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Clinical Studies

Small hospitals matter: insights from the emergence and spread of vancomycin-resistant enterococci in 2 public hospitals in inner Brazil

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ABSTRACT

Although vancomycin-resistant enterococci (VRE) are reported in Brazil since 1996, data on their impact over settings of different complexity are scarce. We performed a study aimed at identifying determinants of VRE emergence and spread in a public hospital consortium (comprising 2 hospitals, with 318 and 57 beds) in inner Brazil. Molecular typing and case–control studies (addressing predictors of acquisition or clonality) were performed. Among 122 authocthonous isolates, 106 were *Enterococcus faecium* (22 clones), and 16, *Enterococcus faecalis* (5 clones). Incidence was greater in the small-sized hospital, and a previous admission to this hospital was associated with greater risk of VRE colonization or infection during admission to the larger one. Overall risk factors included comorbidities, procedures, and antimicrobials (piperacillin-tazobactam, cefepime, and imipenem). Risk factors varied among different hospitals, species, and clones. Our findings demonstrate that VRE can spread within low-complexity facilities and from these to larger hospitals.

1. Introduction

Despite decades of extensive research, the prevention and control of multidrug-resistant organisms (MDROs) within healthcare settings are still a major challenge (Siegel et al., 2007). The difficulties multiply in developing countries, where the application of basic infection control routines is undermined by such factors as understaffing, physical structure, and poor microbiology resources (Allegranzi et al., 2011). In this context, the case for vancomycin-resistant enterococci (VRE) in Brazil is exemplary.

The first reports of VRE infection in Brazil date back from 1996 (Dalla Costa et al., 1998; Zanella et al., 1999). In the following years, VRE spread through hospitals in several Brazilian states (Bender et al., 2009; Conceição et al., 2010; da Silva et al., 2012; Moretti et al., 2011). In a recent report from a Latin American program for monitoring resistance, 27% of enterococci isolates from Brazil were VRE (Jones et al., 2013).

The epidemiology of VRE is intricate, involving cross-transmission, selection by antimicrobials, and environmental reservoirs (DeLisle and Perl, 2003). On the other hand, hospitals in Brazil vary widely in their size, complexity, and target population. In 2013, there are 6226 hospitals distributed in the vast Brazilian territory, two thirds of them with less than 50 beds (data from CNES, Brazilian's National Database of Healthcare Settings; cnes.datasus.gov.br). Many among those small-sized hospitals harbor intense surgical activity (Padoveze et al., 2010). It is therefore challenging to approach VRE epidemiology in such a variety of settings. However, that challenge is worth facing, in order to increase our understanding and identify targets for control strategies. This was the rationale of our study.

In the present study, we attempted to identify determinants of VRE emergence and spread in 2 public hospitals from a consortium: one that provides tertiary (high complexity) care and other that admits less severe medical patients. We mixed molecular strain typing and observational epidemiological designs (Foxman and Riley, 2001), in order to provide a comprehensive approach to VRE epidemiology in those settings.

In order to approach the complex epidemiology of VRE, we posed several research questions, as follows: What was the incidence of VRE in the 2 study hospitals? How were VRE clones distributed among those facilities? What were the risk factors for VRE acquisition, and how did they vary from one hospital to the other? Among VRE-harboring patients, what were the predictors for species (*Enterococcus faecalis* versus *Enterococcus faecium*) and clonality?

2. Materials and methods

2.1. Study setting

The study was conducted in 2 hospitals in the City of Bauru, São Paulo State, Brazil (22°018′ 53″ S, 49° 03′ 38″ W). That city has approximately 360,000 inhabitants and is located 330 km away from the state capital (São Paulo City). The study hospitals are public and work as a

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consortium, administered by a foundation linked to Faculdade de Medicina de Botucatu (Botucatu Medical Faculty). The Hospital Estadual Bauru (HEB) is a 318-bed facility providing tertiary care for the city of Bauru and surrounding municipalities, an area comprising 1 million inhabitants. It is a modern building, dating from the early 2000s. HEB has 3 intensive care units, 1 burn unit, and several wards caring for medical, surgical, and pediatric patients. Most rooms in wards for noncritical patients comprise 1 or 2 beds. The Hospital Estadual "Manuel de Abreu" (HEMA) has 57 beds and admits adult patients with infectious diseases or chronic noninfectious disorders. It was built in the 1950s with the original intent of admitting patients with tuberculosis. Most rooms comprise 3 or 4 beds. It is worth noting that both hospitals share the same microbiology laboratory and infection control committee.

2.2. Study design and subjects

We conducted a descriptive and case–control studies. The study subjects were patients who had VRE recovered from clinical or surveillance cultures from January 2010 to June 2012. The case patients were divided in 3 groups. The first 2 groups comprised "autochtonous cases", i.e., patients who acquired VRE while staying in HEB and HEMA, respectively. A case patient was assigned to 1 of the study hospitals when he/she had stayed in that hospital for more than 2 days previously to the collection of cultures. Whenever 1 patient transferred from HEB to HEMA (or vice versa) presented positive cultures during the first 2 days of admission, it was assigned to the original hospital. The third group consisted of "imported cases", patients from whom VRE was recovered during the first 48 hours of admission and who had no history of hospital admission or were transferred from hospitals other than HEB and HEMA.

