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Paper Poster Session
Problems in antimicrobial susceptibility testing

Evaluation of the Phoenix automated microbiology system to detect polymyxin-resistant strains enterobacteria : failure to detect heteroresistant Enterobacter cloacae isolates

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Background: Increasing antibiotic resistance in gram-negative bacteria has recently lead to an increasing use of colistin. However, methods routinely used to determine colistin susceptibility (disk diffusion method, Etest strips) are not reliable (high rate of false-susceptibility), while the dilution methods considered as the reference technique are tedious and time-consuming. No study has evaluated the performance of the Phoenix automated system to detect colistin resistance among gram negatives rods. The objective of this study was to evaluate the accuracy of the Phoenix system for the colistin susceptibility testing by comparing three methods : Phoenix automated system (BD Diagnostic Systems), broth microdilution (BMD) method and the newly-developed Rapid Polymyxin NP test.

Material/methods: This study was carried out on a total of 69 non-duplicate clinical isolates of various enterobacterial species (*E. coli*, *K. pneumoniae*, *E. cloacae*, *C. freundii*, *H. alvei*, *S. marcescens*, *M. morganii, Proteus* spp., *Providencia* spp.) isolated from clinical samples and recovered worldwide. The collection included 12 colistin-susceptible isolates and 57 colistin-resistant isolates (intrinsic, chromosomic or plasmid-mediated resistance). Colistin susceptibility testing was determined by using broth microdilution according to CLSI, by using Phoenix system according to manufacturer's guidelines, and by using the Rapid Polymyxin NP test that is based on a rapid culture in presence of a defined concentration of colistin and a defined culture medium. The first two methods requiring an incubation of 16 to 20 hours at 35°C, while the Rapid Polymyxin NP test results are obtained in less than two hours. Results were interpreted according to EUCAST and were compared to results obtained with the BMD method (gold standard). *Escherichia coli* ATCC 25922 and a *M. morgannii* strain naturally resistant to colistin were included in all experiments as control strains.

Results: The specificity of the Phoenix system and the Rapid Polymyxin NP test were excellent (100 %). The sensibility of the Phoenix system to detect colistin resistant enterobacterial strains was only of 82 % (9 isolates falsely susceptible), while an excellent specificity of 98 % was found for the Rapid Polymyxin NP test (only 1 isolate falsely susceptible). Heteroresistance was defined if skipped wells (i.e. wells that exhibit no growth although growth does occur at higher concentrations) were observed with the BMD method. Analysis of discrepancies revealed that the Phoenix system displayed low sensitivity in the detection of the heteroresistant subpopulations of *E. cloacae*, while Polymyxin NP test detected these strains in two hours.

Conclusions: This study revealed that the Phoenix system is a reliable tool to determine susceptibility to colistin in enterobacterial isolates of genera that do not exhibit colistin heteroresistance. For isolates

of genera known to exhibit heteroresistance such as *Enterobacter* spp. and *K. pneumoniae*, the Rapid Polymyxin NP test or dilution methods are more reliable.