* (ATCC 27853) were used as reference strains.

Data analysis

Patients were considered eligible for analysis when at least one consecutive sample was taken following the sample upon admission and when the length of stay was ≥ 5 days. Samples collected once weekly during hospital stay were categorized as "during stay". When no sample was taken on the day of discharge from the ICU, the last sample taken during stay was considered as being representative for the colonization and resistance status at discharge and was therefore categorized as such. When patients died during stay on the ICU, the last sample taken was also categorized as "during stay".

The percentage of patients colonized with resistant bacteria at different times was calculated for each antibiotic. We selected one isolate per patient with the most resistant result for that particular antibiotic. To test for differences in resistance frequencies between the different times, we used the chi-square test or Fisher's exact test (in case of low (≤ 5) cell numbers).

Multidrug resistance was defined as oropharyngeal and/or rectal colonization with at least one isolate that was resistant to three or more of the antibiotics tested. The chi-square test was used to test for differences in multidrug resistance.

We used logistic regression analysis to assess whether resistance frequencies in bacteria were associated with length of stay in the ICU. *P* values of < 0.05 were considered significant. The data were analyzed by using SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois) and GraphPad Prism version 3.0 for Windows (GraphPad Software, San Diego, California).

RESULTS

Inclusion and follow-up of the study population

A total of 200 ICU and 319 general ward (GW) patients were included in this prospective study. The data for 17 ICU and 91 GW patients were not analyzed. The reasons for withdrawal from the analyses were a length of stay of less than 5 days (for the ICU group, $n = 9$; for the GW group, $n = 52$) and no collection of a second specimen following admission (for the ICU group $n = 8$; for the GW group $n = 39$).

Table 1 shows the characteristics of the two enrolled populations. The number of ICU and GW patients analyzed at the different times are presented in Table 2. Patients were lost to follow-up as a result of death, transfers, and withdrawal from the study.

Thirty-six patients (19.6%) were already hospitalized on a GW before admission to the ICU (Table 1). The median length of stay on the participating units was 12 days (range, 5 to 119 days) for the ICU patients (n = 183) and 10 days (5 to 116 days) for the GW population (n = 228). Patients who died during stay on the ICU had a median length of stay of 22 days (range, 7 to 119 days; n = 25). The median length of stay in hospital after discharge from the ICU was 15 days (range, 0 to 281 days; n = 158).

Bacterial strains

In total, 3,316 specimens from 411 patients were collected. From these specimens 6,069 gram-negative bacteria (3,941 *E. coli*, 755 *Klebsiella* spp, 497 *Enterobacter* spp, 243 *Pseudomonas* spp, 249 *Serratia marcescens* and 384 other Enterobacteriaceae) were isolated, identified and tested for antimicrobial resistance.

Table 1. Patient characteristics upon admission

Characteristic	Intensive care units (n = 183)	General wards (n = 228)
Age (mean ± SD), yrs	55.9 ± 15.6	57.1 ± 14.0
Men, *n (%)*	120 (65.6)	135 (59.2)
Reason for admission, n (%)		
Surgery		
Abdominal	53 (29.0)	2 (0.9)
Neurosurgery	32(17.5)	67 (29.4)
Transplantation	14 (7.7)	-
Urologic surgery	-	46 (20.2)
Other surgery	20 (10.9)	-
Trauma	29 (15.8)	1 (0.4)
Medical		
Abdominal	-	20 (8.8)
Neurologic disorder	13 (7.1)	1(0.4)
Obstructive pulmonary disease	5 (2.7)	54(23.7)
Other medical disorder	17(9.3)	37 (16.2)
Residential status 48 hours before admission, *n (%)*		
Community	145 (79.2)	228 (100)
General ward at Erasmus MC	29 (15.8)	-
General ward at other hospital	7 (3.8)	-
Other	2 (1.1)	-
SAPS II on admission (mean ± SD)[a]	33.5 (13.9)	-
Admissions with comorbidity[b], *n* (%)	54 (29.5)	84 (36.8)
Hospitalization 3 months prior to admission	101 (55.2)	52 (23)
Use of antibiotics 3 months prior to admission	42 (23)	57 (25)

[a] Simplified Acute Physiology Score(27)

[b] According to the definitions of the Dutch National Intensive Care Evaluation (www.stichting-nice.nl). Comorbidity was defined as at least one of the following disorders: decreased immunity, respiratory insufficiency class IV according to New York Heart Association (NYHA), cardiovascular insufficiency class IV according to NYHA, cirrhosis, hematologic malignancy, AIDS, neoplasm with metastases, or chronic renal failure.

Species distribution during and after hospitalization.

ICU-population. A total of 1,580 specimens, 788 of which were fecal and 792 of which were oropharyngeal, were studied. The percentage of fecal samples with detectable aerobic gram-negative bacteria varied from 27% for samples collected during stay on the ICU to 84% for samples collected 3 months after discharge from the hospital (Table 2). A significant increase in the frequencies of *Klebsiella* spp ($p = 0.001$) and *P. aeruginosa* ($p < 0.001$) and a significant decrease in the frequency of *E. coli* ($p < 0.001$) were found in the intestinal microflora at discharge from the ICU. At discharge from the hospital, the frequencies of the different species were the same as those upon admission to the ICU, with the exception of *P. aeruginosa*.

Upon admission of patients to the ICU, 18% of the oropharyngeal samples yielded gram-negative bacteria. For samples collected during stay in the ICU, at discharge from the ICU, at discharge from the hospital, and 1 and 3 months after discharge from the hospital, these percentages were 43, 27, 42, 31 and 33 %, respectively. During hospitalization, the frequency of *E. coli* in oropharyngeal samples decreased significantly from 43.1% upon admission, to 26.9% at discharge from the ICU ($p = 0.001$) and finally to 13.0% ($p < 0.001$) at discharge from the hospital. One month after discharge from the hospital, the frequency of *E. coli* had already increased to 28.3% ($p = 0.006$). The frequency of *P. aeruginosa* in oropharyngeal samples increased significantly during hospitalization, from 6.0% upon admission to 13.2% at discharge from the ICU ($p = 0.02$) but did not decrease during the first 3 months after discharge from the hospital. The frequency of *S. marcescens* increased significantly during hospitalization, from 1.0% at discharge from the ICU to 14.6% at discharge from the hospital ($p < 0.001$), but decreased to 4.1% in the 3 months after discharge from the hospital ($p = 0.004$).

General ward population. A total of 1,736 specimens, 881 of which were fecal and 855 of which were oropharyngeal, were studied. The number of fecal samples with detectable aerobic gram-negative bacteria varied from 43.8% for samples collected weekly during stay on the general ward to 74.8% for samples collected 3 months after discharge from the hospital (Table 2). A significant increase in the frequency of *Klebsiella* spp and a significant decrease in the frequency of *E. coli* were found in the intestinal microflora at discharge from the general wards (Table 2). However, 3 months after discharge from the hospital, the frequencies of the different species were almost the same as those upon admission.

Upon admission of patients to the general ward, 1.1 % of the oropharyngeal samples yielded detectable gram-negative bacteria, whereas for samples collected during stay in the hospital, at discharge from the hospital, and 1 and 3 months after discharge hospital, these percentages were 3.4, 12.4, 19.4 and 20.3 %, respectively. From these oropharyngeal samples, 57 *E. coli* isolates, 348 other *Enterobacteriaceae* isolates and

Table 2. Frequencies of aerobic gram-negative bacteria in the intestinal microflora during and after hospitalization

	ICU					General ward			
	Admission	Discharge ICU	Discharge Hospital	1 month after discharge hospital	3 months after discharge hospital	Admission General ward	Discharge hospital	1 month after discharge hospital	3 months after discharge hospital
Patients, *n*	183	150	62	82	73	228	205	185	167
Samples with detectable aerobic Gram-negative bacteria, *n* (%)	67 (37)	51 (34)	42 (67)	66 (81)	61 (84)	112 (49)	113 (55)	131 (71)	125 (75)
Isolates, *n*	335	254	206	332	304	524	562	639	586
gram-negative bacteria *(% of isolates)*:									
Enterobacter spp	2.4	3.1	1.5	3.6	2.3	3.4	2.7	2.5	3.6
Escherichia coli	83.9	68.5[a]	84.0	79.2	85.2	89.5	83.8[b]	85.6	87.7
Klebsiella spp	9.0	20.1[a]	5.8	10.5[c]	7.6	3.1	9.3[b]	6.3[c]	3.1[d]
Pseudomonas spp	-	6.3[a]	2.4[b]	-[c]	-[d]	0.6	0.2	-	0.5
Other	4.7	2.0	6.3	6.7	4.9	3.4	4.0	5.6	5.1

The relative frequencies were compared in defined pairs (see a-d), using the chi-square test or Fisher's exact test as applicable ($P < 0.05$ was considered significant).

[a] significant difference for discharge from ICU versus admission to participating ICU

[b] significant difference for discharge from hospital versus admission to participating ICU or general ward

[c] significant difference for 1 month after discharge from hospital versus discharge from hospital

[d] significant difference for 3 months after discharge from hospital versus discharge from hospital

Table 3. Percentages of patients colonized with resistant *Escherichia coli* in the intestinal microflora during and after hospitalization[a, b]

	ICU					General ward			
Antibiotic(s)	Admission ICU (*n* = 183)	Discharge ICU (*n* = 150)	Discharge Hospital (*n* = 62)	1 Month after discharge hospital (*n* = 82)	3 Months after discharge hospital (*n* = 73)	Admission general ward (*n* = 228)	Discharge hospital (*n* = 205)	1 Month after discharge hospital (*n* = 185)	3 Months after discharge hospital (*n* = 167)
Ampicillin	10.5	12.7	22.6[d]	18.1	22.2	14.6	13.7	21.1	17.8
Piperacillin	5.0	6.7	9.7	6.0	8.3	9.0	8.3	9.4	8.3
Amoxicillin-clavulanic acid	1.1	2.7	3.2	2.4	1.4	1.9	0.5	0.6	1.9
Piperacillin-tazobactam	0.6	0.7	0	2.4	0	0.5	0.5	0	0
Meropenem	0	0	0	0	0	0	0	0	0
Cephalothin	2.2	4.0	8.1[d]	6.0	6.9	4.7	2.9	5.0	8.9
Cefuroxime sodium	0.6	2.0	1.6	2.4	2.8	0.5	1.0	1.1	1.0
Ceftazidime	0.6	1.3	0	1.2	0	0.5	0.5	0	0
Gentamicin	0.6	0	0	0	0	0.5	0.5	1.7	1.3
Ciprofloxacin	0.6	0	0	0	0	0.5	1.0	1.1	2.5
Trimethoprim-sulfamethoxazole	4.4	2.0	8.1	10.8	19.4	8.5	8.3	16.1[e]	10.8

[a] NCCLS breakpoints were applied.

[b] One isolate per patient was included, giving the most resistant result for each antibiotic

[c] The resistance frequencies were compared in pairs, using the chi-square test or Fisher's exact test as applicable (*P* values < 0.05 were considered significant).

[d] Significant difference for discharge from hospital versus admission to participating ICU

[e] Significant difference for 1 month after discharge from hospital versus discharge from hospital.

42 *P. aeruginosa* isolates were cultured. The number of isolates from the oropharyngeal samples was too small to compare the species distribution over time.

Percentage of patients colonized with resistant *E. coli* during and after hospitalization.

The percentage of ICU patients colonized with ampicillin- and/or cephalothin-resistant fecal *E. coli* was significantly increased at discharge from the hospital and did not return to baseline in the three months after discharge (Table 3). Trimethoprim-sulfamethoxazole resistance fluctuated remarkably, with the lowest resistance rate after discharge from the ICU and the highest rate 3 months after discharge from the hospital.

For the general ward population, no significant differences in resistance frequencies were found during hospitalization (Table 3). However, the percentage of patients colonized with trimethoprim-sulfamethoxazole-resistant *E. coli* increased significantly from 8.3% at discharge from the hospital to 16.1% 1 month later. For both populations, the number of *E. coli* isolated from oropharyngeal samples at the times were too small to allow a comparison of resistance frequencies over time.

Effect of length of stay on antibiotic resistance in *E. coli* during hospitalization

Resistance in *E. coli* isolated from oropharyngeal swabs was significantly associated with the length of stay in the ICU (by logistic regression, the *P* values were < 0.001

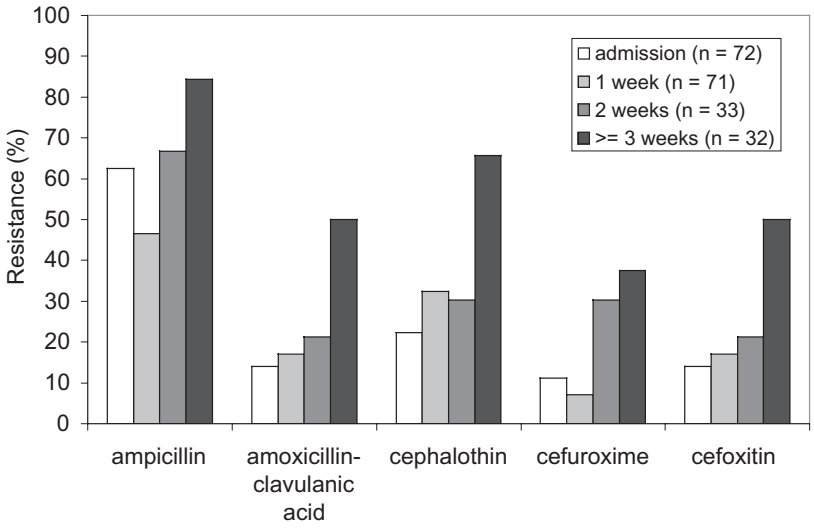

Figure 1. Effect of length of stay on antibiotic resistance in Escherichia coli isolates from the oropharynxes of patients admitted to the intensive care unit.

for ampicillin, amoxicillin-clavulanic acid, cephalothin, cefuroxime and cefoxitin) (Figure 1). Similar results were found for *E. coli* strains isolated from rectal swabs that were taken weekly. Additionally, for rectal *E. coli*, the length of stay was also significantly associated with resistance to ceftazidime. However, we did not notice a significant trend of increasingly resistant *E. coli* strains with increasing lengths of stay among general ward patients.

Table 4. Frequency of common co-resistance patterns in *E.coli* isolated on admission and during hospitalization[a,b]

	Intensive care unit (ICU) (No. of patients (%))		General ward (No. of patients (%))	
Antibiotic(s)	Upon admission (*n* = 183)	During stay in ICU[c] (*n* = 183)	Upon admission (*n* = 228)	During stay in general ward[c] (*n* = 228)
AMP	12 (6.6)	17 (9.3)	10 (4.4)	15 (6.6)
CEF	-	3 (1.6)	-	-
SXT	3 (1.6)	4 (2.2)	3 (1.3)	3 (1.3)
AMP PIP	5 (2.7)	9 (4.9)	7 (3.1)	9 (3.9)
AMP SXT	8 (4.4)	10 (5.5)	5(2.2)	14 (6.1)
AMP CEF	2 (1.1)	3 (1.6)	-	-
AMP CIP	-	2 (1.1)	-	-
AMP PIP SXT	2 (1.1)	7 (3.8)	8 (3.5)	11 (4.8)
AMP PIP CEF	-	2 (1.1)	3 (1.3)	-
AMP AMC CEF FOX	-	3 (1.6)	-	-
AMP AMC CEF FOX CXM	-	5 (2.7)	-	
AMP AMC CEF FOX CXM PIP	-	2 (1.1)	-	
AMP AMC CEF FOX CXM CAZ	-	2 (1.1)	-	

[a] For each location and time resistance patterns are presented that occurred in at least 1% of the patients.

[b] The antibiotics tested were ampicillin (AMP), piperacillin (PIP), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), meropenem (MEM), cephalotin (CEF), cefuroxime (CXM), cefoxitin (FOX), ceftazidime (CAZ), cefepime (FEP), gentamicin (GEN), ciprofloxacin (CIP) and trimethoprim-sulfamethoxazole (SXT).

[c] Including samples at discharge

Multidrug resistance of *E.coli* on admission and during stay on ICU and general wards

Upon admission to the ICU, 25 (14%) patients were colonized with at least one *E. coli* isolate that was resistant to one or more antibiotics. During stay in the ICU, the number increased to 50 (28%) patients. Resistance was most often observed against ampicillin, ampicillin and trimethoprim-sulfamethoxazole, or ampicillin and piperacillin (Table 4). Multidrug resistance (resistance to three or more antibiotics) was observed in five (3%) patients. Five *E. coli* strains, all from one patient, were resistant to all penicillins and cephalosporins and were producers of extended spectrum beta-lactamases. During stay in the ICU, the number of patients colonized with multidrug-resistant *E. coli* increased significantly, to 28 (15%) ($p < 0.001$).

