COMPARISON BETWEEN AUTOMATED SYSTEM AND PCR-BASED METHOD FOR IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF CLINICAL Enterococcus spp

Luciana FURLANETO-MAIA(1), Kátia Real ROCHA(2), Vera Lúcia Dias SIQUEIRA(3) & Márcia Cristina FURLANETO(2)

SUMMARY

Enterococci are increasingly responsible for nosocomial infections worldwide. This study was undertaken to compare the identification and susceptibility profile using an automated MicrosScan system, PCR-based assay and disk diffusion assay of Enterococcus spp. We evaluated 30 clinical isolates of Enterococcus spp. Isolates were identified by MicrosScan system and PCR-based assay. The detection of antibiotic resistance genes (vancomycin, gentamicin, tetracycline and erythromycin) was also determined by PCR. Antimicrobial susceptibilities to vancomycin (30 μg), gentamicin (120 μg), tetracycline (30 μg) and erythromycin (15 μg) were tested by the automated system and disk diffusion method, and were interpreted according to the criteria recommended in CLSI guidelines. Concerning Enterococcus identification the general agreement between data obtained by the PCR method and by the automatic system was 90.0% (27/30). For all isolates of E. faecium and E. faecalis we observed 100% agreement. Resistance frequencies were higher in E. faecium than E. faecalis. The resistance rates obtained were higher for erythromycin (86.7%), vancomycin (80.0%), tetracycline (43.35) and gentamicin (33.3%). The correlation between disk diffusion and automation revealed an agreement for the majority of the antibiotics with category agreement rates of > 80%. The PCR-based assay, the van(A) gene was detected in 100% of vancomycin resistant enterococci. This assay is simple to conduct and reliable in the identification of clinically relevant enterococci. The data obtained reinforced the need for an improvement of the automated system to identify some enterococci.

KEYWORDS: Enterococcus; MicrosScan system; PCR assay.

INTRODUCTION

Enterococci are implicated in a wide diversity of infections and are the third most common pathogen isolated from several infections worldwide. According to a recent epidemiological survey conducted in Brazil, Enterococcus spp accounted for 4.5% of all nosocomial bloodstream infections (BSIs), resulting in 49.5% crude mortality. Enterococci infections’ greater mortality rates and antibiotic resistance are associated with prolonged hospitalization and increased health-care costs. It has recently been reported that inappropriate and delayed antibiotic therapy present an independent risk factor for mortality caused by enterococcal bacteraemia. Besides, the difficulty in treating enterococci infections, particularly with respect to vancomycin resistance isolates, emphasizes the need for safe and therapeutic guidance for rapid identification and effective management.

In this context, the employment of automated systems, that provide rapid identification and susceptibility testing, may lead to a significant reduction of patient morbidity, mortality and cost. However, the identification and susceptibility testing of microorganisms usually takes 24-48 h after initial growth in a routine laboratory. In addition, automated systems may present problems in the identification of members of the genus Enterococcus in clinical laboratories. Currently, several studies have compared the direct and standard methods for different automated systems.

The employment of polymerase chain reaction (PCR)-based assay in the identification of enterococci and detection of antibiotic resistance genes offered a specific and rapid alternative to standard tests, providing essential information concerning the effective management and appropriate therapy of enterococcal bacteraemia.

In this study, we compared for the first time the MicrosScan® system versus PCR-based approach for identification as well as the susceptibility profile of clinical Enterococcus sp.

MATERIAL AND METHODS

Isolates: A total of 30 Enterococcus clinical isolates were obtained from January 2008 to June 2010, from patients of the University Hospital of State University of Maringá (UEM). The origins of the isolates were...
Antimicrobial susceptibility testing: Susceptibility testing of four antimicrobial agents (vancomycin, 30 μg; tetracycline, 30 μg; erythromycin, 15 μg; and gentamicin 120 μg) (Laborclin) was performed by the disk diffusion assay on Muller Hinton agar plates. After 18 or 24 h of incubation at 37 °C, inhibition zone diameters around each disc were measured and the diameters of inhibition zones were interpreted according to the criteria recommended by the Clinical and Laboratory Standards Institute, 2011. Staphylococcus aureus 25923 ATCC was used as a control strain. MicroScan® system was used on the same antimicrobial agents for the antimicrobial susceptibility testing.

