Rapid detection of polymyxin resistance in Enterobacteriaceae: the Rapid Polymyxin NP test

P. Nordmann, A. Jayol2, L. Poirel2

1Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Switzerland, 2HFR - Hôpital Cantonal de Fribourg, Fribourg, Switzerland

INTRODUCTION

Enterobacterial strains resistant to colistin (CS) are increasingly reported worldwide (1). Currently available polymyxins susceptibility methods are fastidious, time-consuming (24 h) and some methods are not reliable (2). They are poorly adapted to the clinical need and to the prevention of the dissemination of those multidrug resistant isolates.

Purpose

Our aim was to develop a rapid, reliable and cost-effective test to detect polymyxin resistant Enterobacteriaceae.

PRINCIPLE

This test is based on the detection of the glucose metabolism related to bacterial growth in presence of a defined concentration of colistin. Formation of acid metabolites consecutive to the glucose metabolism was evidenced by a color change (orange to yellow) of a pH indicator (red phenol).

CONCLUSIONS

• The Rapid Polymyxin NP test is easy to perform, rapid, reliable, cheap, sensitive (99.3%, specific (92.3%) and implementable worldwide.
• It detects colistin-resistant enterobacterial strains from any species regardless the molecular mechanism of resistance to polymyxins (intrinsinc, chromosomic and/or plasmid-mediated).
• It will change the overall management of the infected/colonized patients. It will be industrialized soon.

PREPARATION OF THE POLYMIXYN NP TEST

1. Reagents and solution.
• To prepare the Rapid Polymyxin NP solution, 6.25 g of Mueller Hinton Broth adjusted in cation (MHB-CA) powder, 0.0125 g of phenol red and 225 ml of distilled water were mixed.
• The pH of the solution was adjusted to 6.7.
• The solution was then autoclaved at 121°C for 15 min.
• After cooling the solution to room temperature, 25 ml of D(+)-glucose anhydre 10 % sterilized by filtration, was added.
• Colistin was added extemporaneously to the solution.

2. Bacterial inoculum.
• A standardized enterobacterial inoculum was prepared using freshly-obtained (overnight) bacterial colonies grown on Luria-Bertani or Mueller-Hinton plates.
• The bacterial colonies were resuspended into 10 ml of sterile NaCl 0.85 % to obtain a 3 to 3.5 McFarland optical density (ca. 109 CFU/ml).

3. Tray inoculation
• For each isolate, 2 wells are inoculated in parallel with the bacterial suspension, respectively with or without colistin.
• After mixing the bacterial suspension to the reactive medium, the final concentration of bacteria was ca. 108 CFU/ml and the final concentration of colistin was 3.75 µg/ml.

4. Tray incubation
The inoculated tray was incubated up to 4h at 35±2°C in ambient air, not sealed and without agitation.

EVALUATION OF THE POLYMIXYN NP TEST

• Performance of the rapid test was evaluated with a total of 200 enterobacterial isolates.
• Five isolates were from intrinsically polymyxin-resistant species, 130 isolates of various genus (E. coli, Klebsiella, Enterobacter, Hafnia) exhibited acquired resistance to polymyxins, and 65 isolates of various species were polymyxin-susceptible.
• Some of those strains had well-characterized mechanisms of resistance, such as mutations in the MmrAB and PhoPQ two-component systems (E. coli, Klebsiella pneumoniae), alterations in the mcrB gene or its promoter sequences (Klebsiella spp.), or produced the plasmid-mediated MCR-1 colistin resistance determinant (E. coli).
• MICs of polymyxins were determined using the broth microdilution reference method according to the CLSI guidelines and results were interpreted according to the EUCAST breakpoints.

RESULTS

• As expected, isolates that were intrinsically resistant to colistin gave a positive test result.
• Enterobacterial isolates with acquired resistance to colistin (CS MICs ranging from 4 to >128 µg/ml) gave also positive results, except a single colistin-resistant E. coli isolate with CS MIC at 8 µg/ml and an unknown mechanism of resistance that gave a negative result (false-negative result).
• Colistin-susceptible enterobacterial isolates (CS MICs ranging from 0.12 to 2 µg/ml) gave negative results, except for three isolates with CS MICs of 1 to 2 µg/ml that gave a positive result (false-positive result).
• A high correlation was obtained between colistin resistance and positivity of these Rapid Polymyxin NP and conversely, colistin susceptibility and negativity of the test. The sensitivity and the specificity of the Polymyxin NP test were excellent, being 99.3 and 92.3 %, respectively, as compared to the broth microdilution method taken as the gold standard.
• It was rapid (less than 2h) and reproducible.