# Chapter 7

# Ecological replacement of *Enterococcus faecalis* by multi-resistant clonal complex 17 *Enterococcus faecium*.

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## Abstract

The proportion of enterococcal infections caused by ampicillin-resistant *Enterococcus faecium* (AREfm) in a European hospital increased from 2% in 1994 to 32% in 2005, with prevalence rates of AREfm endemicity of up to 35% in at least six hospital wards. Diabetes mellitus, three or more admissions in the preceding year, and use of β-lactams and fluoroquinolones, were all associated with AREfm colonisation. Of 217 AREfm isolates that were genotyped, 97% belonged to clonal complex 17 (CC17). This ecological change mimics events preceding the emergence of vancomycin-resistant *E. faecium* (VREF) in the USA and may presage the emergence of CC17 VREF in European hospitals.


## Introduction

Molecular epidemiological studies of *Enterococcus faecium* have revealed the existence of host-specific genogroups, including an ampicillin-resistant genetic lineage, labelled clonal complex 17 (CC17), which are associated with nosocomial outbreaks and infections in five continents (1). In European hospitals, rates of infection with vancomycin-resistant *E. faecium* have been rising since the year 2000 (EARSS Annual Report 2004; http://www.rivm.nl/earss), suggesting that the emergence of vancomycin-resistant *E. faecium* in Europe may be following the pattern observed in the USA, but with a 10-year delay. The emergence of vancomycin-resistant *E. faecium* in the USA was preceded by the emergence of ampicillin resistance in *E. faecium* (2,3).

## Material and methodes

Stimulated by an increase in ampicillin-resistant *E. faecium* (AREfm) bloodstream infections during 2003, the present study analysed trends in enterococcal infection and colonisation at the University Medical Centre, Utrecht, The Netherlands (1042 beds). The prevalence of invasive enterococcal infections was assessed retrospectively using microbiological data for 1994–2005. Invasive infections were defined as infectious episodes with enterococci isolated from normally sterile specimens, e.g., blood, abdominal fluid, intravascular catheter tips, cerebrospinal fluid, pus and wound specimens. Enterococci isolated from urine were not considered to represent invasive infections. Yearly proportions of *Enterococcus faecalis* and *E. faecium* among enterococcal bloodstream infections were determined on the basis of the first 20 enterococcal blood culture isolates (one per patient) per year. The intestinal AREfm reservoir was measured by monthly point-prevalence studies between August 2005 and January 2006 in

**Table 1**. Risk-factor analysis for colonization with ampicillin-resistant *Enterococcus faecium*

| Variable | Cases(%) (n=43) | Controls(%) (n=93) | OR | 95%CI | p value[a] |
|---|---|---|---|---|---|
| Univariate analysis | | | | | |
| Demographic and clinical data | | | | | |
| Medical speciality (nephrology) | 72.1 | 39.8 | 3.91 | 1.78-8.57 | <0.001 |
| Age, mean years (±SD) | 57.4 (±14.4) | 54.4 (±16.0) | | | 0.29[b] |
| Male gender | 51.2 | 43.0 | 1.39 | 0.67-2.87 | 0.38 |
| Length of stay, median of days (range) | 10 (1-78) | 7 (1-55) | | | 0.19[c] |
| Number of readmissions | | | | | |
| in preceding year | | | | | |
| 0 | 32.6 | 59.1 | | | <0.001 |
| 1-2 | 37.2 | 35.5 | | | |
| ≥3 | 30.2 | 5.4 | | | |
| CAPD | 27.9 | 9.7 | 3.613 | 1.39-9.41 | 0.006 |
| Haemodialysis | 25.6 | 9.7 | 3.208 | 1.22-8.47 | 0.02 |
| Kidney transplantation | 27.9 | 14 | 2.382 | 0.98-5.79 | 0.05 |
| Recent surgery | 34.9 | 24.7 | 1.630 | 0.74-3.57 | 0.22 |
| Malignancy | 4.7 | 8.7 | | | 0.25 |
| Immunecompromised state | 50 | 27.2 | 2.680 | 1.25-5.73 | 0.01 |
| Systemic use of corticosteroids | 48.8 | 29 | 2.333 | 1.11-4.93 | 0.03 |
| Cirrhosis of the liver | 9.3 | 5.4 | 1.805 | 0.46-7.09 | 0.39 |
| Crohn's disease | 2.3 | 5.4 | .419 | 0.05-3.7 | 0.42 |
| Colitis ulcerosa | 0 | 2.2 | | | 0.33 |
| Diabetes mellitus | 23.3 | 8.6 | 3.220 | 1.17-8.87 | 0.02 |
| Antibioticusage | 76.7 | 49.5 | 3.372 | 1.49-7.63 | 0.003 |
| β-Lactams | 65.1 | 37.6 | 3.09 | 1.46-6.58 | 0.004 |
| Co-trimoxazol | 25.6 | 8.6 | 3.652 | 1.35-9.9 | 0.008 |
| Macrolides | 4.7 | 3.2 | 1.463 | 0.24-9.1 | 0.68 |
| Vancomycin | 2.3 | 3.2 | .714 | 0.07-7.07 | 0.77 |
| Quinolones | 18.6 | 5.4 | 4.023 | 1.23-13.15 | 0.02 |
| Aminoglycosides | 4.7 | 10.8 | .405 | 0.09-1.93 | 0.24 |
| Multivariate analysis[d] | | | | | |
| CAPD | | | 2.75 | 0.82-9.20 | 0.10 |
| Haemodialysis | | | 3.44 | 0.96-12.36 | 0.06 |
| Kidney transplantation | | | 0.44 | 0.09–2.24 | 0.32 |
| Immunecompromised state | | | 1.14 | 0.22-5.95 | 0.88 |
| Systemic use of corticosteroids | | | 5.68 | 1.18-27.31 | 0.30 |
| Diabetes mellitus | | | 8.59 | 2.08-35.44 | 0.003 |
| β-Lactams | | | 2.97 | 1.09-8.09 | 0.03 |
| Co-trimoxazol | | | 2.38 | 0.58-9.71 | 0.23 |
| Quinolones | | | 5.23 | 1.22-22.48 | 0.03 |
| Number of readmissions | | | | | |
| in preceding year | | | | | |
| 0 | | | 1 | Reference | 0.001 |
| 1-2 | | | 1.75 | 0.62–4.91 | 0.29 |
| ≥3 | | | 14.84 | 3.44–64.10 | <0.001 |

