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Expression of pathogenicity factors by *Enterococcus* strains isolated from inpatients with bloodstream infection in Belo Horizonte, Minas Gerais, Brazil

Expressão de fatores de patogenicidade por amostras de Enterococcus *isoladas de pacientes com infecção de corrente sanguínea em Belo Horizonte, Minas Gerais, Brasil*

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ABSTRACT

In this investigation, we addressed the pathogenicity profile of 35 *Enterococcus* samples isolated from inpatients with bloodstream infections (BSI) at hospitals in Belo Horizonte (MG), Brazil. The most prevalent species was *E. faecalis* (27/77.14%). The ability to produce gelatinase and cytolysin was detected in 14/40% of the samples and presented a heterogeneous distribution. Biofilm production was observed in 27 strains (77.14%) and classified as weak (8/22.86%), moderate (18/51.42%), or strong (1/2.86%). This study adds to knowledge of the pathogenicity profile of *Enterococcus* strains isolated from BSI, aiming to increase understanding about virulence of the circulating strains in Brazil.

Key words: *Enterococcus*; virulence; gelatinases; hemolysin proteins; biofilms.

INTRODUCTION

Bloodstream infections (BSI) are associated with high morbidity and mortality rates worldwide[1]. Cases related to *Candida* spp., *Pseudomonas aeruginosa* and *Enterococcus* spp. are frequently fatal[2].

Bacteria of the *Enterococcus* genus are members of the indigenous intestinal microbiota. Initially, the group was not recognized as having great clinical relevance, but has lately emerged as an important nosocomial pathogen. These microorganisms can synthesize the enzymes gelatinase and cytolysin, as well as adhesion proteins, which confer capacity of biofilm formation to the samples. These abilities contribute to their capacity to survive for long periods in surfaces and can help explain their dissemination in hospital settings[3, 4].

The objective of this study was to confirm the identification of *Enterococcus* samples isolated from BSI patients in hospitals of Belo Horizonte (MG), Brazil, to the species level. Another objective was to assess the expression of pathogenicity factors that are important for the infectious process associated with the microorganism.

MATERIAL AND METHOD

Thirty-five *Enterococcus* samples isolated during 2008 and 2009 from blood cultures of inpatients with BSI in different hospitals of Belo Horizonte, Minas Gerais, Brazil, were included in the study. The project was approved by the Ethics Research Committees of the participant hospitals and of Universidade Federal de Minas Gerais (UFMG) (ETIC 114/08).

The species-level biochemical and physiological identification (Vitek® 2) of bacteria was confirmed by polymerase chain reaction (PCR), using the conditions and the starters described by Dutka-Malen *et al*.[5]. Moreover, the

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samples identified as *E. casseliflavus* by the physiological and biochemical method were tested for pigment production in tryptic soy agar (TSA) with 5% horse blood[6].

In order to assess gelatinase production, bacteria were inoculated into nutrient agar with 3% gelatin; enzyme production was identified by the presence of clear zones around the colonies[7]. In order to investigate cytolysin production, samples were inoculated into TSA with 5% horse blood, and the enzyme was detected by the presence of hemolysis zones[7].

The assay to assess biofilm production was based on modifications in a protocol described by López-Salas *et al*.[4]. The inoculum was adjusted to the turbidity of a 0.5 McFarland standard, and then was diluted 1:40 in tryptic soy broth with 2% glucose. Suspensions were inoculated into 96-well microplates and incubated. Then, plates were processed and stained with crystal violet solution. Excess crystal violet was removed, the stain adhering to the microplate was solubilized, and the optical density of the solution was measured at 570 nm. The samples were classified as biofilm producers (absorbance > 0.5) or non-biofilm producers (absorbance ≤ 0.5); production, in its turn, was classified as strong (absorbance > 2), moderate (1 < absorbance ≤ 2), or weak (0.5 < absorbance ≤ 1).

RESULTS

The distribution of *Enterococcus* species, considering the method of genotypic identification, was *E. faecalis* – 27 samples (77.14%), *E. faecium* – five samples (14.29%), *E. casseliflavus* – two samples (5.71%), and *E. gallinarum* – one sample (2.86%) (**Figure A**). There was agreement of 97.14% (34/35) in species-level identification between the genetic technique and the physiologic and biochemical method. Both samples of *E. casseliflavus* produced pigment, forming yellow colonies. PCR result was considered definitive.

Gelatinase production was detected in 14 (40%) samples of *E. faecalis* (**Figure B**). Capacity for cytolysin production was also detected in 14 (40%) samples, although with a distinct distribution (**Figure C**), and among which just two samples of *E. faecalis* were classified as beta-hemolytic. Five samples, all *E. faecalis*, were able to synthesize both enzymes.

Biofilm production was observed in most samples (27/35, 77.14%). Among them, 18 (51.42%) were included in the category of moderate production; one (2.86%) presented strong biofilm production; and eight (22.86%), weak production (**Figure D**).

