High acquisition and environmental contamination rates of CC17 ampicillin-resistant Enterococcus faecium in a Dutch hospital

Marieke J. A. de Regt1*, Lotte E. van der Wagen1, Janetta Top1, Hetty E. M. Blok1, Titia E. M. Hopmans1, Adriaan W. Dekker2, Ronald J. Hené3, Peter D. Siersema4, Rob J. L. Willems1 and Marc J. M. Bonten1,5

1Department of Medical Microbiology, University Medical Centre Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands; 2Department of Haematology, University Medical Centre Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands; 3Department of Nephrology, University Medical Centre Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands; 4Department of Gastroenterology and Hepatology, University Medical Centre Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands; 5Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

Received 11 July 2008; returned 5 August 2008; revised 12 August 2008; accepted 18 August 2008

Background: Enterococcus faecium has rapidly emerged as a nosocomial pathogen worldwide, and the majority of these isolates belong to clonal complex-17 (CC17). In Europe, CC17 isolates are usually ampicillin-resistant, but most are still vancomycin-sensitive. We aimed to study ampicillin-resistant E. faecium (ARE) epidemiology in our hospital.

Methods: In a 3 month study, 210 of 358 admissions (59%) to haematology and gastroenterology/nephrology were screened for rectal ARE colonization on admission (<48 h) and 148 of 210 (70%) also at discharge (<72 h). In a second (3 month) study, environmental swabs from eight predetermined sites were obtained from ARE-colonized haematology patients once weekly. All ARE isolates were genotyped by multiple-locus variable-number tandem repeat analysis (MLVA).

Results: ARE admission prevalence was 10% and 16% and acquisition rates were 39% and 15% in haematology and gastroenterology/nephrology, respectively. Carriage on admission was associated with previous admission <1 year (OR 5.0, 95% CI 1.8–14.0) and acquisition with β-lactam (OR 2.7, 95% CI 1.1–6.7) and quinolone use (OR 3.1, 95% CI 1.1–8.2). Five of the 57 (9%) colonized patients developed invasive ARE infections. Genotyping revealed 12 genotypes (all CC17) with two MLVA types responsible for 94% of acquisitions. In 18 of the 19 colonized patients, the environment was contaminated with ARE. Sites most often contaminated were the toilet seat (43%), over-bed table (34%) and television remote control (28%).

Conclusions: CC17 ARE epidemiology is characterized by high admission (10% to 16%), acquisition (15% to 39%) and environmental contamination (22%) rates, resulting from cross-transmission, readmission and antibiotic pressure. A multifaceted infection control approach will be needed to curtail further spread.

Keywords: VRE, epidemiology, nosocomial infections

Introduction

Enterococcus faecium has emerged as a worldwide nosocomial pathogen, particularly affecting immunocompromised patients. In the USA, nosocomial infections caused by ampicillin-resistant E. faecium (ARE) increased in the 1980s, followed by the emergence of vancomycin-resistant E. faecium (VREF) in the 1990s. In Europe, VREF prevalence rates have been rising since the turn of the century. Molecular epidemiological and population biology-based studies using multilocus sequence typing (MLST) identified a globally dispersed genetic lineage of multiresistant E. faecium

*Correspondence address. Department of Medical Microbiology, University Medical Centre Utrecht, G04.614, PO Box 85500, 3508 GA Utrecht, The Netherlands. Tel: +31-88-7555006; Fax: +31-88-7555426; E-mail: m.deregt@umcutrecht.nl

© The Author 2008. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org
designated clonal complex-17 (CC17), characterized, among other things, by ampicillin and ciprofloxacin resistance and the presence of a putative pathogenicity island containing the enterococcal surface protein (esp) virulence gene, which is associated with hospital outbreaks and infections.4–8 This evidence strongly suggests that widespread emergence of ARE in hospitals has preceded the dramatic emergence of VREF.2,9,10

Recently, we reported an increase in invasive ARE infections during the last decade in our hospital, as well as in several other Dutch hospitals, demonstrating that polyclonal endemicity with CC17 E. faecium has been established in hospital wards in a European country, previously known to be highly successful in curtailing the spread of multiresistant nosocomial pathogens.11,12

In this study, we aimed to quantify the transmission, acquisition and environmental contamination rates of ARE in two wards where colonization was endemic.

Materials and methods

Setting

The University Medical Centre Utrecht (UMCU) is a 1042 bed, tertiary-care hospital. Two wards were selected for this study where, based on previously performed point-prevalence studies, colonization with ARE was endemic: the haematology ward (21 beds) and the combined gastroenterology/nephrology ward (32 beds).11

Design

In both study wards, rectal swabs were obtained from all patients admitted between 7 November 2005 and 10 February 2006. An admission swab was obtained within 48 h after admission and a discharge swab within 72 h before discharge. The latter was only obtained if an admission swab had been taken. The following patient-specific data were collected: age, gender, length of stay, number of pre-admissions in the previous year, status of ARE colonization during previous admissions, invasive infections [defined as infectious episodes with ARE isolated from normally sterile body sites (blood, pus, wounds etc.)], mortality, the presence of underlying diseases and co-morbidity, immune status and the use of antibiotics, steroids and immunosuppressive drugs during admission to the study wards. During a second period, between 5 March 2007 and 23 May 2007, we aimed to determine environmental contamination with ARE. For this, all patients admitted to the haematology ward were again screened for ARE colonization within 48 h of admission. In case of colonization or suspected colonization (readmission of previously colonized patient), the environment of the patient was screened for ARE contamination at eight predetermined sites—blood pressure cuff, over-bed table, television remote control, bed rails, inside handle bathroom door, soap dispenser, toilet seat and control panel infusion pump—by rubbing a rayon swab moistened with PBS over a 50 cm² area once a week until discharge. In the UMCU, for ARE, no hygiene and infection control measures are taken. During both study periods, the regular standard dry cleaning protocol was followed, in which patient rooms were cleaned every weekday using moistened microfibre cloths for bathrooms and patient’s environment (without addition of cleansing agents) and disposable cloths for the floors. Per room, 8–14 min (dependent on size) were reserved for cleaning. Throughout both studies, hygiene and infection prevention policies did not change.

Ethics approval

As both studies were part of the regular infection control surveillance programme conducted by the Department of Hospital Hygiene and Infection Control, for which the need for written informed consent was waived by the Institutional Review Board, only verbal patient consent was required.

Microbiology, species identification and genotyping

Rectal and environmental swabs were analysed for ARE by inoculating the swabs in Enterococcosel Enrichment Broth (Becton–Dickinson, Cockeysville, MD, USA) supplemented with 75 mg/L aztreonam for 48 h and subsequently cultured on Enterococcosel Agar plates (Becton–Dickinson) supplemented with ampicillin (16 mg/L) for 24–48 h. Resistance was confirmed by amoxicillin Etests (AB Biodisk, Solna, Sweden). Species identification of all isolated ARE was performed using a species-specific PCR, based on the adl gene of E. faecium.13 To determine the genetic relatedness among the recovered ARE isolates and with the previously identified CC17, all ARE isolates were genotyped using multiple-locus variable-number tandem repeat analysis (MLVA) as described previously.14 The genetic relatedness of MLV A type (MT) 159 was confirmed with MLST on a subset of representative isolates.15 The presence of esp was assessed by PCR as described previously.16

Statistics

Categorical variables were compared by χ² test or Fisher’s exact test, and continuous data by Mann–Whitney test. Variables with a P value of <0.1 in univariate analysis were used for binary logistic regression to establish independent risk factors for acquisition of ARE colonization. Statistical analysis of the data was performed using SPSS 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA). The genetic diversity of ARE isolated from patients on admission and 48 h after admission were calculated following the methods described previously, using the EpiCompare software (Version 0.99 Ridom GmbH, Germany).17,18

Results

Patients

During the first study period, 358 patients were admitted: 127 (35.5%) to the haematology ward and 231 (64.5%) to the gastroenterology/nephrology ward, of which 75 (59%) and 135 (58%) were screened for ARE colonization on admission, respectively. The most important reasons for not including patients were failure to obtain swabs within 48 h of admission during weekends (n = 32) and over a 2 week holiday period (n = 31). On weekdays, 70% of all admissions to both wards were included. On these days, short duration of hospital stay—with patients being discharged or transferred before a swab was obtained—was the most frequent reason for failure to be included. Median lengths of stay of included and not included patients were 9 and 2.5 days, respectively (P < 0.001) in the haematology ward and 6 and 4.5 days, respectively (P = 0.159) in the gastroenterology/nephrology ward. Discharge swabs were obtained from 148 (70.5%) of the 210 patients included.

Colonization with ARE

On admission, 25 (11.9%) of the 210 patients were colonized with ARE, and colonization persisted in all patients (n = 17)
from whom a discharge swab was taken. Of these 25 patients, 10 (40%) had been identified as ARE carriers during a previous hospital stay (6 with a similar MT, 1 with a completely different MT and in 3 patients previous isolates had not been typed with MLVA). Time between previous culture-positivity and readmission ranged from 14 to 226 days. Six of the 25 patients (24%) had been screened during earlier hospitalization but were then found not to be colonized with ARE. The remaining nine patients (36%) had not been tested before. Twenty-two of the 25 patients colonized on admission had been admitted to the study wards at least once during the past year (7 once, 6 twice and 9 three or more times) compared with 102 of the 185 ARE-negative admissions (43 once, 22 twice and 37 three or more times). The odds ratio for ARE colonization on admission was 5.0 (95% CI 1.8–14.0) for patients with a previous admission to the study wards in the past year, when compared with patients with no hospitalization in the previous year.