2.3. Screening for VRE-harboring patients

The first case of VRE infection was diagnosed in January 16, 2010. From that moment on, a strategy for screening patients for VRE colonization was implemented. Briefly, it consisted in the collection of rectal swabs for i) every patient transferred from other hospitals, ii) every patient admitted to 1 of the intensive care unit (ICUs) (upon admission and weekly thereafter), and iii) weekly for every patient from hospital units where a VRE case was identified in the previous 15 days. This latter rule (iii) means that, whenever a VRE case was identified, collection of swabs for all patients admitted to the same unit was performed and repeated on a weekly basis until 2 weeks after the identification of the last case. In addition to screening, we recorded every clinical culture that was positive for VRE. All patients from whom VRE was recovered were placed in isolation precautions for contact transmission.

2.4. Microbiology methods

Rectal swabs were plated on BBL™ Enterococcosel™ Agar (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Clinical specimens were cultured following current procedures and later plated on the same medium (Zimbro et al., 2009). Species identification was carried out with specific kit (Probac, São Paulo, Brazil). Susceptibility tests were performed through disk diffusion, following guidelines from the Clinical Laboratory Standards Institute (CLSI, 2012). Resistance to vancomycin was confirmed in E-test™ (BioMerieux SA, Marcy l'Etoile, France).

2.5. Molecular strain typing

The first VRE isolate from each case patient (either autochtonous or imported) was submitted to strain typing. Pulsed-field gel electrophoresis (PFGE) was performed according to the protocol described by Bedendo and Pignatari (2000). *Sma*I enzyme (Roche Diagnostics, Québec, Canada) was used for DNA restriction. Electrophoresis was using a CHEF-DR III System (BioRad Laboratories, Hercules, California, USA).

The analysis of band patters was performed in Bionumerics software (AppliedMaths, Sint-Martens-Latem, Belgium). Dendrograms were created using unweighted pair group method with arithmetic mean method. The similarity of band patterns was analyzed using the Dice coefficient. A similarity coefficient of 0.8 or greater was used for definition of clones.

2.6. Descriptive analysis and case–control studies

We provide a descriptive analysis of the emergence of VRE in the study hospitals. Monthly and aggregate incidence rates were calculated and compared using the mid-*P* exact test in the OpenEpi software (Emory University, Atlanta, GA, USA).

Several case–control studies were performed, varying the cases and controls. Case–control study 1 was designed to identify general risk factors for VRE acquisition in HEB or HEMA. For each case, 2 controls were selected among patients admitted to the same unit in the same period. Case–control studies 2 and 3 included subgroups of cases and controls from HEB and HEMA, respectively. For the case–control studies 4, 5, and 6, only VRE-positive subjects were included. The outcomes of interest were *E. faecalis* (as opposed to *E. faecium*), belonging to the predominant clones (comprising the greatest number of isolates) or to 1 of the major clones (i.e., clones comprising 5 or more isolates).

For all those studies, patient data were recovered from charts and laboratory files. A VRE case was defined as a subject from whom *E. faecalis* or *E. faecium* was recovered, regardless of the sample. Thus, we included subjects who had VRE recovered from either surveillance or clinical cultures.

We recorded data on demographics, comorbidities (including the Charlson comorbidity index) (Charlson et al., 1987), procedures, and use of antimicrobials during "time at risk". Time at risk was defined as the number of days from admission up to the positive culture (for VRE-positive subjects) or to hospital discharge (for VRE-negative controls).

Data from cases and controls were recorded in EPI INFO 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and analyzed with SPSS 19.0 (IBM, Armonk, NY, USA). All data were initially submitted to univariate analysis. Chi-square or Fisher exact test was applied for dichotomous data, and the Mann–Whitney *U* test was used for comparisons of numeric data. In the second phase, multivariable analysis (logistic regression) was performed. We used a "change in estimate" approach for selecting variables (Greenland, 1989). Briefly, the first logistic regression model included all variables that achieved $P < 0.1$ in the univariate analysis. A second step selected only those that resulted significant ($P < 0.05$) in the previous model. Other steps added (alternately) all the other variables. Those that changed the odds ratio of any statistically significant variable in more than 10% were included in the final model.

3. Results

During the study period, we identified 130 subjects harboring VRE, of whom 122 were diagnosed as autochthonous for the study hospitals. Among those subjects, 109 had the first VRE isolate recovered from rectal swabs. Other 13 subjects had VRE recovered from urine (10), blood (2), and tracheal aspirate (1). Since this number was small to warrant a separate analysis, all the patients harboring VRE were analyzed as a single group.

According to the criteria discussed above, 78 and 44 patients were assumed to have acquired VRE in HEB and HEMA, respectively. The overall incidence of VRE was higher in HEMA, as compared to HEB (12 versus 4 per 10,000 patients-day, $P < 0.001$). The relation of *E. faecium* to *E. faecalis* was 69:9 for HEB and 37:7 for HEMA. The remaining 8 subjects were transferred from other hospitals and already harbored VRE (7 *E.* faecium and 1 *E. faecalis*) upon admission. Table 1 presents differences of subjects harboring VRE in the study hospitals. Coherently with the nature and complexity of settings, patients from HEB were more likely to have comorbidities (except AIDS) and to have been exposed to invasive procedures or devices.