Upon admission to the general ward, 37 (16%) patients were colonized with at least one *E. coli* isolate that was resistant to one of more antibiotics. During stay

on the general ward, the number increased to 44 (19%) patients. Resistance was most often observed against ampicillin, ampicillin and piperacilin, ampicillin and trimethoprim-sulfamethoxazole, or ampicillin, piperacillin and trimethoprim with sulfamethoxazol (Table 4). Multidrug resistance was found in 17 (7%) patients and did not change during hospitalization.

Multidrug resistance of *Enterobacter* spp, *Klebsiella* spp and *P. aeruginosa* during stay in the ICU and general wards

During stay in the ICU, five (3%) patients were colonized with at least one *Enterobacter* spp that was resistant to three or more antibiotics. An analysis of susceptibility patterns showed that almost half of these *Enterobacter* isolates showed combined resistance to cefuroxime, piperacillin, ceftazidime, piperacillin/tazobactam and ciprofloxacin. Multidrug resistance in *Klebsiella* spp was detected in two patients, whereas four patients carried multidrug resistant *P. aeruginosa*.

During stay on the general wards, resistance towards three or more antibiotics was only detected for three *Klebsiella* spp isolated from one patient.

Antibiotic use before, during and after hospitalization

The percentages of ICU and general ward patients receiving antibiotics during the 3 months preceding hospitalization were 23 and 25% respectively (Table 1). Before hospitalization, the ICU and general ward population used 7 and 5 DDD/100 inhabitant-days, respectively. The total use of antibiotics during stay in the ICU was 180 DDD/100 bed days. The use of ampicillin and piperacillin, amoxicillin/clavulanic acid and piperacillin/tazobactam, cephalosporins, third generation cephalosporins, carbapenems, fluoroquinolones and aminoglycosides represented 8%, 53%, 5%, 2%, 3%, 11% and 5% of total consumption, respectively, in the ICU. After discharge from the ICU, 71 DDD/100 bed days was used in the general ward. The general ward population used 67 DDD/100 bed days during stay on the general ward. During the 3 months after discharge from the hospital, the general ward population consumed 7 DDD/100 inhabitant-days whereas the ICU population used 5 DDD/100 inhabitant-days.

DISCUSSION

This study documents several interesting features of the colonization and resistance dynamics of aerobic gram-negative bacteria in the intestinal and oropharyngeal microflora of patients admitted to ICUs and general wards in a tertiary care hospital.

Intestinal colonization rates. Large differences in the prevalence of intestinal colonization by gram-negative bacteria were observed at different times. The lowest colonization rate was observed during hospital stays both in the ICU and in the general ward population. Colonization rates increased after discharge from the hospital. Several factors may have contributed to the differences in gram-negative colonization rates. First, the use of antibiotics might have suppressed the gram-negative bacteria to concentrations below the detection limit (31). In that case, the results would reflect hospital practice. This hypothesis is supported by the fact that the colonization rates at different times and between the two populations were inversely related to the measured levels of antibiotic use. Thus, the higher the levels of antibiotics used, the lower the gram-negative colonization rate. Secondly, either fresh stool specimens or rectal swabs were collected, and a bias may have been introduced at this point. For the ICU population, all patient samples upon admission and at discharge from the ICU were collected with rectal swabs, whereas at discharge from the hospital, stool specimens were collected. For the general ward population, the percentages of cultures that were as rectal swabs upon admission and at discharge from the hospital were 77 and 45%, respectively. For both populations, stool specimens were collected 1 and 3 months after patients were discharged from the hospital. We collected these different specimens on the assumption that no differences in recovery exist between the two sampling methods (32). In a pilot study that was performed on healthy individuals prior to the present investigation, no differences in the predominant flora recovered from both types of specimens were found (data not shown). Although clear instructions for the collection of swabs from the rectum were given, one cannot exclude that swabs may occasionally have been taken from the perineum. Thirdly, the recovery of gram-negative bacteria from frozen specimens might have been suboptimal. However, the observations of Bonten et al.(32) do not support this theory. Other studies have also reported low colonization rates of gram-negative or gram-positive bacteria in hospitalized patients (33-35).

Oropharyngeal colonization rates. For both patient populations, the prevalence of oropharyngeal colonization by gram-negative bacteria increased during hospitalization, and these rates were still increased three months after discharge from the hospital. This phenomenon has not been reported previously. We are unaware of any studies that have documented colonization rates with gram-negative bacteria after the discharge of patients from intensive care units and general wards. The very

low colonization rate upon admission in general ward patients is consistent with the results of a recent study demonstrating that healthy individuals rarely carry oropharyngeal gram-negative bacteria (36). Previously, the severity of underlying disease, mechanical ventilation, and the presence of nasogastric feeding tubes were associated with oropharyngeal gram-negative colonization (37-39). Differences in colonization rates between the ICU and general ward population and the high colonization rate in specimens obtained during stay on the ICU confirm these earlier findings.

Species distribution. A change in species distribution among the dominant microflora was observed during hospitalization. Suppression of the normal intestinal flora was more pronounced in the ICU population than in the general ward population. The normal intestinal and oropharyngeal microflora was frequently replaced with *Klebsiella spp*, *Enterobacter spp, S. marcescens* and *P. aeruginosa*. Previous studies have also found that hospitalization has an impact on the species distribution of the aerobic gram-negative fecal flora (20-23).

Prevalence of resistance. For this study, we analyzed the resistance data from a population perspective. A limited number of changes in the prevalence of resistance were found among the different time points. The percentage of ICU patients colonized with ampicillin- and/or cephalothin-resistant fecal *E. coli* was significantly increased at discharge from the hospital. These frequencies did not change during the 3 months after discharge. In The Netherlands, resistance rates to most antibiotics are among the lowest in Europe (40). Ampicillin/amoxicillin, piperacillin, first generation cephalosporins and trimethoprim-sulfamethoxazole are commonly used antibiotics in the hospital and/or in the community (41, 42). Amoxicillin is the most frequently used first-line antibiotic in Dutch primary health care (41). The first generation cephalosporin cefazolin is the drug of choice for surgical prophylaxis (43). The persistence of ampicillin and cephalothin resistance in fecal *E. coli* strains in ICU patients might have consequences for the empirical regimens and surgical prophylaxis used by these patients up to 3 months after discharge. For the general ward population no significant differences in resistance frequencies were found during hospitalization. In a previous study, we assessed the prevalence of resistance of *E. coli* in the intestinal flora of surgical patients upon admission to the hospital, at discharge, and 1 and 6 months after discharge. We found no changes between the different time points (34). As far as we know, no other recent study has measured antibiotic resistance in the intestinal or oropharyngeal microflora during and after hospitalization.

It might be interesting to look for changes in resistance patterns at the individual patient-level. The observed increase in multidrug resistance during stay in the ICU and the increase in resistance with increasing lengths of stay for these patients are supportive of this approach. If changes in resistance do occur at the level of indi-

vidual patients, then an examination of risk factors for the acquisition of antibiotic resistance can be performed (44). It is likely that the more frequent use of antibiotics in the ICU population results in the development of resistance at the level of individual patients (45).

Selection bias. For the present study, different numbers of patients were present at the various time points examined. There is some risk of bias due to the differential loss of follow-up of sicker individuals. In a previous study, it was demonstrated that as the length of ICU stay increased, the incidence of nosocomial infections and antibiotic resistance also increased (46). The observed increasing resistance with increasing length of stay in the ICU may therefore be due to the selection of sicker individuals who use more antibiotics. Three months after discharge, specimens were collected from 61% of patients with an ICU stay of shorter than 12 days and from 38% of patients with a stay of more than 12 days. Moreover, 44 ICU patients and 133 general ward patients could be tracked right through the entire project (all specimens were taken appropriately). We recalculated the resistance rates for these subpopulations. For both populations the same "trends" were observed as those for the entire study population. Therefore, the selection bias appears to be limited.

External validity. Whether the results of this study are representative of the conditions in other hospitals mainly depends on the studied population and the effectiveness of infection control measures taken to prevent cross-colonization. We suppose that most of the observed trends will be universal and can be extrapolated to other hospitals. However, we expect changes to be more extreme with higher consumption of antibiotics. Our findings suggest only modest changes, but this may be due to the stringent usage of antibiotics and intensive infection control measures used in our hospital and in Dutch hospitals in general (41, 42, 47).

For the present study, only patients with a length of stay of 5 days or longer were included, since we hypothesized that these patients were at risk for the acquisition of resistant strains. In addition, patients with human immunodeficiency virus infection, tuberculosis and cystic fibrosis were excluded. It is known that these patients use large volumes of antibiotics and are at high risk for the development of resistant microorganisms. The attribution of this relatively small group of patients to the dissemination of resistant stains into the community by the total hospital population was not taken into account.

In summary, the results of this study show that in patients in the ICU as well as in general ward patients, intestinal colonization rates with gram-negative bacteria are low during hospitalization and increase after discharge from the hospital. Suppression of the normal intestinal microflora is more pronounced for ICU patients than for general ward patients and is reversible. Oropharyngeal colonization rates increase during hospitalization and remain high during the 3 months after discharge from the

hospital. From a population perspective, the risk of dissemination of resistant gram-negative bacteria in the community through hospitalized patients appears to be low for general ward patients but is noticeably higher among ICU patients. Antimicrobial resistance that emerges during the hospitalization of ICU patients may persist after discharge for at least 3 months. Whether bacterial, host or community determinants are responsible for this phenomenon remains to be determined.

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CHAPTER 3.4

Change in colonization and resistance of *Enterococcus* species in patients during and after hospitalization

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ABSTRACT

The colonization and resistance dynamics of *Enterococcus* species in the intestinal microflora of patients admitted to intensive care units (ICU) and general wards were investigated during and after hospitalization. A total of 1,669 specimens were obtained from patients upon admission, once weekly during hospitalization, at discharge from the ICU, at discharge from the hospital, and 1 and 3 months after discharge from the hospital. Five colonies per specimen were selected for identification and susceptibility testing. We observed changes in species distribution during ICU hospitalization only. The isolation of *E. faecium* increased from about 10 to 25% of all enterococcal isolates, at the expense of *E. faecalis*. In general ward patients about 25% were *E. faecium* at the different times. The percentage of ICU patients colonized with high-level gentamicin (HLG) resistant *E. faecalis* increased during hospitalization and decreased in the 3 months after discharge from the hospital. In the IC population the percentage of patients colonized with ampicillin resistant *E. faecium* and HLG resistant *E. faecalis* significantly increased with length of stay. PFGE typing revealed that the majority of these resistant strains were not detected at previous sampling times. This suggests selection of resistant strains rather than development of resistance within strains. The risk of dissemination of resistant *Enterococcus* species from the hospital into the community appears to be low for general ward patients but is noticeably higher among ICU patients

INTRODUCTION

The enterococci have emerged as important causes of nosocomial infections (15). The most frequent hospital infections caused by enterococci are urinary tract infections, bloodstream infections, intra-abdominal infections, skin and soft tissue infections and endocarditis (20). Most clinical infections are due to either *E. faecalis* or *E. faecium* (18). Historically, the ratio of infections due to *E. faecalis* to those due to all other *Enterococcus* species was approximately 10:1 (18). In recent years several studies reported on a decline in this ratio (10, 15, 41). It has been suggested that this shift may be explained by a relatively high increase in the frequency of antibiotic resistance in *E. faecium* (10, 18).

Enterococci possess intrinsic resistance to a diverse range of antimicrobial agents including penicillin, cephalosporins, aminoglycosides (low-level), clindamycin and trimethoprim with sulfamethoxazole (20). In addition, enterococci may acquire resistance to many other antimicrobial agents. Three major forms of acquired resistance emerged over the last decades: (1) ampicillin resistance; (2) high-level gentamicin (HLG) resistance and (3) vancomycin resistance (19). Numerous studies determined the prevalence of resistance in enterococci in the community or hospital (10, 15, 24-27). Of great concern is the emergence of multiple-drug resistance that limits treatment options (11, 19, 24). The spread of multi-resistant enterococci between hospital wards and also inter-hospital (4, 16) may, in part, be explained by the ability of the *Enterococcus* species to colonize the gastro-intestinal tract and skin and to persist in the environment of the human host (3, 6). The relevance of transmission of resistant *Enterococcus* species from hospitals into the community has so far not been studied. The aim of the present study was therefore to accurately assess the epidemiology of *Enterococcus* species colonization and antibiotic resistance in the intestinal microflora of hospitalized patients from admission up to three months after discharge from the hospital.

MATERIALS AND METHODS

Patients and study design

Setting. Erasmus MC is a 1,200-bed university, referral hospital in Rotterdam, the Netherlands. The study was conducted at two ICUs (surgical and neurosurgical) and five general wards, including the departments of internal medicine, pulmonology, neurosurgery, urology and gastroenterology. Patients were enrolled into the study between November 2000 and July 2003. The study was approved by the Medical

Ethics Committee, and informed consent was provided before participation in the study.

Inclusion and exclusion criteria. Patients were eligible for the study if they met all of the following criteria: ≥18 years of age, the length of stay was expected to be ≥ 5 days; and informed consent was given by the patient or his/her representative. The exclusion criteria for patients on general wards were as follows: preceding admission to an ICU or another general ward during the same hospitalization, the presence of an ileostoma, and a diagnosis of human immunodeficiency virus infection, tuberculosis, or cystic fibrosis. The same criteria were applicable for ICU patients except that preceding admission to a general ward was allowed.

Collection of specimens. Rectal swabs (or stool specimens) were obtained within 48 h of admission, once a week if the length of stay was more than 7 days, at discharge from the ICU (ICU patients only), and at discharge from the hospital. At discharge from the hospital and 1 and 3 months after discharge, patients were contacted by mail to send in a stool specimen.

Data collection. The following data were collected: age; gender; reason for admission; severity of acute illness upon admission, graded according to the simplified acute physiologic score for ICU patients (14); co-morbidity upon admission according to the definitions of the Dutch National Intensive Care Evaluation (http://www.stichting-nice.nl); residential status 48 h before admission; hospitalization history in the 3 months prior to admission; and the consumption of antibiotics during hospitalization. Community pharmacies were asked for information about the use of antibiotics in the 3 months prior to and after hospitalization. Patients provided written informed consent for the acquisition of these medication lists.

Antibiotic use during hospitalization was expressed as the number of defined daily doses (DDD) per 100 bed days whereas the use before and after hospitalization was expressed as DDD per 100 inhabitant days. We used the DDD of antibiotics for systemic use listed in the Anatomical Therapeutic Chemical (ATC) Classification System 2003 (40).

Microbiological methods

Isolation of Enterococcus spp. Stool specimens were diluted 1:10 in physiological saline containing 20% glycerol and stored at –20°C. After thawing of the stool specimens, swabs were taken from the 10^{-1} dilutions. Rectal swabs were transported in Amies transport medium, diluted in 1 ml of Stuart transportmedium, stored at –80°C, and thawed before use. All swabs were cultured in a selective, esculin-containing enrichment broth supplemented with 75 mg of aztreonam (Bristol Myers Squibb, Princeton, N.J.) per liter (7). Fifty µl of the esculin-positive broth cultures was subcul-

tured on Colombia bloodagar. The plates were incubated aerobically at 37°C and examined after 18-24 h for growth and colony characteristics. An algorithm was defined for the selection of five colonies on the basis of morphology. This method led to proportional sampling of the microorganisms. When no differences in morphology were observed, five colonies were randomly selected. This study was designed to assess the dominant *Enterococcus* species in the intestinal microflora.

Identification. Identification of the colonies was done with the VITEK 2 system (bioMerieux, Marcy l'Etoile, France) and proprietary data management software (version: VT2-R02.03) by using ID-GPC cards after subculturing on Colombia blood agar.

Determination of susceptibility. MICs were determined with the VITEK 2 system using AST-P524 cards. The breakpoints of the National Committee for Clinical Laboratory standards (NCCLS) were applied (22). The Advanced Expert System of the VITEK 2 system (version: AES.R02.00N) was used for all reading and interpretation of susceptibility results. *E. faecalis* ATCC 29212 was used as reference strain.

Pulsed field gel electrophoresis (PFGE). PFGE was performed as described previously (37). In brief, colonies of an overnight culture, grown on a bloodagar plate, were mixed and suspended in EET buffer (100 mM Na_2EDTA, 10 mM EGTA, 10 mM Tris-HCl; pH 8.0). This suspension was mixed with 100 µl of 1% agarose. Cells in the plugs were lysed, washed, stabilized and restricted with *SmaI*. Electrophoresis was performed and the gel was stained with ethidium-bromide before photography under UV irradiation. The PFGE patterns were interpreted according to previously described criteria (31).

Data analysis

Patients were considered eligible for analysis when at least one consecutive sample was taken following the sample taken upon admission and when the length of stay was ≥ 5 days. Samples collected once weekly during hospital stays were categorized as "during stay". When patients died during stay on the ICU, the last sample taken was also categorized "during stay".

