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Implementation of the Rapid Polymyxin™ NP test directly to positive blood cultures bottles

Ergina Malli, Constantinos C. Papagiannitsis, Stelios Xitsas, Katerina Tsilipounidaki, Efi Petinaki *

Department of Microbiology, University Hospital of Larissa, Larissa, Greece

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ABSTRACT

The present study assessed the performance of Rapid Polymyxin™ NP test to detect colistin resistance directly from 132 blood cultures found positive for *Enterobacterales* by FilmArray. Additionally, colistin MICs of isolated microorganisms were determined by the commercial broth microdilution method ComASP™ Colistin, used as the gold standard comparator. The Rapid Polymyxin™ NP test correctly detected all colistin-resistant isolates. However, the test has misidentified as resistant 4 colistin-susceptible isolates (1 *Escherichia coli* and 3 *Klebsiella pneumoniae*). Molecular characterization of colistin-resistant isolates showed that *K. pneumoniae*, which belonged to ST15 and ST147, carried alterations in the *mgrB*. Moreover, the first Greek *mcr-1*–positive colistin-resistant *E. coli* was detected. The rapidity (2–3 h) of the results, combined with its excellent negative predictive value (100%), allows implementation of the test for routine testing of blood cultures mainly in the clinical settings that are endemic for carbapenem-resistant bacteria, avoiding misuse of colistin and preventing the spread of colistin-resistant bacteria.

1. Introduction

Bloodstream infections (BSIs), which can progress to severe sepsis, are an important cause of morbidity and mortality and are associated with prolonged hospitalization as well as high costs for health care systems (Pittet et al. 1994; Angus et al. 2001). Carbapenem-resistant Gram-negative bacteria, *Enterobacterales, Pseudomonas*, and *Acinetobacter*, are frequently the causative agents for BSI (Mayr et al. 2014). Polymyxins (polymyxin B and colistin) are "last resort" antimicrobial agents for the treatment of serious infections caused by multidrug-resistant *Enterobacterales*, in particular carbapenem-resistant *Enterobacterales* (CRE) (Biswas et al. 2012; Nation et al. 2015). Unfortunately, immediately after the reintroduction of polymyxins, resistance to colistin has emerged either via chromosomal mutations in genes involved in lipopolysaccharide synthesis or via the plasmid-mediated colistin resistance *mcr-1* to *mcr-9* genes (Olaitan et al. 2014; Liu et al. 2016; AbuOun et al. 2018; Wang et al. 2018; Yang et al. 2018; Carroll et al. 2019).

To increase the survival rate of the patients with BSI, early administration of appropriate antimicrobials is vital for favorable outcome (Retamar et al. 2012). The problem is that the traditional methods, which include isolation of the microorganism and antimicrobial susceptibility test, are time-dependent processes that often require 2 to 4 days before final test results are available (van Belkum and Dunne Jr 2013). Recently, a variety of methods have been developed for the rapid identification and antibiotic susceptibility test directly from positive blood cultures (Granato et al. 2018; Kim et al. 2018; Lutgring et al. 2018). In 2019, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has published recommendations for short-incubation (4, 6 and 8 h) antimicrobial susceptibility testing directly from positive blood culture bottles (EUCAST 2019). The method used is the disk diffusion method (DD) with a specific panel of antimicrobials for each microorganism; although DD helps to establish antibiotic stewardship, it is not suitable for determining MICs. On the other hand, colistin is not included in the panel as the reference method for susceptibility testing retains the broth microdilution method; however, this method is time consuming, with a turnaround time of approximately 24 h (Humphries 2015).

To shorten the time for the detection of susceptibility or resistance to colistin, an additional test, the Rapid Polymyxin NP test, has been developed and has been used for the detection of colistin resistance within 2 h (Nordmann et al. 2016; Poirel et al. 2018; Dalmolin et al. 2019). Its principle is based on the detection of the glucose metabolism of *Enterobacterales* upon culture and differentiates colistin-susceptible from colistin-resistant isolates by supplementing the growth medium with a given concentration of colistin. An industrial version of the Rapid Polymyxin™ NP test (ELITechGroup, Puteaux, France) is now available and can be used either from colonies or directly from positive blood cultures, according the instructions of the manufacturer.

* Corresponding author. Tel: +302413502517; fax: +302413501570.
E-mail address: petinaki@med.uth.gr (E. Petinaki).

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The purpose of the present study was to assess the performance of this industrial Rapid Polymyxin™ NP test to detect colistin resistance directly from blood cultures positive for *Enterobacterales*. Moreover, during this study period, we have found and characterized the first Greek *mcr-1*–positive, colistin-resistant *Escherichia coli*.

