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ARTICLE

Environmental survival of vancomycin-sensitive ampicillin-resistant *Enterococcus faecium* (AREfm)

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Abstract Ampicillin-resistant *Enterococcus faecium* (AREfm) has gained increased footholds in many hospital intensive care units (ICUs) and belongs to specific hospital-adapted *E. faecium* sub-populations. Three AREfm strains survived in an in vitro survival setting for approximately 5.5 years. These findings have important consequences for the epidemiology of AREfm in hospital settings and stress the importance of maintaining a good level of hospital hygiene.

Introduction

Antibiotic-resistant *Enterococcus faecium*, including vancomycin- and ampicillin-resistant *E. faecium* (VREFm) and vancomycin-sensitive and ampicillin-resistant *E. faecium* (AREfm) that belong to a specific hospital-adapted sub-population previously designated clonal complex (CC) 17—clade A strains according to current typing techniques—have become prominent in nosocomial infections, especially in the intensive care units (ICUs) of many hospitals [1–3]. This phenomenon was also observed in The Netherlands [3, 4], including the Atrium Medical Centre Parkstad (Parkcity) in the South Limburg region. The dynamics of CC17 have been described previously [2–4].

AREfm are responsible for hospital infections, including bacteraemias [2, 3], which necessitate vancomycin therapy. In contrast to VREFm, no specific targeted precautions against AREfm were suggested by the Dutch Working Group on Infection Prevention (WIP) [5]. In the ICU of our hospital, AREfm of CC17 have been isolated on more than 50 occasions in three episodes in different years, with irregular and prolonged intervals. This occurred predominantly in patients with duration of ICU stay of more than 2 weeks. Due to hygienic interventions, the transmission of AREfm could be contained.

High acquisition and environmental contamination rates and survival characteristics of *E. faecium* that may increase the risk for acquisition of this opportunistic pathogen in hospital settings have previously been described [3, 6, 7]. In a previous study, we have found extensive in vitro survival of VREfm for approximately 4 years [8]. This prompted us to assess the survival dynamics of three contemporary AREfm strains originating from the period 2003–2007, when the proportion of enterococcal infections caused by AREfm increased, in a way comparable to the survival test described previously for VREFm that originated from just before this period [8].

Materials and methods

From three different outbreak events in different years, representative AREfm isolates obtained from ICU patients were selected. The three CC17 AREfm isolates belonged to different multi-locus variable-number tandem repeat analysis (MLVA) genotypes (MT-16 from

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2003, MT-214 from 2005 and MT-159 from 2007) [1]. Based upon multi-locus sequence typing (MLST) [4], the 2003 and 2005 isolates were both ST16, while the 2007 isolate was ST78.

All three strains were resistant to ampicillin [Etest minimum inhibitory concentrations (MICs) of >32 mg/l] and susceptible to vancomycin, with Etest MIC values of <4 mg/l.

For the survival experiment, suspensions of the AREfm strains containing approximately 10^{10} colony-forming units (cfu/ml) were prepared in sterile phosphate-buffered saline (PBS; pH 7.2) in a 100-ml screw-top glass bottle, as described previously [8, 9]. In short, a 1-ml sample of each suspension was transferred to 104 identical bottles for each strain and allowed to dry. All bottles were plugged with cotton wool to allow free communication with the hospital environment in air and were kept in a surrounding with indirect sunlight to simulate the most hidden and inaccessible places of the ICU. After 10 days, the suspensions in these bottles with a flat bottom of area circa 12 cm^2 had completely dried and sampling began 4 days later (point zero), i.e. 14 days after the suspensions had been prepared. Survival was initially measured with weekly intervals and, in the final stage of the experiment, with up to 3-month intervals. The effect of desiccation was studied by recovering remaining viable bacteria with the addition of 1 ml of PBS to the bottle. After vigorous vortexing, the suspension was spread on blood agar plates in different dilutions, including undiluted, 10^{-2} and 10^{-4} dilutions, and incubated for 48 h at 37 °C. In addition, 50 ml of peptone broth was added to the empty bottle and incubated for 72 h at 37 °C, which was sub-cultured on a blood agar plate only if there was no growth observed on the blood agar plates from the different dilutions of the suspension. Each individual bottle was, thereafter, discarded. Colony counts were rounded up to the nearest first decimal place on the $\log_{10}$ scale. For all strains, survival counts were made until extinction. Thereby, the last day of viability (DOV) was approximately determined.

