Evaluation of two rapid phenotypical tests—Alifax rapid AST colistin test and Rapid Polymyxin NP test—for detection of colistin resistance in Enterobacterales

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Abstract
Our study evaluates the performance of two rapid phenotypical tests to detect colistin resistance in Enterobacterales: Alifax rapid AST colistin test using the HB&L system and Rapid Polymyxin NP test prepared in-house. A collection of well-characterized 53 colistin-susceptible and 66 colistin-resistant Enterobacterales isolates was used. The results obtained using both rapid tests were compared to the reference broth microdilution. Overall categorical agreement was 81.5% for Alifax test and 98.3% for Rapid Polymyxin NP test. Based on our results, the Rapid Polymyxin NP test is superior to the Alifax test that performed inadequate for Enterobacter spp.

Keywords Colistin resistance · Rapid diagnostic test · Enterobacterales · susceptibility testing

Introduction
Colistin use in clinical practice is mainly restricted to treatment of severe infections caused by multidrug-resistant (MDR) Gram-negative bacilli (GNB) [1]. Hence, it is rarely included in routine susceptibility panels in medical microbiology laboratories. In addition, colistin susceptibility testing is methodologically challenging due to its inherent properties [1–3]. The reference method chosen by the CLSI-EUCAST joint subcommittee is broth microdilution (BMD) [4]. Because colistin susceptibility testing is usually performed on demand when it becomes a treatment option results are delayed for up to 24 h. To reduce the time to results and provide simpler testing, several rapid tests have been developed [5–7].

This study evaluates the performance of two rapid tests that detect colistin resistance in Enterobacterales isolates and compares the results with the reference BMD.

Methods
To evaluate the two rapid tests, a total of 119 well-characterized Enterobacterales isolates were used; the majority were collected prospectively at the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana between February 2017 and
June 2018 [14, 15]. Isolates were recovered in accordance with our standard laboratory protocol and identified using matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI TOF MS) (Microflex LT, Bruker Daltonics, Bremen, Germany). Isolates were stored at −80 °C. Non-selective culture medium Columbia agar plate (Oxoid, Vienna, Austria) was used for reviving.

Colistin MIC was determined in accordance with the joint CLSI-EUCAST Polymyxin Breakpoints Working Group recommendation. MIC results were interpreted in accordance with EUCAST guidelines [16].

Our collection consisted of 53 colistin-susceptible isolates and 66 isolates with acquired colistin resistance. The colistin-resistant strains were 29 Escherichia coli, of which 15 were MCR-producers from an international collection (14 mcr-l and a single mcr-2 isolate), 16 Klebsiella pneumoniae, 19 Enterobacter spp., and two Citrobacter spp. (Appendix) [17–20].

To perform mcr gene detection, genomic DNA was isolated using Instant Gene Matrix (Bio-Rad Laboratories, Hercules, USA) following the manufacturer’s instructions. Detection of mcr-1, mcr-2, mcr-3, mcr-4, and mcr-5 was performed on isolates with colistin MIC > 2 mg/l using multiplex PCR as previously described [14, 15, 21].

To perform the susceptibility testing of Enterobacteriales isolates using the Alifax rapid COL-AST test, a 10-μl loopful of fresh overnight bacterial culture was carried out by transferring from non-selective culture medium Columbia agar plate (Oxoid) into a vial containing 3 ml of HB&L culture kit (Alifax) enrichment broth. Vials were loaded into the HB&L system (Alifax) and bacterial growth was automatically monitored; a notification on the screen appeared when 0.5 McFarland optical density was reached. Meanwhile, lyophilized colistin powder (Alifax) was dissolved in 2 ml of regenerating solution. Two vials containing enrichment broth were prepared for each bacterial isolate following the manufacturer’s instructions; into the vial for susceptibility testing, 200 μl of colistin suspension was added; the other vial served as a reference. Into each of the two vials, 100 μl of 0.5 McFarland of the bacterial suspension was transferred. The vials were inserted into the HB&L system (Alifax) following the manufacturer’s protocol for 5 h. Bacterial growth was automatically monitored while the logarithm of growth was calculated and compared between the two vials. The final results for the bacterial isolate were reported by the HB&L system as colistin-susceptible and colistin-resistant using EUCAST breakpoints (2 mg/l for Enterobacteriales).

The Rapid Polymyxin NP Test was prepared in-house as previously described [5]. The trays were visually inspected after 10 min, followed by hourly inspection after 1 h, 2 h, 3 h, and 4 h for color change.