2. Methods

2.1. Study population

This prospective study was conducted at the University Hospital of Larissa, a 600-bed tertiary care hospital in Central Greece. During the study period, the types of blood culture bottles used were BACTEC Plus Aerobic/F and Anaerobic/F culture bottles, which were processed using a BACTEC FX automated incubation system (Becton Dickinson Company, NJ). All sets of positive blood cultures, recovered during the period from September 2018 to February 2019 and obtained from nonduplicate patients with sepsis, which were detected as positive by the BACTEC FX system and stained as Gram-negative rods were included.

2.2. FilmArray™ blood culture combined with Rapid Polymyxin™ NP test

The FilmArray blood culture panel (bioMérieux, Marcy-l'Étoile, France) was preliminarily used for identification of the causative microorganism to species level directly from the bottles according to the guidelines of the manufacturer. Bottles that were found to be positive with any of *Enterobacterales* species included in FilmArray blood culture panel were tested for susceptibility or resistance to colistin using the Rapid Polymyxin™ NP test. Bottles positive with more than 1 microorganism were excluded. Briefly, 300 μL was obtained from each positive bottle following the instructions for use. Results were interpreted by 2 individual readers after 2 or 3 h of incubation at 37 °C. A positive test corresponded to an orange-to-yellow color change, whereas an orange color corresponded to a negative test.

2.3. Traditional microbiological protocol

In parallel, positive blood culture samples were processed using routine methods. Drops from the vials were inoculated into MacConkey agar and blood agar plates (aerobically and anaerobically). Isolated microorganisms were identified to species level and tested for susceptibility to various antimicrobial agents by Vitek-2 automated system (bioMérieux, Marcy-l'Étoile, France). MICs of colistin were determined by the commercial broth microdilution method ComASP™ Colistin (Liofilchem®). Interpretation of the susceptibility results was based on EUCAST criteria (www.eucast.org).

2.4. Detection of mechanisms for colistin resistance

All microorganisms that were isolated from blood cultures characterized as colistin positive according to the Rapid Polymyxin™ NP test were further analyzed for the presence of various mechanisms for colistin resistance (mutations and/or *mcr1–5* genes). Additionally 7 other microorganisms that were isolated from blood cultures characterized as colistin negative by the test and which had MICs 1–2 mg/L were also investigated. The detection of carbapenemase-encoding genes (bla_{KPC}, bla_{VIM}-like, bla_{NDM}-like, and bla_{OXA-48}-like) and plasmid mediated colistin resistance genes *mcr-1* to *mcr-5* was performed by polymerase chain reaction (PCR) (Malli et al. 2018; Rebelo et al. 2018). In addition, PCR amplification of the *mgrB* was carried out, and PCR products were analyzed using an ABI 377 sequencer (Applied Biosystems, Foster City, CA) (Malli et al. 2018). Molecular typing of isolates was based on multilocus sequence typing. Finally, from the sole microorganism that was found positive for *mcr*-1, 1 *Escherichia coli* strain genomic DNA was extracted and was sequenced by CeMIA (Larissa, Greece) using the Ion Torrent™ platform Ion PGM.

2.5. Statistical analysis

SPSS version 19.0 (SPSS, Chicago, IL) software was used for data analysis. Specificity, sensitivity, and positive and negative predictive values (PPV and NPV) were calculated in order to assess the diagnostic performance of NP test. The gold standard comparator for colistin resistance detection was the broth microdilution method performed with the isolated microorganism (ComASP™ Colistin, Liofilchem®).

3. Results

One hundred thirty-two blood cultures bottles found positive for *Enterobacterales* by FilmArray were tested by the Rapid Polymyxin™ NP test; 75 were positive for *E. coli*, 42 for *Klebsiella pneumoniae*, 11 for *Enterobacter spp*., 2 for *Proteus mirabilis*, and 2 for *Serratia marcescens*.

One hundred fifteen bottles gave negative results by the Rapid Polymyxin™ NP test, demonstrating that they contained bacteria susceptible to colistin. Indeed, the microorganisms, after their isolation, were characterized as colistin susceptible according to their MIC values using the EUCAST criteria (MICs ranged from 0.25 to 2 mg/L). Seven isolates (6 *K. pneumoniae* and 1 *Enterobacter cloacae*), which had MICs 1–2 mg/L, were further investigated for the presence of *mcr* genes and/or chromosomal mutations and were found to be negative.

Seventeen bottles gave positive results by the Rapid Polymyxin™ NP test. Among them, 4 were positive with isolates belonging to species that are naturally resistant to polymyxins (*P. mirabilis* and *S. marcescens*). Nine grew bacteria, 1 *E. coli* and 8 *K. pneumoniae,* that were characterized as colistin resistant according to their MICs values (MICs from 4 to 16 mg/L) and that were found to possess any mechanism of resistance (see below). The remaining 4 bottles gave falsely positive results since they grew 3 *K. pneumoniae* isolates, which had MICs values of 0.25, 0.25, and 1 mg/L, respectively, and 1 *E. coli* isolate with MIC 1 mg/L, all susceptible to colistin according to EUCAST breakpoints. The repetition of the Rapid Polymyxin™ NP test and the broth microdilution method gave similar results, while none of them carried *mcr* genes and/or chromosomal mutations. Inoculation of these discrepant isolates into agar plates containing 2 mg/L colistin-sulfate and incubation at 37 °C for 48 h did not reveal colistin-resistant subpopulations.