[a]Chi-square test.

[b]*t* -test

[c]Mann-Whitney test.

[d]Logistic regression on variables with p<0.100.

CAPD, continuous ambulatory peritoneal dialysis

seven hospital wards (haematology, 21 beds; gastroenterology/nephrology, 25 beds; adult intensive care unit (ICU), 24 beds; paediatric ICU, 47 beds; geriatrics, 15 beds; general surgery, 30 beds; and dermatology, 12 beds). The AREfm reservoir in the community was investigated using faecal samples from 650 outpatients with abdominal discomfort who visited general practitioners in the Utrecht region during 2004.

Risk-factors for colonisation with AREfm were determined using clinical and demographical data for patients in the mixed gastroenterology/nephrology ward. Statistical analysis was performed with SPSS v.12.0.1 (SPSS Inc., Chicago, IL, USA). The clinical impact of AREfm was determined by analysis of clinical, demographical and outcome data for all patients with an invasive AREfm infection between May 2001 and November 2005.

Enterococcosel enrichment broth and agar plates (Becton Dickinson, Cockeysville, MD, USA), supplemented with aztreonam 75 mg/L and amoxycillin 16 mg/L, were used to obtain isolates of AREfm. Resistance was confirmed by amoxycillin Etests (AB Biodisk, Solna, Sweden). A species-specific multiplex PCR, based on the *ddl* gene of *E. faecalis* and *E. faecium*, was used for speciation (4). Susceptibilities to ampicillin and imipenem were determined by inoculation of

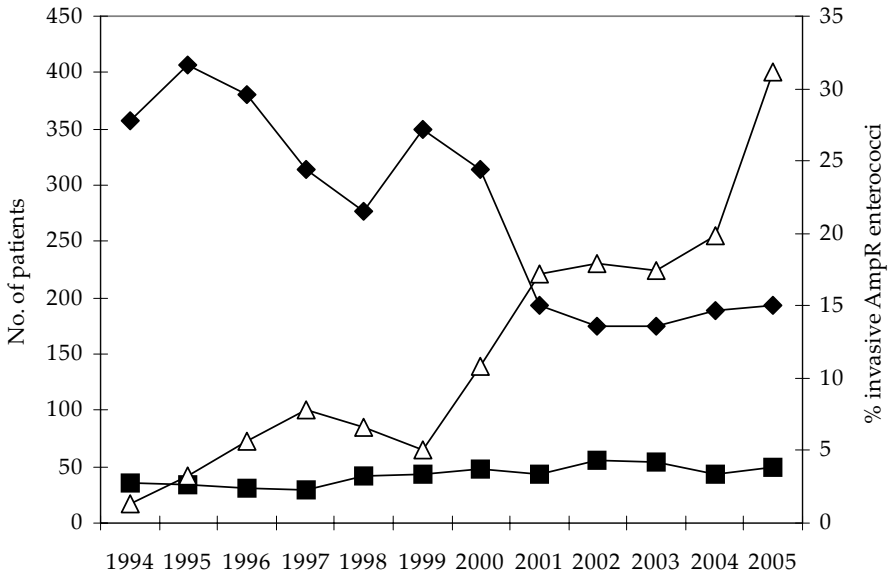


**Figure. 1**. Invasive enterococcal infections, 1994–2005. ■, No. of patients with enterococcal bloodstream infections.♦, No. of patients with other invasive enterococcal infection. Δ, Percentage of invasive ampicillin-resistant(AmpR) enterococci among the total number of invasive enterococci.