RESULTS

In the present study, we firstly evaluated the genetic similarities of the Enterococcus isolates using the RAPD-PCR analysis. The fingerprinting revealed no clonal lineage (unrelated strains) among tested isolates (data not shown).

As shown in Table 2, for 27 out of 30 (90%) isolates the identification was concordant between the automated system and the molecular method. All 20 isolates identified as E. faecium and seven isolates as E. faecalis by automation system were confirmed by PCR assay. Figure 1 illustrated the amplicon size of Enterococcus sp. Among the isolates tested, E. faecium (76.7%) had a much higher incidence rate followed by the PCR assay.

The disagreement was observed in the identification of three isolates. The species classified by automation as E. gallinarum (isolate 817) and E. durans/hirae (isolate 917 and 1000) were all identified as E. faecalis by the PCR assay.
Antibiotic susceptibility phenotypes and resistance genes profile, detected by PCR, of the enterococcal isolates are shown in Table 3. The presence of resistance genes \textit{erm}(B), \textit{tet}(L), \textit{vanA} and \textit{aac(6')}\textit{Ie}-\textit{aph}(2'') were 86.7%, 23.3%, 80.0% and 66.7%, respectively. Several isolates harbored resistance genes to more than one antibiotic. Of significance were \textit{tet}(L)/\textit{erm}(B) to \textit{E. faecalis} (42.8%) and \textit{erm}(B)/\textit{aac(6')}\textit{Ie}-\textit{aph}(2'') to \textit{E. faecium} (69.6%).

The presence of the \textit{vanA} gene was detected in three isolates of \textit{E. faecalis} and twenty-two of \textit{E. faecium}, corresponding to 42.8% and 96.6% of the isolates, respectively. The \textit{vanA} gene was detected in 100% of vancomycin resistant enterococci (Table 3), however, five isolates harbored the \textit{vanA} gene and presented vancomycin susceptibility phenotype.

Enterococci have been implicated in severe human infections as a consequence of associated determinants of virulence and antimicrobial resistance. Accurate identification and rapid analysis of the antibiotic susceptibility pattern of the causative microbial agent leads to earlier targeting of antibiotic therapy and may be lifesaving.

In this study, we describe a comparison between automatic and PCR-based assay for identification of \textit{Enterococcus} spp. Our results showed 90% agreement in the identification of clinically relevant enterococcal species, revealing that the PCR method is reliable and convenient for rapid identification and has potential for use in clinical microbiology laboratories.

Besides, one isolate was identified as \textit{E. gallinarum} and two were

Table 2
Identification of clinical enterococci isolates by automated systems and molecular method

| Strain | origin          | Identification automated system | PCR-based assay |
|--------|----------------|--------------------------------|-----------------|
| 802    | urine          | \textit{E. faecalis}            | \textit{E. faecalis} |
| 817    | rectal swab    | \textit{E. gallinarum}         | \textit{E. faecium} |
| 840    | blood          | \textit{E. faecalis}           | \textit{E. faecalis} |
| 848    | urine          | \textit{E. faecalis}           | \textit{E. faecalis} |
| 872    | orotracheal fluid | \textit{E. faecalis}    | \textit{E. faecalis} |
| 906    | urine          | \textit{E. faecalis}           | \textit{E. faecalis} |
| 917    | urine          | \textit{E. durans/hirae}       | \textit{E. faecium} |
| 924    | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 925    | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 928    | urine          | \textit{E. faecalis}           | \textit{E. faecalis} |
| 973    | urine          | \textit{E. faecium}            | \textit{E. faecium} |
| 1000   | urine          | \textit{E. durans/hirae}       | \textit{E. faecium} |
| 1035   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 1053   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 1062   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 1076   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 1097   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 1112   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 1114   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 1115   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 1227   | urine          | \textit{E. faecium}            | \textit{E. faecium} |
| 1231   | urine          | \textit{E. faecium}            | \textit{E. faecium} |
| 1246   | urine          | \textit{E. faecium}            | \textit{E. faecium} |
| 1280   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 1295   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |
| 1298   | rectal swab    | \textit{E. faecium}            | \textit{E. faecium} |