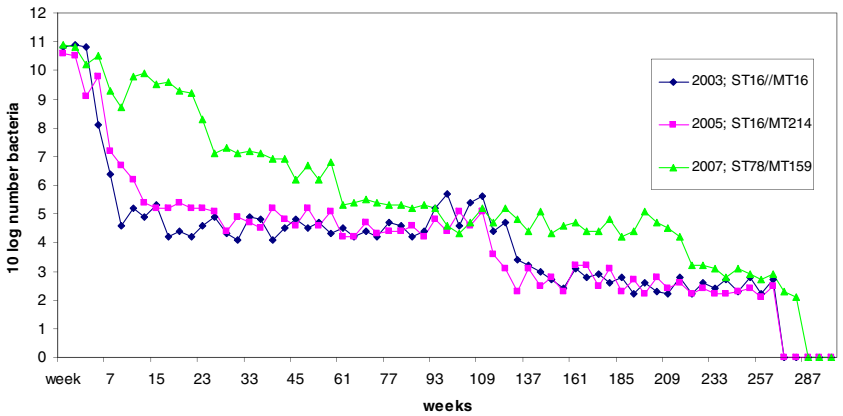
Results

The survival dynamics of the three AREfm strains are shown in Fig. 1. A difference was observed between the strain with ST78 (MT159) and the other two strains with ST16 (MT16 and MT214). While only a gradual decrease of survival time was observed for the ST78 strain, with colony counts dropping to 10^7 cflu/ml, a steep decline of survival during the first year was observed for both ST16 isolates, with colony counts dropping to 10^4 cflu/ml. The following 30 months of colony counts gradually declined to 10^2 to 10^5 cfu/ml for all three strains. During the whole test period, strain colony counts for ST78 were the highest. After 4 years, survival was still detected. More than 1 year later, no further survival could be noted, representing last DOVs of 1925 days for both ST16 strains and 2009 days for the ST78 strain. The time period between the measurements was gradually extended due to the shortage of bottles as a result of the unexpected prolonged survival to prevent running out of bottles before arriving at the target of the approximate DOV.

Discussion

Hospital environmental survival of nosocomial pathogens contributes to their transmissibility within hospital wards. It has been reported previously that two methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak strains survived with last DOVs between 318 and 379 days and an *Acinetobacter baumannii* outbreak strain survived nearly as long as the MRSA strains (last DOV 329 days) [7, 9]. These survival times were, however, remarkably shorter than the survival times of two VREFm (DOVs of approximately 1400 days) on which we have reported previously [8].

The three AREfm strains described in the current study survived even longer, up to approximately 2000 days, which may indicate that these contemporary AREfm are even better equipped for prolonged survival on inanimate surfaces than



MLVA; multiple-locus variable-number tandem repeat analysis (MT). MLST; multi-locus sequence typing (ST).

Fig. 1 Survival of outbreak-related ampicillin-resistant *Enterococcus faecium* (ARE) strains typed by MLVA/MLST in an intensive care unit during three different years (legend: year, type). *MLVA* multi-locus variable-number tandem repeat analysis (MT), *MLST* multi-locus sequence typing (ST)

the VRE isolates from the year 2000. It remains to be elucidated which factors contribute to this prolonged survival of *E. faecium* in general and of the ST78 isolate in particular.

In hindsight, given the long survival time of both ST16 isolates, it is conceivable that the isolate recovered from an ICU patient in 2005 was not a new, independent introduction but the same strain that was already found in 2003 and persisted for 2 years either as an asymptomatic coloniser in patients or staff, or survived for 2 years on inanimate surfaces at the ICU and subsequently recolonised a patient.

Previously, Kramer et al [7] reviewed studies investigating the survival of nosocomial pathogens on surfaces. They reported on studies in which the survival times for enterococci were significantly shorter (5 days to 4 months) than what we found. The shorter survival may be explained by different experimental circumstances, such as choice of strains, density of the inoculum, influences of sun or artificial light, temperature and humidity. As the strains used by Kramer et al. were not included in our study, it is difficult to speculate as to why we found longer survival times [7]. The survival pattern of this enterococcal species is distinctively different from a number of virulent types of the related group A streptococcus, which survived in the same experimental setting for only 4 weeks [10].

This study suggests that prolonged survival is an intrinsic property of *E. faecium* and underlines the ability of this species to sustain itself in the hospital environment. The results do not prove anything about the transmission of this organism from person to person. To be successful in a modern busy hospital, an organism only needs to survive for a few hours before the next patient occupies the room. Thus, long persistence is important for this species. Persistence may possibly be a marker of something else that is being selected, e.g. bacterial cell wall thickness, capsule expression, ability to form biofilm during drying, resistance to biocides etc., that just happens to lead to a persistence of the phenotype.

Indeed, the survival of these AREfm strains is the longest documented among non-spore-forming hospital pathogens. This means that this bacterium can re-emerge from hidden places in a presumed 'AREfm-free ward' by colonising susceptible patients. This stresses the importance of adequate environmental decontamination of patient rooms to break the chain of patient-to-patient cross-transmission of multidrug-resistant nosocomial pathogens [6].

Conflict of interest The authors declare that they have no conflict of interest.

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