Resistance analyzed at the population level. The percentages of patients colonized with resistant *E. faecalis* and *E. faecium* at different times were calculated for ampicillin, HLG and vancomycin. For each species, we selected one isolate per patient with the most resistant result for that particular antibiotic.

Resistance analyzed at the individual patient level during hospitalization. Fecal samples of the first 100 consecutive ICU and general ward patients, collected on admission, during hospitalization and at discharge from the ICU (only ICU patients) or discharge from the hospital (only general ward patients), were included in this

analysis. For each patient, a number of observational intervals were determined. An observational interval was defined as the time period between two consecutive fecal samples. Each of these intervals was evaluated for the occurrence of a transitional event. A transitional event was defined as 1) a more than twofold increase in the log MIC of a bacterial species, isolated at two consecutive sampling times, for at least one antibiotic or 2) resistance for at least one antibiotic, in a species that was not isolated at previous points in time (breakpoints of the NCCLS were applied). We studied results of susceptibility testing of *E. faecalis* and *E. faecium* to ampicillin, HLG and vancomycin. If a transitional event of the first type was observed, all preceding susceptible and resistant isolates of that particular patient were analyzed by PFGE to assess whether resistance had been developed in indistinguishable isolates or not.

Statistics. Before starting the inclusion we hypothesized that the number of patients with HLG resistant *E. faecalis* and/or ampicillin resistant *E. faecium* might be approximately 5% on admissions and may increase to 15% at discharge from the hospital. Power analysis ($\alpha = 0.05$; ß = 0.2) revealed that approximately 100 patients were needed for each sampling time. To achieve this, we decided to include 200 patients in each arm. To test for differences in resistance frequencies between the different times, we used the chi-square test or Fisher's exact test (in cases of low (≤ 5) cell numbers).

We used logistic regression analysis to assess whether the percentages of patients colonized with resistant bacteria were associated with the length of stay in the ICU or general ward.

P values of < 0.05 were considered significant. The data were analyzed by using SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois) and GraphPad Prism version 3.0 for Windows (GraphPad Software, San Diego, California).

RESULTS

Inclusion and follow-up of study population

A total of 200 ICU and 319 general ward patients were included in this prospective study. The data for 17 ICU and 91 general ward patients were not analyzed. The reasons for withdrawal from the analyses were a length of stay less than 5 days (for the ICU group, $n = 9$; for the general ward group, $n = 52$) and no collection of a second specimen following admission (for the ICU group, n = 8; for the general ward group, n = 39).

Table 1 shows the characteristics of the two enrolled populations. Although, in both populations almost 50% of the patients were admitted for surgery, the types of surgery leading to admission were different. ICU patients were most often admit-

ted for abdominal surgery, transplantation or neurosurgery, whereas general ward patients were admitted for neurosurgery (at the most 24h post-operative ICU stay) and urologic surgery. The numbers of ICU and general ward patients analyzed at the different times are presented in Table 2. Patients were lost to follow-up as a result of death, transfers, and withdrawal from the study.

Thirty-six patients (19.6%) were already hospitalized on a general ward before admission to the ICU (Table 1). The median length of stay on the participating units was 12 days (range, 5 to 119 days) for the ICU patients (n = 183) and 10 days (range, 5 to 116 days) for the general ward population (n = 228). Patients who died during stay on the ICU had a median length of stay of 22 days (range, 7 to 119 days; n = 25). The median length of stay in the hospital after discharge from the ICU was 15 days (range, 0 to 281 days; n = 158).

Table 1. Patient characteristics on admission

Characteristic	Intensive care units (n = 183)	General wards (n = 228)
Age (mean ± SD), y	55.9 ± 15.6	57.1 ± 14.0
Men, *n (%)*	120 (65.6)	135 (59.2)
Reason for admission, *n* (%)		
Surgery		
Abdominal	53 (29.0)	2 (0.9)
Neurosurgery	32(17.5)	67 (29.4)
Transplantation	14 (7.7)	-
Urologic surgery	-	46 (20.2)
Other surgery	20 (10.9)	-
Trauma	29 (15.8)	1 (0.4)
Medical		
Abdominal	-	20 (8.8)
Neurological disorder	13 (7.1)	1(0.4)
Obstructive pulmonary disease	5 (2.7)	54(23.7)
Other medical disorder	17(9.3)	37 (16.2)
Residential status 48 hours before admission, *n (%)*		
Community	145 (79.2)	228 (100)
General ward Erasmus MC	29 (15.8)	-
General ward, other hospital	7 (3.8)	-
Other	2 (1.1)	-
SAPS II on admission (± SD)[a]	33.5 (13.9)	-
Admissions with comorbidity[b]	54 (29.5)	84 (36.8)
Hospitalization 3 months prior to admission	101 (55.2)	52 (23)
Use of antibiotics 3 months prior to admission	42 (23)	57 (25)

[a] Simplified Acute Physiology Score(14)

[b] According to the definitions of the Dutch National Intensive Care Evaluation (www.stichting-nice.nl). Co-morbidity was defined as at least 1 of the following disorders: decreased immunity, respiratory insufficiency class IV according to New York Heart Association (NYHA), cardiovascular insufficiency class IV according to NYHA, cirrhosis, hematological malignancy, AIDS, neoplasm with metastases, chronic renal failure.

Table 2. Frequencies of *Enterococcus* species isolated from the intestinal microflora during of ICU and general ward patients during and after hospitalization

	ICU					General ward			
	Admission ICU	Discharge ICU	Discharge Hospital	1 month after discharge hospital	3 months after discharge hospital	Admission General ward	Discharge hospital	1 month after discharge hospital	3 months after discharge hospital
Patients, *n*	183	150	62	82	73	228	205	185	167
Samples with detectable *Enterococcus* species, *n* (%)	86 (47)	91 (61)	43 (69)	57 (69)	56 (76)	107 (47)	133 (64)	113 (61)	104 (62)
Isolates, *n*	424	455	215	282	280	506	654	554	491
Enterococcus species *(%)*:									
E. faecalis	76.7	73.4	56.3[a]	53.9	50.7	61.9	61.5	57.6	59.3
E. faecium	10.8	13.6	23.7[a]	26.2	25.9	22.1	24.0	26.5	23.4
E. hirae	5.4	3.9	7.0	6.0	9.2	6.9	5.7	6.9	7.3
E. gallinarum / E. cassiflavum	2.6	4.6	10.2[a]	6.0	6.7	4.9	7.3	5.8	6.7
E. durans	1.4	1.1	2.8	3.5	4.3	3.6	1.1[a]	1.4	3.3[c]
E. avium	3.1	3.5	-[a]	4.3[b]	3.2[c]	0.6	0.5	1.8[b]	-

For each unit the frequencies were compared in defined pairs (see a-c), using the chi-squared analysis or Fisher's exact test as applicable ($p<0.05$ was considered significant).

[a] significant difference for discharge from hospital versus admission on ICU or general ward

[b] significant difference for 1 month after discharge from hospital versus discharge from hospital

[c] significant difference for 3 months after discharge from hospital versus discharge from hospital

Bacterial strains

In total, 1,669 specimens from 411 patients were collected. From these specimens 5,121 *Enterococcus* species (3,304 *E. faecalis*, 1,034 *E. faecium*, 280 *E. hirae*, 317 *E. gallinarum/ E. casseliflavus*, 98 *E. durans and 88 E. avium)* were isolated, identified and tested for antimicrobial resistance.

Species distribution during and after hospitalization

ICU population. A total of 797 fecal specimens were studied. The percentage of fecal samples with detectable *Enterococcus* species varied from 47% for samples collected on admission to 76% for samples collected 3 months after discharge from the hospital (Table 2). In the ICU population the frequency of *E. faecium* in the intestinal microflora was low on admission (10.8%) and increased significantly to 23.7% at discharge from the hospital ($p < 0.001$), whereas the frequency of *E. faecalis* decreased from 76.7% upon admission to 56.3% at discharge from the hospital ($p < 0.001$). Both frequencies did not change during the first 3 months after discharge from the hospital (Table 2).

General ward population. A total of 872 fecal specimens were studied. Upon admission of patients to the general ward, 47% of the fecal specimens yielded detectable *Enterococcus* species, whereas for samples collected at later times these percentages varied between 61 and 64% (Table 2). No significant changes in the frequencies of the different *Enterococcus* species in intestinal microfloras were observed during and after hospitalization. Only upon admission and at discharge from the ICU, the frequencies of the different *Enterococcus* species in the ICU patients differed from the frequencies found for general ward patients (Table 2).

Percentage of patients colonized with resistant *Enterococcus* species during and after hospitalization

In both patient populations, no significant differences in the percentages of patients colonized with ampicillin-, HLG- or vancomycin resistant *Enterococcus* species were found at the different times (Table 3). Ampicillin resistance was low in both *E. faecalis* and *E. faecium*, and vancomycin resistance was not detected at all. The percentage of patients colonized with HLG resistant *E. faecalis* at discharge from the hospital was significantly higher in the ICU than in the general ward population (11.3 and 3.4%, $p = 0.02$).

The percentages of patients colonized with gentamicin resistant *E. faecalis* strains (Figure 1a) or ampicillin resistant *E. faecium* strains (Figure 1b) were significantly associated with the length of stay in the ICU (by logistic regression, the P values were < 0.001). However, we did not notice a significant trend of increasingly resistant *Enterococcus* species with increasing lengths of stay among general ward patients.

Table 3. Proportion of ICU and general ward patients colonized with resistant *Enterococcus faecalis* and *Enterococcus faecium* in the intestinal microflora during and after hospitalization[a, b, c]

	ICU					General ward			
Antibiotic (s)	Admission ICU (*n*= 183)	Discharge ICU (*n* = 150)	Discharge Hospital (*n* = 62)	1 Month after discharge hospital (*n* = 82)	3 Months after discharge hospital (*n* = 73)	Admission General ward (*n* = 228)	Discharge hospital (*n* = 205)	1 Month after discharge hospital (*n* = 185)	3 Months after discharge hospital (*n* = 167)
E. faecalis									
Ampicillin	0.5	0.7	3.2	-	-	-	1.0	-	-
Vancomycin	-	-	-	-	-	-	-	-	-
Gentamicin high level resistance	6.0	10.0	11.3	8.5	5.5	2.6	3.4[d]	2.7	2.4
E. faecium									
Ampicillin	2.2	2.7	4.8	1.2	4.1	1.8	3.9	2.7	3.0
Vancomycin	-	-	-	-	-	-	-	-	-
Gentamicin high level resistance	0.5	-	-	-	-	-	-	-	-

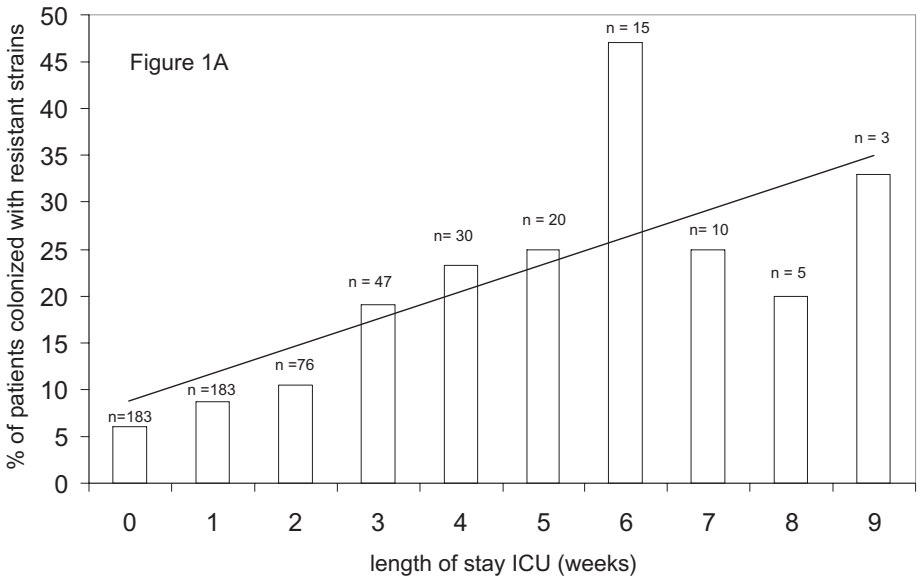
[a] NCCLS breakpoints were applied.

[b] One isolate per patient was included, the most resistant result for each antibiotic.

The proportions of colonized patients were compared in defined pairs (c-d), using the chi-squared analysis or Fisher's exact test as applicable ($p<0.05$ was considered significant).

[c] No significant differences were found in resistance rates when comparing discharge versus admission ICU, discharge hospital versus admission ICU or general ward, 1 month after discharge versus discharge hospital and 3 months after discharge versus discharge hospital.

[d] Significant difference for discharge hospital (general ward) versus discharge hospital (ICU)



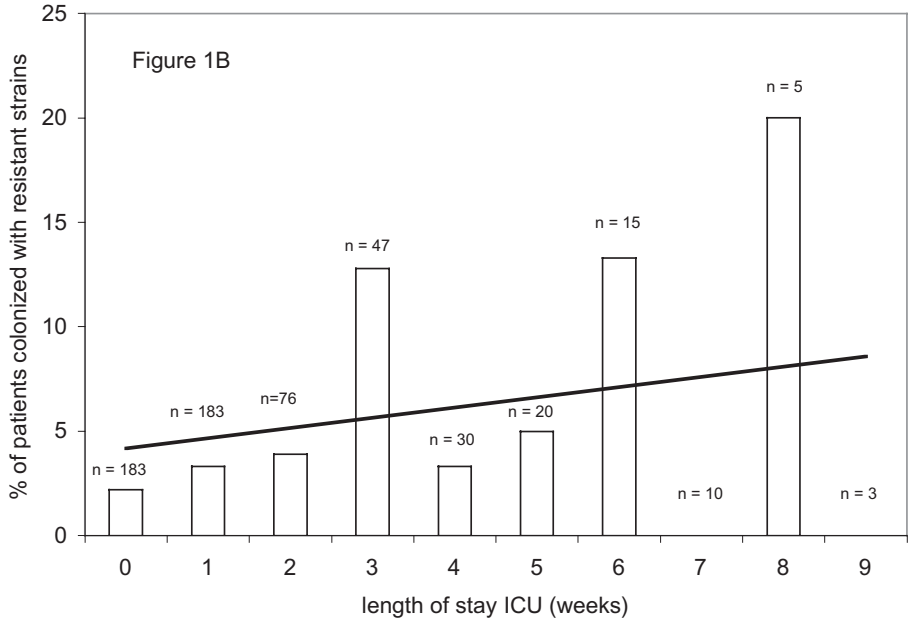


Figure 1. Effect of length of stay on high level gentamicin resistant Enterococcus faecalis (1A) and ampicillin resistant Enterococcus faecium (1B) isolated from the intestinal microflora of patients admitted to the ICU.

Table 4. Genetic relatedness and emergence of resistance among *E. faecalis* and *E. faecium* isolated during hospitalization.

				PFGE type[a]							
Enterococcus species	Patient No.	Patient population[b]	Admission	During stay ICU resp. general ward (no. of weeks)						Discharge ICU	Discharge hospital
				1	2	3	4	5	6		
	1	IC		aaabb	a	CCCCC					
High level	2	IC	aaaa	aaa	aaaa	aaaa	BB				
gentamicin	3	IC	aaa	AAAAA							
resistance in	4	IC	aaaaa	bbbbb	ccccc				DDDDD		
E. faecalis	5	IC	a							BBBBB	
	6	GW	aaaaa								BBBBB
	2	IC		aa			bbb		C		
	7	IC	ab	CCC							
Ampicillin	8	IC			a				Bb		
resistance in	9	IC				aaaaa			Bbbbb		
E. faecium	10	GW	ab	Cd							
	11	GW		aa							bCDc
	12	GW	a	bBBBB							

[a] Genetic relatedness has been assessed at the patient level. Different genotypes within one patient were designated with different letters; lower case letters for susceptible strains and capital letters for resistant strains. No comparisons can be made between strains of different patients.

[b] IC = intensive care; GW = general ward

Genetic relatedness and resistance among *E. faecium* and *E. faecalis* isolated during hospitalization

In 21 patients (for the ICU group, n = 15; for the general ward group n = 6) HLG resistant *E. faecalis* emerged during hospitalization in the ICU or general ward. In 15 of these patients gentamicin susceptible *E. faecalis* isolates were not detected at any of the previous sampling times and in 6 patients HLG susceptible *E. faecalis* isolates were found on at least one of the previous sampling times. Only in 1 out of these 6 patients resistance towards HLG developed in a susceptible *E. faecalis strain*s (Table 4).

