27th ECCMID Vienna, Austria 22 – 25 April 2017

The congress of ESCMID

Session: P007 MIC and disc diffusion methods - revisited

Category: 3c. Susceptibility testing methods

22 April 2017, 15:30 - 16:30
P0149

What are we missing when epidemiological cut-off are not used to evaluate ampicillin phenotypes among Enterococcus faecium?

Carla Novais*[1], Ana Raquel Freitas[1], Ana P. Pereira[2], Barbara Duarte[2], Flavia Mendes[2], Teresa Coque[3], Luisa Peixe[4]

[1]*Ucibio/Requimte, Faculty of Pharmacy, University of Porto; Microbiology*

[2]*Faculty of Pharmacy, University of Porto; Microbiology*

[3]*Ramón Y Cajal University Hospital; Ramón Y Cajal Health Research Institute (Irycis) & Network Research Centre in Epidemiology and Public Health (Ciber-Esp); Microbiology*

[4]*Requimte/Ucibio, Faculdade de Farmácia Da Universidade Do Porto; Microbiology*

Background: Ampicillin-resistance (AmpR) associated with mutated PBP5 is considered a marker of hospital-adapted-*Enterococcus faecium (Efm)* clones (BAPS-3.3.a.1, 3.3.a.2, 2.1a) and is sporadically observed in most of non-clinical environments. The PBP5 consensus regions defining AmpR (PBP5-R) and ampicillin susceptibility-AmpS (PBP5-S) were recently proposed (PMID:21576454). However, our previous studies (PMID:27766095) showed the occurrence of PBP5-R in *Efm-pbp5*-transconjugants classified as AmpS by clinical-breakpoints (CLSI guidelines; MIC<16mg/L). The incongruence between phenotypes and genotypes in wild-type strains is still unknown. Here we hypothesized that current methods might underestimate the occurrence of *Efm*-PBP5-R, being our goal to study the suitability of current CLSI and EUCAST guidelines to interpret ampicillin-susceptibility by diffusion disk tests to detect such strains.

Material/methods: *Efm* (n=126) from disparate origins (hospitalized/ambulatory/continuous-care-patients, n=64; healthy-human-volunteers, n=6; raw food of animal origin and vegetables, n=16; pig/poultry/trout producing-setting, n=28; sewage/river, n=12), showing diverse AmpS and AmpR phenotypes (CLSI guidelines) and clonal-relationships (BAPS 1,2,3,5,6,7) were selected from our previous publications. They were recovered during 1995-2016 in Portugal, Spain and Angola. Transconjugants (n=13) acquiring *pbp5* from 5 donor strains (community/clinical origins-BAPs 2 and 3; recipient *Efm*-BM4015RF-MIC_{Amp}=0,5-1mg/L; BAPS-1) were also included. Ampicillin phenotype was studied by disk diffusion (2ug; 10ug), Etest and microdilution (0,125-64mg/L; used here as reference

method) methods, following EUCAST and CLSI guidelines. The identification of PBP5 protein sequences variants was evaluated by PCR and sequencing on those *Efm* with dissimilar ampicillin phenotype classification (D-*Efm*) between guidelines.

Results: AmpR was detected in 60% (83/139) and 51% (71/139) of *Efm* when 2ug (EUCAST; clinical-breakpoint <8mm) or 10ug (CLSI; clinical-breakpoint ≤16mm) disks were used, respectively. Those *Efm* classified as AmpR only by the 2ug-disk were recovered from the clinical-setting, poultry and piggeries (BAPS-2.1b, 7) and also included transconjugants. Additionally, when EUCAST epidemiological-cut-off (ECOFF<10mm) was applied to the 139 isolates, 9 further *Efm* (poultry, pig, trout, healthy human origins; BAPS-1.5, 2.1b, 2.3a, 3.2, 7) were reclassified as non-wild-type isolates. On those D-*Efm*, subpopulations were often observed in the borders of the susceptibility 10ug-disk halo or near/contacting the 2ug-disk. These D-*Efm* showed MICs=2-16mg/L, being some phenotypes classified as wild-type by ECOFF (EUCAST<8mg/L) and as susceptible by the clinical-breakpoints (EUCAST<8mg/L; CLSI<16mg/L). The analysis of the PBP5 variants of such D-*Efm* showed the occurrence of mutations compatible with PBP5-R or hybrid PBP5-R/PBP5-S consensus region.

Conclusions: EUCAST guidelines appear more reliable to detect *Efm* with PBP5-R/hybrid consensus regions. Additionally, the distribution of such *Efm* by diverse non-clinical settings supports the application of ECOFF in surveillance studies, as the incidence of *Efm* with PBP5-R/hybrid could be higher in non-clinical environments than presently acknowledge. Finally, more studies concerning the clinical implications of the presence of AmpR subpopulations hardly detected by clinical breakpoints (either by EUCAST but mostly by CLSI) are urgently needed.