ORIGINAL ARTICLE

Comparison of colistin drop test to broth micro-dilution for the screening of colistin resistance in carbapenem-resistant gram-negative organisms

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ABSTRACT

Background: Colistin is a last-resort agent for treating infections caused by carbapenem-resistant gram-negative bacteria. However, standard susceptibility testing methods are complex and resource-intensive. This study aimed to evaluate the agreement of the colistin drop test with the reference standard for detecting colistin resistance.

Material and Methods: This prospective observational study was conducted from 1st January 2022 to 31st December 2023 at the Aga Khan University Hospital clinical laboratory, Karachi. The colistin drop test was evaluated using three solutions: one prepared from colistin powder and two from elution of colistin and polymyxin B disks. Carbapenem-resistant *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., and *Pseudomonas aeruginosa* isolated from clinical samples (January–December 2022) were included. All methods were compared with breakpoint BMD; discrepant isolates were confirmed by reference BMD and categorized as intermediate or resistant according to CLSI guidelines.

Results: Out of 190 isolates, 85 (44.8%) were colistin-resistant and 105 (55.2%) intermediates by breakpoint BMD. Categorical agreement for colistin powder, colistin disk, and polymyxin disk methods was 97%, 95%, and 98%, respectively. Corresponding positive predictive values were 100%, 95%, and 100%; negative predictive values 96%, 96%, and 97%; sensitivity 95%, 96%, and 96%; and specificity 100%, 96%, and 100%.

Conclusion: Study findings align with previous research. The colistin drop test using polymyxin B disk solution showed optimal performance and offers a simple, reliable method for detecting colistin resistance in Gram-negative organisms in resource-limited settings.

Keywords: Carbapenem-resistant *Enterobacterales*, Carbapenem-resistant *Pseudomonas aeruginosa*, Colistin drop test (CT-DT), Colistin resistance screening, Colistin susceptibility testing

BACKGROUND

Asia is one of the epicenters of antimicrobial resistance (AMR) worldwide leading to a big public health concern. The spread of New Delhi metallo-beta-lactamase-1 (NDM-1) from India to many countries is an example.[1] Due to uncontrolled and over the counter use of antibiotics, antibiotic resistance and emergence of multi drug resistant (MDR) strains have taken place over the last decade. A study described 30% *Escherichia coli* to be resistant to first line antibiotics on phenotypic testing while 32% *Escherichia coli* and 33% *Klebsiella* spp were determined resistant on genotyping.[2] MDR non-fermenter Gram negative bacilli are highly prevalent in Asian countries with rates of carbapenem-resistant *Pseudomonas aeruginosa* as high as 56.9% in China.[3] As a result, colistin is used as a last resort to treat complicated infections with gram-negative bacteria.[4]

Colistin belongs to class of drugs known as polymyxin. It has a polypeptide structure, initially isolated from soil bacterium *Paenibacillus polymyxa*.[5] Polymyxins are cationic polypeptides that consist of a cyclic heptapeptide possessing a tripeptide side chain acylated at the N-terminus by a fatty acid tail. Colistin and polymyxin B differ by only a single amino acid in the peptide ring, with a phenylalanine in polymyxin B and a leucine in colistin.[6]

Currently broth microdilution (BMD) method, colistin broth disk elution (CBDE) and agar dilution are the gold standards for colistin susceptibility testing according to Clinical & Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards.[7, 8] Colistin susceptibility testing using BMD is challenging in laboratories with limited resource as expensive reagents, consumables and technical expertise is required. Apart from that,