In 19 patients (for the ICU group, n = 9; for the GW group n = 10) ampicillin resistant *E. faecium* emerged during hospitalization. In 7 of these patients ampicillin susceptible *E. faecium* isolates were found on at least one of the previous sampling times. In none of these patients indistinguishable or closely related fingerprints of the ampicillin susceptible and resistant *E. faecium* strains were found (Table 4).

Antibiotic use before, during and after hospitalization

The percentages of ICU and general ward patients receiving antibiotics during the 3 months preceding hospitalization were 23 and 25% respectively (Table 1). Before hospitalization, the ICU and general ward population used 7 and 5 DDD/100 inhabitant-days respectively. Total use of antibiotics during stay on the ICU was 180 DDD/100 bed-days. The use of ampicillin and piperacillin, amoxicillin/clavulanic acid and piperacillin/tazobactam, cephalosporins, third generation cephalosporins, carbapenems, fluoroquinolones and aminoglycosides represented 8%, 53%, 5%, 2%, 3%, 11% and 5% of total consumption in the ICU, respectively. After discharge from the ICU, 71 DDD/100 bed-days were used at the general ward. The general ward population used 67 DDD/100 bed-days during stay on the general ward. During the three months after discharge from the hospital the general ward population consumed 7 DDD/100 inhabitant-days whereas the ICU population used 5 DDD/100 inhabitant-days.

DISCUSSION

We extensively studied colonization and resistance dynamics of *Enterococcus* species in the intestinal microflora of adult patients admitted to our intensive care units (ICUs) and several general wards.

Colonization rates. In both groups *Enterococcus* species were less frequently found on admission than during hospitalization. One and three months after discharge colonization rates remained stable and comparable to those during hospitalization. These colonization rates were comparable with those found in a previous study

performed in our hospital, in which *Enterococcus* species were found in about 50% of hospitalized patients (7). In the literature colonization rates vary from 50-60% (13, 26, 35) to 90% (29). Various exogenous influences may have affected the recovery of *Enterococcus* species from our specimens. First, the use of antibiotics might have suppressed the *Enterococcus* species to concentrations below the detection limit (28). Secondly, either fresh stool specimens or rectal swabs were collected and a bias may have been introduced at this point. We collected these different specimens on the assumption that no differences in recovery exist between these two sampling methods (2). Thirdly, the recovery of *Enterococcus* species from frozen specimens might have been suboptimal, although observations of Bonten et al. refute this theory (2).

Species distribution. We observed changes in species distribution during ICU hospitalization only. *E. faecium* isolation increased from about one-tenth to a quarter of all enterococcal isolates, at the expense of *E. faecalis*. In general ward patients, at all sampling times, about a quarter of isolates were *E. faecium*. This relatively high proportion of *E. faecium* is consistent with figures reported in other studies (10, 15, 26, 27). The observed species distribution in patients on admission (specimens collected within 48h of admission) and at discharge from the ICU may reflect the higher selective pressure exerted by antibiotics administered for abdominal surgery, transplantation or empirical therapy for severe infection at the time of admission to the ICU as compared to the general ward population. ICU patients were also more often hospitalized and used more antibiotics in the three months prior to admission as compared to the general ward population. Differences in antimicrobial susceptibilities within the genus might have led to inhibition of susceptible members of the indigenous microflora and facilitate overgrowth of antibiotic-resistant microorganisms.

Prevalence of resistance. In both patient populations no significant differences were found in ampicillin-, HLG - or vancomycin resistant *Enterococcus* species. As resistance percentages were lower as expected, the power of our study is not sufficient to conclude that no minor differences exist between the different sampling times. In particular, in the ICU population an increasing trend in HLG resistance in *E. faecalis* was observed during hospitalization, that decreased after discharge from the hospital.

At discharge from the ICU and general ward, between 2.7 and 4.8% of the patients were colonized with ampicillin resistant *E. faecium*. These results are in accordance with a report from Norway, where 6.9% of hospitalized patients were fecal carriers (9). The prevalence found in Swedish hospitals however was significantly higher (22%) (33). In a study performed in North Carolina, United States, 5.4% of the patients were colonized with ampicillin resistant *E. faecium* on admission and 9.4% of patients acquired ampicillin resistant *E. faecium* during hospitalization (42).

HLG resistance was particularly found in *E. faecalis*. This is also observed by several other investigators (5, 21, 27). Overall 4.1% of patients carried a HLG resistant *E. faecalis* isolate on admission. We observed no significant trend in this rate during hospitalization. In a previous study we assessed the prevalence of resistance of *E. faecalis* in the intestinal flora of surgical patients on admission to the hospital, at discharge, and 1 and 6 months after discharge. We found no changes between the different sampling times (23). Few other studies have examined the carrier rate of HLG resistant *Enterococcus* species in hospitalized patients (9, 17, 34, 43). In Norway, prevalence rates of 3.3-7% were reported (9, 34). In a Turkish hospital 8.2% of the patients were colonized with HLG-resistant *Enterococcus* species (17). The prevalence found in a United States hospital was significantly higher (36.1%) (43).

Vancomycin resistance was not detected in our study. Screening with a highly sensitive method (selective broth) for fecal carriage of vancomycin resistant enterococci (VRE) in ICU's and hematology-oncology wards in nine hospitals in the Netherlands between 1995 and 1998 revealed that only 1.4% of these high-risk patients carried VRE(36). In 2000, outbreaks of VRE occurred in three hospitals in the Netherlands (16, 32, 39). Recently it was found that VRE were isolated from 2.7% of patients repatriated from foreign hospitals to The Netherlands (12). The incidence of vancomycin resistant *Enterococcus* species (VRE) infections in hospitalized patients is low in Europe as compared to the United States (8). As the carrier rates of VRE appears to be very low in The Netherlands, it is not surprisingly that we found no VRE among the dominant microflora in our study.

During hospitalization resistance emerged in ICU (23%) as well as in general ward patients (16%). On patient level, one third of these ICU patients in whom resistance emerged died, and one third lost resistance before discharge. Also, half of the general ward patients who gained a resistant strain during admission appeared to be negative again at discharge from the hospital. Since the absolute number of patients colonized with resistant strains is low, these percentages did not result in significant changes in prevalence at discharge from the hospital. Furthermore, PFGE typing revealed that the majority of resistant strains were not detected on previous sampling times. This suggests selection of a resistant strain rather than development of resistance within strains. Selection may either have occurred within the patients own strains, or patients may acquire resistant strains by horizontal transfer between patients.

The prevalence of ampicillin resistant *E. faecium* and HLG resistant *E. faecalis* significantly increased with length of stay on the ICU. This confirms the findings of a previous study in which duration of ICU stay was positively associated with antibiotic resistance (1).Risk factors for the acquisition of resistant *Enterococcus* species during hospitalization remain to be determined.

Selection bias. In the present study different numbers of patients were sampled at the various sampling times. There is some risk of bias due to differential loss to follow-up of sicker individuals. Our observation of increasing resistance with increasing length of stay on the ICU may be due to selection of sicker individuals who use more antibiotics. In other words, length of stay may be associated with severity of disease. Three months after discharge specimens were collected from 61% of the patients with an ICU stay shorter than 12 days and from 38% of the patients with a stay of more than 12 days. Moreover, 42 ICU patients and 136 general ward patients could be tracked through the entire study period. We recalculated the resistance rates for these subpopulations. For both populations, the same "trends" were observed as those for the entire study population. Therefore, the selection bias appears to be limited.

External validity. In the present study only patients with a length of stay of five days or longer were included since we hypothesized that these patients were at risk for the acquisition of resistant strains. We suppose that most of our epidemiological findings will have universal relevance, and can be extrapolated to other hospitals. However, we expect changes in resistance during hospitalization to be more extreme in settings with higher consumption of antibiotics. The modest changes found in our study might be due to stringent antibiotic policies and the emphasis on infection control measures in our hospital, and in Dutch hospitals in general (30, 38).

In summary, this is the first specifically designed study that examines the epidemiology of colonization and resistance dynamics of *Enterococcus* species in the intestinal microflora of ICU and general ward patients during and after hospitalization. We observed changes in species distribution during ICU hospitalization only. The isolation of *E. faecium* increased from about 10 to 25% of all enterococcal isolates, at the expense of *E. faecalis*. In both patient populations no significant differences were found in the proportion of patients colonized with ampicillin-, HLG- or vancomycin- resistant *Enterococcus* species at different times. However, an increasing trend in resistance during hospitalization, followed by a decrease in resistance after discharge from the hospital has been observed for HLG resistant *E. faecalis* in the ICU population. Moreover, in the ICU population the prevalence of ampicillin resistant *E. faecium* and high-level HLG resistant *E. faecalis* significantly increased with length of stay in the ICU. PFGE typing revealed that the majority of these resistant strains were not detected on previous sampling times. This suggests selection of resistant strains rather than development of resistance within susceptible strains. From a population perspective, the risk of dissemination of resistant *Enterococcus* species from the hospital into the community appears to be low upon discharge of general ward patients but is noticeably higher among ICU patients.

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CHAPTER 3.5

Risk factors for colonization with antibiotic resistant *Enterobacteriaceae* and *P. aeruginosa* in hospitalized patients

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ABSTRACT

Objectives. The incidence and risk factors for the emergence of antibiotic resistance in *Enterobacteriaceae* and *Pseudomonas aeruginosa* were investigated.

Methods. Fecal and oropharyngeal specimens were obtained from 411 patients on admission and weekly during hospitalization. A transitional event was defined as a more than twofold increase in the log MIC of a bacterial species, isolated at two consecutive sampling times, or resistance in a species that was not isolated at a previous sampling time. If a transitional event of the first type was observed, all preceding isolates of the same species were genotyped. Risk factors for transitional events were studied using multivariate Cox's regression analysis.

Results. The incidence of transitional events was almost 3 times higher in ICU patients than in general ward patients. PFGE typing revealed that the majority of resistant strains were not detected at previous sampling times, suggesting horizontal transfer between patients. Admission to the urology department was associated with trimethoprim-sulfamethoxazole resistance in *Escherichia coli* and *Klebsiella* species (adjusted HR (AHR) = 3.7; 95% CI, 1.1 to 12.7) whereas prior fluoroquinolone use was negatively associated (AHR = 0.11; 95% CI , 0.02 to 0.5). Use of ampicillin was associated with ceftazidime resistance in gram-negative bacteria (AHR = 8.8; 95% CI, 1.5-52.6). Risk factors for fluoroquinolone resistance in *P. aeruginosa* were transfer from a general ward (AHR = 0.22; 95% CI, 0.05 to 0.9) and prior use of third generation cephalosporins (AHR = 5.5; 95% CI, 1.1-36.7).

Conclusions. The high number of transitional events and the results of the risk factor analysis warrant two intervention strategies. Besides policies to promote rational use of antibiotics, emphasis must also be placed on infection control measures.

INTRODUCTION

Infections caused by antibiotic resistant microorganisms are associated with greater mortality, morbidity and costs relative to antibiotic-sensitive bacterial infections, particularly when inadequate empiric antimicrobial treatment is administered (1-4). Bacterial colonization is often a first step in the pathogenesis of nosocomial infections (5). Different mechanisms may lead to colonization of hospitalized patients with resistant strains (6, 7). First, these strains may enter the hospital upon the admission of patients already colonized with resistant strains. Secondly, during hospitalization, susceptible bacteria may develop resistance due to genetic mutations or through transfer of resistance genes. Thirdly, resistance may emerge through induction of genes that are already present in susceptible bacterial subpopulations. A lack of hygiene and infection control may facilitate the spread of these resistant bacteria between patients and hospital staff. Colonization with resistant bacteria may remain undetectable within a largely susceptible microflora until, because of the selection pressure exerted by antibiotics, bacterial outgrowth of the resistant pathogen occurs.

Emergence of resistance may lead to inappropriate antibiotic therapy when patients are not switched to regimens effective against the resistant microorganisms. Effort should therefore be directed towards early detection of resistance and the delineation of risk factors for emergence of resistance during hospitalization. Numerous previous studies have identified risk factors for antibiotic resistance. However, most studied clinical isolates and only in few studies case patients were identified through systematic surveillance cultures (8-15). Since bacterial colonization often precedes infections (5) it might be argued that in a case-control study of risk factors for antibiotic resistance, case patients should be defined as all patients colonized with the antibiotic-resistant microorganism (16).

The aim of the present study was, therefore, to investigate risk factors for the emergence of antibiotic resistance in *Enterobacteriaceae* and *Pseudomonas aeruginosa* in the oropharyngeal and fecal microflora of patients during stay in the hospital.

MATERIALS AND METHODS

Study population

The Erasmus MC is a 1200-bed university, referral hospital in Rotterdam, The Netherlands. The study was conducted in two ICU's (surgical and neurosurgical) and in general wards of the departments of internal medicine, pulmonology, neurosurgery, urology and gastro-enterology. Patients were enrolled between November 2000 and July 2003. The study was approved by the Medical Ethics Committee and informed

consent was obtained before participation in the study. Patients were eligible if they met all of the following criteria: ≥18 years of age; expected length of stay ≥ 5 days; informed consent given by the patient or his representative. The exclusion criteria for patients on general wards were: preceding admission to an ICU or another general ward during the same hospitalization; presence of an ileostoma; diagnosis of human immunodeficiency virus infection, tuberculosis or cystic fibrosis. The same criteria were applicable for ICU patients except that preceding admission to a general ward was allowed. Rectal swabs (or stool specimens) and oropharyngeal swabs were obtained within 48 hours of admission and thereafter weekly to the time of discharge from the ICU (ICU-patients) or hospital (general ward patients). Patients were considered eligible for analysis when at least one sample was taken following the sample upon admission and when the length of stay was ≥ 5 days.

Microbiological methods

Stool specimens were diluted 1:10 in physiological saline containing 20% glycerol, and stored at –20°C. After thawing, 10^{-2} and 10^{-4} dilutions in physiological saline were inoculated on chromogenic plates (Chromagar Orientation (Becton Dickenson, Heidelberg, Germany). Swabs were diluted in 1 ml of Stuart transport medium and stored at –80°C until assayed. After thawing the samples were further diluted. Fifty µl of the undiluted suspension and 50 µl of a 10^{-1} dilution in physiological saline were inoculated on chromogenic plates. The plates were incubated aerobically at 37°C and examined after 18-24 hours for growth and colony characteristics. The dilution containing ≤ 100 colonies was used for the selection of five colonies. An algorithm was developed for the selection of these five colonies (17). This method led to a proportional sampling of the microorganisms. E.g., if 60 pink colonies and 40 blue colonies grew on the chromogenic agar, the proposed algorithm resulted in selecting three pink and two blue colonies. Minimal inhibitory concentrations (MICs) were determined by the VITEK 2 system using AST-N010/020 cards (bioMérieux, Marcy l'Etoile, France). Breakpoints of the National Committee for Clinical Laboratory Standards (NCCLS) were applied (18). The Advanced Expert System of the VITEK 2 system (version: AES.R02.00N) was used for all reading and interpretation of susceptibility results. *Escherichia coli* (ATCC 25922 and ATCC 35218) and *P. aeruginosa* (ATCC 27853) were used as reference strains.

Risk factors for resistance during hospitalization

Definition transitional events. Fecal and oropharyngeal samples were analyzed separately. First, the observational intervals were determined for each patient. An

observational interval was defined as the time period between two consecutive fecal or oropharyngeal samples. Each of these intervals was evaluated for the occurrence of one or more transitional events. A transitional event was defined as 1) a more than twofold increase in the log MIC of a bacterial species, isolated at two consecutive sampling times, for at least one antibiotic or 2) resistance for at least one antibiotic, in a species that was not isolated at previous points in time (breakpoints of the NCCLS were applied). If a transitional event of the first type was observed, all preceding susceptible and resistant isolates of that particular patient were genotyped by pulsed field gel electrophoresis (PFGE) as described previously (19). Isolates were considered to be clonally related if their genotype patterns did not differ by more than 3 bands, according to standard criteria (20).

Outcome definition. On the basis of a previous study on the epidemiology of antibiotic resistance in *Enterobacteriaceae* and *P. aeruginosa* (21), and general knowledge of phenotypes and resistant mechanisms (22), we assessed the following five transitional events: 1) amoxillin with clavulanic acid, piperacillin with tazobactam, cefoxitin and/or cefuroxime resistance in *E.coli*, *Klebsiella species* or *Proteus mirabilis* (Group I microorganisms); 2) fluoroquinolone resistance in *E. coli*, *Klebsiella species*, *Proteus species*, *Serratia marcescens*, *Enterobacter species* and *Citrobacter freundii* (Group II microorganisms); 3) trimethoprim with sulfamethoxazole resistance in group I microorganisms; 4) ceftazidime resistance in group II microorganisms or *P. aeruginosa*; and 5) fluoroquinolone resistance in *P. aeruginosa*. For each population and specimen type the incidence of transitional events was expressed as the number of transitional events per 1000 observation days. The number of observation days was defined as the number of days between admission and the day the last specimen was taken.

