Molecular Characterization of multi-resistant Enterococcus faecium causing bloodstream infections in a Northern Spain University Hospital

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Abstract:

Introduction

Multi-resistant CC17 Enterococcus faecium represent an important cause of nosocomial infections. Our main objective is the analysis of the multi-resistant E. faecium local epidemiology in the Hospital Universitario Marqués de Valdecilla. To do this, the clonal relationship, the profile of antimicrobial resistance and the presence of virulence determinants will be determined.

Methods

157 E. faecium were isolated from blood cultures between 2009, January and 2016, August. The identification and antimicrobial resistance profile were determined by "VITEK 2" system. The resistance and virulence genes determination was performed by PCR and sequencing. The clonal relationship between the strains was analyzed by PFGE and MLST.

Results

96% of the studied strains showed high level resistance to β-lactams, 99% to quinolones, 98% to erythromycin and 92% showed high level resistance to streptomycin. Furthermore, 28% of the isolates were tetracycline-R, 7% gentamicin-R, 2% linezolid-R and 2% chloramphenicol-R. Glycopeptide resistance was not detected.

The identified virulence genes were: entA (96%), ermB (96%), aac(6')-aph(2'')-Ia (94%), ant(6')-Ia (94%), acm (96%), aph(3')-III (85%), and aac(6')-aph(2'')-Ia (94% to high level streptomycin resistance), ant(6')-Ia, and aac(6')-aph(2'')-Ia (high level gentamicin resistance), ant(6')-Ia and aac(6')-aph(2'')-Ia (high level streptomycin resistance), respectively. The determination of mutations involved in the resistance to aminoglycosides is now in course.

The detected virulence genes were: entA (96%), aac(6')-aph(2'')-Ia, ant(6')-Ia, and aac(6')-aph(2'')-Ia (high level streptomycin resistance), ant(6')-Ia, and aac(6')-aph(2'')-Ia (high level streptomycin resistance), respectively. The determination of mutations involved in the resistance to aminoglycosides is now in course.

Discussion & Conclusions:

Most of the strains are part of the sub-cluster CC17 that is well adapted to the hospital environment. The multi-resistant E. faecium clone ST117 has emerged and disseminated in our hospital where it has become endemic and represents the 70% of the isolates.

Introduction & Objective:

Clonal Complex 17 (CC17) Enterococcus faecium has increasingly been reported as a nosocomial pathogen worldwide (1, 2). Previous studies in our hospital indicated that most E. faecium isolates from clinical samples recovered between 2005 and 2008 belonged to this clonal complex and clonally-related PFGE types were disseminated among different areas in the hospital (3). Since 2009 a high increase in the number of BSI caused by E. faecium was observed.

The objective of this study was to analyze the local epidemiology of multi-resistant E. faecium population isolated in our hospital during the years 2009 to 2016 to understand the causes explaining the increasing number of BSI caused by E. faecium resistant to at least 4 different antimicrobials (Figure 1), being resistance to macrolides, streptomycin and kanamycin, respectively), E. faecium resistant to streptomycin. Furthermore, 28% of the isolates were tetracycline-R, 7% gentamycin-R, 2% linezolid-R and 2% chloramphenicol-R.

The detection of mobile genetic elements responsible for the acquisition and spread of antibiotic resistance genes in the hospital has been initiated. Most isolates encoded at least six virulence determinants (Figure 3): entA, aac(6')-aph(2'')-Ia and sgrA were the most commonly found (92% of the isolates), followed by ecbA (85%), esp (82%) and hyl (17%).

Antibiotic resistance genes found by PCR included ermB, ermT and msrC for macrolides resistance, aac(6')-aph(2'')-Ia, ant(6')-Ia, aph(3')-III a for aminoglycosides and sat4 for streptothricin, tetL and tetM for tetracycline and cat for chloramphenicol resistance (Figure 2). The quinolone resistance was due to amino acid substitutions S80I or S80R in ParC and S83Y or S83R in GyrA. The determination of mutations involved in the resistance to ampicillin is now in course.

Hypothesis:

A new clone of E. faecium particularly well adapted to the hospital setting has been introduced and spread in our institution, explaining the high increase in BSI by E. faecium since 2009.

Methods:

Between January 2009 and August 2016, 157 E. faecium clinical isolates were routinely recovered from blood cultures in our hospital (HUMV). Identification and susceptibility testing were done with the VITEK 2 System (Biomérieux).

Clonal relatedness was assayed by 1) PFGE, using SmaI as restriction enzyme and FPCQuest 4.5 software (BioRad) for image comparison, and 2) MLST of selected strains with distinct PFGE patterns, using http://efaecium.mlst.net database for PCR protocols and sequence analysis.

Specific antibiotic resistance genes and virulence determinants were tested by PCR as previously described (4).

Results:

Most of the 157 E. faecium isolates recovered in our hospital from blood cultures during this period were multidrug-resistant; 96% of the isolates showed resistance to at least 4 different antimicrobials (Figure 1), being resistance to ampicillin, erythromycin, ciprofloxacin and high level resistance to streptomycin the most common pattern. The percentages of antibiotic resistance of the E. faecium isolates studied are shown in Table 1.

Discussion & Conclusions:

Most of these strains (>90%) belong to CC17, a sub-cluster particularly well adapted to hospital settings and responsible for most nosocomial outbreaks worldwide. The multi-resistant E. faecium clone ST117 has emerged and spread in our hospital where it has become endemic and represents over 70% of the isolates, substituting other E. faecium clones prevalent in the previous study (1). Both major clones, ST117 (clone 1) and ST17 (clone 2), are nowadays considered high risk clones. Most of the strains encode multiple antibiotic resistance and virulence determinants. Surveillance studies and control measures based on this study are being adopted in order to prevent the possible emergence of a vancomycin-resistant E. faecium outbreak in our hospital.

References: