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Confirmation of colistin resistance in *Klebsiella pneumoniae* as detected by VITEK 2 with gradient strip tests and broth microdilution method

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Objectives

Carbapenemase-producing *Klebsiella pneumoniae* isolates are being increasingly reported worlwide. Colistin is one of the few remaining last resort antimicrobials in the treatment of multi-drug-resistant *K. pneumoniae*, however there are already reports of colistin-resistance in *K. pneumoniae*. In order to highlight the difficulties in susceptibility testing of colistin, we conducted this study on carbapenemase-producing clinical *K. pneumoniae* isolates which were initially found as resistant to colistin by VITEK 2 system, an important result limiting the treatment options. For the confirmation of colistin resistance obtained by VITEK 2, we used gradient strip tests and reference broth microdilution method.

Methods

The initial susceptibility testing was performed with VITEK 2 automated antimicrobial susceptibility test system (bioMérieux, France). The carbapenemase genes (IMP, VIM, NDM-1, KPC, and OXA-48) were investigated with an in-house polymerase chain reaction (PCR) test. A total of 70 *K. pneumoniae* isolates which were found to carry one or two carbapenemase gene(s) and nonsusceptible to colistin by VITEK 2 [colistin minimum inhibitory concentration (MIC) ≥2 mg/L] were included in the study.

For these isolates the colistin MICs were determined by gradient strip tests (Liofilchem, Italy) on Mueller Hinton E agar (bioMérieux, France) and the reference broth microdilution (BMD) method using polystyrene microtiter plates.

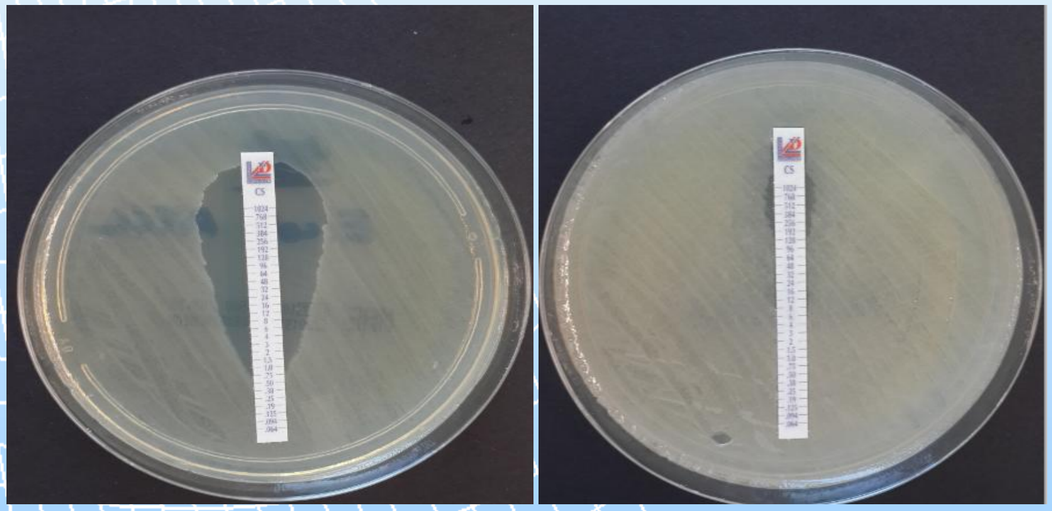
Results

The multiplex PCR test identified the carbapenemase genes as OXA-48 (n=57), NDM-1 (n=8), and OXA-48+NDM-1 (n=5) in the study isolates.

VITEK 2 results showed 97.1% categorical agreement (CA) with the reference BMD method, whereas the gradient strip test results showed 72.9% CA. Essential agreement (EA) could not be calculated for the results obtained by VITEK 2 against BMD since the colistin MIC for 61 out of 70 isolates (87.1%) were expressed by VITEK 2 as ≥16 mg/L.

The EA between gradient strip test results and BMD was found as low as 5.7%. This discordance was mostly due to BMD results being 3-4 dilutions higher than the gradient strip test results.

Colistin MIC testing: U-bottom, polystyrene plate, microdilution method



Colistin MIC testing: gradient strip test

Conclusion

➢The colistin resistant VITEK 2 results showed high CA with the reference method, however the low CA obtained with gradient strip tests means a high risk to report false susceptible colistin results for laboratories relying on gradient strip tests for colistin susceptibility testing.

➢The difficulties with colistin susceptibility testing have already been demonstrated in other studies, all phenotypic methods, including the reference BMD method, have been shown to have problems for colistin.

➢Colistin is not a suitable antimicrobial agent for disc diffusion testing which requires the determination of the MIC value for colistin.

➢The widely used methods for this purpose are liquid-based automated systems and the gradient strip tests. For the evaluation of results the comparisons were made against the reference BMD method but the best reference method for colistin susceptibility testing is yet to be established.