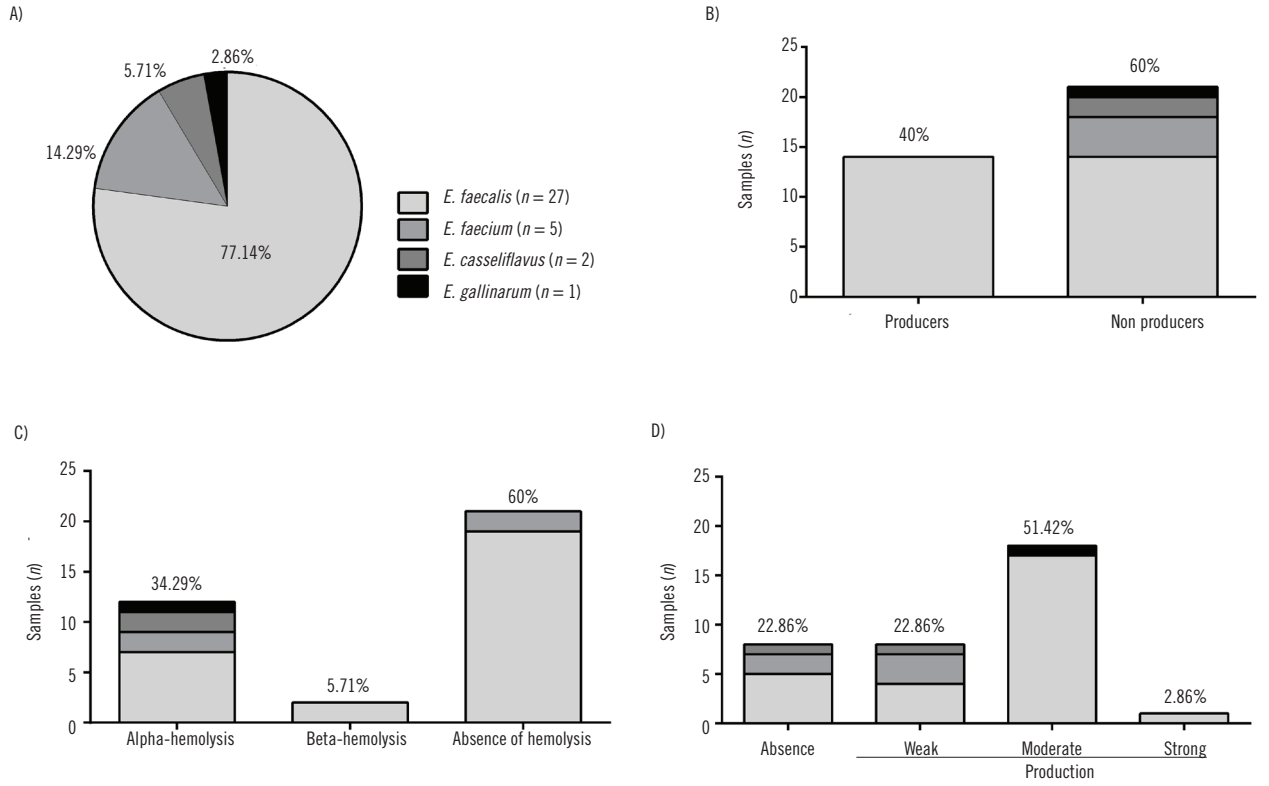

FIGURE – *Distribution of* Enterococcus *species (A) and evaluated production of gelatinase (B), cytolysin (C) and biofilm (D) by the samples included in the study*

DISCUSSION

Results indicated higher prevalence of *E. faecalis* species (77.14%), as well as high agreement between the genotypic (PCR) and automated phenotypic (Vitek® 2) methods. Considering the high accuracy of the employed genetic technique, identification by PCR was chosen to define the species[8, 9]. Thus, a sample initially identified as *E. casseliflavus* was reidentified as *E. gallinarum*. The result indicates that the employed phenotypic method requires complementary tests, such as assessment of pigment production, to enable accurate species identification[6, 9]. The data obtained in this study are in agreement with previous reports, which show elevated prevalence of *E. faecalis* in enterococci infections, including in Brazil[10, 11].

The pathogenicity factors of *Enterococcus* contribute to enhance fitness and persistence of the pathogen in the environment. Among them can be cited those related to adhesion (biofilm formation) and synthesis of enzymes, such as gelatinase and cytolysin/hemolysin[12]. Knowledge about the presence of a specific pathogenicity factor and its real implication in enterococci infectious processes in human beings is still limited. In this regard, the present investigation is even more significant, once data on virulence of *Enterococcus* lineages circulating in Brazil are so far scarce[13].

The expression of gelatinase was observed in 40% of the studied samples. In the literature, values close to the ones of this investigation have already been reported[12, 14]. Cytolysin activity was also observed in 40% of the samples. Values equal to or close to this have been reported in studies evaluating samples from diverse origins, including blood cultures[15, 16].

In this study, more than 75% of the samples were able to produce biofilm, predominantly included in the category moderate production. Among the five samples of *E. faecium*, two were classified as non-producers; and three, with low capacity for biofilm production. These results are similar to other already described in the literature, which also observed that most samples of *E. faecalis* were strong or moderate producers, while most samples of *E. faecium* were non-biofilm producers[17, 18].

The data provided by this study represent a contribution for the characterization of Brazilian samples of *Enterococcus*, considered of great relevance as infectious disease agent in our country.

RESUMO

Neste trabalho, avaliou-se o perfil de patogenicidade de 35 amostras de Enterococcus *isoladas de pacientes com infecção de corrente sanguínea (ICS) em diferentes hospitais de Belo Horizonte (MG), Brasil.* E. faecalis *foi a espécie mais prevalente (27/77,14%). A produção de gelatinase e citolisina, detectada em 14/40% das amostras, apresentou distribuição heterogênea. A produção de biofilme foi observada em 27 amostras (77,14%) e classificada como fraca (8/22,86%), moderada (18/51,42%) ou alta (1/2,86%). Este estudo contribui para o conhecimento do perfil de patogenicidade de amostras de* Enterococcus *isoladas de ICS, buscando auxiliar na compreensão da virulência das amostras circulantes no Brasil.*

Unitermos: Enterococcus*; virulência; gelatinases; proteínas hemolisinas; biofilmes.*

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