28th ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
Madrid, Spain
21 – 24 April 2018

P2484 Improved methodological conditions that accurately detected Enterococcus faecium with low-level ampicillin resistance

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Background: Resistance to high-levels of ampicillin (AmpR; PBP5-mutations) is associated with *E. faecium* isolates from hospitalized humans (clade-A1) and at lesser extent with community-based isolates (clade-A2 comprising strains of human and animal origin; clade-B comprising human-commensal strains). *E. faecium* populations with resistance to low concentrations of ampicillin (Efm-LC-AmpR; MIC=4-8mg/L; 8-10mm/2μg-disk) are variably classified as susceptible/intermediate according to EUCAST guidelines. This difficulty on predicting the presence of acquired resistance mechanisms is often associated with occurrence of heteropopulations within the inhibition zone of disk diffusion methods (ECCMID-2016-P0149). This study aimed to characterize the genetic background of Efm-LC-AmpR and to improve their detection using a 2μg-disk-diffusion method.

Materials/methods: Efm-LC-AmpR-n=41 and Efm-susceptible-n=14 (Efm-AmpS; MIC=1-2mg/L; control strains) recovered from animal-production-setting (pig, poultry, trout)-n=42, human (hospitalized-patients/long-term-care-patients/healthy-volunteers)-n=11 and environment (hospital-sewage/river)-n=2 (1997-2016; Portugal/Spain/Angola) were included. Clonality was assessed by MLST; ampicillin susceptibility (disk-diffusion) by standard (EUCAST) and modified conditions [including Brain-Heart-Infusion-agar (BHI); 48h incubation; 2-Mcfarland inoculum; 30ºC], in strains (14 Efm-LC- AmpR and Efm-AmpS each) of diverse sources and ST. The PBP5 genetic context was characterized by PCR/sequencing.

Results: Efm-LC-AmpR were included in clade-A2 (n=31; 20-ST; animal and human origins), clade-B (n=1) and non-identified (n=9). Efm-AmpS were from clades-A2 (n=10) and B (n=5) (14-ST; animal/human/environmental origins). The best-modified conditions to easily identify Efm-LC-AmpR were BHI+2-McFarland+37ºC+48h and BHI+0,5 or 2 McFarland+30ºC+48h, with absence of an inhibition zone for all strains. Most Efm-LC-AmpR (n=11/14) had the PBP5-C23 variant (PMID:27766095) also detected in 54 Efm genomes available at the Genbank (all clade-A2; 1961-2012, Europe/South and North America, AmpR/AmpS). Most of strains had an IS*Ef1* upstream *pbp5*-C23. Using the above conditions, three Efm-AmpS isolates (clade-A2; human/animal origins) were classified as Efm-LC-AmpR, one of them with a PBP5-C23 variant without ISEf1 upstream. The Efm-AmpS phenotype was confirmed for the remaining isolates, with PBP5 variants different of C23.

Conclusions: We described the improved detection of Efm-LC-AmpR using BHI+2-McFarland+48h. This method may impact the AmpR rates in surveillance studies and could enhance the accuracy to predict the occurrence of acquired resistance mechanisms in *E. faecium*. The data also suggests that AmpR-*E. faecium* might be more frequent in clade-A2 and community settings than previously thought.