Egyptian Journal of Medical Microbiology Volume 32 / No.3 / July 2023 109-115 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Comparative Evaluation of Colistin Susceptibility Testing Using Agar Dilution and Broth Microdilution in Multidrug-resistant and Extensively Drug-resistant Gram-Negative Isolates

Abeer K. Abu-El-Azayem, Reham A. Dwedar, Nihal Nashaat[1], Rania Y. Shash*
Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo, Egypt

Key words:
Gram-negative bacteria; MDR; XDR; colistin; broth microdilution; agar dilution

Corresponding Author:
Rania Yahia Shash
Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo, Egypt
Tel.: 01005240101
raniayahiashash@cu.edu.eg

ABSTRACT

Background: *Emergence of multidrug resistance (MDR) and extensively drug resistance (XDR) among Gram-negative bacteria has obliged the clinical re-use of old antimicrobial agents, such as colistin. The increase in the use of colistin has necessitated reliable, and accurate methods for in vitro antimicrobial susceptibility testing to allow proper antimicrobial therapy. Up till now the most commonly used method in laboratories for colistin susceptibility testing is the disk diffusion test which is considered unreliable.* ***Objectives:*** *This study aimed to evaluate and compare two methods for colistin susceptibility testing using agar dilution and broth microdilution among MDR and XDR Gram-negative isolates.* ***Methodology:*** *This study included 62 Gram-negative isolates (38 MDR and 24 XDR) obtained from the Medical Microbiology and Immunology Department, Cairo University, Egypt. Colistin susceptibility was tested for these isolates by agar dilution and broth microdilution methods.* ***Results:*** *Our study revealed a significant statistical difference between colistin MIC by using broth microdilution and the agar dilution method. In comparison to broth microdilution, the agar dilution method had 100% sensitivity, 85.88 % specificity, 25% positive predictive value, 100% negative predictive value and 86.21% accuracy.* ***Conclusions:*** *The agar dilution method is unreliable for the detection of colistin resistance. Therefore, we could depend on colistin susceptibility testing by using standard broth microdilution even in case of limited resources. This accurate susceptibility testing may help us to maintain the therapeutic usefulness of colistin until newer treatment choices are available.*

INTRODUCTION

Resistance to antibiotics is recognized as a worldwide threat by the World Health Organization (WHO). A major concern is the continuous escalation of antimicrobial resistance among Gram-negative bacteria resulting in the endemic presence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens. This concern is raised by the detection of MDR/XDR Gram-negative bacteria in both hospitalized and community patients and as causative agents for many infections[1].

Nosocomial infections caused by MDR and XDR Gram-negative bacteria are considered a main threat worldwide[2]. A group of bacteria abbreviated as ESKAPE are made up of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. These bacteria are major causes of life-threatening and severe infections as well as the development of drug-resistance mechanisms[3].

One of the last resort antibiotics is a revived antimicrobial of the 1970s, colistin, which has proved promising effectiveness in critically ill host[4]. However, because of the exaggerated colistin usage, resistance is more rapidly increasing[5].

Colistin is an antimicrobial cationic peptide isolated from *Bacillus polymyxa* in 1947. It was used clinically in 1958. Unfortunately, colistin usage was inhibited in the 1970s because of nephrotoxic and neurotoxic reported cases. Later, the reuse of colistin was necessary because of the spread of MDR Gram-negative pathogens[2].

Colistin antimicrobial action depends on the interaction of the cationic peptide and the bacterial cell membrane negatively charged lipopolysaccharide, resulting in enhancement of the permeability of the cell membrane and ultimately cell death[4].

