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Note

An alternative disk diffusion test in broth and macrodilution method for colistin susceptibility in *Enterobacteriales*

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ABSTRACT

Colistin and polymyxin B are old drugs that have been reintroduced to treat Gram-negative infections lacking other treatment options; however, colistin resistance have been reported. To know the correct susceptibility pattern is mandatory for multidrug resistant bacteria. Broth microdilution method is the gold standard to evaluate colistin and polymyxin B susceptibility; nevertheless, it is time consuming and needs expertise to be performed. Disk diffusion method on Müeller-Hinton agar is no longer recommended to evaluate polymyxins susceptibility. In this study we evaluated two methods (disk diffusion in broth and broth macrodilution) as alternative options to identify polymyxin resistance in an easy way. A total of 536 *Enterobacteriales* isolates were assessed for colistin susceptibility. All non-wild type *Enterobacteriales* (41) were chosen and 31 wild type bacteria were randomly selected, were used to perform disk diffusion tests in broth and for broth macrodilution tests. We found 100% of concordance between both tested methods and broth microdilution.

In conclusion, these two methods are reliable and easier options that complement as initial screening susceptibility for colistin in *Enterobacteriales* in microbiology laboratories lacking personnel and infrastructure to perform broth microdilution method.

1. Introduction

Polymyxins, including polymyxin B and colistin, are lipopeptide antibiotics produced by the bacterium *Paenibacillus polymyxa* (Yahav et al., 2012). They selectively bind to the lipopolysaccharide (LPS) of Gram-negative bacteria, and also, they displace divalent cations that bridge adjacent LPS molecules inducing expansion of the outer bacterial membrane and loss of integrity of the inner membrane. Bacterial death comes as a consequence of leakage of the intracellular content (Baron et al., 2016; Hancock and Chapple, 1999). The increase of multidrug resistant Gram-negative bacteria has promoted the reuse of these antibiotic family members as a last resource option to treat patients. Nonetheless, resistance to this drugs in Gram-negative bacteria has been reported and it is mediated by chromosomal mutations and by genes located in mobile elements, such as *mcr-1* (Landman et al., 2008; MacNair et al., 2018; Peterson et al., 1985). Laboratory tests to assure susceptibility or resistance to polymyxins have been in a continuous change, primarily by colistin poor diffusion on agar media (Poirel et al., 2017; Velkov et al., 2010). The gold standard method is the broth microdilution test and international standards organizations such as the Clinical and Laboratory Standards Institute (M100, 2018) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017.), recommend do not to perform it using gradient tests, agar disk diffusion or semi-automated devices such as Vitek 2®, Phoenix® or MicroScan® due of their unreliable results. Most of microbiology laboratories around the world lack of trained staff and enough time to perform this particular methodology (Gwozdzinski et al., 2018; Matuschek et al., 2017). Optional techniques, such as macrodilution methods, have been accepted as adequate tests to evaluate susceptibility in microorganisms such as in *Pseudomonas aeruginosa* (Turlej-Rogacka et al., 2018).

The aim of this study was to test the macrodilution method and a modified disk diffusion method as alternatives to screen colistin susceptibility in *Enterobacteriales,* both methods were compared with

Abbreviations: LPS, Lipopolysaccharide; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, Minimal inhibitory concentration; Bmd, Broth microdilution; BMD, Broth macrodilution

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microdilution broth such as gold standard.

2. Material and methods

2.1. Bacterial strains

536 clinical strains from *Enterobacteriales* were selected from a seven years period (2011–2017). We performed the broth microdilution susceptibility method for colistin (Colistin sulfate salt, Sigma Aldrich C-4461. USA) to each isolate as gold standard and comparison method in order to validate our experimental assays according to CLSI M07 2018 methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically (M07, 2015). Susceptibility interpretation was made according to M100 Performance standards for antimicrobial susceptibility testing (M100, 2018). All non-wild type *Enterobacteriales* isolates were included, and we randomly selected (https://www.random.org/lists/) the same number of wild-type microorganisms to compare them. Bacteria distribution were as follow: 33 *Enterobacter cloacae,* 21 *Escherichia coli,* 16 *Klebsiella pneumoniae* and 2 *Klebsiella aerogenes* strains. All determinations were made by triplicate.