CUSP pattern of clinical Enterococcus spp. Rev. Inst. Med. Trop. Sao Paulo, 56(2): 97-103, 2014.

DISCUSSION

Enterococci have been implicated in severe human infections as a consequence of associated determinants of virulence and antimicrobial resistance. Accurate identification and rapid analysis of the antibiotic susceptibility pattern of the causative microbial agent leads to earlier targeting of antibiotic therapy and may be lifesaving.

On the other hand, antimicrobial susceptibility phenotype was detected even in the absence of the respective resistance gene for two isolates to \textit{erm}(B), 10 to \textit{tet}(L) and three to \textit{aac(6')}\textit{Ie}-\textit{aph}(2'')-\textit{Ia} gene.

Additionally, antimicrobial susceptibilities to erythromycin, tetracyclin, vancomycin, and gentamicin were analyzed by disk diffusion. Evaluation revealed excellent agreement for all of the antibiotics with category agreement rates > 80% between automatized method and disk diffusion. Major error rates were for erythromycin, vancomycin and tetracycline with 20.7%, 7% and 16.7% respectively. Minor error rates were found as 12.1% for gentamicin.

Resistance rates obtained by disk diffusion were as follows: 86.7% for erythromycin, 80.0% for vancomycin, 43.35% for tetracycline and 33.3% for gentamicin. Resistance frequencies were higher in \textit{E. faecium} than \textit{E. faecalis}.

![Fig. 1 - Amplification gel pictures characteristic of polymerase chain reaction (PCR) amplification of Enterococcus sp gene. Lanes: (1) Enterococcus spp. (112 pb), (2) \textit{E. faecalis} (941 pb), (3) \textit{E. faecium} (550 pb). M - Ladder 1kb plus (Invitrogen).](image-url)
Table 3

