Preliminary evaluation of the SensiTest Colistin, a broth microdilution based method to evaluate colistin MICs

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Background: Colistin is often the last option to treat severe infections caused by multidrug-resistant microorganisms, such as Pseudomonas aeruginosa, Acinetobacter baumannii and carbapenem-resistant enterobacteriaceae. Recently a plasmid-mediated resistance (mcr-1) was described: this plasmid was found in many countries, becoming rapidly matter of concern worldwide. Almost contemporarily, international committees pointed out that antimicrobial susceptibility testing (AST) for colistin should be performed only by using broth microdilution (BMD) techniques, because other methods, including agar dilution, disk diffusion and gradient diffusion, have been demonstrated to be unreliable. Therefore, the development of commercial test for colistin AST based on BMD is highly desirable. We evaluate the pre-commercial version of a BMD test, the SensiTest™ Colistin (Liofilchem, Italy), a miniaturized microwells system that evaluates 15 different dilutions of the drug (range: 0,008-128 mg/L) and one positive control.

Material/methods: colistin susceptibility was tested on 5 Acinetobacter baumannii, 5 Pseudomonas aeruginosa and 75 Enterobacteriaceae (39 Escherichia coli, 23 Klebsiella pneumoniae, 5 Proteus mirabilis, 3 Hafnia alvei, 3 Salmonella species, 1 Serratia marcescens and 1 Enterobacter cloacae) using BMD according to CLSI, SensiTest™ Colistin and Phoenix 100™ (PHX, Becton Dickinson, USA). SensiTest™ Colistin and PHX were performed according to the manufacturers’ recommendations. All the strains were previously collected from clinical samples. 20 out of the 62 E. coli and K. pneumoniae were known to be resistant to colistin; at least three E. coli isolates harbored the mcr-1 gene. Four strains were replicated eight times with SensiTest™ Colistin, to assess the reproducibility of the method.

Results: SensiTest™ Colistin showed a good agreement with BMD-CLSI for 83 out of the 85 strains tested (97,6%) – 45 strains same MIC value, +/- 1 log for 38 isolates. For two K. pneumoniae, a 2 log MICs difference between the two methods was documented, without changing the strains’
categorization. Also PHX showed a 97.6% agreement with the BMD-CLSI: in this case, the two discordant strains resulted in a very major error (susceptible instead resistant, one *K. pneumoniae* and one *Salmonella* species). Two out the 4 isolates that were replicated 8-times gave always the same MIC, whereas in one case one replicate had a single value differing for 1 log, and for the last strain there was 2 determinations which differed from 1 log (overall agreement among replicates, 33/36, 91.7%).

**Conclusions:** our analysis demonstrated that SensiTest™ Colistin is a simple but highly reliable test to assess the colistin susceptibility, providing a true MIC with a large range. It can be postulated that definitive formulation of the test will comprise only seven dilutions, allowing to test isolates of different patients in the same panel. This device may be an excellent response to the needs of clinical microbiologists for having a backup test for this critical antibiotic.