2.2. Broth macrodilution method screening

We decided to use only one concentration such as screening test. Briefly, we prepared colistin aliquots with a final concentration of 4 μg.mL^{-1} (Colistin sulfate salt, Sigma Aldrich C-4461. USA) with Müeller-Hinton (MH) broth supplemented with cations as CLSI refers (M100, 2018). Aliquots were kept at −20 °C until its use. In a 5 mL tube, 1 mL of isotonic saline solution was verted and then 6.6 μL were taken. Bacterial suspension was adjusted at 0.5 of McFarland scale. From that suspension 6.6 μL were taken and deposited into saline solution. Afterwards, 1 mL of MH broth cation adjusted with 4 μg.mL^{-1} of colistin was added to have a final concentration of 5 × 10^5 UFC.mL^{-1} and a final colistin concentration of 2 μg.mL^{-1}. *P. aeruginosa* ATCC 27853 was used as susceptible control and *Proteus vulgaris* ATCC 6380 as intrinsically resistant control. Every sample and control were done by triplicate assays. Non-wild type result was defined as optical turbidity visually, wild type result was defined as turbidity absence, and both were read after 18 h of incubation at 37 °C.

In order to know the colistin MIC we prepared 10 double dilutions from 0.125 to 64 μg.mL^{-1} on MH broth adjusted with cations, as referred to in CLSI M07 and CLSI M100 (M100, 2018). 11 tubes of 5 mL were taken, 1 mL of isotonic saline was placed for each dilution and a tube for growth control, 6.6 μL was removed. Bacterial suspensions were made by adjusting them to 0.5 McFarland; 6.6 μL of this suspension was taken and deposited in the saline tubes (dilution 1: 150). Subsequently, 1 mL of the different dilutions of colistin with MH was added, in descending form (64–0.125 μg / mL) and ending with the growth control. *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as sensitive controls and *Proteus vulgaris* ATCC 6380 and *S. marcescens* AC 17–1291 as resistant controls; they were incubated from 18 to 24 h. MIC interpretation was defined visually, in the concentration where turbidity was no longer observed and classified according to the CLSI M100 (M100, 2018) guidelines in wild or non-wild type.

2.3. Disk diffusion in broth method

10 μg colistin disks (BD BBL, USA) were used to perform the test. A 5 mL tube was filled with 2.5 mL of isotonic saline solution, then 16.7 μL were removed. A colistin disk was set and then the tubes were incubated at 37 °C for 1 h to allow colistin diffusion. Bacterial suspension was adjusted at 0.5 of McFarland scale and 16.7 μL were deposited to the tube previously incubated. Then, 2.5 mL of MH broth were added with a final concentration of 2 μg.mL^{-1} of colistin in each tube having a final concentration of 5 × 10^5 UFC.mL^{-1}. *P. aeruginosa* ATCC 27853 was used as susceptible control strain and *P. vulgaris* ATCC 6380 as resistant control. Each test for clinical and control samples were done by triplicate. Non-wild type result was defined as optical turbidity, wild type result was defined as turbidity absence, both were read at 18 h.

To know the MIC by this method we used 10 μg colistin disks (BD BBL, USA) to prepare 10 dilutions, starting at a concentration of 64 μgmL^{-1}, prepared in MH broth adjusted with cations, adding colistin discs and incubated at 37 °C for 1 h to allow the diffusion of colistin. After this time, double dilutions of concentrations from 64 to 0.125 μg.mL^{-1} and a growth control were performed, according to the CLSI M07 (M07, 2015) and CLSI M100 (M100, 2018). Bacterial suspensions were made by adjusting them to 0.5 McFarland; 6.6 μL of this suspension was taken and deposited in the saline tubes (1:150 dilution) 5 × 10^5 CFU.mL^{-1}. Subsequently, 1 mL of the different dilutions of colistin with MH was added, in descending form (64–0.125 μg.mL^{-1}) and ending with the growth control. *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as sensitive controls and *P. vulgaris* ATCC 6380 and *S. marcescens* AC 17–1291 as resistant controls. They were incubated for 18–24 h. The MIC result was visually defined in the concentration where turbidity was no longer observed and classified according to the CLSI M100 (M100, 2018)guidelines in wild or non-wild type.

To confirm colistin susceptibility in the wild-type strains, we centrifuge the broth from the macro-dilution test and the disk diffusion test in broth and then we growth the pellet on blood agar plates to corroborate bacteria viability.

2.4. Bacterial DNA extraction

DNA extraction of all strains, wild type and Non-wild type, were performed by thermal shock using Chelex resin (BioRad, USA). It was carried out taking two colonies of the pure culture and dissolved in 100 μL of Chelex 5%, the vial was placed at 95 °C for 30 min, then centrifuged at 9600*g* for 5 min. The supernatant was quantified in NanoDrop (Thermo Fischer, USA) and a dilution was made until a final concentration of 20 ng.μL^{-1} was obtained.