The results obtained with both rapid methods were compared to the reference BMD method and categorized as follows: categorical agreement (CA) between the rapid test and the reference method, major error (ME, defined as false-resistant compared to the reference BMD method), and very major error (VME, defined as false-susceptible compared to the reference BMD method), as described elsewhere [22, 23]. Positive predictive value (PPV), negative predictive value (NPV) sensitivity, and specificity were calculated.

Results

Using the Alifax rapid COL-AST test, CA was attained in 97/119 isolates (81.5%). Among the colistin-susceptible isolates CA was 100%, and among the 66 colistin-resistant isolates CA attained 66.7% (44/66 isolates). CA determined per species/genus was 97.8% for Escherichia coli, 97.8% for K. pneumoniae, 51.6% for Enterobacter spp., and 100.0% for Citrobacter spp. Overall VME was detected in 22 out of 119 Enterobacteriaceae: 1/46 for E. coli, 6/32 for K. pneumoniae, and 15/31 for Enterobacter aerogenes. Detailed results are shown in Table 1.

Using the in-house Rapid Polymyxin NP test, CA was attained in 117/119 isolates (98.3%). Among the colistin-susceptible isolates, CA was 100%. Among the 66 colistin-resistant isolates, CA determined per species/genus was 100.0% for E. coli, 100.0% for K. pneumoniae, 88.9% for Enterobacter spp., and 100.0% for Citrobacter spp. In total, VME was detected in 2/119 Enterobacteriales; both were Enterobacter spp. Detailed results are shown in Table 1.

Discussion

A total of 119 isolates were included in this evaluation of two rapid phenotypical tests to detect colistin resistance in Enterobacteriales.

The performance of the Rapid Polymyxin NP test was excellent, with overall 98.3% CA and 2.17% VME (two colistin-resistant Enterobacter spp. isolates with colistin MICs of 32 and 128 mg/l tested false-colistin-susceptible using this test), which is in accordance with previous evaluations [5, 8–10].

The HB&L system yielded an 81.5% overall CA and no ME; however, 18.5% of the evaluated isolates tested as false-colistin-susceptible (VME), including one E. coli (not an MCR producer), six K. pneumoniae,
and 15 of 31 Enterobacter spp. isolates. Although the test performed well for E. coli and to a lesser extent for K. pneumoniae, the performance of susceptibility testing for Enterobacter spp. was inadequate. We have noted that growth of colistin-resistant Enterobacter spp. isolates was actually present in the vials upon visual inspection after the test was completed (turbid broth); however, the system algorithm failed to detect resistance (no growth present was reported). Perhaps with an improved algorithm the performance of the HB&L system for Enterobacter spp. will improve. Another possibility for such a discrepancy could be that Enterobacter spp. testing requires a longer time. However, the Rapid Polymyxin NP test performed much better with the same isolates of this challenging genus even with a shorter test time (Alifax 5 h, Rapid Polymyxin NP 2 h), and so this explanation is unlikely [24]. Of note is also the previously described trend toward forming a heteroresistant subpopulation for colistin in Enterobacter spp.; the smaller bacterial inoculum used for the HB&L system (Alifax) compared to the one used in the Rapid Polymyxin NP test could potentially result in a smaller number of resistant bacterial cells. In combination with a shorter incubation time in comparison with standard MIC testing, this may lead to false-susceptibility results. Interestingly, false-negative results were described among Enterobacter spp. when evaluating the Rapid Polymyxin NP Test [10]. Among the 31 Enterobacter spp. isolates included in our performance evaluation, 22 were Enterobacter aerogenes and nine isolates belonged to the Enterobacter cloacae complex. In a study conducted by Simar et al., a higher number of Enterobacter spp. isolates were included in the evaluation with a different proportion of E. cloacae and E. aerogenes (in favor of E. aerogenes), which could be an explanation for the difference in the results [10].

Based on our experience, the Alifax rapid COL-AST test requires longer hands-on time compared to the Rapid Polymyxin NP test due to its two-step course. Interpretation of the results is automated in the HB&L system (Alifax); in contrast, the Rapid Polymyxin NP test results are based on color change and are visually inspected, which can lead to subjective interpretation. In our test, the color change was well-pronounced and interpretation after 2 h was not problematic. Furthermore, readout optimization with an ELISA reader has been described, which enhances the objectivity of the results [25].