To assess risk factors for antibiotic resistance, separate analyses were performed for each of the transitional events. Results of susceptibility testing of species isolated from fecal as well as oropharyngeal samples were included in the analysis. For all case patients, the observation continued from time of admission to the occurrence of a transitional event. Patients who did not reach an outcome status were censored at time of discharge or death.

Independent variables. Potential risk factors were selected on the basis of a systematic review of previous studies assessing risk factors for colonization or infection with various (multi)resistant microorganisms (10, 12, 15, 23-46). The following baseline patient characteristics were considered as potential determinants of the occurrence of transitional events during hospitalization: age, gender, reason for admission, residential status 48 hours before hospitalization, co-morbidity on admission according to the definitions of the Dutch National Intensive Care Evaluation (http://www.stichting-nice.nl), severity of acute illness on admission graded according to the simplified acute

physiologic score (SAPS II) (47) and the type (ICU or general ward) and medical specialty of the ward the patient was admitted to. We also considered colonization with the particular resistant microorganism on admission as a potential risk factor for the occurrence of the particular transitional event during hospitalization. The exposure to different subgroups of antibiotics during hospitalization was assessed: ampicillin and/or piperacillin; amoxicillin with clavulanic acid and/or piperacillin with tazobactam; flucloxacillin; first, second and third generation cephalosporins; carbapenems; aminoglycosides; fluoroquinolones; and trimethoprim with sulphamethoxazole. Also treatment with steroids and stress-ulcer prophylactic agents were considered as risk factors. Finally, exposure to medical devices are included as potential risk factors. The following invasive devices were assessed: mechanical ventilation tube, central and peripheral venous catheters, arterial catheters, urinary catheters, nasogastric catheters, extra ventricular or liquor drain and parenteral nutrition.

Statistical analysis. Risk factors for the occurrence of transitional events during hospitalization were identified by means of bivariate and subsequent multivariate Cox-regression analysis. The multivariate models included all factors from bivariate analysis that were associated with the occurrence of a transitional event at a P value < 0.1. Statistical significance was accepted at a two sided P value of 0.05. Results are reported as (adjusted) hazard ratios (HR) with 95% confidence intervals (CI). All statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois).

RESULTS

Inclusion of the study population. A total of 411 patients were included. Important characteristics of the study population are shown in Table 1. One hundred and eighty-three patients were admitted to the ICU and 228 patients to the general ward. Thirty-six ICU patients were already hospitalized on a general ward before admission to the ICU. Median length of stay on the participating units was 12 (5-119) days for the ICU patients and 10 (5-116) days for the general ward population. Seventy-five percent of the patients had been exposed to antibiotics (Table 2). Amoxicillin-clavulanic acid is the most commonly used antibiotic. Antibiotic use varied largely between the different ward locations (Table 2). Total systemic use in the surgical ICU was approximately three times higher than in the urology ward.

Species distribution on admission and during hospitalization. 2273 specimens from 411 patients yielded 3328 gram negative bacteria. A significant increase in the isolation frequencies of *Enterobacter* spp, *Klebsiella* spp, *P. aeruginosa* and *S. marcescens*, and a significant decrease in the frequency of *E. coli* was found in the fecal

Table 1. Descriptive characteristics of patient cohort

Characteristics	Total cohort (*n* = 411)
Male gender	255 (62%)
Age (mean ± SD), yrs	56.6 ± 14.7
Reason for admission, n (%)	
Surgical	234 (56.9%)
Trauma	30 (7.3%)
Medical	147 (35.8%)
Residential status 48 hours before admission, *n (%)*	
Community	373 (90.8%)
General ward	36 (8.8%)
Other	2 (0.5%)
Type of hospital ward	
ICU	183 (44.5%)
General ward	228 (55.5%)
SAPS II on admission (mean ± SD)[a]	33.5 ± 13.9
Admissions with comorbidity[b], *n* (%)	138 (33.6%)
Steroid treatment[c]	109 (54.5%)
Treatment with H_2 blockers, antacids, proton pump inhibitors[c]	132 (66.0%)
Presence of invasive devices[c]	
Mechanical ventilation tube	90 (45.0%)
Peripheral venous catheters	174 (87.0%)
Central venous catheters	56 (28.0%)
Arterial catheters	105 (52.5%)
Nasogastric catheters	94 (47.0%)
Urinary catheter	122 (61.0)
Extra ventricular or liquor drain	18 (9.0%)
Parenteral nutrition	32 (16.0%)

[a] Simplified Acute Physiology Score (47); only determined in ICU patients

[b] According to the definitions of the Dutch National Intensive Care Evaluation (www.stichting-nice.nl). Comorbidity was defined as at least one of the following disorders: decreased immunity, respiratory insufficiency class IV according to New York Heart Association (NYHA), cardiovascular insufficiency class IV according to NYHA, cirrhosis, hematologic malignancy, AIDS, neoplasm with metastases, or chronic renal failure;

[c] Assessed in the first 100 consecutive ICU and general ward patients.

microflora of patients during their stay in the ICU. In samples collected at the general wards, a significant increase in the frequency of *Klebsiella* spp. and a significant decrease in the isolation frequency of *E. coli* were found.

Upon admission of patients to the ICU, 18% of the oropharyngeal samples yielded gram-negative bacteria whereas during stay in the ICU the recovery rate increased significantly to 37%. During hospitalization in the general wards, the number of oropharyngeal samples with gram negative bacteria also increased significantly, from 1% on admission to 8% during hospitalization. Among the species cultured, *E. coli* was prevalent but less than compared to the fecal microflora. The frequencies of *P. aeruginosa* and *S. marcescens* in oropharyngeal samples from ICU patients increased significantly during hospitalization, whereas that of *E. coli* decreased.

Resistance upon admission to the hospital. Upon admission to the ICU, 25 (14%)

Table 2. Exposure to antibiotics for systemic use at the individual patient and hospital ward level[a]

Antibiotics	Individual exposures to antibiotics *(no. of patients (%))*	Exposure to antibiotic use at ward level (2002) *(DDD/100 bed days)*						
		Surgical ICU	Neuro surgical ICU	Pulmonology	Gastro-enterology	Internal medicine	Neuro-surgery	Urology
Ampicillin and/or piperacillin	26 (6.3)	3.9	13.4	14.0	7.3	5.9	6.9	3.5
Amoxicillin-clavulanic acid and/or piperacillin-tazobactam	132 (32.1)	75.2	66.4	37.4	45.9	32.4	9.2	10.6
Flucloxacillin	82 (20.0)	8.4	28.3	6.6	3.5	9.3	14.1	2.4
First generation cephalosporins[b]	75 (18.2)	0.5	0.1	0.04	-	-	-	2.3
Second generation cephalosporins	38 (9.2)	3.5	3.6	0.6	1.6	1.3	0.6	3.7
Third generation cephalosporins	18 (4.4)	4.9	7.8	5.8	0.8	0.4	4.2	0.1
Carbapenems	27 (6.6)	10.5	2.3	2.3	1.8	1.5	5.2	0.1
Aminoglycosides	74 (180)	17.2	4.5	12.4	2.5	3.0	1.0	3.1
Fluoroquinolones	76 (18.5)	25.7	10.0	10.4	11.0	7.4	14.0	17.3
Trimethoprim-sulfamethoxazole	52 (12.7)	2.8	4.7	2.8	1.4	4.7	4.9	9.6
Antibiotics for systemic use (total)	306 (74.5)	173.6	147.1	100.6	83.1	81.7	63.7	54.3

[a] from the 2002 edition of the ATC/DDD system

[b] administered at operating theatre, thus not counted at ward level

Table 3. Incidence of transitional events within patients in the faecal and oropharyngeal microflora of ICU and general ward patients (number of transitional events per 1000 observation days[a]).

	ICU patients (*n* = 183)		General ward patients (*n* = 228)	
Transitional event	Fecal	Oropharyngeal	Fecal	Oropharyngeal
Amoxillin with clavulanic acid, piperacillin with tazobactam, cefoxitin and/or cefuroxime resistance in group I microorganisms[b]	7.2	4.4	2.1	0.4
Trimethoprim with sulfamethoxazole resistance in group I microorganisms[b]	3.4	1.2	5.7	0.4
fluoroquinolone resistance in group II microorganisms[c]	2.8	3.7	1.8	-
ceftazidime resistance in group II microorganisms[c] or *P. aeruginosa*	1.9	2.5	0.4	-
fluoroquinolone resistance in *P. aeruginosa*	0.9	2.5	-	-

[a] The number of observation days in the ICU and general ward population was 3202 and 2798, respectively.

[b] Group I microorganisms : *E. coli*, *Klebsiella* spp or *P. mirabilis.*

[c] Group II microorganisms: *E. coli*, *Klebsiella* spp, *Proteus* spp, *Serratia marcescens*, *Enterobacter* spp or *C. freundii.*

patients were colonized with at least one *E. coli* isolate that was resistant to one of more antibiotics whereas for general ward patients this number was 37 (16%). Resistance was most often observed against ampicillin and trimethoprim with sulfamethoxazole. Twenty-three (12.6%) ICU and 32 (14.0%) general ward patients were colonized with ampicillin resistant *E. coli* whereas 12 (6.6%) ICU and 18 (7.9%) general ward patients were colonized with trimethoprim with sulfamethoxazole resistant *E. coli*. In both populations the percentage of patients colonized with piperacillin with tazobactam-, ceftazidime-, meropenem-, gentamicin- or ciprofloxacin-resistant *E. coli* on admission was less than 1% whereas resistance rates of amoxicillin with clavulanic acid and cephalotin were approximately 2 and 4%, respectively.

No significant differences were observed between patients colonized with ampicillin- or trimethoprim with sulfamethoxazole-resistant *E. coli* and patients not colonized with these microorganisms with regard to baseline demographics and exposure to antibiotics in the 3 months preceding admission. The number of patients colonized with resistant *Enterobacteriaceae* other than *E. coli* was too small to allow such analysis.

Resistance during hospitalization: transitional events. In the total cohort of patients, 148 transitional events were observed during 6000 observation days. The incidence of the transitional events was almost 3 times higher among ICU patients as compared to general ward patients (Table 3). Only the emergence of trimethoprim with sulfamethoxazole resistance in group I microorganisms was more commonly observed in general ward patients. The most frequent event in the ICU population was the emergence of amoxicillin with clavulanic acid, piperacillin with tazobactam , cefoxitin or cefuroxime resistance in group I microorganisms. About 50% of the transitional events concerning emergence of ceftazidime resistance occurred in the oropharyngeal microflora and 80% of emergence of fluoroquinolone resistance in *P. aeruginosa* was found in the oropharyngeal microflora. The other transitional events were detected most often in the fecal microflora.

In the risk factor analysis we excluded 33 transitional events since in the case of multiple identical transitional events for a patient (e.g. ceftazidime resistance in *P. aeruginosa* at day 7 and again at day 28), we only included the first event in the analysis. The remaining 115 events, consisted of changes in resistance in 175 antibiotic-microorganism combinations (Table 4). In 74 (= 52 transitional events) of these 175 changes, a susceptible isolate of the same species was detected at the previous sampling time (type I event). In about half of these changes, the more than twofold increase in MIC resulted in a susceptible or intermediate resistant isolate and not in a resistant isolate as defined by the NCCLS. Genotyping revealed that in 24 of these 52 events indistinguishable or closely related fingerprints of the susceptible and resistant strains were found.

Risk factors. Results for the bivariate and multivariate risk factor analysis are outlined in Table 5. In bivariate analysis previous exposure to trimethoprim with sulfa-

Table 4. Transitional events: microorganisms, antibiotics and type of resistance emergence[a]

Microorganism	Antibiotic towards resistance emerged	Transitional event type I [b]						Transitional event type II [c]
		Total no. of changes	Change in resistance category[b]					Total no. of changes
			S → S	S → I	S → R	I → I	I → R	R → R	
E. coli	Amoxicillin-clavulanic acid	10	3	4	2		1		12
	Cefuroxime	7		5	2				9
	Cefoxitin	1		1					9
	Trimethoprim-sulfamethoxazole	14			13			1	14
	Norfloxacin	4	1		2	1			7
	Ciprofloxacin	3	1	1	1				6
	Ofloxacin	6	3	1	2				7
	Ceftazidime	1			1				3
Klebsiella spp	Amoxicillin-clavulanic acid	2	1	1					1
	Piperacillin-tazobactam	1		1					1
	Cefuroxime	2	1		1				4
	Cefoxitin	1			1				3
	Trimethoprim-sulfamethoxazole	-							5
	Norfloxacin	4	1	1			1	1	-
	Ciprofloxacin	3	1	1			1		-
	Ofloxacin	3	1				2		-
Enterobacter spp	Ceftazidime								4
P. aeruginosa	Ceftazidime	1			1				2
	Norfloxacin	2		1	1				3
	Ciprofloxacin	3	1		2				3
	Ofloxacin	3		1	1		1		3
S. marcescens	Norfloxacin	1	1						3
	Ciprofloxacin	1	1						1
	Ofloxacin	1		1					3
Citrobacter spp	Ceftazidime	-							2
Total		74	16	19	31	1	6	2	101

[a] The following five transitional events were assessed:1) amoxillin with clavulanic acid, piperacillin with tazobactam, cefoxitin and/or cefuroxime resistance in *E. coli*, *Klebsiella* spp (Group I microorganisms); 2) fluoroquinolone resistance in *E. coli*, *Klebsiella* spp, *S. marcescens*, *Enterobacter* spp. and *C. freundii* (Group II microorganisms); 3) trimethoprim with sulfamethoxazole resistance in group I microorganisms; 4) ceftazidime resistance in group II microorganisms or *P. aeruginosa*; and 5) fluoroquinolone resistance in *P. aeruginosa*. Each transitional event was only counted once per patient.

[b] Transitional event type I: a more than twofold increase in the log MIC of a bacterial species, isolated at two consecutive sampling times, for at least one antibiotic;

[c] Transitional events type II: resistance emergence for at least one antibiotic, in a species that was not isolated at previous points in time (breakpoints of the NCCLS were applied).

Table 5. Risk factors associated with transitional events during stay ICU and general ward

Transitional event / characteristic	Case patients	Control patients	HR_{crude} [a] (95% CI)	*P* value	$HR_{adjusted}$ [b] (95% CI)	*P* value
Amoxillin with clavulanic acid, piperacillin with tazobactam, cefoxitin and/or cefuroxime resistance in group I microorganisms[c]	*n* = 38	*n* = 373				
Type of hospital ward						
ICU	30	153	1.00			
General ward	8	220	0.30 (0.14-0.66)	0.003		
Medical specialty						
Surgical ICU	20	99	1.00			
Neurosurgery	2	68	0.22 (0.05-0.96)	0.040		
Trimethoprim with sulfamethoxazole resistance in group I microorganisms[e]	*n* = 33	*n* = 378				
Specialty of hospital ward admitted						
Surgical ICU	9	110	1.00			
Urology	6	40	4.96 (1.68-14.64)	0.004	1.00	
Fluoroquinolones	2	64	0.15 (0.03-0.61)	0.009	3.67 (1.06-12.7)	0.046
Trimethoprim with sulfamethoxazole	10	38	2.55 (1.21-5.41)	0.014	0.11 (0.02-0.50)	0.003
Fluoroquinolone resistance in group II microorganisms[d]	*n* = 21	*n* = 362				
Type of hospital ward admitted						
ICU	16	150	1.00			
General ward	5	212	0.42 (0.15-1.16)	0.090		
Reason for admission						
Surgical	12	206	1.00	0.090		
Trauma	5	22	2.48 (0.86-7.15)			
Medical	4	134	0.53 (0.17-1.66)			
Nasogastric tube[e]	7	87	4.58 (1.02-20.6)	0.050		
Ceftazidime resistance in group II microorganisms[d] or *P. aeruginosa*	*n* = 13	*n* = 370				
Type of hospital ward admitted						
ICU	12	154	1.00			
General ward	1	216	0.09 (0.01-0.68)	0.020		
Residential status 48 h before admission						
General ward	5	29	1.00			
Community	6	341	0.18 (0.05-0.61)	0.006		
Ampicillin or piperacillin	3	19	4.46 (1.20-16.6)	0.030	6.03 (1.21-29.93)	0.028
First generation cephalosporins	6	58	3.83 (1.27-11.53)	0.020		
Nasogastric tube[e]	9	85	7.99 (1.01-63.45)	0.049		
Parenteral nutrition	5	27	3.98 (1.12-14.1)	0.032		
Fluoroquinolone resistance in *P. aeruginosa*	*n* = 10	*n* = 218				
Residential status 48 h before admission						
General ward	4	22	1.00		1.00	
Community	5	195	0.23 (0.06-0.86)	0.029	0.25 (0.07-0.95)	0.041
Second generation cephalosporins	3	24	4.01 (1.02-15.79)	0.047		
Third generation cephalosporins	3	10	4.74 (1.20-18.79)	0.027	4.50 (1.08-18.77)	0.039

[a] Only bivariate risk factors with a *P* value < 0.1 are shown in this table.