2.5. mcr-1 amplification

It was performed by using PCR endpoint; briefly: 25 ng of DNA, 10 mmol L^{-1} of dntp's, 1 mmol L^{-1} of $MgCl_2$, 10 pmol of each oligonucleotide (*mcr-1*-F 5′CGGTCAGTCCGTTTGTTC-3′ and *mcr1*-R 5′-CTT GGTCGGTCTGTAGGG-3′)(Chang et al., 2017), a unit of AmpliTaq Gold (Thermo Fisher Scientific, USA). Program conditions were: 94 °C for 5 min, followed by 35 cycles of 94 °C 30 s, 55 °C 30 s and 72 °C for 40 s, finally 72 °C for 10 min, the thermocycler used was Veriti (Applied Biosystems, USA). Positive control was kindly given by Instituto Nacional de Salud Pública (Garza-Ramos et al., 2018). The 335 bp PCR products were visualized on a 1% agarose gel, using SYBR Green 1 as an intercalating agent.

3. Results

We found 41 *Enterobacteriales* isolates with colistin MIC > 2 μg.mL^{-1} during the study period. When we performed the disk diffusion in broth method, it showed 100% concordance with gold standard, both wild type and non-wild type results. Macrodilution method showed the same results as it was observed with the disk diffusion in broth method. The MIC verification of both methods was equal as broth microdilution method when it was used as screening test, none result was different; however, the final MIC in both methods showed positive differences compared to broth microdilution (Table 1). We performed *mrc-1* PCR in order to identify genetic resistance to colistin; however, there were not positive results in susceptible or resistant samples.

Table 1
Colistin susceptibility results among the three different tests in *Enterobacteriales* strains.

Strain	Bacteria	MIC-Bmd ($\mu g.mL^{-1}$)	MIC-BMD ($\mu g.mL^{-1}$) screening	MIC – BMD Verification ($\mu g.mL^{-1}$)	MIC-Disk ($\mu g.mL^{-1}$) screening	MIC – Disk Verification ($\mu g.mL^{-1}$)	Interpretation
EB116	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB773	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB1581	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB1822	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB1823	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB1825	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB1855	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB1986	*E. cloacae*	≤0.25	≤2	0.25	≤2	0.25	Wild type
EB2234	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB2779	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB3115	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB3119	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB3123	*E. cloacae*	1	≤2	0.5	≤2	0.5	Wild type
EB3128	*E. cloacae*	1	≤2	1	≤2	2	Wild type
EB3704	*E. cloacae*	≤0.25	≤2	0.5	≤2	0.5	Wild type
EB3747	*E. cloacae*	≤0.25	≤2	0.5	≤2	0.5	Wild type
EB443	*E. coli*	0.5	≤2	0.5	≤2	0.5	Wild type
EB791	*E. coli*	0.5	≤2	0.5	≤2	0.25	Wild type
EB840	*E. coli*	≤0.25	≤2	0.25	≤2	0.25	Wild type
EB3669	*E. coli*	≤0.25	≤2	0.125	≤2	0.25	Wild type
EB3671	*E. coli*	≤0.25	≤2	0.125	≤2	0.125	Wild type
EB3681	*E. coli*	≤0.25	≤2	0.125	≤2	0.125	Wild type
EB3722	*E. coli*	≤0.25	≤2	0.125	≤2	0.125	Wild type
EB270	*K. aerogenes*	≤0.25	≤2	0.125	≤2	0.125	Wild type
EB1719	*K. aerogenes*	1	≤2	0.5	≤2	0.5	Wild type
EB365	*K. pneumoniae*	≤0.25	≤2	0.125	≤2	0.125	Wild type
EB478	*K. pneumoniae*	≤0.25	≤2	0.125	≤2	0.125	Wild type
EB628	*K. pneumoniae*	≤0.25	≤2	0.25	≤2	0.125	Wild type
EB3698	*K. pneumoniae*	≤0.25	≤2	0.125	≤2	0.125	Wild type
EB3733	*K. pneumoniae*	≤0.25	≤2	0.25	≤2	0.25	Wild type
EB3751	*K. pneumoniae*	≤0.25	≤2	0.25	≤2	0.25	Wild type
EB001	*E. cloacae*	≥ 64	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB024	*E. cloacae*	0.125	< 2	≤0.125	< 2	≤0.125	Non-wild type
EB043	*E. cloacae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB081	*E. cloacae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB487	*E. cloacae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1502	*E. cloacae*	8	> 2	8	> 2	8	Non-wild type
EB1575	*E. cloacae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1604	*E. cloacae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1635	*E. cloacae*	16	> 2	16	> 2	16	Non-wild type
EB1672	*E. cloacae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1899	*E. cloacae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1977	*E. cloacae*	16	> 2	16	> 2	16	Non-wild type
EB1981	*E. cloacae*	16	> 2	16	> 2	16	Non-wild type
EB2074	*E. cloacae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB2216	*E. cloacae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB2898	*E. cloacae*	> 2	> 2	4	> 2	4	Non-wild type
EB3020	*E. cloacae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB300	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB566	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB581	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB791	*E. coli*	8	> 2	8	> 2	8	Non-wild type
EB840	*E. coli*	8	> 2	8	> 2	8	Non-wild type
EB1234	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1407	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1568	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1603	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1934	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB2075	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB2541	*E. coli*	4	> 2	4	> 2	4	Non-wild type
EB2544	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB2891	*E. coli*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB478	*K. pneumoniae*	8	> 2	8	> 2	8	Non-wild type
EB882	*K. pneumoniae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB927	*K. pneumoniae*	4	> 2	4	> 2	4	Non-wild type
EB1105	*K. pneumoniae*	8	> 2	8	> 2	8	Non-wild type
EB1337	*K. pneumoniae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1432	*K. pneumoniae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1602	*K. pneumoniae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB1791	*K. pneumoniae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB2394	*K. pneumoniae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
EB2761	*K. pneumoniae*	> 2	> 2	≥ 64	> 2	≥ 64	Non-wild type
ATCC 27853	*P. aeruginosa*	0.5	≤2	0.5	≤2	0.5	Susceptible