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other potential limitations include chances of cross-contamination, and non-interpretable minimum inhibitory concentrations (MICs) due to the presence of skip wells (i.e., wells that exhibit no growth, whereas growth is observed in wells with higher antibiotic concentrations). Colistin broth disk elution recommended by CLSI is a comparatively simpler technique, however, it is also time consuming and expensive and can be cumbersome and economically unfeasible.[9] Agar dilution can also be difficult to perform mainly due to preparation of serial dilutions of antimicrobials which are incorporated in agar and is not cost effective.[10] Maintaining the recommended optimum testing parameters like pH, temperature, media, and length of incubation are additional challenges. Break point BMD method can be employed in resource limited settings as it is a modified form of BMD with colistin concentrations of 1, 2, & 4 µg/mL for test and control strains.[11] This method has been validated and adopted in our institute for colistin susceptibility testing. Despite being simpler than the reference BMD, breakpoint BMD is also not feasible for many laboratories in resource limited settings.

Due to the above discussed drawbacks, need of simpler methods for colistin susceptibility testing has been advocated.[12] Colistin drop test was initially developed by Halling et al and was performed in *Brucella abortus* for the determination of polymyxin B resistance.[13] Jouy et al[14] tested 52 colistin susceptible and 89 resistant *E. coli* strains with colistin drop test and demonstrated 100% categorical agreement with BMD and genotyping. Another study conducted by Pasteran et al, showed 96% categorical agreement with BMD using colistin drop test in 37 *Acinetobacter* spp. 195 *Enterobacterales*, and 39 isolates of *P. aeruginosa*.[15]

Considering the concordant results of previous studies, we validated this simpler method in gram negative strains from Pakistan where NDM-1 production is a major mechanism of carbapenem resistance.[16] We hypothesized that as different bacterial isolates from various geographic regions exhibit distinct antibiotic susceptibility patterns and resistance mechanisms, the results of colistin drop test may be different in our population.[17] As CLSI does not recommend testing *Acinetobacter* spp. using colistin broth disk elution method, we did not include *Acinetobacter* spp. in our study.

MATERIAL AND METHODS

This prospective observational study was conducted from 01st January 2022 - 31st December 2023..Isolate collection and bench work started after obtaining Ethics Review Committee (ERC) exemption (letter no 2022-6737-20848) on 08th March 2022 at the Aga Khan University Hospital clinical laboratory, Karachi, Pakistan. Sample size for this study was determined for assessing colistin susceptibility by Colistin Drop Test (COL-DT), using World Health Organization sample size software. According to antibiogram of Aga Khan University hospital microbiology lab, prevalence of colistin resistant Gram-negative rods, isolated from clinical specimens, categorized as resistant by breakpoint broth microdilution method (BMD), is 1%, 19%, 18% and 1% for *Escherichia coli, Enterobacter* spp., *Klebsiella* spp. and *Pseudomonas aeruginosa* respectively. We estimated that a minimum sample size of 230 clinical isolates (24 *Escherichia coli*, 93 *Enterobacter* ,89 *Klebsiella* and 24 *Pseudomonas aeruginosa)* will be needed for assessing agreement between breakpoint BMD and COL-DT for detecting colistin resistance, keeping 95% confidence interval, margin of error 4% for *Escherichia coli* and *Pseudomonas aeruginosa and* 8% for *Enterobacter and Klebsiella*, and using the equation below.

$$n=\frac{1.96^2\,p(1-p)}{d^2}=\frac{(1.96)^2\times0.01\times(1-0.01)}{(0.04)^2}=24$$ *Escherichia coli*

$$n=\frac{1.96^2\,p(1-p)}{d^2}=\frac{(1.96)^2\times0.19\times(1-0.19)}{(0.08)^2}=93$$ *Enterobacter*

$$n=\frac{1.96^2\,p(1-p)}{d^2}=\frac{(1.96)^2\times0.18\times(1-0.18)}{(0.08)^2}=89$$ *Klebsiella*

$$n=\frac{1.96^2\,p(1-p)}{d^2}=\frac{(1.96)^2\times0.01\times(1-0.01)}{(0.04)^2}=24$$ *Pseudomonas aeruginosa*

All carbapenem resistant *Pseudomonas aeruginosa and Enterobacterales* in which colistin susceptibility was determined using breakpoint BMD were selected using a convenience sampling. Duplicate isolates obtained from the same patient and specimen source were excluded from the study. In addition, isolates showing discrepant results between the colistin drop test and broth microdilution (BMD) breakpoint testing were excluded if confirmatory testing could not be performed. All other isolates were included. Laboratory Methods: Three different solutions were prepared for colistin susceptibility testing (colistin powder, colistin disk and PB disk). After preparation, all three solutions were stored at 2-8°C.