The antibacterial spectrum of colistin includes activity *in vitro* against, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Klebsiella* spp., *E. coli*, *Salmonella* spp., *Shigella* spp., *Enterobacter* spp., *Citrobacter* spp. and *Yersinia pseudotuberculosis*. Furthermore, colistin has substantial activity against *Stenotrophomonas* spp., whereas it is inactive against some Gram-negative aerobic bacilli, including *Burkholderia cepacian*, *Burkholderia mallei*, *Proteus* spp., *Morganella*

morganii, *Providencia* spp., *Brucella* spp., *Serratia* spp. and *Edwardsiella* spp.[6].

Expectedly, colistin resistance has been detected in Gram-negative bacteria like *Pseudomonas* spp., *Acinetobacter* spp., *E. coli*, and *Klebsiella* spp. Colistin resistance was due to chromosomal mutations in addition to genes such as the mobilized colistin resistance 1 (mcr-1) and mcr-2. This emphasizes the critical need for standardized *in vitro* colistin susceptibility testing by laboratories of microbiology for patient care and epidemiological surveillance[7].

Carbapenem-resistant *Enterobacteriaceae* (CRE) became resistant through various mechanisms, the most important mechanism is by producing carbapenemases which are enzymes encoded on mobile genetic elements. Class A *Klebsiella pneumoniae* carbapenemases (KPCs), class B metallo-β-lactamases (MBLs) and class D OXA β-lactamases are the most common carbapenemases[8].

Polymyxins, including polymyxin B and colistin (polymyxin E), are known as 'last resort' antibiotics being sometimes the only available agent active against CRE. However, along with chromosomal mutations associated with polymyxin(s) resistance, increasing reports document the global spread of resistance-conferring plasmids, posing the risk that, if present together with carbapenemases, microorganisms may become untreatable with most or all antibiotics[9].

The increased usage of colistin has necessitated the urgent need for accurate, rapid, and reliable methods to test for antimicrobial susceptibility to ensure proper decisions for therapy. The disk diffusion method, which is commonly used in microbiology laboratories, is considered defective and not reliable due to the poor colistin diffusion in agar because of electrostatic interactions with acid or sulfate groups of the agar, resulting in a smaller diameter of zones of inhibition [10]. This poor colistin diffusion results in higher inaccuracy rates in comparison to the method of broth microdilution for the determination of minimum inhibitory concentration (MIC)[11]. At present there is no standardized disk diffusion method to test colistin susceptibility to be used in the laboratories of microbiology[12].

This study aimed to evaluate and compare 2 methods for colistin susceptibility testing using agar dilution and broth microdilution among MDR and XDR Gram-negative isolates.

METHODOLOGY

This observational cross-sectional study was conducted from April 2022 to October 2022. The research was authorized by the Ethics Committee of the Institutional Review Board on April 17, 2022 (Code: MS-56-2022), Faculty of Medicine, Cairo University, Egypt.

Bacterial isolates:

Sixty-two Gram-negative isolates (38 MDR and 24 XDR isolates) were obtained from the strain bank of the Medical Microbiology and Immunology Department, Cairo University.

MDR was considered when there was resistance to at least one antimicrobial agent in three or more classes, XDR was considered when there was resistance to at least one antimicrobial agent in all but two or fewer classes[2].

26 isolates were *Klebsiella* spp. (11XDR and 15 MDR), 14 isolates were *E. coli* (7 XDR and 7 MDR), 17 isolates were *Pseudomonas* spp. (5 XDR and 12 MDR), 4 isolates were MDR *Acinetobacter* spp. and 1 isolate was XDR *Enterobacter* spp.

Detection of MIC of colistin using agar dilution method for Gram-negative bacteria:

According to the CLSI guidelines, MICs of colistin were detected by agar dilution using Mueller Hinton agar (MHA) (Oxoid, UK) [13]. Ten serial dilutions of colistin were prepared using the powder of colistin sulphate (6 million I.U /gm). The range of colistin serial dilutions was from 640 µg/ml to 1.25 µg/ml. Each one of the ten dilutions was added (by a percentage of 10% of the agar volume while it is still warm) to the Mueller Hinton agar plates separately and distributed into five sets. Therefore, the final concentrations at 1:10 dilution in agar plates were (64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125µg/ml) **(**Figure 1).