(*continued on next page*)

Table 1 (*continued*)

Strain	Bacteria	MIC-Bmd ($\mu g.mL^{-1}$)	MIC-BMD ($\mu g.mL^{-1}$) screening	MIC – BMD Verification ($\mu g.mL^{-1}$)	MIC-Disk ($\mu g.mL^{-1}$) screening	MIC – Disk Verification ($\mu g.mL^{-1}$)	Interpretation
ATCC 25922	*E. coli*	0.25	< 2	0.25	< 2	0.25	Wild type
CI 17–1291	*S. marcescens*	≥ 64	> 2	≥64	> 2	≥ 64	Intrinsically resistant
ATCC 6380	*P. vulgaris*	8	> 2	8	> 2	8	Intrinsically resistant

MIC-Bmd: Minimal inhibitory concentration broth microdilution; MIC-BMD: Minimal inhibitory concentration broth macrodilution method; MIC-Disk: Minimal inhibitory concentration broth diffusion disk. CI, confirmed linical isolate.

4. Discussion

Gram-negative multidrug resistance is a dynamic and emerging public health problem around the world. Colistin in several cases is the last resource antibiotic; however, physicochemical characteristics reduce the possibility to do simple susceptibility tests. The most commonly used method in routine laboratories, disk diffusion, has been shown to be unreliable due to poor colistin diffusion in agar. Recently, CLSI and EUCAST suggest performing microdilution broth susceptibility method in order to get reliable results. *Enterobacteriales* infections are a global concern, and some reports show the presence of resistance to colistin in this bacterial family (Moffatt et al., 2010). Even though there are only epidemiological break points for *Enterobacteriales*, there is a great concern for the adequate antibiotic profile identification, as polymyxins are drugs used in some countries where new beta-lactamase inhibitors do not exist yet or when metallo beta-lactamase is present leaving polymyxins as the only one option; so, the adequate susceptibility identification is critical and necessary. Not all microbiology laboratories are able to perform broth microdilution, since it needs expertise and supplies, especially in developing countries. Here we evaluate either broth macrodilution and disk diffusion in broth methods showing concordance with broth microdilution with the difference that those were less time consuming and elaborate. As we showed, using these methods the MIC of either wild typa and non-wild type bacteria corresponded to the gold standard method. The main difference between the use of disk diffusion in broth instead of using agar is that in broth, the molecule can elute from the disk to the broth. In the case of disk diffusion in broth method, it could be applied in any laboratory where broth microdilution method cannot be done.

We were not be able to identify all polymyxin resistance mechanisms, though no-one clinical strains, wild type and Non-wild type had presence of *mcr-1*. The tests were not performed in other *Enterobacteriales* members, however among the four species the results were reproducible.

5. Conclusion

We found that disk diffusion in broth method and broth microdilution are equal in reproducibility, robustness and ease of use when they are compared to the currently recommended broth dilution method tested here for colistin MIC determination in *Enterobacteriales*, and it might be used as initial screening susceptibility method for colistin.

Ethical approval was not required for this study.

Significance and Impact of the study

Colistin susceptibility has been difficult to perform in clinical fields because of particular drug characteristics; the medical impact of not having an unfailing test might be giant.

Having reliable and easy tests for colistin susceptibility different to broth microdilution is important for all clinical microbiology laboratories in order to assess its clinical utility as another consistent option.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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References

Baron, S., Hadjadj, L., Rolain, J.M., Olaitan, A.O., 2016. Molecular mechanisms of polymyxin resistance: knowns and unknowns. Int. J. Antimicrob. Agents 48, 583–591.

Chang, Y.-F., Lima Barbieri, N., Nielsen, D.W., Wannemuehler, Y., Cavender, T., Hussein, A., Yan, S.-g., Nolan, L.K., Logue, C.M., 2017. mcr-1 identified in Avian Pathogenic Escherichia coli (APEC). PLoS One 12, e0172997.

EUCAST, 2017. EUCAST Warnings Concerning Antimicrobial Susceptibility Testing Products or Procedures.

Garza-Ramos, U., Tamayo-Legorreta, E., Arellano-Quintanilla, D.M., Rodriguez-Medina, N., Silva-Sanchez, J., Catalan-Najera, J., Rocha-Martinez, M.K., Bravo-Diaz, M.A., Alpuche-Aranda, C., 2018. Draft genome sequence of a multidrug- and Colistin-resistant mcr-1-producing *Escherichia coli* isolate from a swine farm in Mexico. Genome announcements. 6.

Gwozdzinski, K., Azarderakhsh, S., Imirzalioglu, C., Falgenhauer, L., Chakraborty, T., 2018. An improved medium for colistin susceptibility testing. J. Clin. Microbiol. 56.

Hancock, R.E., Chapple, D.S., 1999. Peptide antibiotics. Antimicrob. Agents Chemother. 43, 1317–1323.

Landman, D., Georgescu, C., Martin, D.A., Quale, J., 2008. Polymyxins revisited. Clin. Microbiol. Rev. 21, 449–465.

M07, C, 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 10th Ed. CLSI suplement M07-A10.

M100, C, 2018. Performance Standards for Antimicrobial Susceptibility Testing. 28th Ed. CLSI Suplement M100. Vol. 2018.

MacNair, C.R., Stokes, J.M., Carfrae, L.A., Fiebig-Comyn, A.A., Coombes, B.K., Mulvey, M.R., Brown, E.D., 2018. Overcoming mcr-1 mediated colistin resistance with colistin in combination with other antibiotics. Nat. Commun. 9, 458.

Matuschek, E., Ahman, J., Webster, C., Kahlmeter, G., 2017. 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. https://doi.org/10.1016/j.cmi.2017.11.020. (Epub 2017 Dec 5).

Moffatt, J.H., Harper, M., Harrison, P., Hale, J.D., Vinogradov, E., Seemann, T., Henry, R., Crane, B., St Michael, F., Cox, A.D., Adler, B., Nation, R.L., Li, J., Boyce, J.D., 2010. Colistin resistance in Acinetobacter baumannii is mediated by complete loss of lipopolysaccharide production. Antimicrob. Agents Chemother. 54, 4971–4977.

Peterson, A.A., Hancock, R.E., McGroarty, E.J., 1985. Binding of polycationic antibiotics and polyamines to lipopolysaccharides of Pseudomonas aeruginosa. J. Bacteriol. 164, 1256–1261.

Poirel, L., Jayol, A., Nordmann, P., 2017. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin. Microbiol. Rev. 30, 557–596.

Turlej-Rogacka, A., Xavier, B.B., Janssens, L., Lammens, C., Zarkotou, O., Pournaras, S., Goossens, H., Malhotra-Kumar, S., 2018. Evaluation of colistin stability in agar and comparison of four methods for MIC testing of colistin. Off. Pub. Eur. Soc. Clin. Microbiol. Infect. Dis. 37, 345–353.

Velkov, T., Thompson, P.E., Nation, R.L., Li, J., 2010. Structure–activity relationships of polymyxin antibiotics. J. Med. Chem. 53, 1898–1916.

Yahav, D., Farbman, L., Leibovici, L., Paul, M., 2012. Colistin: new lessons on an old antibiotic. Off. Pub. Eur. Soc. Clin. Microbiol. Infect. Dis. 18, 18–29.