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The evaluation of the rapid polymyxin NP test for the detection of colistin resistance in Enterobacteriaceae

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Background: Challenges in susceptibility testing of colistin continues and leave routine laboratories no other choice but broth dilution which is not feasible for most of the laboratories. In our study we aimed to evaluate the performance of the recently described rapid polymyxin NP test (RPNPT) to address the need for phenotypic detection of colistin resistance.

Material/methods: *Klebsiella pneumoniae* isolates (n=64) that were found as resistant to colistin with VITEK 2 instrument (bioMérieux, France) were further tested with gradient test (Liofilchem, Italy) and broth microdilution (BMD) methods. RPNPT was performed as follows: “Polymyxin stock solution” containing 0.2 mg/mL colistin sulfate in Mueller-Hinton broth (MHB) and “rapid polymyxin NP solution” (pH 6.7) containing MHB, phenol red, distilled water and D-glucose were prepared. Just before testing, the solutions were mixed to obtain a final colistin concentration of 5 mg/mL. A suspension matched to 3-3.5 McFarland standard was prepared in %0.85 NaCl using colonies grown on Mueller-Hinton agar. Testing was performed in 96-well polystyrene plates (Figure 1). Following inoculation, the plates were incubated at 35°C for 4 hours. The wells containing %0.85 NaCl were examined for contamination (the orange color should be preserved), and colistin susceptible and resistant isolates were identified by examining the change of color in the wells (bacterial growth in the presence of colistin causes acidification of the medium and thus a change of the original orange color to yellow due to pH indicator phenol red). Additionally, all isolates were investigated for the presence of *mcr-1* gene with PCR method.

Results: The BMD method revealed that only one isolate had colistin MIC in the susceptible range among 64 isolates that were initially found as resistant to colistin with VITEK 2. The results of RPNPT

were evaluated against the results obtained by the BMD method which is regarded as the only valid method for the determination of colistin susceptibility. In concordance with BMD, the RPNPT gave negative result for the colistin susceptible isolate and positive results were obtained for 58 out of 63 colistin resistant isolates (92.1%). Low MIC values were obtained by the gradient test method and the categorical agreement between the gradient test method and BMD was determined as 7.8%. None of the isolates were found to harbor the *mcr-1* gene.

Conclusions: The hands-on time for RPNT is approximately 15 minutes and after 4-hour incubation the results can be evaluated. These features make RPNPT an alternative to BMD for the investigation of colistin resistance. The colistin resistant results obtained by VITEK 2 exhibited high concordance with BMD, however the discrepant results obtained by gradient test method proves that – until the problems are solved – these method should not be used for the determination of colistin susceptibility.

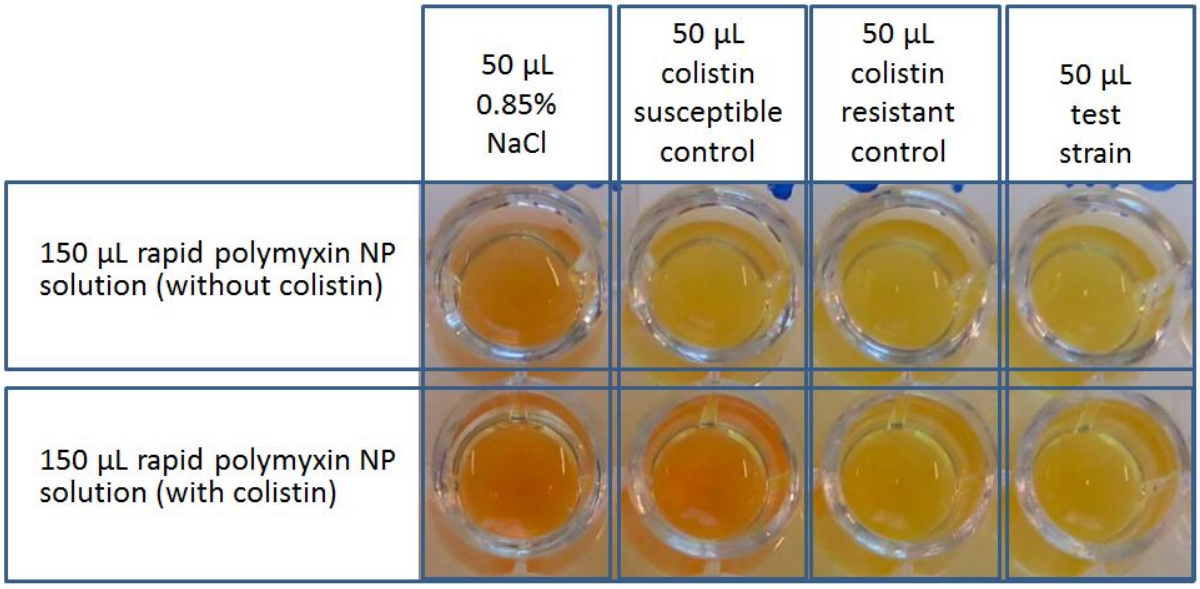

Figure 1. Distribution of solutions and bacterial suspension into wells for the rapid polymyxin NP test and positive result obtained with a colistin resistant test isolate.