Colistin powder solution was obtained by dissolving colistin sulphate powder (Sigma-Aldrich) in cation adjusted Mueller-Hinton broth (CA-MBH) (Thermo Scientific™ Sensititer™ Cation Adjusted Mueller-Hinton Broth) to obtain 16 µg/ml solution. (Calculations were done according to CLSI recommendations)[7].Colistin disk solution was obtained by disk elution method. Eight, 10-µg colistin disks (Becton, Dickinson and Company) were added in 5ml CA-MHB. Disks were left in tubes for at least 30 minutes but no longer than 60 minutes for proper elution. This yielded a 16 µg/ml solution. Disks were then removed aseptically, and solution was filtered with syringe microfiltration membrane (Thermo Scientific™ Nalgene™ Sterile Syringe Filter). Polymyxin B solution was prepared using polymyxin B disks (Oxoid). Eight, 300 IU polymyxin B disks were added in 10ml CA-MHB yielding 30 µg/ml polymyxin solution. Disks were left in tubes for at least 30 minutes but no longer than 60 minutes for proper elution. Disks were then removed aseptically and solution was filtered with syringe microfiltration membrane.[15]

While performing colistin drop test using any of the above solutions, 10 µL of solution was dropped on an agar plate, having an already inoculated lawn of 0.5 McFarland standard inoculum of the strain to be tested. It was allowed to dry for at least 15 minutes and plates were incubated at 37 °C ambient air incubator for 16 to 18 hours. Results were determined based on presence or absence of inhibition zone. Any zone of inhibition was interpreted as intermediate while no zone was interpreted as resistant. Colistin drop test by all three solutions were repeated for isolates with discrepant results between breakpoint BMD and any of the colistin drop tests. If there was still a discrepancy, the reference BMD was performed. *E. coli* ATCC 25922, *E. coli* NCTC 13846 and *P. aeruginosa* ATCC 27853 were used as quality controls[15].

Proportions of colistin intermediate and resistant organisms classified by each method were determined. The proportions were determined for each sample type as well as each organism group.

Categorical agreement was defined as agreement between the test and reference method in the categorization of an isolate as intermediate or resistant. Sensitivity was defined as the proportion of true positives (i.e., correctly identified resistant isolates). Specificity was defined as proportion of true negatives (i.e., correctly identified intermediate isolates). Very major error and major error cannot be calculated as there is no sensitive category for colistin according to CLSI. Proportions with confidence intervals were calculated for isolates which can be reliably classified as colistin intermediate or colistin resistant and the solution which exhibited the most agreement with breakpoint BMD.

RESULTS

Total 230 isolates were tested, out of which four were excluded due to duplicate isolates. Further, 36 isolates with discrepant results, between colistin drop test & breakpoint BMD, were excluded because the isolates either could not be revived or had been discarded after initial testing and hence results could not be confirmed. Hence, a total of 190 isolates were included for the analysis, out of which 106 were *Klebsiella* species, 32 were *Escherichia coli, 25 were Enterobacter* species and 27 were *Pseudomonas aeruginosa.* (Figure-I).

Among the total, 66 (35%), 76 (40%), 18 (9%), 16 (8%), 9 (5%), and 5 (3%) isolates were from blood, urine, respiratory tract (sputum, tracheal, bronchoalveolar), pus, tissue samples, and sterile body fluids/central venous catheter tips respectively *(*Figure-II).

