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

Jorge Eduardo Coba [1,2]

[1] Departamento de Biología Molecular, Universidad de Cantabria, Santander, Spain. [2] Servicio de Microbiología, Hospital Universitario Marqués de Valdecilla, Santander, Spain.

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 isolates clonal relationship, the profile of antibiotic 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% gentamycin-R, 2% linezolid-R and 2% chloramphenicol-R. Glycopeptide resistance was not detected.
The identified resistance genes were: *ermB*, *ermT* and *mrsC* (encoding macrolide-resistance), *ant6-Ia*, and *aph(3')-III* (high level resistance to streptomycin and kanamycin, respectively), *aac(6')-Ie_aph(2')-Ia* (high level gentamycin-resistance), *tetM* and *tetL* (tetracycline-resistance). The determination of mutations involved in the resistance to ampicillin is now in course.
The detected virulence genes were: *entA* (96%), *acm* (96%), *scm* (94%), *pilA* (94%), *sgrA* (92%), *ecbA* (85%), *esp* (82%) and *hyl* (17%).
The isolates were classified in 21 pulse-types, highlighting the presence of two main clones with 111 and 23 isolates, corresponding to MLST sequence types ST117 and ST17, respectively.
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 *E. faecium* BSI detected, specifically determining the clonal relationship, antibiotic susceptibility and the presence of resistance and virulence determinants.

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 FPQuest 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.

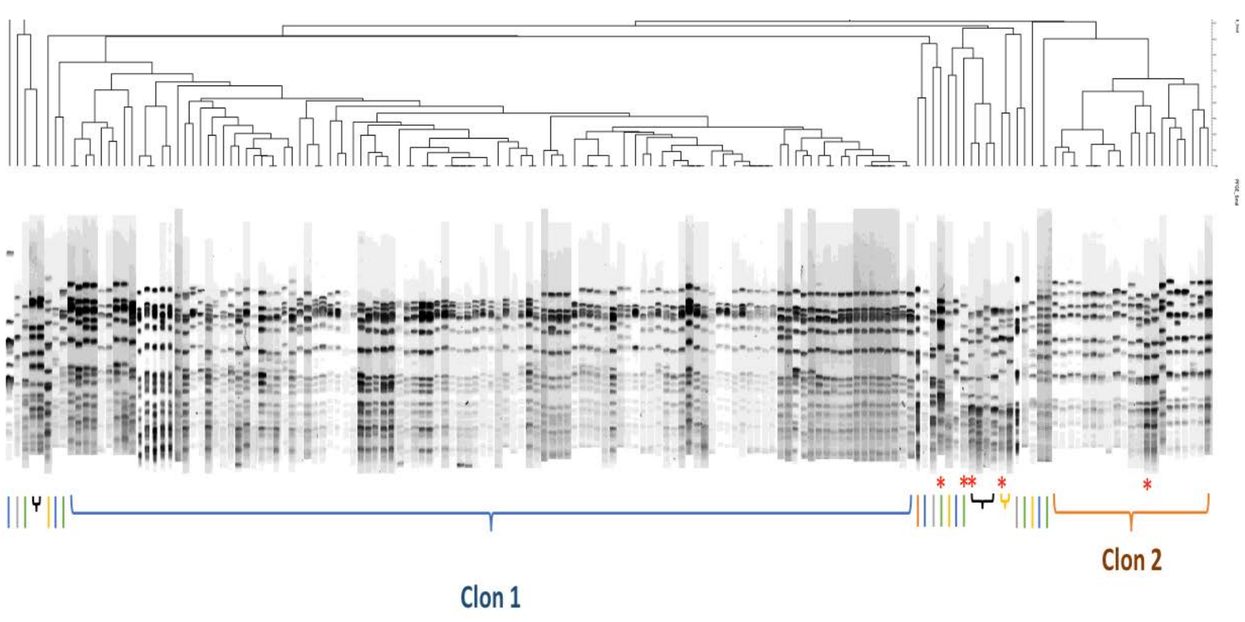

Figure 4. Phylogenetic tree of the 2009-2016 HUMV *E.faecium* clinical isolates, highilighting the presence of two major clones (*) that included 111 and 24 isolates, respectively.

