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ScienceDirect
International Journal of Medical Microbiology 298 (2008) 515–527
IJMM
www.elsevier.de/ijmm

Increasing rates of vancomycin resistance among *Enterococcus faecium* isolated from German hospitals between 2004 and 2006 are due to wide clonal dissemination of vancomycin-resistant enterococci and horizontal spread of *vanA* clusters

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Received 5 March 2007; received in revised form 20 May 2007; accepted 24 May 2007

Abstract

Results of national and international surveillance studies revealed increasing rates of vancomycin-resistant *Enterococcus faecium* (VREF) among German hospital patients since 2003. To investigate the molecular background of *vanA*-type glycopeptide resistance, 51 clinical VREF isolated between 2004 and 2006 and originating from 19 German hospitals representing 10 Federal States have been investigated. Isolates were characterised by multi-locus sequence typing (MLST), SmaI macrorestriction analysis in pulsed-field gel electrophoresis (PFGE), and multiple-locus variable-number tandem repeat analysis (MLVA). Phylogenetic relatedness between strains was identified using BioNumerics and eBURST software. Distribution of virulence markers *esp* and hyl_{Efm} was investigated by PCR. The structure of the *vanA* gene clusters was investigated by PCR, long-template PCR, sequencing and Southern hybridisations. The 51 VREF were rather diverse constituting different strain types, different virulence markers and *vanA* clusters. Within this diversity we found supportive data for a dissemination of related – already vancomycin-resistant – *E. faecium* among various hospitals and Federal States and for spread of identical *vanA* gene clusters among clonally different strain types within single hospitals. In conclusion, the increase in the rates of VREF among German hospital patients within the last 2 years might be rather complex and due to different molecular events and scenarios.

Keywords: Vancomycin-resistant enterococci; Vancomycin resistance; MLST; *vanA*; *Enterococcus faecium*

Introduction

Enterococci are commensal bacteria of the animal and human gut but can also serve as dreaded nosocomial pathogens of life-threatening infections especially among the elderly, immuno-compromised or seriously ill patients. *E. faecalis* still earmarks the majority of enterococcal infections, mostly urinary tract infections, endocarditis and bacteraemia. However, *E. faecium* attracts more and more attention due to its capacity of acquiring multiple antibiotic resistance determinants, especially those encoding glycopeptide resistance and its potential to spread among the nosocomial setting (Courvalin, 2006; Murray, 2000; Top et al., 2007; Woodford, 2001). The population structure of *E. faecium* has been disclosed by methods like amplified-fragment length polymorphism (AFLP) (Willems et al., 2000), multi-locus sequence typing (MLST)

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doi:10.1016/j.ijmm.2007.05.008

(Homan et al., 2002) and most recently comparative genomic hybridisations (Leavis et al., 2007). Within the clonal complex (CC) of human isolates a distinct subcluster (CC17) was identified by all these methods combining strains from clusters of infections and outbreaks among hospital patients from a representative worldwide collection (Homan et al., 2002; Leavis et al., 2006a; Willems et al., 2000, 2005). Characteristics of those so-called hospital-adapted, epidemic-virulent clonal types are, based on logistic regression models, (i) ampicillin resistance, (ii) high-level fluoroquinolone resistance (Leavis et al., 2006b) and (iii) possession of virulence markers (e.g., *esp* gene) (Leavis et al., 2003, 2006a; Willems et al., 2005). The majority of strain collections constituting hospital-adapted isolates involved vancomycin-resistant *E. faecium* (VREF). However, as stated above, possession of vancomycin resistance is not an independent factor of MLST CC17 clonal types. So the current model foresees dissemination of epidemic CC17 isolates among the hospital setting possessing the above-mentioned features and acquisition of determinants encoding vancomycin resistance at a later stage (Kawalec et al., 2001; Leavis et al., 2003; Suppola et al., 1999; Willems et al., 2005). Both, the markers for an enhanced epidemicity, genes for hyaluronidase hyl_{Efm} and enterococcal surface protein *esp* and the *vanA* and *vanB* clusters encoding vancomycin resistance are localised on mobile elements and could either be mobilised or conjugatively transferred when in the appropriate genetic background (Dahl et al., 2003; Johnsen et al., 2005; Leavis et al., 2004; Oancea et al., 2004; Rice et al., 2003; Werner et al., 1997, 2006). Consequently, hospital VREF belonging to MLST CC17 and lacking genes *esp* and *hyl* were also described (Harrington et al., 2004; Leavis et al., 2003; Oh et al., 2005).

The European Antimicrobial Resistance Surveillance System (EARSS) reports about resistance development in invasive bacterial isolates including enterococci and demonstrates a heterogeneous picture for VREF all over Europe (http://www.rivm.nl/earss). For several European countries, rates of VREF among all invasive *E. faecium* infections showed a marked increase between 2001 and 2005 (Germany, Ireland), whereas in other countries rates are still at low levels of a few percent (The Netherlands, Scandinavian countries) or at an already elevated level of 15–25% or higher (Italy, Portugal, Greece, UK; the latter communicated via national surveillance systems). The reasons for these differences might be rather complex (Bonten et al., 2001; Donskey, 2004; Leavis et al., 2006a; Mascini and Bonten, 2005; Tenover and McDonald, 2005; Vander Stichele et al., 2006; Werner and Bronzwaer, 2007). Clonal spread of hospital-adapted, epidemic-virulent *E. faecium* is discussed as an independent prerequisite for a successful country-wide dissemination of VREF (Kawalec et al., 2001; Suppola et al., 1999; Willems et al., 2005). Studies were done on a representative, international strain collection (Leavis et al., 2003; Willems et al., 2005) or independently in several countries. The situation in single countries is characterised by a clonal expansion of distinct types, such as MLST ST-78 in Italy or ST-117 in Germany between 1995 and 1999 (Bonora et al., 2004; Kawalec et al., 2001; Klare et al., 2003, 2005; Stampone et al., 2005; Suppola et al., 1999). Preliminary results for Germany or South Korea showed a dissemination of a various number of clonal types, even within single hospitals, but all belonging to MLST CC17 (Klare et al., 2003, 2005; Ko et al., 2005). In the present study, we expand the coverage of involved hospitals to demonstrate a representative picture of VREF from clusters of infections and colonisations and outbreaks in German hospital patients between 2004 and 2006. We raise the question whether VREF with the same genetic background as defined by MLST appearing in different hospitals and Federal States in Germany might be related and disseminated as VREF or whether they emerged independently from glycopeptide-susceptible ancestors by acquisition of *vanA* gene clusters from different sources.

Materials and methods

Bacterial isolates

Our laboratory receives samples of isolates or single strains for confirmation (antibiotic susceptibility results, other features) or an identification of clonal relatedness in case of supposed outbreaks or clusters of infections and colonisations. Altogether 51 *E. faecium* (one isolate per patient) from 2004 to 2006 from 19 hospitals representing 10 German Federal States were chosen for further analyses. All isolates were vancomycin-resistant and possessed the *vanA* gene. Isolates UW5248, UW5250, UW5251, UW5254, UW5255 and UW5258 originated from a large private laboratory service provider in South-western Germany (Labor Limbach, Heidelberg) with a representative coverage of hospitals in five Federal States. The six isolates were chosen from a larger set of isolates representing clusters of infections and colonisations in five hospitals in 2004 (partly described in Klare et al., 2005). The other 45 nosocomial *E. faecium* originated from 14 other German hospitals and were isolated in 2004–2006. They were chosen as representative when originating from a collection of more than three VREF sampled at the same time from the same hospital (= cluster of infections and colonisations) or from invasive infections (blood). Clonality of isolates was allocated based on SmaI macrorestriction analysis in PFGE. For larger numbers of isolates ($n > 20$) from the same provider