Of the non-colonized patients (n = 185), 131 (70.8%) had a discharge swab taken, of which 32 contained ARE: a nosocomial acquisition rate of 24.4%. Acquisition rates were 39.2% (n = 20) in haematology and 15.0% (n = 12) in gastroenterology/nephrology.

MLVA genotyping of 76 ARE isolates (23 isolated on admission, 48 at discharge and 5 >48 h of admission) revealed 12 different MTs, 5 in the haematology and 11 in the gastroenterology/nephrology ward (4 MTs were recovered on both wards), all belonging to CC17. Of these, MT1 and MT159 were most frequently encountered (both 31 times) and were responsible for 93.8% of the acquisitions. MT12 and MT16 were both isolated from two patients and the other eight MTs were seen only in a single patient. MLST of seven MT159 strains revealed a single clone: sequence type 78. All MT159 isolates carried the esp gene, in contrast to 52% of the MT1 strains. The almost complete dominance of genotypes MT1 and MT159 in ARE acquisition during hospital stay (genetic diversity \( D = 0.548, 95\% \ CI 0.49–0.606 \)) contrasts with the more heterogeneous population of genotypes among ARE recovered from patients on admission, where nine different MTs were found among 23 patients (two isolates were not preserved for genotyping) (Figure 1). Although the genetic diversity (\( D = 0.794, 95\% \ CI 0.673–0.916 \)) among the admission isolates was significantly higher, MT1 and MT159 were also the predominant genotypes, found in nine and six patients, respectively. Epidemiological time curves linking acquisition of MT1 and MT159 with patients colonized with these clones strongly suggest that at least 94% of the acquisitions were exogenous (data not shown).

**Risk factors of acquisition of colonization with ARE**

Length of stay, medical specialty, immunocompromised status, haematological malignancies and exposure to \( \beta \)-lactam antibiotics and quinolones were associated with acquisition of ARE (Table 1). Multivariate logistic regression analysis revealed the use of \( \beta \)-lactam antibiotics (OR 2.7, 95% CI 1.1–6.7) and quinolones (OR 3.1, 95% CI 1.1–8.2) as independent risk factors for the acquisition of ARE.

**Infections with ARE**

Five of the 57 ARE-colonized patients had an invasive infection with ARE [three bacteraemias (2 \( \times \) MT1 and 1 \( \times \) MT159), one cholangitis (MT159) and one pleuritis (isolate not preserved for genotyping)], yielding an infection attack rate of 8.8%. Infections occurred 3–35 days after the first positive rectal swab; however, four of the five patients were already colonized on admission. Based on the MLVA-typing colonizing and infecting, ARE isolates were genetically indistinguishable. All three patients with ARE bacteraemia were neutropenic haematology patients, and ARE was isolated from pleural fluid and bile in two immunocompetent patients. Six of the included patients died; none of them had suffered from an infection with ARE.

**Environmental contamination**

During the second study period, there were 72 admissions to the haematology ward, of which 64 (89%) were screened for ARE on admission. Fourteen patients (22%) were colonized on admission. Monthly screening identified six additional carriers. From 19 of the 20 colonized admissions, representing 15 individual patients, environmental cultures were taken at least once (range: 1–8). The first environmental screening was performed

![Figure 1](image-url)
1–6 days after the admission swab was taken. Median duration of the follow-up was 24 days (range: 4–64).

A total of 450 environmental cultures were obtained, which were frequently colonized with ARE (Table 2). On 40 of the 63 (63%) occasions when cultures were taken and in 18 of the 19 patients, ARE grew on at least one of the environmental samples.

MLVA typing of 20 colonizing and 98 environmental strains demonstrated that MT1 and MT159 dominated also during this study as these MTs were isolated from 16 out of 18 patients and their environment. One patient was colonized with vancomycin-resistant MT287, and in one patient, the colonizing strain was not preserved for genotyping. In 11 patients, a single strain, identical to the colonizing strain, was isolated from the patients’ environment. ARE isolates representing two different MTs were isolated from the environment of three patients (Table 3). In two cases, both strains, albeit on different sample occasions, were also isolated from rectal swabs taken from these patients. Three other cases were colonized with more than one ARE MT, but only one MT was detected in environmental cultures.