| Isolates | Genes detected by PCR | Antibiotic resistance phenotype (MIC μg/mL)* |
|----------|-----------------------|--------------------------------------------|
|          | em(B) | tet(L) | vanA | aac(6’)-Ie-aph(2’)-Ia | ERY | TET | VAN | GEN |
| 802      | +     | +      | +    | -                  | > 4R | > 8 R | ≤ 2 S | ≤ 500 S |
| 817      | -     | -      | +    | +                  | ≤ 0,5 S | > 8 R | 8 I | ≤ 500 S |
| 840      | -     | -      | +    | -                  | > 4 R | ≤ 4 S | ≤ 2 S | ≤ 500 S |
| 848      | +     | +      | -    | -                  | 2     | ≤ 4 S | ≤ 2 S | ≤ 500 S |
| 872      | +     | +      | -    | -                  | > 4 R | > 8 R | ≤ 2 S | ≤ 500 S |
| 906      | +     | +      | -    | -                  | > 4   | > 8 R | ≤ 2 S | ≤ 500 S |
| 917      | +     | +      | -    | -                  | > 4   | > 8 R | ≤ 2 S | ≤ 500 S |
| 924      | +     | -      | +    | +                  | > 4   | ≤ 4 S | > 16 R | ≤ 500 S |
| 925      | +     | +      | +    | +                  | > 4   | ≤ 4 S | > 16 R | ≤ 500 S |
| 928      | +     | -      | +    | +                  | --//-- | > 8 R | ≤ 2 S | --//-- |
| 973      | +     | -      | +    | +                  | > 4   | > 8 R | ≤ 2 S | --//-- |
| 1000     | +     | -      | +    | +                  | > 4   | ≤ 4 S | > 16 R | > 500 R |
| 1035     | +     | -      | +    | +                  | > 4   | > 8 R | > 16 R | > 500 R |
| 1053     | +     | -      | +    | +                  | > 4   | > 8 R | > 16 R | ≤ 500 S |
| 1062     | +     | -      | +    | +                  | > 4   | ≤ 4 S | > 16 R | > 500 R |
| 1076     | +     | -      | +    | +                  | > 4   | > 8 R | > 16 R | ≤ 500 S |
| 1097     | -     | -      | +    | +                  | > 4   | > 8 R | > 16 R | ≤ 500 S |
| 1112     | +     | -      | +    | -                  | > 4   | ≤ 4 S | > 16 R | > 500 R |
| 1114     | +     | -      | +    | +                  | > 4   | ≤ 4 S | > 16 R | > 500 R |
| 1115     | +     | -      | +    | -                  | > 4   | ≤ 4 S | > 16 R | > 500 R |
| 1125     | +     | +      | +    | +                  | > 4   | ≤ 4 S | > 16 R | > 500 R |
| 1143     | +     | -      | +    | -                  | > 4   | ≤ 4 S | > 16 R | > 500 R |
| 1211     | -     | -      | -    | -                  | ≤0,5  | ≤ 4 S | ≤ 2 S | ≤ 500 S |
| 1215     | +     | -      | +    | +                  | > 4 R | > 8 R | > 16 R | ≤ 500 S |
| 1227     | +     | -      | +    | +                  | > 4   | ≤ 4 S | > 16 R | > 500 R |
| 1231     | +     | -      | +    | +                  | > 4   | > 8 R | > 16 R | ≤ 500 S |
| 1246     | +     | -      | +    | +                  | > 4   | ≤ 4 S | > 16 R | > 500 R |
| 1280     | +     | -      | +    | +                  | > 4 R | ≤ 4 S | > 16 R | > 500 R |
| 1295     | +     | -      | +    | +                  | > 4 R | ≤ 4 S | > 16 R | > 500 R |
| 1298     | +     | -      | +    | +                  | > 4 R | > 8 R | > 16 R | ≤ 500 S |

MIC: minimal inhibitory concentration; ERY: erythromycin; TET: tetracycline; VAN: vancomycin; GEN: gentamicin (120 µg/mL); --//--: data not provided; S: sensible; R: resistance; I: intermediate resistance. (*) Result obtained from the automated method.
identified as *E. durans/hirae* by MicroScan, whereas by PCR-based assay all three isolates were identified as *E. faecium*. Similar discrepancy was described by ROBREDO et al., who compared the API 20 STREP and colony hybridization for identification of enterococci obtained from several origins. According to these authors, high agreement was obtained for *E. faecalis* identification, however, for eight isolates identified as *E. durans* and *E. casseliflavus* by API 20 STREP were identified as *E. faecium* according to the molecular method.

Several studies have found differences between automatic and classical or molecular bacterial identification systems. For instance, concerning Gram positive bacteria, no gram-positive cocci showed concordant identification between the direct and standard methods; other discrepancies consisted of misidentification between various species of coagulase-negative staphylococci.

On the other hand, some studies showed the agreement between automatic and classical or molecular bacterial identification systems, D’AZEVEDO et al. compared the automated Vitek system and standard methods for identification of 80 isolates belonging to different species of *Enterococcus*. The general agreement between results was 83.7%. Among isolates of *E. faecalis* and *E. faecium* were observed that the automated system correctly identified 35/40 (87.5%) and 12/14 (85.7%) of the strains, respectively.

CEKIN et al. demonstrated the consistency of automated systems with the conventional methods. They detected as 97.8% to identification of VRE strains using both methods.

Based on the results presented here and the previous report there is a need for improvement in the automated MicrosScan system to identify enterococci.