To conclude, the use of rapid tests to determine colistin resistance is a promising tool to help assess the suitability of using colistin when MDRGNB are isolated in patients with severe infection. The result for colistin can realistically be obtained the same working day the

| n, colistin MIC range in mg/l | Bacteria tested | Enterobacterales (n 119, 0.25–128) | Escherichia coli (n 46, 0.25–8) | Klebsiella pneumoniae (n 38, 0.25–64) | Citrobacter spp. (n 4, 0.25–64) | Enterobacter spp. (n 31, 0.25–128) |
|-----------------------------|----------------|---------------------------------|-------------------------------|---------------------------------|-------------------------------|---------------------------------|
| Alifax rapid AST colistin test (Alifax, Polverara, Italy) | CA (n) | 97 (81.5%) | 45 (97.8%) | 32 (84.2%) | 4 (100%) | 16 (51.6%) |
| | ME (n) | 0 | 0 | 0 | 0 | 0 |
| | VME (n) | 22 (18.5%) | 1 (2.2%) | 6 (15.8%) | 0 | 15 (48.4%) |
| | PPV (%) | 100 | 100 | 100 | 100 | 100 |
| | NPV (%) | 71.1 | 94.7 | 78.6 | 100 | 44.4 |
| | Sensitivity (%) | 66 | 96 | 63 | 100 | 21 |
| | Specificity (%) | 100 | 100 | 100 | 100 | 100 |
| Rapid Polymyxin NP test | CA (n) | 117 (98.3%) | 46 (100%) | 38 (100%) | 4 (100%) | 29 (93.5%) |
| | ME (n) | 0 | 0 | 0 | 0 | 0 |
| | VME (n) | 2 (1.7%) | 0 | 0 | 0 | 2 (6.5%) |
| | PPV (%) | 100 | 100 | 100 | 100 | 100 |
| | NPV (%) | 96.4 | 100 | 100 | 100 | 85.7 |
| | Sensitivity (%) | 97 | 100 | 100 | 100 | 89 |
| | Specificity (%) | 100 | 100 | 100 | 100 | 100 |

n number, MIC minimal inhibitory concentration, AST antimicrobial susceptibility testing, CA categorical agreement, ME major error, VME very major error, PPV positive predictive value, NPV negative predictive value
initial antimicrobial susceptibility is available. Based on the results of our study, the Rapid Polymyxin NP test is superior to the Alifax rapid COL-AST test that performed inadequate in detection of colistin-resistant Enterobacteriaceae spp.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10096-021-04182-w.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Code availability Not applicable.

Conflict of interest The authors declare no conflict of interest.

