P0354
Poster Session I
Susceptibility testing of multiresistant bacteria

EVALUATION OF A NEW PHENOTYPIC METHOD TO REVEAL AMPC PRESENCE AND PORIN LOSS IN ENTEROBACTERIACEAE WITH DECREASED SUSCEPTIBILITY TO CARBAPENEMS.

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OBJECTIVES – The AmpC beta-lactamases are intrinsic resistance mechanisms in several Enterobacteriaceae. The combination of AmpC with porin loss (AmpC-PL) can cause decreased susceptibility to carbapenems. A phenotypic test useful to confirm this kind of resistance is based on the synergistic action of combination of both meropenem/boronic acid and meropenem/cloxacillin compared with the activity of meropenem alone. This approach is commonly used in the diagnosis of the carbapenemase-producing Enterobacteriaceae. However, AmpC-PL+ isolates often show a decreased susceptibility only to ertapenem, whilst the other carbapenems remain susceptible. If so, the above synergy tests result undetermined. The performance of a new phenotypic test, product by Liofilchem® , Italy and consisting in agar diffusion gradient strips of ertapenem (ETP)/ertapenem + boronic acid (ETP/EBO) and ETP/ertapenem + cloxacillin (ETP/ECX) was evaluated.

METHODS – 27 isolates with decreased susceptibility to ertapenem (range: 1 to >32 mcg/ml) were analysed with the Liofilchem® MTS; all strains were susceptible to meropenem. 17 strains were AmpC producers (14 *Enterobacter cloacae*, 2 *Enterobacter aerogenes*, 1 *Citrobacter freundii*) and 4 strains were ESBL+ (3 *Escherichia coli* and 1 *Klebsiella pneumoniae* - KP). All those microorganisms did not harbour resistance genes for carbapenemases, as demonstrated by using a in-house multiplex-PCR. The other strains were 2 KPC+ (1 KP, 1 *E. coli*), 1 NDM+ KP, 1 VIM+ KP, 2 OXA48+ (1 KP, 1 *E. cloacae*).

RESULTS – As expected, all isolates did not show synergistic effect using the meropenem/boronic acid and meropenem/cloxacillin compared with meropenem alone. With regard to Liofilchem® MTS, 16 out of the 17 ampC producer strains showed a clear synergistic effect for both ETP/EBO and ETP/ECX combinations (i.e., MICs ratio of ETP vs ETP/EBO and ETP/ECX was >=8). One *E. cloacae*, with a ertapenem MIC = 1 mcg/ml, provided a non determinable result. Among the other strains, the 4 ESBL+, the NDM+ and the OXA-48 *E. cloacae* showed a synergy with boronic acid, but not with cloxacillin. The same happened for the KP KPC+. The KPC+ *E. coli* strains gave a non determinable result for boronic acid (no synergy with cloxacillin). The VIM+ and the OXA-48 KP did not show any synergy.
CONCLUSION – This phenotypic test was developed to detect AmpC-PL+ strains. Although the detection of AmpC enzymes is not mandatory for average laboratories, infection control considerations can support the need of this test. The new Liofilchem® MTS method described above is in a preliminary phase of development and the results seem promising as demonstrated by 94.1% sensitivity in detecting AmpC-PL+ strains. Enhancement of the ETP/EBO formulation (currently is being improved), as well as larger studies are needed to enhance the specificity of the method (some strains showed an unexpected synergy with boronic acid, whereas ETP/ECX demonstrated always a good performance).