Breakpoint BMD categorized 85 (44.7%) isolates as resistant and 105 (55.2%) as intermediate to colistin.

CT powder solution: Overall, categorical agreement of CT powder solution with breakpoint BMD was 97%. The organism-wise categorical agreement was 97% for *Klebsiella* species, 100% for *E. coli*, 100% for *Enterobacter* spp. and 96% for *Pseudomonas aeruginosa.* The sensitivity was 95% and specificity was 100%. CT disk solution: Overall categorical agreement of CT disk solution with breakpoint BMD was 95%. The organism-wise categorical agreement was 97% for *Klebsiella* species, 100% for *E. coli*, 100% for *Enterobacter* spp. and 81 % for *Pseudomonas aeruginosa.* The sensitivity was 96% and specificity was 96%. PB disk solution: Overall, categorical agreement of PB disk solution with breakpoint BMD was 98%. The organism-wise categorical agreement was 98% for *Klebsiella* species, 100% for *E. coli*, 100% for *Enterobacter* spp. and 96 % for *Pseudomonas aeruginosa.* The sensitivity was 96% and specificity was 100% (Table-I).

Figure-I: Flow diagram of isolates included and excluded from the study.

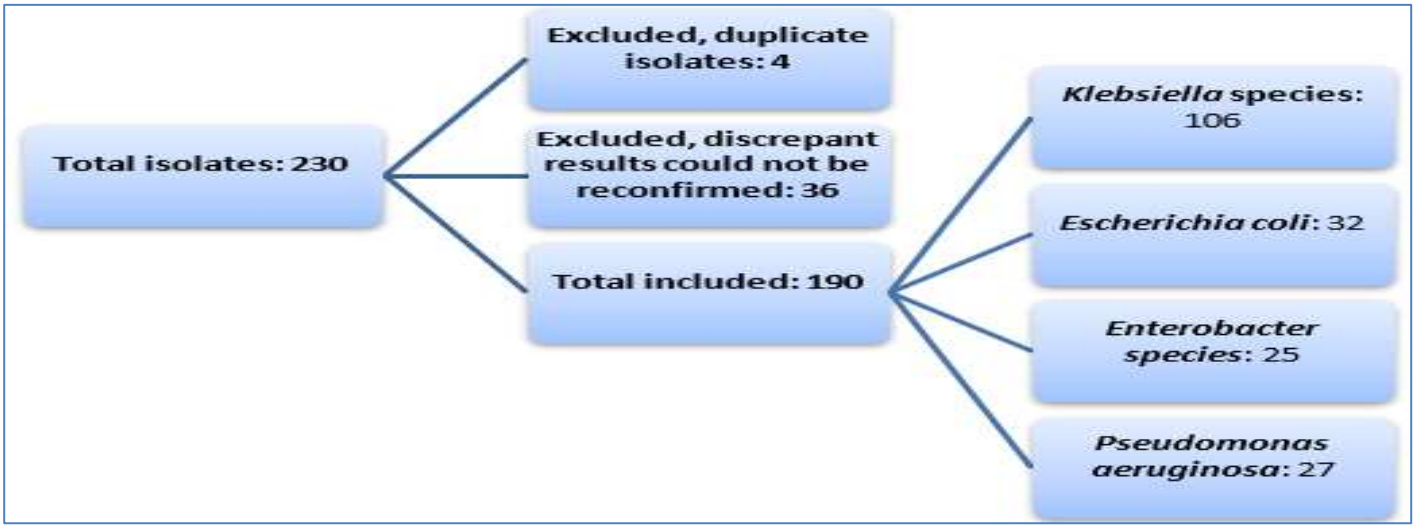

Table-I: Performance of three different colistin testing methods

Method	Organism	Categorical agreement (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
Colistin powder solution	Total	97	100	96	95	100
	Klebsiella species	97				
	Escherichia coli	100				
	Enterobacter species	100				
	Pseudomonas aeruginosa	96				
Colistin disk solution	Total	95	95	96	96	96
	Klebsiella species	97				
	Escherichia coli	100				
	Enterobacter species	100				
	Pseudomonas aeruginosa	81				
PB disk solution	Total	98	100	97	96	100
	Klebsiella species	98				
	Escherichia coli	100				
	Enterobacter species	100				
	Pseudomonas aeruginosa	96				

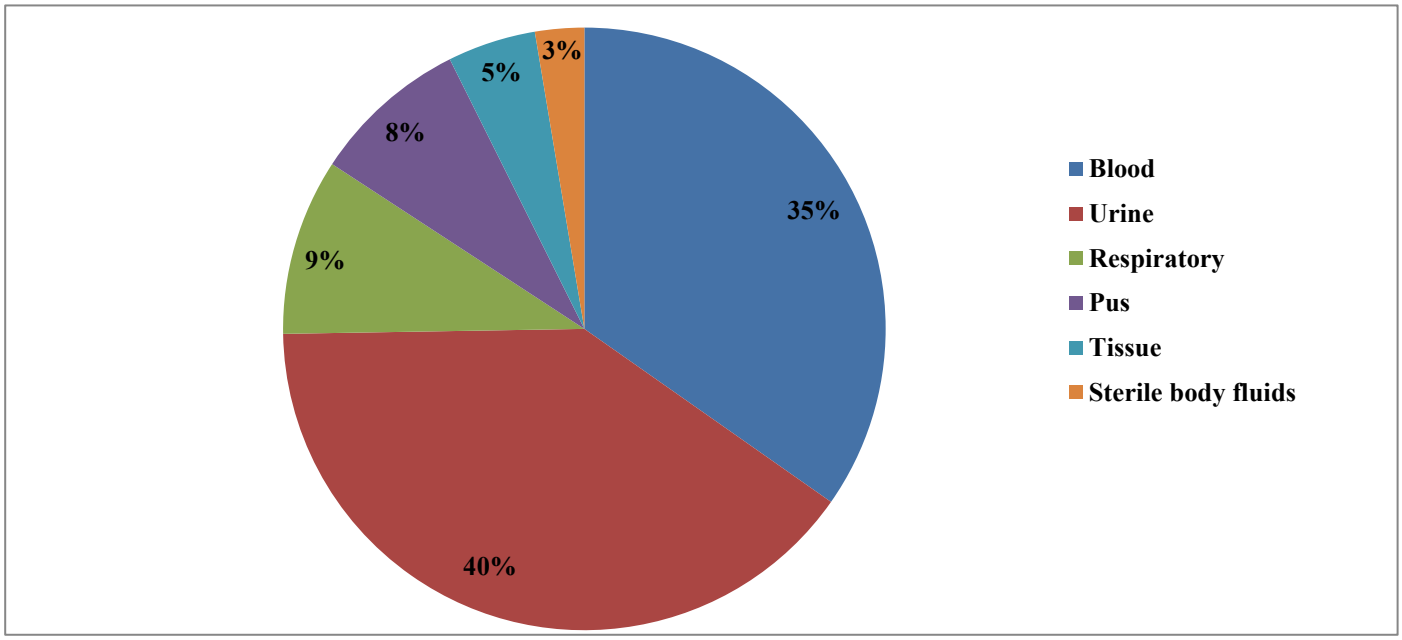

Figure-II: Distribution of carbapenem-resistant isolates by sample type.