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References

1. Poirel L, Jayol A, Nordmann P (2017) Polymyxins antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev 30(2):557–596
2. Matuschek E, Åhman J, Webster C, Kahlmeter G (2018) Antimicrobial susceptibility testing of colistin—evaluation of seven commercial MIC products against standard broth microdilution for Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter spp. Clin Microbiol Infect 24(8):865–870
3. Satlin MJ, Lewis JS, Weinstein MP, Patel J, Humphries RM, Kahlmeter G, Giske CG, Turnidge J (2020) Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing Position Statements on Polymyxin B and Colistin Clinical Breakpoints. Clin Infect Dis 71(9):e523–e529
4. The European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute (2016) Recommendations for MIC determination of colistin (polymyxin E) as recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf. Accessed 30 March 2020
5. Nordmann P, Jayol A, Poirel L (2016) Rapid detection of polymyxin resistance in Enterobacteriaceae. Emerg Infect Dis 22(6):1038–1043
6. Pfennigwerth N, Kaminski A, Korte-Berwanger M, Pfeifer Y, Simon M, Werner G, Jantsch J, Marlinghaus L, Gatermann SG (2019) Evaluation of six commercial products for colistin susceptibility testing in Enterobacteriales. Clin Microbiol Infect 25(11):1385–1389
7. Furniss RCD, Dortet L, Bolland W, Dews O, Sparbier K, Bonnin RA, Filloux A, Kostrzewa M, Mavridou DAI, Larrouy-Maumus G (2019) Detection of colistin resistance in Escherichia coli by use of the MALDI Biotyper Sirius Mass Spectrometry system. J Clin Microbiol 57(12):e01427-19. https://doi.org/10.1128/JCM.01427-19
8. Jayol A, Kieffer N, Poirel L, Guérin F, Guneser D, Cattoir V, Nordmann P (2018) Evaluation of the Rapid Polymyxin NP test and its industrial version for the detection of polymyxin-resistant Enterobacteriaceae. Diagn Microbiol Infect Dis 92(2):90–94
9. Mitton B, Kingsburgh C, Kock MM, Mbelle NM, Strydom K (2019) Evaluation of an in-house colistin NP test for use in resource-limited settings. J Clin Microbiol 57(10):e00501–e00519
10. Simar S, Sibley D, Ashcraft D, Pankey G (2017) Evaluation of the Rapid Polymyxin NP test for Polymyxin B resistance detection using Enterobacter cloacae and Enterobacter aerogenes isolates. J Clin Microbiol 55(10):3016–3020
11. Boland L, Streel C, De Wolf H, Rodriguez H, Verroken A (2019) Rapid antimicrobial susceptibility testing on positive blood cultures through an innovative light scattering technology: performances and turnaround time evaluation. BMC Infect Dis 19(1):94
12. Knaack D, Idelevich EA, Körber-Irrgang B, Kresken M, Becker K (2018) Evaluation of a novel optical assay for rapid detection of methicillin-resistant Staphylococcus aureus in liquid culture. J Microbiol Methods 146:68–70
13. Van den Poel B, Meersseman P, Debavey Y, Klak A, Verhaeghen J, Desmet S (2020) Performance and potential clinical impact of Alfred60AST (Alifax®) for direct antimicrobial susceptibility testing on positive blood culture bottles. Eur J Clin Microbiol Infect Dis 39(1):53–63
14. Gerr J, Seme K, Cerar Kišek T, Teržan T, Mueller Premru M, Križan Hergouth V, Švent Kučina N, Pirš M (2019) Evaluation of a novel epidemiological screening approach for detection of colistin resistant human Enterobacteriaceae isolates using a selective SuperPolymyxin medium. J Microbiol Methods 160:117–123
15. Gerr J, Cerar Kišek T, Kokošar Ularč B, Lejko Zupanc T, Mrvić T, Kerin Povšič M, Seme K, Pirš M (2019) Surveillance cultures for detection of rectal and lower respiratory tract carriage of colistin-resistant Gram-negative bacilli in intensive care unit patients: comparison of direct plating and pre-enrichment step. J Med Microbiol 68(9):1269–1278
16. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.0, 2018. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.0_Breakpoint_Tables.pdf. Accessed 30 March 2020
17. Nordmann P, Jayol A, Poirel L (2016) A universal culture medium for screening polymyxin-resistant Gram-negative isolates. J Clin Microbiol 54(5):1395–1399

18. Del Bianco F, Morotti M, Pedna MF, Farabegoli P, Sambri V (2018) Microbiological surveillance of plasmid mediated colistin resistance in human Enterobacteriaceae isolates in Romagna (Northern Italy): August 2016–July 2017. Int J Infect Dis 69:96–98

19. Hartl R, Kerschner H, Lepuschitz S, Ruppitsch W, Allerberger F, Apfalter P (2017) Detection of the mcr-1 gene in a multidrug-resistant Escherichia coli isolate from an Austrian patient. Antimicrob Agents Chemother 24:61(4)

20. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H et al (2016) Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016. Euro Surveill 21(27). https://doi.org/10.2807/1560-7917.ES.2016.21.27.30280

21. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA, Battisti A, Franco A, Alba P, Perrin-Guyomard A, Granier SA, De Frutos EC, Malhotra-Kumar S, Villa L, Carattoli A, Hendriksen RS (2018) Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Euro Surveill 23(6):17–00672 Erratum in: Euro Surveill 2018;23(7)

22. International Standard Organization. 2006. Clinical Laboratory testing and in vitro diagnostic test systems—susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices—Part 2: evaluation of performance of antimicrobial susceptibility test devices. International Standard ISO 20776-2. International Standard Organization Geneva, Switzerland

23. Garcia LS, Isenberg HD (eds) (2010) Clinical microbiology procedures Handbook, 3rd edn. ASM Press 2516 p

24. Guérin F, Isnard C, Sinel C, Morand P, Dhalluin A, Cattoir V, Giard J (2016) Cluster-dependent colistin hetero-resistance in Enterobacter cloacae. J Antimicrob Chemother 71:3058–3061

25. Belda-Orlowski A, Pfennigwerth N, Gatermann SG, Kortebewanger M (2019) Evaluation and readout optimization of the Rapid Polymyxin NP test for the detection of colistin-resistant Enterobacteriaceae. J Med Microbiol 68(8):1189–1193

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