In the present study, the genotypic basis of the resistance phenotype found in isolates of *E. faecium* and *E. faecalis* was determined by PCR based detection of resistance genes. The majority of *Enterococcus* isolates displayed resistance to at least one antibiotic tested. Our results revealed that the *vanA* gene was predominant in *E. faecium* tests since this gene was detected in 100% of vancomycin-resistant isolates, although *Enterococcus* spp. may harbor other genes (*vanB, vanC-1, vanC-2/3 and vanD*) related to resistance.

Antibiotic resistance has played an essential role in the emergence of *E. faecalis* and *E. faecium* as nosocomial pathogens. Vancomycin is an important therapeutic option for the treatment of severe enterococcal infections and resistance to this type of antibiotic is concerning. Identified risk factors for vancomycin-resistant enterococci (VRE) acquisition include a prolonged hospital stay, exposure to intensive care units or residence on transplant oncology wards, prior exposure to antibiotics, and proximity to other patients infected or colonized with VRE.

In our study we detected the tet(∗L) gene in 23.3% (7/30) of the isolates, while four and five of these presented resistance to tetracycline in automated and disk diffusion method, respectively. Similar prevalence of tet(∗L) gene (21%) in enterococci was described by STOVCIK et al. In contrast, FRAZZON et al. detected the tet(∗L) gene in only 9% of the *Enterococcus* sp isolates. Furthermore, tetracycline resistance phenotype was detected even in the absence of the tet(∗L) gene for 10 isolates. This may be explained by the fact that in enterococci two major groups of tetracycline resistance genes have been identified. One group encoding ribosomal protection proteins include tet(∗M), tet(∗O) and tet(∗S) genes, and the other one that encodes tetracycline efflux pumps includes the tet(∗L) and tet(∗K) genes. Similarly, erythromycin resistance was detected even in the absence of the *ermB* gene. This resistance may be due to the presence of *ermA* and/or *ermC* genes related to erythromycin resistance phenotype.

Gentamicin susceptible phenotype was detected in 36.7% of the isolates. However, 52.6% of these were detected as the *aac(6′)-Ie-aph(2′″)-Ia* gene. Similar results were obtained by POULSEN et al.

In our study, the MicroScan system and disk diffusion method had an agreement of about 80%. Gülmez & Hasçelik compared the Phoenix system and microdilution method and observed an excellent agreement for all of the antibiotics with category agreement rates of > 97%. In contrast, the API method was considered unreliable in detecting high levels of aminoglycoside resistance among *Enterococcus* strains compared to disc diffusion method.

Our data revealed high frequency of *E. faecium* and the occurrence of several multi resistance isolates. Antibiotic resistance appears to have contributed to increasing administration of inadequate antimicrobial therapy for infections, particularly enterococci nosocomial acquired infections, which is associated with greater hospital mortality rates.

Rapid and reliable identification of these antibiotic resistant organisms is crucial for patient management and infection control measures. Enterococci are intrinsically resistant to many antimicrobial agents, and their ability to acquire resistance to other agents such as aminoglycosides, β-lactams and glycopeptides (vancomycin and teicoplanin) is well known, resulting in invasive human enterococcal infections that are extremely difficult to treat.

The primary objective of the study was to determine whether molecular identification and direct antimicrobial susceptibility testing would provide results comparable to those obtained from an automated system in routine use. This study revealed that the PCR assay and disk diffusion method are in agreement with MicroScan automated system employed for identification and test susceptibility, respectively of clinical *Enterococcus* spp.