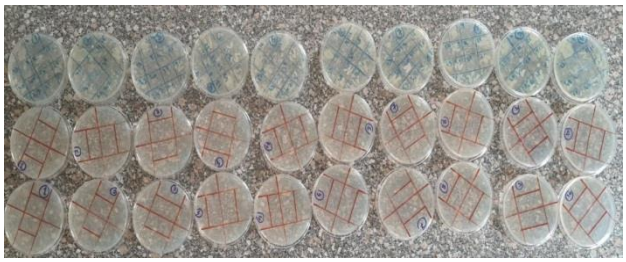

Fig. 1: Detection of MICs of colistin using agar dilution method.

Three sets of plates\ each set has ten plates of the ten tested colistin concentrations.

Bacterial suspensions of each of the isolates were prepared equivalent to 0.5 McFarland and 10 µL of each suspension was inoculated on each set of the prepared plates. The agar plates were incubated at 37 ºC for 24 hours. Colistin MIC was determined as the lowest concentration of the antibiotic with no visible growth of the organism[14]. Results interpretation was performed according to CLSI[13] guidelines. Isolates with MIC ≤ 2 µg/mL were considered sensitive to colistin while isolates with MIC of ≥ 4 µg/mL were considered resistant.

Colistin MIC determination using broth microdilution for Gram-negative bacteria:

Colistin sulphate powder (6 million I.U. /gm.) was used as recommended by CLSI[13].

Inoculum preparation:

For each tested isolate, 3-5 well-isolated colonies of similar morphology from a 24-hour culture were inoculated into four to five ml of Mueller-Hinton broth (Oxoid, UK) and the turbidity of the suspension was adjusted to match the 0.5 McFarland standard (approximately 1 x 10^8 CFU/ml) by diluting with additional broth. Within 15 minutes of preparation of the bacterial inoculum, it was diluted to 1:100 by adding 50 µl of the prepared inoculum to 4.95 ml (4950 µl) of sterile broth so that bacterial concentration in this 2nd inoculum became approximately $1X10^6$ CFU/ml.

Colistin stock solutions preparation:

1 gm of colistin sulphate powder was dissolved in 100 ml of sterile distilled water to get the stock solution of 2000 µg/ml which was subsequently used as outlined in CLSI[13], to prepare ranged serial dilutions from 128 µg/ml to 0.25 µg/ml. To prepare the colistin intermediate dilutions, the formula $C_1*V_1 = C_2*V_2$ was used where C_1 is the stock solution concentration; V_1 is the unknown volume that will be needed to make the intermediate concentration; C_2 is the intermediate concentration required; and V_2 is the intermediate solution volume required[13].

Steps of MIC test[13]:

A volume of 100 µl of colistin dilutions was distributed in wells from 1 to 10 in microtiter plates starting from the highest concentration to the lowest one. A volume of 100 µl of the 2nd inoculum of each tested isolate was added to wells 1 through 10 in each raw using a multi-channel pipette so that the wells from 1 to 10 contained serial colistin dilutions starting from 64 µg/ml to 0.125 µg/ml and a final bacterial concentration of 5×10^5 CFU/ml with a final volume of 200 µl in each well. Column 11 was the growth control, containing 100 µl of broth plus 100 µl of inoculum, with a final volume of 200 µl and column 12 was the sterility control, containing 200 µl of broth only. The microtiter plates were gently tapped for proper mixing. The microtiter plates were incubated at 37 °C for 24 hours and then examined.

Interpretation of results:

MICs were defined as the highest dilution of the antibiotic that visually inhibited the growth of the tested organism as verified by turbidity and reading the turbidity using a microplate reader. For *Enterobacteriaceae*, results interpretation was done following the CLSI cutoff value as wild or non-wild type[13]. For *Pseudomonas* and *Acinetobacter* spp., results were interpreted following the CLSI breakpoints as susceptible or resistant[13].