Mueller–Hinton agar containing ampicillin 8 mg/mL and imipenem 16 mg/mL, according to CLSI (formerly NCCLS) guidelines. Isolates were genotyped using multiple locus variable number tandem repeat analysis (MLVA), which is based on variations in the number of tandem repeats at six different loci (5). MLVA profiles were analysed using BioNumerics v.4.00 (Applied Maths, St-Martins-Latem, Belgium).

## Results

The overall number of patients with invasive enterococcal infections decreased from 393 in 1994 to 243 in 2005, but proportions of ampicillin-resistant enterococcal infections increased from 2% in 1994 to 32% in 2005 ($p < 0.001$) (Figure. 1). *E. faecium* increased from 3% of enterococcal bloodstream infections in 1994–1996 to 30% in 2003–2005 ($p < 0.001$), and 75% of *E. faecium* blood culture isolates were resistant to both ampicillin and imipenem, compared with 0% for *E. faecalis* ($p < 0.001$).

Point-prevalence studies revealed carriage rates ranging from 0% in dermatology to 10.3% in the paediatric ICU, 29% in gastroenterology/nephrology, 29.2% in the adult ICU, 34.6% in haematology and 34.8% in geriatrics. AREfm isolates were obtained from 19 (2.9%) of 650 community-derived faecal samples. No data were available concerning previous hospitalisation or antibiotic use for these patients.

Diabetes mellitus (OR 8.59, 95% CI 2.08–35.44), three or more admissions in the preceding year (OR 14.84, 95% CI 3.44–64.10) and use of β-lactams (OR 2.97, 95% CI 1.09–8.09) or quinolones (OR 5.23, 95% CI 1.22–22.48) were associated independently with AREfm colonisation (Table 1).

Between May 2001 and November 2005, 167 patients had an AREfm infection, with blood ($n$ = 53) and pus ($n$ = 30) as the predominant samples. Overall, 154 (92%) of 167 patients had received antibiotics before developing an infection with AREfm. Nineteen of 20 haematology patients with AREfm infection had bacteraemia. Haematology patients in the studied hospital receive ciprofloxacin prophylaxis during prolonged granulocytopenia, and imipenem is the empirical antibiotic regimen for granulocytopenic fever. During 2002–2005, 7% ($n$ = 12) of all episodes of bacteraemia during granulocytopenia were caused by AREfm. The average period between obtaining blood cultures and commencing appropriate antimicrobial therapy (i.e., vancomycin) was 2 days. Of 167 patients with invasive AREfm infections, 58 (35%) died during hospitalisation, with an average period of 15 days (0–105 days) between identification of the AREfm infection and death.

MLVA typing of 217 AREfm isolates revealed 40 different MLVA types, of which 211 (97%) belonged to CC17 (data not shown). A gradual increase in

infection and colonisation episodes with CC17 *E. faecium* has occurred in this hospital in recent years, with bacteraemia accounting for 32% of all infections, an overall mortality rate of 35%, and CC17 infections being most prevalent among high-risk patients (i.e., transplant or ICU patients).

## Discussion

*E. faecium* CC17 consists mainly of clinical isolates and isolates associated with hospital outbreaks of vancomycin-resistant enterococci (VRE) (1). Strains colonising healthy individuals and animals cluster, almost without exception, outside this complex. It has been postulated that specific adaptations to the hospital environment that facilitate efficient spread are the reasons for the success of this pathogen (1). However, the spread of multiple CC17 subclones, without an existing community reservoir, can only be explained by cross-transmission and selective antibiotic pressure. Increasing rates of infection with AREfm present a therapeutic dilemma, as amoxycillin has been the preferred antibiotic for invasive enterococcal infections. In bone marrow transplant patients (for whom imipenem was first-choice therapy for granulocytopenic fever), surveillance for AREfm carriage has now been implemented and vancomycin has been added to imipenem for the treatment of granulocytopenic fever in AREfm carriers.

A similar rise of AREfm may have preceded the nationwide nosocomial epidemic of VRE in the USA. Three longitudinal microbiology-based studies in the USA reported changes in *E. faecalis*/*E. faecium* ratios in hospital infections (6–8). High prevalence and nosocomial spread of AREfm have also been reported, albeit sporadically, in European countries (9–14). The emergence of AREfm may presage the emergence of VRE, following horizontal transfer of vancomycin resistance genes into AREfm (15,16). In Europe, the prevalence of VRE carriage among healthy individuals decreased to 3% after the ban on the use of avoparcin in the agricultural industry in 1996 (17,18). However, the prevalence of VRE in a cohort of non-hospitalised patients in the Utrecht region during the year 2000 was still 2%(19), which represents a relatively abundant pool of vancomycin resistance genes in the community.

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