**RESUMO**

**Comparação entre o sistema automatizado e PCR na identificação e susceptibilidade de isolados clínicos de Enterococcus spp**

Os enterococos são cada vez mais responsáveis por infecções hospitalares em todo o mundo. Este estudo foi realizado para comparar a identificação e perfil de suscetibilidade entre o sistema automatizado MicroScan e a técnica molecular de PCR em espécies de *Enterococcus* spp. Foram avaliados 30 isolados clínicos de *Enterococcus* spp. Os isolados foram identificados pelo sistema MicroScan® e pela técnica de PCR. A detecção de genes de resistência a antibióticos (vancomicina, gentamicina, tetraciclina e eritromicina) foi determinada por PCR. Suscetibilidades antimicrobianas à vancomicina (30 μg), gentamicina (120 μg), tetraciclina (30 μg) e eritromicina (15 μg), foram testados.
pelos métodos automatizados e pelo disco difusão, de acordo com as orientações do CLSI. No que diz respeito à identificação de *Enterococcus* em geral entre os dados obtidos pelo método de PCR e pelo sistema automático foi de 90,0% (27/30). Para todos os isolados de *E. faecium* e *E. faecalis* observamos concordância de 100%. FreQUências de resistência foi maior em *E. faecium* do que em *E. faecalis*. As taxas de resistência obtidas foi maior para eritromicina (86,7%), vancomicina (80,0%), tetraciclina (43,3%) e gentamicina (33,3%). A correlação entre a técnica de disco difusão e automação revelou-se de acordo para maioria dos antibióticos com taxas > 80%. O gene van(A) foi detectado em 100% dos *Enterococcus* resistentes à vancomicina. O ensaio baseado em PCR é de simples realização e de confiança para identificação de enterococos clinicamente relevantes. Os dados obtidos reforçam a necessidade de melhoria no sistema automatizado para identificar alguns enterococos.

ACKNOWLEDGEMENT

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - Brazil and Fundação Araucária – Paraná, Brazil. Kátia Real Rocha is fellowship holder of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Brazil.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