Comparing the results obtained by the Rapid Polymyxin™ NP test performed directly to positive blood cultures bottles with the MICs values of isolates, we found that the PPV, the NPV, the sensitivity, and the specificity of the test were 76.5%, 100%, 100%, and 96.7%, respectively.

Regarding the molecular characterization of colistin-resistant *Klebsiella pneumoniae*, all were carbapenem resistant and belonged to the STs 15 and 147. They were positive for both bla_{KPC} and bla_{VIM} genes and carried alterations in the *mgrB* gene as previously described (Malli et al. 2018). In ST15 isolates, the *mgrB* sequences revealed the presence of mutations, resulting in premature stop codons at third amino acid. Additionally, in ST147 isolates, the *mgrB* was disrupted by the insertion of IS*Kpn14* element at nnucleotide +43. None of them were positive for any of the *mcr* genes.

The *mcr-1* gene was detected in 1 colistin-resistant *E. coli* (Eco-4849Lar), which had MIC 4 mg/L. Eco-4849Lar exhibited resistance to ampicillin (MIC >32 mg/L), ampicillin-sulbactam (MIC: 16 mg/L), piperacillin (MIC >128 mg/L), ciprofloxacin (MIC >4 mg/L), chloramphenicol (MIC: 64 mg/L), and trimethoprim-sulfamethoxazole (MIC >4 mg/L), while remaining susceptible to other antibiotics (gentamicin, amikacin, tigecycline, cefotaxime, ceftazidime, cefepime, imipenem, meropenem). The isolate was classified as ST156. Sequence analysis showed that Eco-4849Lar harbored a 30.7-kb segment carrying *mcr1.1*; this segment exhibited extensive similarity to IncX4 plasmid p31349 (99% coverage,

99.99% identity), carrying *mcr-1.1* previously characterized among *E. coli* isolates from human feces in Switzerland (Donà et al. 2017).

4. Discussion

The Rapid Polymyxin NP test has been applied in clinical routine for detecting colistin resistance from bacterial colonies (Nordmann et al. 2016; Dalmolin et al. 2019). Jayol et al. have also demonstrated its ability to detect polymyxin-resistant *Enterobacteriaceae* from blood cultures; the test was performed in 73 blood culture sets (either spiked or clinical blood cultures) with various enterobacterial species (Jayol et al. 2016).

The industrial version of the Rapid Polymyxin NP test was already evaluated from colonies (Jayol et al. 2018). In the present study, we have implemented it directly to blood cultures bottles positive for *Enterobacterales*, which were collected in a clinical setting endemic for carbapenemase-producing *Enterobacterales.* According to our results, the test showed good performance for the detection of colistin-resistant enterobacteral isolates, quite similar to that achieved when it was performed to clinical isolates (Jayol et al. 2018). Given the complexity and diversity of polymyxin resistance mechanisms (mutations or *mcr* genes), the test has a better potential for rapid detection of polymyxin-resistant *Enterobacterales* than the molecular assays applied directly to positive blood cultures for *mcr* genes detection. The results were available within 2–3 h versus 48 h by standard antimicrobial susceptibility testing (24 h for obtaining a bacterial culture and an additional 24 h for susceptibility testing). The introduction of the test in the routine laboratory is easy and accurate since all the reagents are available from the manufacturer. The excellent NPV (100%) of the test indicates that the clinicians could initiate therapy with colistin earlier and safety. The implementation of the test for routine testing of blood cultures mainly in the clinical settings that are endemic for carbapenem-resistant bacteria contributes to avoid misuse of colistin and to prevent the spread of colistin-resistant bacteria.

Finally, the identification of even 1 specimen containing the *mcr*-positive *E. coli s*train is of great concern, showing that the use of rapid and sensitive methodologies for the detection of colistin-resistant bacteria is of utmost importance (Nordmann et al. 2016; Malli et al. 2018). ST156 *E. coli* isolates producing MCR-1 have been previously characterized from China and Brazil (Yang et al. 2016; Rossi et al. 2017). Furthermore, sequencing data demonstrated that *mcr-1.1* gene was associated with an IncX4 plasmidic sequence, as previously described in several previous studies, underlining the significant impact of IncX4 *mcr*-positive plasmids in the worldwide dissemination of this resistance determinant (Donà et al. 2017; Dolejska and Papagiannitsis 2018).

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Conflict of interest

The authors declare that they have no conflict of interest.

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