Antimicrobial	% Resistance
Ciprofloxacin/Levofloxacin	99
Erythromycin	99
Ampicillin	96
HLR-Gentamicin	7
HLR-Streptomycin	92
Tetracycline	28
Quinupristin/Dalfopristin	15
Linezolid	2
Chloramphenicol	2
Vancomycin	0
Teicoplanin	0

Table 1. Percentage of antimicrobial resistance of the *E. faecium* clinical isolates, tested by VITEK 2 or disk diffusion.

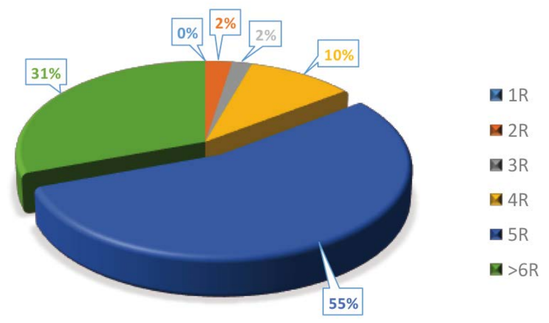

Figure 1. Number of antibiotic resistances (R) present in the studied *E.faecium* isolates and percentage of the isolates showing this resistance pattern.

Antibiotic resistance genes found by PCR included *ermB*, *ermT* and *msrC* for macrolides resistance, *aac(6')-aph(2")-Ia*, *ant(6)-Ia*, *aph(3")-3a* 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.

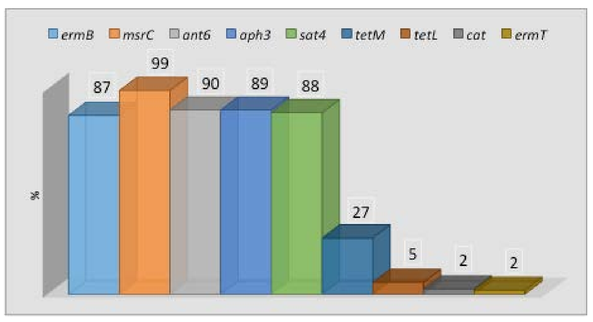

Figure 2. Variety and percentages of antibiotic resistance genes found in the HUMV *E. faecium* clinical isolates.

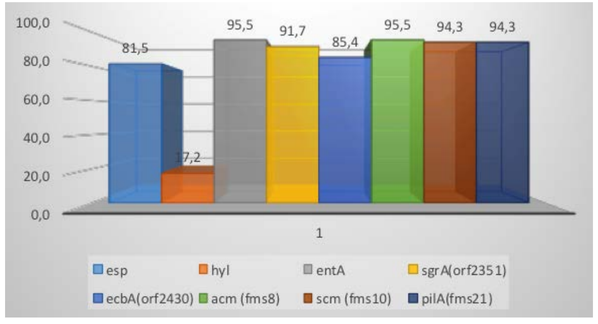

Figure 3. Prevalence of the virulence genes found in our *E. faecium* blood culture isolates.

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, acm, scm, pilA* and *sgrA* were the most commonly found (92% of the isolates), followed by *ecbA* (85%), *esp* (82%) and *hyl* (17%).

The isolates were classified in 20 PFGE types, highlighting the presence of 2 major clones containing 111 and 23 isolates, respectively (Figure 4). MLST analysis (Table 2) displayed 3 known sequence types, ST117 (111 isolates), ST17 (24) and ST18 (1). This analysis is still in progress. According to this data, more than 90% of the isolates belonged to CC17.

Pulse-type	ST	N° Isolates	*atpA*	*ddl*	*gdh*	*purK*	*gyd*	*pstS*	*adk*
1	117	111	9	1	1	1	1	1	1
2	17	24	1	1	1	1	1	1	1
13	17	1	1	1	1	1	1	1	1
3	ND	4	1	1	1	1	1	1	ND
5	ND	2	9	1	1	1	12	1	ND
8	ND	1	9	3	1	6	1	1	ND
10	ND	1	15	1	1	44	ND	20	ND
20	18	1	7	1	1	1	5	1	1

Table 2. MLST analysis of the *E. faecium* clinical isolates, indicating the different STs found and the number of isolates belonging to each one.

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

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