### Discussion

This study demonstrates high admission, high acquisition, high environmental contamination and high infection attack rates of ampicillin-resistant CC17 *E. faecium* in hospitalized patients. The use of quinolones and β-lactams was independently associated with the acquisition of colonization. Genotyping revealed the spread of a limited number of highly related ARE clones, isolated from the environment of three patients (Table 3). In two cases, both strains, albeit on different sample occasions, were also isolated from rectal swabs taken from these patients. Three other cases were colonized with more than one ARE MT, but only one MT was detected in environmental cultures.
Clinical and molecular epidemiology of CC17 *E. faecium*

Table 2. Environmental contamination

<table>
<thead>
<tr>
<th>Location</th>
<th>ARE + swabs/total swabs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toilet seat</td>
<td>27/63 (43)</td>
</tr>
<tr>
<td>Over-bed table</td>
<td>21/62 (34)</td>
</tr>
<tr>
<td>Television remote control</td>
<td>16/57 (28)</td>
</tr>
<tr>
<td>Bed rails</td>
<td>12/60 (20)</td>
</tr>
<tr>
<td>Inside handle bathroom door</td>
<td>8/63 (13)</td>
</tr>
<tr>
<td>Blood pressure cuff</td>
<td>6/47 (13)</td>
</tr>
<tr>
<td>Control panel of infusion pump</td>
<td>3/35 (9)</td>
</tr>
<tr>
<td>Soap dispenser</td>
<td>5/63 (8)</td>
</tr>
</tbody>
</table>

Table 3. MLVA-types colonizing and environmental strains

<table>
<thead>
<tr>
<th>MLVA-type isolated from</th>
<th>Patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rectum</td>
<td>environment</td>
</tr>
<tr>
<td>159</td>
<td>159</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>287</td>
<td>287</td>
</tr>
<tr>
<td>159</td>
<td>1;159</td>
</tr>
<tr>
<td>1;159</td>
<td>1;159</td>
</tr>
<tr>
<td>1;159</td>
<td>159</td>
</tr>
</tbody>
</table>

strongly suggesting that exogenous transmission within wards, via either the hands of healthcare workers and/or the inanimate environment, was the dominant route for ARE acquisition. Continuous introduction of ARE by readmission of colonized patients and frequent nosocomial transmission have, in the presence of antibiotic pressure, resulted in the development of polyclonal endemicity of ARE in several wards in our hospital. With the presence of a reservoir of vancomycin resistance genes in the community and the continuous introduction of these genes into the hospital, the CC17 ARE endemicity poses a serious risk for conversion into VREF endemicity through events of horizontal gene transfer. 

Our findings demonstrate that ARE epidemiology in a Dutch hospital closely mimics the previously observed epidemiology of vancomycin-resistant enterococci (VRE) in American hospitals. Therefore, effective control of this rapidly emerging nosocomial pathogen will be difficult and a multifaceted approach combining rapid screening on admission, isolation of carriers, improved hand hygiene of healthcare workers and enhanced environmental cleaning might be needed.

The use of antibiotics, particularly β-lactams and fluoroquinolones, have frequently been demonstrated to be associated with acquisition and colonization with ARE and VRE. In this study, β-lactam and quinolone use was associated with a three times higher risk of acquisition of colonization with CC17 ARE, which is characterized by ampicillin and ciprofloxacin resistance. Acquisition further depends on colonization pressure within the wards, which is influenced by the continuous admission of colonized patients. During this study, 12% of patients were colonized with ARE on admission, most of them having a history of previous hospitalization. This finding is in line with other studies demonstrating that recent hospitalization is a risk factor for ARE colonization.

In contrast to the high nosocomial prevalence, ARE was found in only 2.9% of the community-derived faecal samples in the hospital catchment area. The virtual absence of a community reservoir, in combination with the presence of highly related strains belonging to one clonal complex in hospitals worldwide, strongly suggests that exogenous transmission within the hospital setting is the main acquisition route of CC17 ARE.

Enterococci can survive for prolonged periods of time on different materials in the hospital. Recently, two studies confirmed the importance of the inanimate environment as a reservoir in the spread of multiresistant enterococci. Similarly, we demonstrated in this study that in our haematology ward colonizing strains can frequently be isolated from several places in the direct environment of ARE-positive patients, on both frequently cleaned and almost never-cleaned spots that are often touched by patients and healthcare workers, forming a possible source of exogenous transmission.

Acknowledgements

Parts of this work were presented at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy and at the 18th European Congress of Clinical Microbiology and Infectious Diseases. We would like to thank the nursing staff of the haematology and gastroenterology/nephrology wards for their assistance with the data collection.

Funding

This work was supported by a grant from the European Union Sixth Framework Programme under contract LSHE-CT-2007-037410. M. J. M. B. was supported by NWO-VICI 918.76.611.

Transparency declarations

None to declare.

References