1. Acar JF. Consequences of bacterial resistance to antibiotics in medical practice. Clin Infect Dis. 1997;24(suppl 1):517-8.
2. Bell IM, Paton JC, Turnidge J. Emergence of vancomycin-resistant enterococci in Australia: phenotypic and genotypic characteristics of isolates. J Clin Microbiol. 1998;36:2187-90.
3. Bruins MJ, Blommebergen P, Ruijs GJHM, Wolfhagen MJHM. Identification and susceptibility testing of *Enterobacteriaceae* and *Pseudomonas aeruginosa* by direct inoculation from positive BACTEC blood culture bottles into Vitek 2. J Clin Microbiol. 2004;42:7-11.
4. Cekin Y, Ozhak Baysan B, Mutlu D, Sepin Özen N, Öngut G, Dönmez L, et al. Evaluation of VITEK 2, (A) foi detectado em 100%.
5. Cekin Y, Ozhak Baysan B, Mutlu D, Sepin Özen N, Öngut G, Dönmez L, et al. Comparison of Phoenix automated system, API ID 32 Strep system and lightcycler *Enterococcus* MGRADE system in the identification of clinical *Enterococcus* isolates. Mikrobiyol Bul. 2013;47:141-6.
6. Chow JW, Fine MJ, Shales DM, Quinn JP, Hooper DC, Johnson MP, et al. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy. Ann Int Med. 1991;115:585-90.
7. CLSI-Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational Supplement Approved standard M100-S21, v. 31. 2011. Wayne; 2011.
8. Courvalin P. Vancomycin resistance in gram-positive cocci. Clin Infect Dis. 2006;42(Suppl 1):S25-S34.
9. Cueto M, Ceballos E, Martinez-Martinez L, Perea EJ, Pascua A. Use of positive blood cultures for direct identification and susceptibility testing with the Vitek 2 system. J Clin Microbiol. 2004;42:3734-8.
10. Dukta-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol. 1995;33:24-7.
11. Facklam RR, Sahn D, Teixeira LM. *Enterococcus*. In: Murray PR, Baron EJ, Pfaffer MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology. 7. ed. Washington: ASM Press; 1999. p. 297-305.
12. Fontanals D, Salceda F, Hernandez J, Sanfeliz I, Torra DM. Evaluation of wider systems for direct identification and antimicrobial susceptibility testing of gram-negative bacilli from positive blood culture bottles. Eur J Clin Microbiol Infect Dis. 2002;21:693-5.
13. Frazzon APG, Gama BA, Hermes V, Bierhals CG, Pereira RI, Guedes AG, et al. Prevalence of antimicrobial resistance and molecular characterization of tetracycline resistance mediated by tet(M) and tet(L) genes in *Enterococcus* spp. isolated from food in Southern Brazil. World J Microbiol Biotechnol. 2010; 2:365-70.
14. Gevers D, Danielsen M, Huys G, Swings J. Molecular characterization of tet(M) genes in *Lactobacillus* isolates from different types of fermented dry sausage. Appl Environ Microbiol. 2003;69:1270-9.
15. Gholizadeh Y, Courvalin P. Acquired and intrinsic glycopeptide resistance in *Enterococcus*. Int J Antimicrob Agents. 2000;16(Suppl 1):S11-7.
16. Gülmez D, Hasçelik G. Comparison of microdilution method and Phoenix automated system for testing antimicrobial susceptibilities of *Enterococcus* strains. Mikrobiyol Bul. 2011;45:21-7.
17. Hansen DS, Jensen AG, Norskov-Lauritsen N, Skov R, Bruun B, Direct identification and susceptibility testing of enteric bacilli from positive blood cultures using VITEK (GNE/GNS-GA). Clin Microbiol Infect. 2002;8:38-44.
18. Huys G, D’Haene K, Collard JC, Swings J. Prevalence and molecular characterization of tetracycline resistance in *Enterococcus* isolates from food. Appl Environ Microbiol. 2004;70:1555-62.
19. Jensen LB, Ahrens P, Doms L, Jones RN, Hammerum AM, Aarestrup FM. Molecular analysis of the *Tn 1546* in *Enterococcus faecium* isolated from animals and humans. J Clin Microbiol. 1998;36:437-42.
20. Jin WY, Jang SJ, Lee MJ, Park G, Kim MJ, Kook JK, et al. Evaluation of VITEK 2, MicroScan, and Phoenix for identification of clinical isolates and reference strains. Diag Microbiol Infect Dis. 2011;70:442-7.
21. Ke D, Picard FJ, Martinerez F, Meinard PHR, Ouellette M, Bergenor MG. Development of a PCR assay for rapid detection of *Enterococci*. J Clin Microbiol. 1999;37:3497-503.
22. Kobashi Y, Hasebe A, Nishio M, Uchiyama H. Diversity of tetracycline resistance genes in bacterial isolated from various agricultural environmental. Microbes Environment. 2007;22:44-51.
23. Leibovici L, Shraga I, Drucker M, Konigsberger H, Samra Z, Piltik SD. The benefit of appropriate empirical antibiotic treatment in patients with bloodstream infection. J Int Med. 1998;244:379-86.
24. Lindén PK. Clinical implications of nosocomial Gram-positive bacteremia and superimposed antimicrobial resistance. Am J Med. 1998;104(5A):24-33.
25. Lupetti S, Barnini B, Castagna B, Nibbering PH, Campa M. Rapid identification and antimicrobial susceptibility testing of Gram-positive cocci in blood cultures by direct inoculation into the BD Phoenix system. Clin Microbiol Infect. 2010;16:986-91.
26. Marra AR, Camargo LFA, Pignatari ACC, Sukienik T, Behar PR, Medeiros EA, et al. Nosocomial bloodstream infections in Brazilian Hospitals: analysis of 2,563 cases from a prospective nationwide surveillance study. J Clin Microbiol. 2011;49:1866-71.
27. Marques EB, Suzart S. Occurrence of virulence-associated genes in clinical Enterococcus faecalis strains isolated in Londrina, Brazil. J Med Microbiol. 2004;53:1069-73.