[b] Only multivariate risk factors with a *P* value < 0.05 are shown in this table.

[c] Group I microorganisms : *E.coli*, *Klebsiella* spp or *P. mirabilis.*

[d] Group II microorganisms: *E.coli*, *Klebsiella* spp, *Proteus* spp, *Serratia marcescens*, *Enterobacter* spp or *C. freundii.*

[e] Assessed in the first 100 consecutive ICU and general ward patients

methoxazole was significantly associated with an increased risk of resistance of the group I microorganisms toward this antibiotic (hazard ratio (HR) = 2.6; 95% CI, 1.2 to 5.4). However, after multivariable analyses it was not independently associated. We observed that admission to the urology department was a risk factor for trimethoprim with sulfamethoxazole resistance in group I microorganisms (adjusted HR (AHR) = 3.7; 95% CI, 1.1 to 12.7) whereas fluoroquinolone use was negatively associated (AHR = 0.11; 95% CI , 0.02 to 0.5).

Previous treatment with ampicillin and/or piperacillin was independently associated with colonization with ceftazidime resistant group II microorganisms or *P. aeruginosa* (AHR = 6.0; 95% CI, 1.2-29.9). The use of third generation cephalopsporins was not a risk factor for the emergence of ceftazidime resistance. However, we found that previous use of these antibiotics were associated with colonization with fluoroquinolone resistance in *P. aeruginosa* (AHR = 4.5; 95% CI, 0.8-18.8). Patients admitted from the community had a lower risk of colonization with fluoroquinolone resistance in *P. aeruginosa* compared to patients who were transferred from a general ward (AHR = 0.25; 95% CI, 0.07 to 0.95). The presence of a nasogastric tube was found to be a risk factor for fluoroquinolone and ceftazidime resistance in group II microorganisms and *P. aeruginosa* in bivariate analysis. No independent associations were observed for the presence of invasive devices.

DISCUSSION

In the present study we assessed risk factors for the emergence of antibiotic resistance in the hospital by analyzing transitional events in the oropharyngeal and fecal miroflora of patients. We found that the incidence rate of transitional events was highest in ICU patients. Transitional events concerning ceftazidime resistance in gram negative bacteria and fluoroquinolone resistance in *P. aeruginosa* were mainly observed in the oropharyngeal microflora. The other transitional events were most often detected in the fecal microflora.

It is remarkable that of the prior antibiotic exposures, only few exposures were independently associated with the emergence of antibiotic resistance. Exposure to ampicillin was independently associated with the isolation of ceftazidime resistant group II microorganismen and *P. aeruginosa*. This might be related to induction of AmpC ß-lactamases since it is known that ampicillin has an high induction potential compared to piperacillin, cephalosporins and clavulanic acid (48). An association between prior exposure to ampicillin and *Enterobacteriaceae* resistant to multiple beta-lactam antibiotics has been reported elsewhere (31). We also found a negative association between the use of fluoroquinolones and colonization with trimethoprim

with sulfamethoxazole resistant group I microorganisms. Such an association has not been previously demonstrated. In a recent study analyzing risk factors for emergence of resistance to broad-spectrum penicillins among *Enterobacter* spp, fluoroquinolone therapy was also found to be a risk factor (32). An explanation might be that fluoroquinolones eliminate or strongly reduce intestinal *Enterobacteriaceae* (49). Patients exposed to third generation cephalosporins were at high risk of colonization with fluoroquinolone resistant *P. aeruginosa*. This might be due to up-regulation of MexAB-OprM (50). These associations deserve further investigation.

In a recent editorial in this journal recommendations were made for case-control studies of risk factors for emergence of antibiotic resistance. In the present study we aimed to meet these criteria (16). Case patients were defined as all patients colonized with the antibiotic-resistant microorganism by conducting a prospective surveillance of fecal and oropharyngeal specimens rather than just assessing clinical specimens. Control patients were drawn from the same population as case patients and we adjusted for time at risk and severity of illness (51, 52). A limitation of the present study is that we did not perform separate analysis of patients with genotypically distinct organisms versus those with genotypically similar organisms. We identified transitional events within observational intervals and subsequently characterized all preceding susceptible and resistant isolates of that particular species by PFGE. However, the results of PFGE revealed that 75% of the resistant strains were not detected on previous sampling times. This suggests acquisition of a resistant strain by horizontal transfer between patients or hospital staff rather than development of resistance within strains already detected on admission. However, we can not exclude that selection may have occurred within the patients' own, previously undetected, microflora.

We did not compare genotypes between patients. Since development of resistance within identical species was low and the overall resistance rates were also low, we decided not to conduct separate analysis for patients with genotypically distinct and similar organisms. Since the relatively low number of transitional events, we also had to assess emergence of resistance in groups of microorganisms rather than performing separate analysis for each microorganism.

This study may be limited by a relatively small number of cases that may have hampered our ability to detect significant risk factors. In a previous study we showed that resistance rates were very low in our hospital population (21). Whether the results of this study are representative of the conditions in other hospitals mainly depends on the studied population, the antibiotic policies applied, and the effectiveness of infection control measures taken to prevent cross-colonization. We suppose that most of the results will be universal and can be extrapolated to other hospitals. However, we expect a higher incidence of transitional events, with higher consumption of antibiotics. We identified only a few risk factors, but this may be due to

the stringent usage of antibiotics and intensive infections control measures in our hospital and in Dutch hospitals in general (53-55).

In summary, the results of this study show that the incidence rate of transitional events was highest in ICU patients, except for SXT resistance that we observed predominantly in patients admitted to the urology department. Specific antibiotics were found to be independent risk factors for the emergence of resistance. Therefore, in order to minimize the risk of emergence of resistance during hospitalization, rational antibiotic prescribing targeted towards these risk factors appears to be warranted. Patients transferred from general wards to the ICU had a 5 times higher risk for fluoroquinolone resistance in *P. aeruginosa* as compared to patient admitted from the community. This should be taken into account in the empiric treatment of ICU patients. Furthermore, we found that 75% of the resistant strains were not detected on previous sampling times. This suggests acquisition of a resistant strain by horizontal transfer between patients or hospital staff rather than development of resistance within strains already detected on admission. However, we can not exclude that selection may have occurred within the patients' own, previously undetected, microflora.

Therefore, two intervention strategies may be indicated. Besides policies to promote rational use of antibiotics, emphasis must also be placed on infection control measures directed at the reduction of transmission of resistant microorganisms between patients and hospital staff.

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CHAPTER 4.

Summary and general discussion

INTRODUCTION

The general aim of this thesis was to explore the current emergence of antibiotic resistance in hospitals. Since there is substantial evidence for the link between resistance and use, studies described in chapter 2 addressed the surveillance of quantitative antibiotic use. The principle theme in these studies was the interpretation of the data with regards to resistance risks. In chapter 3, the epidemiology of colonization with antibiotic resistant bacteria in patients during and after hospitalization was assessed in order to identify risk factors for resistance emergence and to determine the relevance of transmission of resistance from the community into the hospitals and vice versa. In this chapter the main findings are discussed, some methodological issues are considered that appears to be relevant in the different studies and perspectives for future studies are presented.

MONITORING OF QUANTITATIVE ANTIBIOTIC USE IN HOSPITALIZED PATIENTS

Interpretation of antibiotic use data expressed in different units of measurement

Data of epidemiological surveillance studies on antibiotic use might be used for planning, implementing and evaluating interventions in order to optimize antibiotic use in a specific setting (1). Over the last decade several national surveillance systems on antibiotic use have been set up (2-5). Critical assessment of the units of measurement used to quantify antibiotic use and discussions about the interpretation of these units are, however, rarely presented in the scientific literature (6-8). Most of the surveillance systems use the number of DDD per 100 patient days, as recommended by WHO, to compare consumption rates over time, and between hospitals, geographical regions and countries (9).

The study described in chapter 2.1 aimed to demonstrate the importance of units of measurement in the interpretation of trends in antibiotic use with regards to resistance risks. In this study trends in antibiotic use between 1997 and 2001 in 59 Dutch hospitals were analyzed. Total systemic use significantly increased when use was expressed in defined daily doses (DDD) per 100 patient days whereas expressed in DDD per 100 admissions it remained constant. Also for some individual antibiotics increases in DDD per 100 patient days were not accompanied by increases in DDD per 100 admissions and vice versa. Between 1997 and 2001 the mean number of admissions, bed days and length of stay decreased significantly. A slightly decrease (not significant) in total consumption (expressed in DDD) was observed.

Thus, hospitalized patients in the Netherlands did not receive more antibiotics between 1997 and 2001. Since they remained in the hospital for fewer days, the

number of DDD per 100 patient days increased. An increase in DDD per 100 bed days is often interpreted as worrisome with regards to the potential of antibiotic resistance development. However in this study, a constant use per patient and a decrease in the number of admissions are indicative for lowering of the selection pressure. Moreover, an intensification of therapy suggests short duration of therapy that may lead to less selection of resistant micro organisms (10).

From this study it is concluded that DDD per 100 admissions can be better used to compare antibiotic consumption rates over time or between different hospitals, geographical regions or countries. This indicator is less sensitive to changes in hospital resource indicators over time. The question remains whether DDD per 100 patient days best represents the selection pressure exerted by antibiotics. To best estimate the effect of observed trends in antibiotic use on the potential of antibiotic resistance development, it is advised to present antibiotic use data in DDD per 100 bed days as well as in DDD per 100 admissions and to register changes in hospital resource indicators.

In chapter 2.2 trends in antibiotic use in Dutch hospitals between 1997 and 2002 were analyzed and interpreted as recommended in the previous paragraph. Total systemic use was 58.5 DDD per 100 patient days in 2002. The first results of an European surveillance program demonstrates that the Nordic countries and The Netherlands all show a low total antibiotic use compared with other European countries, with a three to four fold difference (11). Such a difference is suggestive for at least inappropriate use of antibiotics in some countries. In most countries there is a strong correlation between the characteristics and extent of use in hospital and ambulatory care. This indicates that common determinants influence antibiotic use within countries.

Regarding trends in antibiotic use over the years in The Netherlands, five main categories could be distinguished. For macrolides, lincosamides and fluoroquinolones we found a significant increase over the years for use expressed in DDD per 100 patient days as well as in DDD per 100 admissions. With regards to resistance risks, an increase in both units of measurement might be an indicator for in-depth audits to the quality of antibiotic use. For total systemic use, combinations of penicillins including betalactamase inhibitors, betalactamase sensitive penicillins, cephalosporins and glycopeptides a significant increase in DDD per 100 patient days and a constant use in DDD per 100 admissions has been observed. Between 1997 and 2002 the total number of admissions and DDD remained constant whereas the number of patient days and length of stay significantly decreased. As in the previous paragraph explained, it is arguable whether in this case an increase in DDD per 100 patient days means an increase in selection pressure. For other subclasses we found no significant change or a significant decrease in both units of measurement.

Chapter 2.1 and 2.2 show that it is difficult to answer the question whether the selection pressure exerted by antibiotics is increased or not over the years. When antibiotic resistance development is the issue then the units of measurement should be a reflection of the selection pressure exerted. The main question that remains is what unit of measurement gives the best correlation with antibiotic resistance and thus best represents the selection pressure? It is now time to design a study that answers this question. In the past it has been postulated that at the hospital population level three factors seems to be important with respect to the selection pressure exerted by antibiotics (12). First, the total amount of an antibiotic used in a particular geographical area (i.e. the entire hospital or a ward or unit) over a certain period of time. Secondly, the number of patients treated with the antibiotic (because they serve as the major "sources" of resistant bacteria) and thirdly, the density of these patients, i.e. the proportion of patients on antibiotics in the hospital. Together these factors represent the selection density. Neither DDD per 100 bed days nor DDD per 100 admissions indicates the number of patients exposed or the proportion of patients on antibiotics. Only a few unpublished studies have compared measurement units with respect to relationships to resistance (13). Most of these studies focused on differences in relationship between grams per 100 patient days, DDD per 100 patient days and the mean daily dose. The latter consistently disagreed with the other measures .

Applying time series analysis might be the most optimal study design to determine the relationship between antibiotic use expressed in different measurement units and resistance (14). This requires for example 5 years of monthly data on use and resistance. Antibiotic use might be expressed as DDD per 100 bed days, DDD per 100 admissions, the proportion of patients exposed to antibiotics, the proportion of bed days on which antibiotics are administered etc. It should also be interesting to assess whether the unit of measurement that best correlates with resistance is the same for different antibiotic-microorganism combinations. Moreover, this experiment should be repeated in a number of other hospitals with preferably different sets of hospital resource indicators (number of admissions, bed days, length of stay).

Determinants of antibiotic use in hospitals

Antibiotic use surveillance data may be helpful in identifying hospitals with a low or elevated level of antibiotic use and that are potential candidates for in depth studies to the quality of antibiotic prescribing. Insight is therefore needed into hospital characteristics that predict quantitative antibiotic use. In chapter 2.3 a multivariate linear regression model was constructed with total systemic use of antibiotics as the dependent outcome variable and institutional, population and care provider characteristics as independent explanatory variables. The number of hospital pharmacists,

medical microbiologists and infectious disease physicians per 100,000 patient days appeared to be significant determinants of quantitative antibiotic use. These variables explained 40% of the variation in antibiotic use.

This result is understandable considering that the relative number of such specialists in a hospital strongly correlates with the hospital's case mix of patients regarding infectious diseases. We assessed many indirect population characteristics in order to include a meaningful indicator of the patient case-mix of hospitals. The inclusion of more disease related variables in the regression model might have explained a higher proportion of the variability in antibiotic use.

It is desirable to extend studies like this to further delineate the determinants that explain variability in antibiotic use. Indicators of the case-mix of hospitalized patients with infectious diseases should be developed. A point prevalence study might be the preferred study design to obtain insight into the indications where antibiotics are prescribed for in the individual hospitals. Other variables that might explain the variability in use might be those related to local antibiotic policies including the presence of guidelines, restricted lists of antibiotics and presence of antibiotic committees. Moreover, data on local resistance levels might be included in the model.

EPIDEMIOLOGY OF COLONIZATION WITH ANTIBIOTIC RESISTANT BACTERIA IN PATIENTS DURING AND AFTER HOSPITALIZATION

There are several reasons for assessing bacterial colonization with resistant strains (15). Bacterial colonization is an important step in the pathogenesis of infections. Many of the bacteria that comprise the gastro-intestinal microflora may cause infections (16-18). Knowledge of the prevalence and degree of antibiotic resistance in the fecal and orophayngeal microflora on admission and during hospitalization may therefore facilitate the choice of empirical therapy in nosocomial infections (19). Bacterial colonization is of further interest since fecal bacteria might act as a reservoir for resistance determinants, plasmids, transposons and other moving genes (20-22). The epidemiology of bacterial colonization is of interest since the digestive tract is the source from where resistant bacteria can spread and cause hospital epidemics. After discharge from the hospital, patients may remain colonized with resistant bacteria acquired in the hospital and resistance may disseminate into the community, nursing homes or other institutes.

We therefore performed a large prospective observational study to accurately assess the epidemiology of aerobic gram negative bacteria and *Enterococcus* species in the oropharyngeal and intestinal microflora of hospitalized patients from admission up to three months after discharge.

Colonization rates and species distribution

In the ICU as well as in the general ward population the aerobic gram negative colonization rates in oropharyngeal specimens increased during hospitalization and did not decrease in the three months after discharge (chapter 3.3). In rectal specimens, colonization rates were low during hospitalization and increased in the 3 months after discharge. A change in species distribution among the dominant oropharyngeal and intestinal microfloras was observed during stay at the ICU. *Klebsiella* spp, *Enterobacter* spp, *Serratia marcescens* and *Pseudomonas aeruginosa* were more often isolated during hospitalization, whereas the frequency of *E. coli* declined. At discharge from the hospital, the frequency of *P. aeruginosa* and *S. marcescens* in the oropharyngeal microflora was increased. In the general ward population, a significant increase in the frequency of *Klebsiella* spp and a significant decrease in the frequency of *E. coli* were found in the intestinal microflora at discharge from the hospital. Three months after discharge, these frequencies returned to the levels observed on admission.

Enterococcus species were less frequently isolated on admission than during hospitalization (chapter 3.4). One and three months after discharge colonization rates remained stable and comparable to those during hospitalization. The frequency of isolation of *E. faecium* in the ICU population, significantly increased during hospitalization, whereas the frequency of

E. faecalis decreased. Both frequencies did not change in the three months after discharge and were comparable to those observed in the general ward population.