DISCUSSION

Our results showed more than ≥95% agreement of colistin drop test with breakpoint BMD using all three solutions. PB disk solution had the highest categorical agreement for all tested organisms compared to the two other solutions. Our results are in alignment with previously published studies. In a study reported in 2016, Joue et al.[14] tested 8 micrograms per ml colistin solution with 54 colistin resistant and 52 colistin susceptible *Escherichia coli* and demonstrated a 100% categorical agreement with reference BMD. Compared to our study, Joue et al tested veterinary *Escherichia coli* isolates only and a lower concentration of colistin solution. Another study conducted by Pasteran et. al.[15] in 2020, showed a categorical agreement of 96.2% with colistin powder and colistin disk solution while 93.4% categorical agreement with PB disk solution in 271 isolates of Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. This study also determined presence of mcr 1 gene in 132 isolates. We did not determine molecular determinants of colistin resistance in our study. Another study conducted by Perez et al[18] in 2020, tested 560 isolates of *Klebsiella spp., Acinetobacter baumannii complex, Pseudomonas aeruginosa,* and *Enterobacter cloacae complex* with PB disk solutions of 2, 4 and 8 µg/mL and showed a categorical agreement of 92% 95% and 94.8% respectively. In this study 78.3% strains were susceptible, and 21.7% isolates were resistant to polymyxin B and 80% of the total isolates were *Klebsiella pneumoniae* carbapenemase (KPC)-producing K. pneumoniae while our study has almost equal representation of both resistant and intermediate strains.

Our study had some limitations, first, all isolates were not compared with reference BMD, only the discrepant ones were, however they were compared with a validated method against the reference BMD.[11] Second, we did not perform any genotypic method to determine genetic resistance markers in the study isolates.

CONCLUSION

In conclusion, categorical agreement, sensitivity and specificity of colistin drop test with PB disk solution in comparison with breakpoint BMD turned out to be the highest i.e. 98%, 96% and 100%. Therefore, we would recommend using PB disk solution for colistin susceptibility testing in resource limited settings.

CONFLICT OF INTEREST

None

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AUTHOR CONTRIBUTION

Syeda Rija Zehra: Conceptualization of the research, isolate collection and storage, bench work, statistical analysis, manuscript writing, final approval, accountable for all aspects of publication

Imran Ahmed: Manuscript writing, calculations, and statistical analysis, final approval, accountable for all aspects of publication

Mohammad Umer Khan: Bench work data collection, manuscript writing and editing, final approval, accountable for all aspects of publication

Tooba Raheem: Bench work and isolate collection and storage, manuscript, final approval, accountable for all aspects of publication

Syed Abbas Kazmi: Bench work, data collection, manuscript writing and editing, final approval, accountable for all aspects of publication

Sadaf Zaka: Bench work, isolate collection and storage, data collection, manuscript writing and editing, final approval, accountable for all aspects of publication

Kauser Jabeen: Supervision of all bench work, conceptualization of the research, manuscript writing, statistical analysis, and final editing of the manuscript, final approval, accountable for all aspects of publication.

REFERENCES

1. Kang CI, Song JH. Antimicrobial resistance in Asia: current epidemiology and clinical implications. Infect Chemother. 2013;45(1):22-31. DOI: https://doi.org/10.3947/ic.2013.45.1.22
2. Bilal H, Khan MN, Rehman T, Hameed MF, Yang X. Antibiotic resistance in Pakistan: a systematic review of past decade. BMC Infect Dis. 2021; 21(1): 244. DOI: https://doi.org/10.1186/s12879-021-05906-1
3. Lee K, Kim MN, Kim JS, Hong HL, Kang JO, Shin JH, et al. Further increases in carbapenem-, amikacin-, and fluoroquinolone-resistant isolates of *Acinetobacter* spp. and *P. aeruginosa* in Korea: KONSAR study 2009. Yonsei Med J. 2011;52(5):793-802. DOI: https://doi.org/10.3349/ymj.2011.52.5.793
4. Hassan J, El-Gemayel L, Bashour I, Kassem II. On the edge of a precipice: The global emergence and dissemination of plasmid-borne *mcr* genes that confer resistance to colistin, a last-resort antibiotic. In: Hashmi MZ, editor. Antibiotics and Antimicrobial Resistance Genes in the Environment. Amsterdam: Elsevier; 2019. p. 155-182. DOI: https://doi.org/10.1016/B978-0-12-818882-8.00010-3