28. Poeta P, Costa D, Sáenz N, Klibi N, Ruiz-Larrea F, Rodrigues J, et al. Characterization of antibiotic resistance genes and virulence factors in faecal enterococci of wild animals in Portugal. J Vet Med B Infect Dis Vet Public Health. 2005;52:396-402.

29. Poulsen L, Biigaard M, Son NT, Trung NV, An HM, Dalgaard A. Enterococcus and Streptococcus spp. associated with chronic and self-medicated urinary tract infections in Vietnam. BMC Infect Dis. 2012;12:320.

30. Ratnasaswan W, Iwen PC, Hinrichs SH, Rupp ME. Bacteremia due to motile Enterococcus species: clinical features and outcomes. Clin Infect Dis. 1999;28:1175-7.

31. Robredo B, Singh KV, Baquero F, Murray BE, Torres C. Vancomycin-resistant enterococci isolated from animals and food. Int J Microbiol. 2000;54:197-204.

32. Rubin RJ, Harrington CA, Poon A, Diretrich K, Greene A, Moiuddin A. The economic impact of Staphylococcus aureus infection in New York city hospitals. Emerg Infect Dis. 1999;5:9-17.

33. Seo JY, Kim PW, Lee JH, Song JH, Peck KR, Chung DR, et al. Evaluation of PCR-based screening for vancomycin-resistant enterococci compared with a chromogenic agar-based culture method. J Med Microbiol. 2011;60:945-9.

34. Sirin MC, Adiçoğlu AK. Comparison of five antimicrobial susceptibility tests in detecting high level aminoglycoside and vancomycin resistances in hospital acquired Enterococcus isolates. Clin Lab. 2011;57:157-62.

35. Stovcik V, Javorsky P, Pristas P. Antibiotic resistance patterns and resistance genes in enterococci isolated from sheep gastrointestinal tract in Slovakia. Bull Vet Inst Pulawy. 2008;52:53-7.

36. Suppli M, Aabenhus R, Harboe ZB, Andersen LP, Tvete M, Jensen J-US. Mortality in enterococcal bloodstream infections increases with inappropriate antimicrobial therapy. Clin Microbiol Infect. 2011;17:1078-83.

37. Torres C, Escobar S, Portillo A, Torres L, Rezusta A, Ruiz-Larrea F, et al. Detection of clonally related vanB2- containing Enterococcus faecium strains in two Spanish hospitals. J Med Microbiol. 2006;55:1237-43.

38. Top J, Willens R, Bonten M. Emergence of CC17 Enterococcus faecium: from commensal to hospital-adapted pathogen. FEMS Immunol Med Microbiol. 2008;52:297-308.

39. Tritz DM, Iwen PC, Woods GL. Evaluation of MicroScan for identification of Enterococcus species. J Clin Microbiol. 1990;28:1477-8.

40. Usacheva EA, Ginocchio CC, Morgan M, Maglanoc G, Mehta MS, Tremblay S, et al. Prospective, multicenter evaluation of the BD gene Ohm vanR assay for direct, rapid detection of vancomycin-resistant Enterococcus species in perilanal and rectal specimens. Am J Clin Pathol. 2010;134:219-26.

41. Vakulenko SB, Donabedian SM, Vorkresenskiy AM, Zervos MJ, Lerner SA, Chow JW. Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. Antimicrob Agents Chemother. 2003;47:1423-6.

42. Waites KB, Brookings ES, Moser SA, Zimmerman DBL. Direct susceptibility testing with positive BacT/Alert blood cultures by using Micro-Scan overnight and rapid panels. J Clin Microbiol. 1998;36:2052-6.

43. Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A, et al. Emergence and spread of vancomycin resistance among enterococci in Europe. Euro Surveill. 2008;13:1-11.

44. Willey BM, Jones RN, McGee A, Witte W, French G, Roberts RB, et al. Practical approach to the identification of clinically relevant Enterococcus species. Diag Microbiol Infect Dis. 1999;34:165-71.

Received: 5 November 2012
Accepted: 5 September 2013