We hypothesize that the high use of specific antibiotics in the ICU suppressed the fecal microflora to concentrations below the detection limit in a large number of patients (23, 24). In other ICU patients changes in species distribution were observed during hospitalization. These changes that occur during hospitalization underscore the importance of well-tailored empiric antibiotic policies, in particular in ICU patients. The persistence of *P. aeruginosa and S. marcescens* colonization in the oropharyngeal microflora of ICU patients after discharge may have implications for the empirical treatment of infections that occur during the first months after discharge from the hospital.

Resistance dynamics

In chapter 3.1 the impact of hospitalization on the prevalence of resistant *E. coli* in the intestinal microflora of patients admitted to the surgical wards of three Dutch university-affiliated hospitals was analyzed. Prevalence of resistance was determined on admission, at time of discharge, and 1 and 6 months after discharge.

Despite the variation in patient characteristics and antibiotic use between the three hospitals, no significant differences in the prevalence of antibiotic resistant *E. coli* at the different times were observed, except for the overall higher prevalence of cefazolin-resistant *E. coli* in patients of the university hospital Maastricht. In this population a significant decrease in cefazolin resistance between the time of discharge and 6 months after discharge was observed. The low prevalence of resistance in these surgical populations may be due to a relatively low antibiotic use in these patients. In hospital populations with a higher consumption of antibiotics, the effect on the prevalence of resistance is likely to be more pronounced.

We showed in chapter 3.3 that the percentage of ICU patients colonized with ampicillin and/or cephalothin resistant fecal *E. coli* was significantly increased at discharge from the hospital and did not further change in the three months after discharge. In view of the low resistance rates in The Netherlands (25), amoxicillin and first generation cephalosporins are commonly used antibiotics in the hospital and/or the community. Amoxicillin is the most frequently used first-line antibiotic in Dutch primary health care (2). The first generation cephalosporin cefazolin is the drug of choice for surgical prophylaxis (26). The persistence of ampicillin and cephalothin resistance in fecal *E. coli* strains in ICU patients is an important observation and may influence the empirical regimens and surgical prophylaxis in these patients up to 3 months after discharge.

Emergence of multi-drug resistance was observed in many gram-negative species during stay on the ICU. Resistance frequencies in *E. coli* significantly increased with length of stay on the ICU. For the general ward population, no significant changes in resistance frequencies were found during hospitalization. This is consisted with the results of the study in chapter 3.1.

In chapter 3.5 it was found that the incidence of changes in resistance patterns, the so called "transitional events" during hospitalization was almost three times higher in ICU patients compared to general ward patients. The overall incidence of transitional events in the ICU population was 31 per 1000 observation days. This means, with a mean length of stay of 12 days, that transitional events occurred in approximately 1 out of 3 patients. This study shows that several antibiotics are found to be independent risk factors for the emergence of resistance. No independent associations were observed for the presence of invasive devices. However, the results of PFGE revealed that 75% of the resistant strains were not detected on previous sampling times. This suggests acquisition of a resistant strain by horizontal transfer between patients or hospital staff rather than development of resistance within strains already

detected on admission. However, we can not exclude that selection may have occurred within the patients' own, previously undetected, microflora.

Therefore, two intervention strategies may be indicated. Besides policies to promote rational use of antibiotics, emphasis must also be placed on infection control measures directed at the reduction of transmission of resistant microorganisms between patients and hospital staff.

The percentage of ICU patients colonized with high-level gentamicin (HLG) resistant *E. faecalis* increased during hospitalization and decreased in the 3 months after discharge from the hospital (chapter 3.4). The percentage of patients colonized with ampicillin resistant *E. faecium* and high-level gentamicin resistant *E. faecalis* significantly increased with length of stay on the ICU. Genotyping revealed that the majority of these strains were not detected at previous sampling times. This also suggests horizontal transfer or selection of a resistant strain from the patients' own microflora rather than development of resistance within strains.

From the studies in chapter 3.1, 3.3, 3.4 and 3.5 we conclude that the risk of dissemination of resistant gram-negative bacteria and *Enterococcus* species into the community through hospitalized patients appears to be low in general ward patients, but is noticeably higher among ICU patients. Our study confirms previous observations that susceptibility trends vary between ICU and general ward patients (27-31).

In contrast with our expectations, the highest resistance rates were not found at discharge from the ICU but at discharge from the hospital. In the general ward populations, however, the highest resistance rates were not observed at discharge from the hospital but 1-month afterwards. A possible explanation is that due to exposure to antibiotics with a very broad-spectrum during hospitalization, the intestinal microflora has been eliminated or suppressed below the detection limit. If subsequently, the selection pressure is partly removed and antibiotic treatment is streamlined to antibiotics with a smaller spectrum, this may result in (re)appearance of initially suppressed resistant bacteria. When the selection pressure is completely removed, the resistant bacteria will be eventually replaced by the susceptible bacteria and result in a decline of the resistance percentages.

It appears from our data that most effort to optimize antibiotic prescribing should be directed towards the ICU population. First, the incidence of transitional events during hospitalization is almost 3 times higher for patients admitted to the ICU compared to patients admitted to the general ward. This may not only lead to treatment failure in the patients, but also interfere with the treatment of other ICU patients as

a result of cross colonization. Secondly, the majority of ICU patients are transferred to a general ward before discharge from the hospital. The highest resistance rates in the ICU population were found at discharge from the hospital. This means that these formerly ICU patients are an important source of resistant bacteria that may transfer to other patients admitted to the general ward. Thirdly, as the highest resistance levels are found at discharge from the hospital and did not change in the 3 months after discharge, there is also a substantial risk for dissemination of resistant strains that were acquired during the stay in the ICU, into the community.

Prevention of colonization and infection with resistant bacteria should be tailored to the relative importance of the different acquisition routes: exogenous by cross-colonization or endogenous caused by the selective pressure of antibiotics. We did not characterize the genotypes of all fecal and oropharyngeal isolates. As we included patients over a long period of time and from seven hospital departments, we did not expect to find evidence for cross colonization. Besides, the genotyping of more than 10,000 microorganisms would have been a very laborious and costly exercise. Thus, we were not able to distinguish between the two routes of transmission. We did however, determine the genetic relatedness of strains within patients and evidence was found that the majority of resistant isolates is not preceded by the isolation of an identical, although susceptible, strain, thereby suggesting the possibility of acquisition by cross-colonization. If cross-colonization is prevalent, hygiene and infection control measures are indicated. However, in the case of endogenous acquisition of resistant organisms due to selection pressure, an antibiotic restriction intervention is most likely to stem the tide. As the routes of transmission are unknown, it is important that an in-depth analysis of the antibiotic prescribing policies is performed. Otherwise, antibiotics may be restricted unnecessarily, which might deny appropriate therapy to patients with life-threatening infections. At times, the two intervention strategies may be indicated.

METHODOLOGICAL CONSIDERATIONS

The studies in this thesis addressed a number of interesting methodological issues.

Variability in quantitative antibiotic use between hospitals: prescribed daily doses (PDD) versus DDD

The aim of the study described in chapter 2.3 was to assess determinants of total systemic antibiotic use in Dutch hospitals. Data on the use of antibiotics in acute care hospitals in 2001 was expressed in DDD per 100 patient days. To calculate the

number of DDD it was decided to use the ATC/DDD classification from WHO, version 2005, rather than the version of 2001 (9). In 2005, the WHO changed the DDD of parenteral amoxicillin-clavulanic acid from 1 to 3 grams.

From our database it is obvious that there is a large variability in the use of amoxicillin-clavulanic acid between Dutch hospitals (data not shown). In some hospitals amoxicillin-clavulanic acid is recommended for a broad range of indications, whereas in other hospitals this antibiotic is rarely advised and other antibiotics are preferred. Theoretically, variability in use of amoxicillin-clavulanic acid between hospitals doesn't affect the total sum of DDD of antibiotics used in these hospitals. However, since the DDD of 1 gram in previous years did not correspond with the actual PDD (in most hospitals 4 grams), total systemic use in hospitals with a high usage of amoxicillin-clavulanic acid was out of proportion as compared to hospitals with a low usage. This may bias the study to determinants of antibiotic use. Therefore, we decided to use the ATC/DDD classification, version 2005. In summary, a major difference between the DDD and the PDD of an antibiotic of which the extent of use varies greatly between hospitals, may lead to a high variability in total systemic use between these hospitals.

Methodology of assessing the gram negative fecal microflora

Methods that have been frequently used for the detection of gram-negative resistant strains in the intestinal microflora include 1) selective plating, using antibiotic containing agar plates and 2) the so called "colony picking methods" in which a number of colonies are selected on morphology and minimal inhibitory concentrations (MICs) are determined by using an agar dilution method. In the study in chapter 3.1 antibiotic containing agar plates were used, whereas in the study in chapter 3.2 a colony picking method was applied. An advantage of the use of antibiotic containing plates is that it allows to determine the frequency of resistant/susceptible strains in each sample whereas the colony picking method has the advantage that it is possible to quantify the susceptibility of a bacterium against different antibiotics by means of the MIC. So far, there has been one report that compared these two methods. Österblad *et al*. did not found a significant difference in resistance detection between the replica plating (a method in which exactly the same colonies were plated onto both antibiotic-containing and antibiotic free plates by using a replicator) and a five-colony picking method (32).

MacConkey agar is the most widely used medium for the isolation and differentiation of aerobic gram negative bacteria. In the past years, several chromogenic agars have been introduced that claimed to allow the presumptive identification of aerobic gram negative bacteria on the basis of colony morphology and distinctive

color patterns (33-35). Accurate identification of *E. coli* on colony morphology and color may considerably reduce time and costs of fecal screening programs. In the study in chapter 3.2 we therefore tested three different chromogenic agars and MacConkey agar for the detection of aerobic gram negative bacteria in stool specimens and the accuracy of these chromogenic agars for the direct identification of *E. coli*. No significant differences in detection rates were found and high positive predictive values were found for all chromogenic media. The Chromogenic UTI medium and the Chromagar Orientation were preferred media because of higher negative predictive values. As we aimed to study a proportional sample of the dominant microflora, an algorithm was defined for the selection of five colonies on colony characteristics. The choice of picking a number of five colonies has been made on the previous described study by Österblad (32) and on a study in which nearly 80% of the serologic groups of *E. coli* identified after an examination of 15 to 25 colonies were present in the first 5 colonies studied (36).

Resistance rates: percentage of patients colonized with resistant bacteria

In chapter 3.3 and 3.4, the epidemiology of antibiotic resistance was analyzed at different times during and after hospitalization. It was decided to report the percentage of patients colonized with resistant bacteria rather than the percentage of antibiotic resistant isolates within species. This was preferred as from these data one can estimate the health and economic impact of antibiotic resistance (37). Due to lost to follow up as a result of death, transfers and withdrawal from the study, different numbers of patients were present at the various sampling times. At each sampling time we included all patients from which specimens were collected. To validate our data we also calculated the resistance rates for a subpopulation of patients that could be followed through the entire project. No differences in observed trends were observed between these two methods. Therefore, selection bias due to the differential follow-up of patients appears to be limited.

The percentage of patients colonized with resistant bacteria at different times was calculated for each antibiotic. We selected one isolate per patient with *the most resistant* result for that particular antibiotic. One may argue that for this reason the results were likely to be potentially biased towards showing more resistance. However, this sensitive method was well considered as we assume that the presence of one resistant bacterium in the dominant intestinal microflora may result in a clinical infection with that resistant isolate.

Transitional events

In the study described in chapter 3.5 a new concept regarding the dynamics of resistance in intestinal bacteria during hospitalization has been introduced: the so-called "transitional event". For each patient the number of observational periods was determined. An observational interval was defined as the time period between two consecutive samples. Each of these intervals was evaluated for the occurrence of transitional events. A transitional event was defined as 1) a more than twofold increase in the log MIC of a bacterial species, isolated at two consecutive sampling times, for at least one antibiotic, or 2) resistance for at least one antibiotic, in a species that was not isolated at previous points in time.

In many studies, emergence of resistance is defined as 1) a change from susceptible to resistant, using the breakpoints of the National Committee for Clinically Laboratory Standards (NCCLS), or 2) the isolation of a new resistant (NCCLS-breakpoint) strain. This is an efficient method to compare various settings. However, it does not fully reflect resistance dynamics of the intestinal bacterial population during hospitalization. We hypothesized that many bacteria become less sensitive to antibiotics as a result of hospitalization without being necessarily resistant as defined by NCCLS criteria. Insight in the incidence of these transitional events and clarification of risk factors for the occurrence of these events may help to further elucidate the mechanisms of resistance emergence in hospitalized patients.

However, the risk factors analysis is not straightforward. We had to make several assumptions and evaluate associations with various types of events as outcome. We decided to perform analyses "on patient level" and not by observational periods. As a consequence, in the case of multiple specific transitional events for a patient, we only included risk factors in the time to the first event in the analysis, and left out the remaining length of stay of that patient in the hospital. Alternatively, we could have analyzed our data on the level of observational periods, and define risk factors per period only. This type of analysis assumes that all observational periods, also within a patient, are independent of each other, which is unlikely. Besides, it assumes that the lag time between exposure and resistance is short, which may be true for some exposures and events (e.g. selection of resistant strains during exposure to ciprofloxacin), but may also be longer for others. In our "patient level" analysis we decided to include the exposure to risk factors between the day of admission and the last day of the observational period.

Methodological issues concerning studies on risk factors for antibiotic resistance

In chapter 3.5 we describe a prospective cohort-study that aims at investigating risk factors for colonization with antibiotic resistant gram negative bacteria during stay

in the hospital. For that purpose patients who got colonized with resistant bacteria were compared with patients from the same cohort who did not (controls). In a recently published series of papers major emphasis has been given to three methodological principles for studies on risk factors for antibiotic resistance: method of control group selection, adjustment for time at risk and adjustment for comorbid illnesses. We addressed these principles in our study design.

Control group selection. Control patients should be selected from the same source population that the cases originated from. Most studies on risk factors for antibiotic resistance select patients with susceptible bacteria as controls. This may overestimate the contribution of the resistance-defining antibiotic in the development of resistance, because of underdetection of exposure in the source population.

Time at risk. The time at risk represents the duration of the at risk period both for exposure to antibiotics and for acquisition of the antibiotic resistant bacterium. Patients who are admitted to the hospital for longer periods are more likely to receive (several) antibiotics, and are also more likely to become colonized with a resistant bacterium. The period at risk needs to be measured and controlled in a stratified or multivariate analysis or by study design (matching). In our study we defined the time at risk for case patients as the duration between admission and the index (event) date, whereas for control patients exposure was assessed during the total observational period. The period of time at risk was controlled for in the multivariate analysis.

Comorbid illnesses. Patients with comorbid conditions, like immunocompromised states, likely have an increased risk of acquiring an antibiotic resistant bacterium and to have received an antibiotic that is risk factor of interest. Therefore comorbid illnesses also need to be measured and controlled for in a stratified or multivariate analysis, or by study design (matching). Comorbidity was measured using the definitions of the Dutch National Intensive Care Evaluation (http://www.stichting-nice.nl) and controlled for in multivariate analysis.

FINAL REMARKS

The general aim of this thesis was to explore the current emergence of antibiotic resistance in hospitals. This aim has been addressed in two research projects.

The first project concerned the optimization of surveillance of quantitative antibiotic use in hospitals. Studies were performed in which the interpretation of these data were the central theme. These studies had an exploratory character and also aimed at opening discussions at the international level about what units of measurement can be best used to compare antibiotic use between countries and what unit

of measurement do best reflect antibiotic resistance. Future research should assess the relationship between different antibiotic use measures and resistance. Up to now determinants of antibiotic use has hardly been studied. We conclude that the case-mix of hospitalized patients with infectious diseases appears to be a very important determinant and recommend developing a good indicator for this case-mix.

The second project concerned research on the epidemiology of colonization with antibiotic resistant bacteria in patients during and after hospitalization. New insight is obtained in the incidence of resistance in our hospital and in the exchange of resistant bacteria between the hospital and the community. Risk factors for development of antibiotic resistance were determined. Future studies in our hospital should focus on the relative importance of the different colonization routes since interventions should be tailored on the relative contribution of these routes to the resistance problem. These routes may be assessed by microbiological surveillance in combination with genotyping but also by mathematical modelling (38). In our hospital, such studies may best be conducted at the ICU as our resistance data points to a highly dynamic bacterial population in these patients. In contrast with the presented studies in this thesis, such a study should include all patients in a relatively small geographical area in a sufficiently long time series. Furthermore, it would be interesting to assess the epidemiology of resistance in the intestinal microflora in other hospitals. Confirmation of our findings with respect to the delay between use and the emergence of resistance (the highest resistance levels in the ICU population were not found at discharge from the ICU but at discharge from the hospital) and the persistence of resistance in the months after discharge from the hospital, is needed. Moreover, we expect resistance problems, also in the general wards, to be more pronounced in settings with a higher consumption of antibiotics.

Ultimately, it is expected that future interventions based on the findings in this thesis will help to reduce the problem of antimicrobial resistance.

