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Letter to Editor

Microbiology Section

Challenges, Issues and Warnings from CLSI and EUCAST Working Group on Polymyxin Susceptibility Testing

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Dear Editor,

Colistin is being increasingly used for treating patients with Multi-Drug Resistant (MDR) Gram-negative infections. Rapid and reliable colistin Susceptibility Testing (ST) is necessary for routine testing and appropriate therapeutic decision making. In vitro ST of colistin is challenging and is influenced by: i) multicomponent composition of colistin; ii) cationic property of colistin attributes for adherence to the microtiter plate; iii) heteroresistance to colistin [1]; and iv) in vitro Colistimethate Sodium (CMS), an inactive prodrug, hydrolysis to active colistin during incubation and results in three to eight folds higher Minimum Inhibitory Concentration (MIC) than colistin sulfate. Hence, ST should be performed only with colistin sulfate [2].

For colistin testing, Disc Diffusion (DD) method is not reliable. Colistin (large molecule) diffuses poorly into the agar medium and leads to inaccurate results with high error rate and poor reproducibility [1]. Until 2016, Clinical and Laboratory Standards Institute (CLSI) guidelines recommended DD (colistin or polymyxin B) for *Pseudomonas* species. Recently in 2017, CLSI guidelines has removed DD testing for colistin, whilst European Committee on Antimicrobial Susceptibility Testing (EUCAST) has never recommended DD for colistin ST. Both CLSI and EUCAST guidelines has recommended Broth Microdilution (BMD) as the reference method for colistin testing.

Adherence of colistin or polymyxin B to microtiter plates is reported as the major technical issues by many investigators across the world [3]. The addition of Polysorbate-80 (P-80), a surfactant, prevents to some extent the binding of polymyxin to microtiter plate [4]. However, CLSI or EUCAST guidelines never approved the use of P-80 for colistin or polymyxin B ST. It has been demonstrated that P-80 might exhibit synergistic effect with colistin [5]. In addition, P-80 acts as a surfactant and has mild antibacterial activity of its own [6]. This antimicrobial activity was further explored with the combined effect of polymyxin B and P-80 in *P. aeruginosa* [5,6]. Initially, polymyxin B binds to lipid A and leads to destabilization of the outer membrane of bacteria. This membrane destabilization allows P-80 to get into the cell and promoting the rupturing of inner membrane leading to cell lysis [7]. Moreover, isolates that are resistant to polymyxin compromise the entry of P-80 into cells. As a result, cell membrane remains intact and yields higher MIC values, while performing ST (colistin or polymyxin B) in the presence of P-80. However, further studies are essential to warrant the precise interaction between P-80 and polymyxin antibiotics.

Among the commercially available methods, colistin gradient Epsilometer test (E-test) is convenient for routine day-to-day testing, but the validity of MICs is not well established. Several studies have reported underestimated MIC values by one or more two fold dilutions, especially for concentrations of ≥2 µg/mL, leading to false susceptible results {Very Major Error (VME)} up to 32% [8–10]. Recently in 2016, EUCAST has given "warning" for testing colistin susceptibility with E-test [11]. However, marginally better result is observed with gradient E-test in EUCAST compliant Mueller-Hinton E (MHE) agar (bioMérieux). Currently, the available colistin gradient E-test is not reliable for routine testing with non-MHE agar. Remarkably, the joint CLSI-EUCAST polymyxin breakpoint working group does not recommend DD, Agar Dilution (AD), and gradient diffusion tests for colistin ST.

For colistin ST, the recommended Quality Control (QC) strains were *Escherichia coli* ATCC® 25922™ (0.25-2 µg/mL) and *Pseudomonas aeruginosa* ATCC® 27853™ (0.5-4 µg/mL). Most of the discordant results between BMD and E-test were reported with MIC of ≥2 µg/mL, but the suggested MICs of QC strains were lower. Currently, EUCAST has advised to include colistin resistant *E. coli* NCTC 13846 (mcr-1 positive) as resistant QC strain. For *E. coli* NCTC 13846, the colistin MIC target value is between 4 µg/mL and 8 µg/mL [11].

Currently, there is no US Food and Drug Administration (FDA) approved breakpoint for colistin. Colistin ST using semi-automated systems has been evaluated in limited studies. It tests mainly the performance of VITEK® 2 system (bioMérieux). However, the findings were conflict, implying that it was either unreliable compared with AD or comparable in terms of agreement with BMD [12,13]. Hence, EUCAST has suggested including QC strains (susceptible and resistant) for instrument's better performance.

Majority of the laboratories depend on either automated susceptibility testing system or gradient E-test. BMD is not performed day-to-day in patient care settings. Molecular methods are not feasible in detecting colistin resistance and are multifactorial. In this circumstance, the newly described and reliable rapid polymyxin Nordmann/Poirel (NP) test is found to be useful and adjunct test in detecting colistin resistance. This rapid polymyxin NP test is based on the detection of bacterial growth in the presence of polymyxin. In this test, bacterial growth is indicated by carbohydrate metabolism. In Enterobacteriaceae, carbohydrate metabolism results in acid production and is indicated by the colour change of pH indicator. This test is rapid and easy to perform with the turnaround time of <2 hours [14]. This test was found with the sensitivity and specificity of 100% [15]. This could be used as an alternative test for detection of colistin resistance in Enterobacteriaceae.

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