5. Petrosillo N, Taglietti F, Granata G. Treatment Options for Colistin Resistant *Klebsiella pneumoniae*: Present and Future. J Clin Med. 2019; 8(7): 934. DOI: https://doi.org/10.3390/jcm8070934
6. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev. 2017; 30(2): 557-96. DOI: https://doi.org/10.1128/cmr.00064-16
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. 33rd ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2023. Available from: https://clsi.org/standards/products/microbiology/documents/m100/
8. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020. Available from: https://www.eucast.org
9. Sujatha SR DR, Tejashree A, Sai S. Evaluation of Colistin broth disk elution and colistin agar test: A study from tertiary care hospital, South India. J Pure Appl Microbiol 2022; 16(2): 885-90. DOI: https://doi.org/10.22207/JPAM.16.2.08
10. Varela NP, Friendship R, Dewey C, Valdivieso A. Comparison of agar dilution and E-test for antimicrobial susceptibility testing of *Campylobacter coil* isolates recovered from 80 Ontario swine farms. Can J Vet Res. 2008; 72(2): 168-74. DOI: https://doi.org/10.1128/cmr.00064-16
11. Ahmed I, Laiq S, Shaheen N, Wahab K, Farooqi J, Shahid A, et al. 'Breakpoint broth microdilution plate' for susceptibility testing of Gram-negative bacilli against colistin sulfate. Pract Lab Med. 2020; 22: e00192. DOI: https://doi.org/10.1016/j.plabm.2020.e00192
12. Mitton B, Kingsburgh C, Kock MM, Mbelle NM, Strydom K. Evaluation of an In-House Colistin NP Test for Use in Resource-Limited Settings. J Clin Microbiol. 2019; 57(10): e00501-19. DOI: https://doi.org/10.1128/JCM.00501-19
13. Halling SM, Jensen AE, Olsen SC. Defensin susceptibility and colonization in the mouse model of AJ100, a polymyxin B-resistant, *Brucella abortu*s RB51 isolate. Curr Microbiol. 2008; 56(3): 274-8. DOI: https://doi.org/10.1007/s00284-007-9074-8
14. Jouy E, Haenni M, Le Devendec L, Le Roux A, Châtre P, Madec JY, et al. Improvement in routine detection of colistin resistance in E. coli isolated in veterinary diagnostic laboratories. J Microbiol Methods. 2017; 132: 125-7. DOI: https://doi.org/10.1016/j.mimet.2016.11.017
15. Pasteran F, Danze D, Menocal A, Cabrera C, Castillo I, Albornoz E, et al. Simple phenotypic tests to improve accuracy in screening chromosomal and plasmid-mediated colistin resistance in gram-negative bacilli. J Clin Microbiol. 2020; 59(1): e01701-20. DOI: https://doi.org/10.1128/jcm.01701-20
16. Suay-García B, Pérez-Gracia MT. Present and future of carbapenem-resistant Enterobacteriaceae (CRE) Infections. Antibiotics (Basel). 2019; 8(3):122. DOI: https://doi.org/10.3390/antibiotics8030122
17. Legenza L, McNair K, Gao S, Lacy JP, Olson BJ, Fritsche TR, et al. A geospatial approach to identify patterns of antibiotic susceptibility at a neighborhood level in Wisconsin, United States. Sci Rep. 2023;13(1):7122. DOI: https://doi.org/10.1038/s41598-023-33895-5
18. Perez LRR, Carniel E, Narvaez GA, Dias CG. Evaluation of a polymyxin drop test for polymyxin resistance detection among non-fermentative gram-negative rods and enterobacterales resistant to carbapenems. APMIS. 2021; 129(3): 138-42. DOI: https://doi.org/10.1111/apm.13096