Statistical methods

Coding and entering the data were done by means of the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Summarization of data was done using mean, median, standard deviation, minimum and maximum for quantitative data and by using frequency (count) and relative frequency (percentage) for categorical data. For studying and relating categorical data, the Chi-square ($\chi 2$) test was used. An exact test was used instead when the frequency predicted is below 5[15]. Standard diagnostic indices including sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and diagnostic efficacy were calculated as demarcated by Galen[13]. p-values of less than 0.05 were counted as statistically significant.

RESULTS

Out of the 62 Gram-negative isolates obtained from the Microbiology Department, 61.3% were MDR (38/62), and 38.7% were extensive drug-resistant (XDR) (24/62 isolates).

26 isolates were *Klebsiella* spp. (11XDR and 15 MDR), 14 isolates were *E. coli* (7 XDR and 7 MDR), 17 isolates were *Pseudomonas* spp. (5 XDR and 12 MDR), 4 isolates were MDR *Acinetobacter* spp. and 1 isolate was XDR *Enterobacter* spp.

Colistin MIC (agar dilution vs broth microdilution method for Gram-negative bacteria):

In the current investigation, by means of the agar dilution test, MIC done to all MDR and XDR bacterial Gram-negative isolates, 25.8% were resistant to colistin (16/62 isolates) and 74.2% were sensitive to it (46/62 isolates). From the 16 colistin-resistant Gram-negative bacteria; 25.0% were *E.* coli (4/16 isolates, 3 MDR and 1 XDR isolate), 50% were *Klebsiella spp.* (8/16 isolates, 6 MDR and 2 XDR isolates), 18.75% were *Pseudomonas* spp. (3/16 isolates, 1MDR and 2 XDR isolates) and 6.25% were *Acinetobacter* spp. (1/16, 1 MDR isolate) (Table 1 and Figure 2,3).

Table 1: Prevalence of colistin resistance by agar dilution MIC method among the Gram-negative isolates

		Colistin resistant isolates	
		No.	**%**
Organisms	***E.* coli**	4	25.0%
	***Klebsiella* spp.**	8	50.0%
	***Pseudomonas* spp.**	3	18.75%
	***Acinetobacter* spp.**	1	6.25%

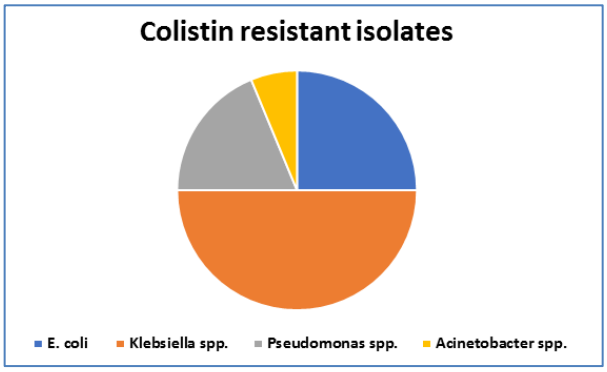

Fig. 2: Prevalence of colistin resistance by agar dilution MIC method among the Gram-negative isolates

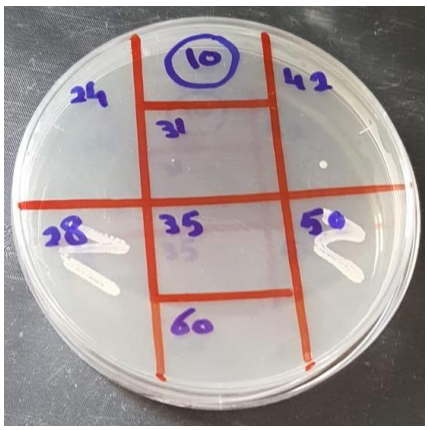

Fig. 3: Detection of colistin MIC using agar dilution method**.**

Mueller Hinton medium at colistin concentration 0.125 µg/mL, growth was seen in samples no.28 and 50

However, 6.5% of the Gram-negative isolates were colistin non-susceptible by the method of broth microdilution (4/62) while 93.5% were colistin susceptible by the same method (58/62). Out of the four isolates that were resistant to colistin; 25.0% were *E. coli* (1/4, 1 MDR isolate) and 75% were *Klebsiella* spp. (3/4 isolates, 2 MDR and 1 XDR isolate) *(*Table 2). These 4 isolates were also colistin-resistant by the agar dilution method.

Table 2: Prevalence of colistin resistance by broth microdilution MIC method among the Gram-negative isolates

		Colistin resistant isolates	
		No.	**%**
Organisms	***E. coli***	1	25.0%
	***Klebsiella* spp.**	3	75.0%

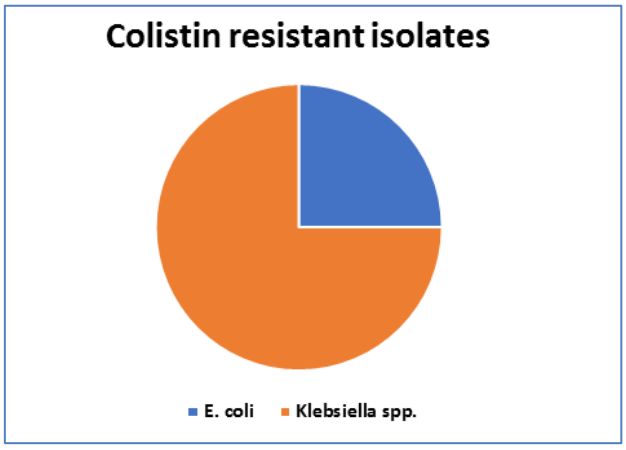

Fig. 4: Prevalence of colistin resistance by broth microdilution MIC method among the Gram-negative isolates

There is a significant statistical difference between colistin MIC using the method of broth microdilution compared to agar dilution method with a P value of 0.001 as demonstrated in Tables 3 and 4.

Table 3: Comparing the accuracy of colistin MIC using agar dilution method and broth microdilution method

		Colistin broth microdilution MIC				
		Resistant		Sensitive		P value
		No.	%	No.	%	
Colistin agar MIC	**Resistant**	4	100.0%	12	14.1%	0.001
	Sensitive	0	0.0%	50	85.9%	

Table 4: Accuracy of colistin agar dilution method

Statistic	Value	95% CI
Sensitivity	100.00%	39.76% to 100.00%
Specificity	85.88%	76.64% to 92.49%
Positive Predictive Value	25.00%	16.48% to 36.03%
Negative Predictive Value	100.00%	-
Accuracy	86.52%	77.63% to 92.83%

DISCUSSION

Globally, the spread of multi-drug resistance in bacteria like carbapenem-resistant *Enterobacteriaceae* (CRE), extended-spectrum β-lactamase (ESBL) producers and methicillin-resistant *Staphylococcus aureus* (MRSA), have dramatically increased in the past two decades. This poses a serious challenge for physicians to treat and often leads to failure of treatment and increased mortality[17].

In the current study, we attempted to study the method of broth microdilution versus the agar dilution method for detecting colistin MIC.

In our study, out of the 62 isolates Gram-negative isolates 61.3% were MDR (38/62), and 38.7% were extensive drug-resistant (XDR) (24/62 isolates). 26 isolates were *Klebsiella* spp. (11XDR and 15 MDR), 14 isolates were *E. coli* (7 XDR and 7 MDR), 17 isolates were *Pseudomonas* spp. (5 XDR and 12 MDR), 4 isolates were MDR *Acinetobacter* spp. and 1 isolate was XDR *Enterobacter* spp.