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SAMENVATTING

Antimicrobiële resistentie maakt infecties moeilijk behandelbaar en kan de duur en de ernst van de ziekte verergeren. In Nederland is het percentage antibiotica-resistente micro-organismen laag vergeleken met andere Europese landen en de Verenigde Staten. De resistentiepercentages nemen de laatste jaren echter toe en regelmatig treden uitbraken met multi-resistente micro-organismen op in de Nederlandse ziekenhuizen. Maatregelen zijn nodig om de ontwikkeling van resistentie en de verspreiding ervan tegen te gaan. Het doel van het onderzoek beschreven in dit proefschrift is om meer inzicht te krijgen in de huidige ontwikkeling van resistentie in ziekenhuizen. De onderzoeken in dit proefschrift richten zich op het meten van kwantitatief antibioticagebruik in de Nederlandse ziekenhuizen en op de epidemiologie van kolonisatie met resistente bacteriën tijdens en na opname van patiënten in het ziekenhuis. **Hoofdstuk 1** is een algemene inleiding tot dit proefschrift.

Om het verband tussen resistentie ontwikkeling en antibioticagebruik op lokaal, regionaal, nationaal en internationaal niveau nader te kunnen onderzoeken, zijn allereerst resistentie en gebruiksgegevens noodzakelijk. Pas daarna is het mogelijk de effecten van een goed antibioticagebruik en preventieve maatregelen te onderzoeken en vervolgens onderbouwde maatregelen te nemen. In de jaren negentig hebben diverse autoriteiten de aanbeveling gedaan een nationaal bewakingssysteem op te zetten voor intramuraal antibioticagebruik.

In het onderzoek in **hoofdstuk 2.1** wordt het belang van gebruikte meeteenheden om antibioticagebruik uit te drukken onderstreept. Het antibioticagebruik in de Nederlandse ziekenhuizen tussen 1997 en 2001 is uitgedrukt in defined daily doses (DDD) /100 patiëntdagen. Het gebruik van deze meeteenheid wordt door de World Health Organization aanbevolen. In dit onderzoek wordt daarnaast een nieuwe maat geïntroduceerd, namelijk het aantal DDD/100 opnamen. Gesteld wordt dat het alleen weergeven van het aantal DDD/100 patiëntdagen niet voldoende is omdat die maat gevoelig is voor veranderingen in de zogenaamde kengetallen van ziekenhuiszorg, met name voor veranderingen in de gemiddelde ligduur en het aantal opnamen. Deze kengetallen beïnvloeden de grootte van het noemergetal (aantal patiëntdagen) aanzienlijk. Zo is het totaal antibioticumgebruik uitgedrukt in DDD/100 patiëntdagen gestegen van 47.2 naar 54.7 DDD/100 patiëntdagen terwijl het aantal DDD/100 opnamen in dezelfde periode niet is gestegen. Het verschil in deze twee trendlijnen is te verklaren door een afname in de gemiddelde ligduur per opname (deze was 8,2 dagen in 1997 en 6,9 dagen in 2001) en een daling van het aantal opnamen met 10% in deze periode.

In **hoofdstuk 2.2.** ligt de nadruk op de interpretatie van de gemeten trends in antibioticumgebruik uitgedrukt in DDD/100 patiëntdagen en in DDD/100 opnamen. In dit hoofdstuk worden trends in antibioticumgebruik over de jaren 1997 en 2002

gemeten. Ook hier vinden we een stijging in totaal antibioticagebruik wanneer het wordt uitgedrukt in DDD/100 patiëntdagen en een constant gebruik in DDD/100 opnamen. Een zelfde trend wordt waargenomen voor amoxicilline met clavulaanzuur, beta-lactamase gevoelige penicillines, cephalosporines en de glycopeptiden. Dit kan als volgt worden geïnterpreteerd: per opname, dat wil zeggen per patiënt, worden niet meer antibiotica voorgeschreven, maar omdat de patiënt gemiddeld steeds korter in het ziekenhuis verblijft neemt het aantal DDD/ patiëntdagen wel toe. Men zou kunnen stellen dat wel sprake is van toegenomen selectiedruk, immers er worden meer antibiotica per ligdag gebruikt, maar op ziekenhuisniveau neemt daarmee de selectiedruk alleen toe als het aantal bedden en de bedbezetting constant zou zijn gebleven. Dit is echter niet het geval.

Voor de macroliden, de lincosamiden en de fluorochinolonen is het aantal DDD/100 patiëntdagen en het aantal DDD/100 opnamen gestegen. Voor deze middelen is vermoedelijk wel sprake van een toegenomen selectiedruk.

Het antibioticagebruik in de Nederlandse ziekenhuizen is laag in vergelijking met de meeste Europese landen. De variatie in antibioticagebruik tussen de Nederlandse ziekenhuizen is echter aanzienlijk (in 2001 is het mediaan totaal antibioticagebruik 45.6 DDD/100 patiëntdagen (laagste waarde = 26,1; hoogste waarde =65.9).

Hoofdstuk 2.3 beschrijft een onderzoek naar determinanten van antibioticagebruik. In dit onderzoek zijn onafhankelijke variabelen meegenomen die het instituut, het soort patiënten en de intensiteit van zorg door (mede) behandelaars weergeven. Het blijkt dat een toename van het aantal ziekenhuisapothekers, arts-microbiologen en internist-infectiologen per 100.000 verpleegdagen gepaard gaat met een hoger gemiddeld antibioticagebruik (R^2 = 0,40). De in het onderzoek meegenomen variabelen lijken niet afdoende te zijn voor het beschrijven van de populatie patiënten met infectieziekten. Het ontwikkelen van een goede indicator voor deze casemix moet dan ook prioriteit hebben bij toekomstig onderzoek.

Om verschillende redenen is het belangrijk onderzoek te doen naar kolonisatie met resistente bacteriën. Allereerst kunnen bacteriën die normaal voorkomen in het maagdarmkanaal soms ook aanleiding geven tot klinische infecties. Kennis van de mate van resistentie van bacteriën in de mond-keel- en darmflora kan de keuze van empirische therapie vergemakkelijken. Daarnaast kunnen fecale bacteriën een reservoir vormen voor plasmiden, transposons en andere mobiele genetische elementen die in verschillende soorten bacteriën resistentie voor antibiotica veroorzaken. Tenslotte is de epidemiologie van kolonisatie met resistente bacteriën interessant omdat het maagdarmkanaal vaak de bron is van waaruit resistente bacteriën zich in een ziekenhuis verspreiden en uitbraken van infecties veroorzaken die moeilijk behandelbaar zijn.

In **hoofdstuk 3.1** wordt de invloed van opname in het ziekenhuis op het voorkomen van resistente *E. coli* bacteriën in de darmflora van patiënten prospectief onderzocht. De studiepopulatie bestond uit patiënten die op chirurgische afdelingen van de academische ziekenhuizen in Groningen, Maastricht en Rotterdam werden opgenomen. Van deze patiënten werd ontlasting verzameld bij opname in het ziekenhuis, bij ontslag, en 1 en 6 maanden na ontslag uit het ziekenhuis.

In het algemeen werden lage percentages resistentie gemeten en werden geen verschillen gevonden in het voorkomen van resistentie op de verschillende afnametijdstippen. Deze lage percentages resistentie kunnen mogelijk worden verklaard door het relatief lage gebruik van antibiotica in deze patiëntenpopulatie.

Hoofdstuk 3.2 beschrijft de ontwikkeling van een eenvoudige methode voor het screenen van de darmflora op resistente Gram-negatieve bacteriën. Hiertoe werden drie verschillende chromogene media en MacConkey agar vergeleken met betrekking tot de detectie van verschillende soorten aerobe Gram-negatieve bacteriën. Daarnaast is bepaald welke van de drie chromogene media het meest geschikt is voor directe identificatie van *E. coli* op basis van de kleur van de kolonie. Omdat *E. coli* de meest voorkomende aerobe Gram-negatieve bacterie is in de darmflora kan een dergelijke identificatie kostenbesparend zijn. Er werd geen verschil gevonden in detectievermogen. De positief voorspellende waarde verschilden eveneens niet. De negatief voorspellende waarde was hoger voor Chromogenic UTI medium en Chromagar Orientation in vergelijking met Chromagar *E. coli* /Coliform. Verder wordt geconcludeerd dat het met de chromogene media mogelijk is een algoritme te ontwikkelen waarmee de dominante darmflora naar kwantitatief voorkomen van de verschillende darmbacteriën kan worden bemonsterd.

De epidemiologie van kolonisatie met aerobe Gram-negatieve bacteriën en *Enterococcus* species is onderzocht tijdens en na opname van patiënten in het ziekenhuis **(hoofdstuk 3.3 en 3.4)**. De onderzoekspopulatie bestond uit 228 verpleegafdeling patiënten en 183 patiënten opgenomen op intensive care afdelingen. Rectale uitstrijken of feces, en uitstrijken uit de mond-keelholte, werden verzameld bij opname, tijdens opname, bij ontslag uit het ziekenhuis en 1 en 3 maanden na ontslag. In totaal werden 6069 Gram-negatieve bacteriën en 5121 enterococcen geïsoleerd, geïdentificeerd en getest voor antimicrobiële resistentie.

Bij opname en ontslag van de IC was slechts in 35% van de patiënten het darmkanaal gekoloniseerd met aerobe Gram-negatieve bacteriën en in 50-60% met enterococcen.

Bij opname op de IC was 20% van de patiënten in de mond-keelholte gekoloniseerd met Gram-negatieve bacteriën; bij en na ontslag van de IC was dit 30-40%. Zowel in de darmflora als in de mond-keelholte werd bij ontslag van de IC een verschuiving waargenomen in het relatieve voorkomen van *E. coli* ten opzichte van

andere *Enterobacteriacea* en *P. aeruginosa*. Deze verschuiving was reversibel. Tussen opname en ontslag van de IC werd een verschuiving gezien van *E. faecium* naar *E. faecalis*. Over het algemeen waren de resistentiepercentages laag. Ampicilline en cephalotine resistentie in *E. coli* namen toe tijdens ziekenhuisopname en bleven verhoogd tot 3 maanden na ontslag. Het percentage patiënten met resistente bacteriën (*E. coli*, *E. faecalis*, *E. faecium*) nam toe met de opnameduur op de IC. Dit is eveneens het geval voor het percentage patiënten met multiresistente *E. coli*.

De patiënten opgenomen op de verpleegafdelingen zijn bij opname niet gekoloniseerd met Gram-negatieve bacteriën. Echter bij en na ontslag uit het ziekenhuis is 20% gekoloniseerd. In deze patiëntenpopulatie werd geen verschuiving waargenomen in relatief voorkomen van verschillende bacteriesoorten tijdens en na opname in het ziekenhuis. De gemeten resistentiepercentages waren laag en veranderden niet tijdens of na verblijf in het ziekenhuis.

Geconcludeerd wordt dat het risico van verspreiding van resistente Gram-negatieve bacteriën en enteroccoccen van het ziekenhuis naar de open bevolking laag is bij de verpleegafdeling patiënten, maar aanzienlijk hoger is bij patiënten opgenomen op de intensive care afdeling. Het is aanbevelenswaardig om dit onderzoek te herhalen in andere ziekenhuizen om de generaliseerbaarheid van het onderzoek vast te stellen. Het is te verwachten dat in ziekenhuizen met een hoger antibioticagebruik, de ontwikkeling van resistentie tijdens verblijf hoger zal zijn, niet alleen op de intensive care afdelingen maar ook op de verpleegafdelingen.

In het onderzoek beschreven in **hoofdstuk 3.5** zijn risicofactoren voor het ontstaan van antimicrobiële resistentie in *Enterobacteriaceae* en *P. aeruginosa* tijdens ziekenhuisopname onderzocht. De onderzoekspopulatie is gelijk aan die in de hoofdstukken 3.3 en 3.4. De ontwikkeling van resistentie tijdens opname wordt uitgedrukt in het aantal "transitional events" per 1000 observatiedagen. In de IC populatie is de incidentie van transitional events drie keer hoger dan in het cohort van verpleegafdeling patiënten.

Genotypering met behulp van pulsed field gel electroforese laat zien dat de meerderheid van de resistente stammen niet op voorgaande afname tijdstippen werd waargenomen. Dit suggereert een horizontale verspreiding van resistente bacteriën tussen patiënten in het ziekenhuis. Opname op de afdeling urologie is geassocieerd met cotrimoxazol resistentie in *E. coli* en *Klebsiella spp*. Het gebruik van de fluorochinolonen is hiermee negatief geassocieerd. Het gebruik van ampicilline verhoogde het risico van ceftazidim resistentie in Gram-negatieve bacteriën. IC-patiënten die overgeplaatst waren van een verpleegafdeling hadden een verhoogd risico op fluorochinolonen resistentie in *P. aeruginosa,,* evenals patiënten die derde generatie cefalosporinen hadden gebruikt.

De resultaten van dit onderzoek rechtvaardigen twee interventie strategieën: maatregelen die de selectieve druk van antibiotica verminderen moeten gecombineerd worden met maatregelen die de verspreiding van resistente micro-organismen tegengaan.

In **hoofdstuk 4** worden de belangrijkste resultaten samengevat en besproken. Daarnaast worden een aantal methodologische aspecten beschreven. Vervolgens worden aanbevelingen gedaan voor toekomstig onderzoek.

In conclusie, de studies in dit proefschrift hebben laten zien dat de keuze van de meeteenheid bepalend kan zijn voor gemeten trends in antibioticagebruik over de jaren. Toekomstig onderzoek dient zich te richten op de vraag welke meeteenheid het meest geschikt is voor vergelijking van kwantitatief antibioticagebruik tussen geografische locaties en over de tijd, en welke meeteenheid een goede correlatie geeft met de ontwikkeling van antibioticaresistentie. Verder is vastgesteld dat prioriteit gegeven moet worden aan het ontwikkelen van een goede indicator voor de case-mix van patiënten met infectieziekten in een ziekenhuis, teneinde het kwantitatieve gebruik van antibiotica in een individueel ziekenhuis te kunnen beoordelen.

Daarnaast hebben we laten zien dat de resistentieproblematiek in ziekenhuizen zich hoofdzakelijk beperkt tot intensive care patiënten. Echter, de resistentiepercentages in deze populatie zijn relatief laag. Het is aanbevelenswaardig het onderzoek naar de epidemiologie van resistentie tijdens en na ontslag in het ziekenhuis te herhalen in een ziekenhuis met een hoger gebruik van antibiotica. Een dergelijke studie kan onze bevindingen bevestigen dat resistentiepercentages het hoogst zijn bij ontslag uit het ziekenhuis (IC patiënten) en 1 maand na ontslag uit het ziekenhuis (verpleegafdeling patiënten), en vervolgens verhoogd blijven gedurende de drie maanden na ontslag uit het ziekenhuis.

Uiteindelijk zullen de uitkomsten van toekomstige interventiestudies, gebaseerd op onder meer de bevindingen in dit proefschrift, moeten leiden tot een reductie van de resistentieproblematiek in ziekenhuizen.

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Margreet Filius
september 2005

CURRICULUM VITAE

Margreet Filius werd op 30 mei 1969 te Ede geboren. In 1987 behaalde zij het VWO diploma aan het Heldring College te Zetten en begon ze met de studie Farmacie aan de Universiteit Utrecht. In 1992 werd het doctoraalexamen afgelegd en startte ze met de opleiding tot apotheker. In 1994 heeft ze stage gelopen in het kader van een Erasmus-uitwisselingsprogramma in de General Infirmary te Leeds, Groot-Brittanië. In 1994 werd het apothekersdiploma behaald.

Daarna werkte zij als apotheker in verschillende ziekenhuizen en werd zij opgeleid tot ziekenhuisapotheker in het Zaans Medisch Centrum te Zaandam (opleider drs F.A. Boom) en het VU Medisch Centrum te Amsterdam (opleider drs A.C. van Loenen) (1996-2000).

In 2000 trad ze als onderzoeker in dienst bij de afdeling Medische Microbiologie en Infectieziekten van het Erasmus MC te Rotterdam (prof. dr. H.A. Verbrugh). Het onderzoek werd uitgevoerd in samenwerking met de afdeling apotheek van het Erasmus MC (prof. dr. A.G. Vulto) en heeft geleid tot het voorliggende proefschrift.

Sinds 2001 is zij tevens werkzaam als coördinator van de werkgroep "Surveillance antibioticagebruik" van de Stichting Werkgroep Antibioticabeleid (SWAB). Zij is "national representative" in de European Study on Antibiotic Consumption (ESAC) en is bestuurslid van de European Study Group on Antibiotic Policies (ESGAP).

Ze werkt thans als ziekenhuisapotheker bij de afdeling apotheek van het Erasmus MC. Zij woont samen met Rob Aarnoutse en is de trotse moeder van hun zoon Joep.

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