Similarly, another study conducted at Alexandria University Hospital, Egypt by *Ismail et al.* [18] stated that 55.1% were MDR (54/98 isolates) and 34.7% were XDR (44/98 isolates).

A different study conducted in South India by *Kathirvel et al.* [19] stated that out of 150 isolates, 66% were MDR or XDR.

In Oman, a study done by Sannathimmappa et al. [20] wasn't in line with our study where it stated that only 36% of the strains were MDR Gram-negative pathogens (63/175 isolates).

The widespread indiscriminate use of broad-spectrum antibiotics is the major factor that leads to selective pressure and the emergence of MDR pathogens. The increasing prevalence of these MDR pathogens is worrying because they limit antibiotic choice and may lead to the worst outcome. Countries which implemented strict antibiotic control policies and caution to the use of antibiotics have shown lower MDR prevalence levels.

In the present study, we found a statistically significant difference between colistin MIC using the broth microdilution method and agar dilution methods where the agar dilution method in comparison to broth microdilution had sensitivity of 100%, specificity of 85.88 %, 25% positive predictive value, 100% negative predictive value and 86.21% accuracy.

One explanation for this could be colistin powder poor diffusion in the agar.

A study conducted by Gales et al.[21] showed a relative analysis between the reference tests for colistin dilution using a set of 35 isolates. Measuring colistin MICs by broth microdilution revealed results that is in parallel with that of agar dilution method, demonstrating an essential agreement of 94.3% (±1-log2 dilution). Only two organisms showed a higher MIC values of agar dilution test of fourfold increase than MICs of broth microdilution.

Another study done by Tan and Ng[22] stated that colistin broth microdilution MICs is more preferred because colistin powder diffuses poorly in agar, resulting in small inhibition zones.

Another study done at Alexandria University Hospital, Egypt by Asser and Kholeif[23] stated that comparative evaluation between the commonly used antibiotic susceptibility methods to colistin showed that the broth microdilution method revealed 100% categorical agreement with Vitek-2.

A study done by Turlej-Rogacka et al.[7] disagreed with us. They stated in their research that agar dilution test is better in terms of accessibility and reproducibility in comparison to the suggested broth microdilution test methods used for detecting colistin MIC. They investigated the solubility, stability, and distribution of colistin in agar plates by testing the same bacterial strain in different regions of the agar plate and comparing it with the obtained MIC values. They suggested that providing the equal distribution of colistin in the agar plates and the good stability of antibiotic in MH agar under the proper storage conditions may help in the bulk production of MIC plates which is commonly done in microbiology laboratories for increased cost-effectiveness and efficiency[7].

It is worth mentioning a study done at Mansoura University; Egypt by Elshaer et al.,[24] who stated that CHROMID Colistin R agar is a reliable culture medium that can be used effectively for rapid screening of Colistin-resistant Gram-negative bacteria. There was a very good agreement with the vitek-2 system in the detection of Colistin resistant Gram-negative bacteria.

CONCLUSIONS

Since the disk diffusion method is unreliable for detecting colistin resistance, we should depend on susceptibility testing using other more accurate methods. We concluded that the agar dilution method is unreliable for detection of colistin resistance. Therefore, we should depend on susceptibility testing by standard broth microdilution even in case of limited resources. This accurate susceptibility testing may help us to maintain the therapeutic usefulness of colistin until newer treatment choices are available.

Recommendations

Further studies comparing colistin susceptibility using broth dilution and agar dilution methods are needed. Programs for the rational usage of antibiotics and strict infection control policies should be applied to reduce the spread of MDR and XDR pathogens.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as an author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and they have approved the manuscript as submitted.

REFERENCES

1. Reynolds D, Burnham JP, Vazquez Guillamet C, et al. The threat of multidrug-resistant/extensively drug-resistant Gram-negative respiratory infections: another pandemic. Eur Respir Rev. 2022 Oct 19;31(166):220068. doi: 10.1183/16000617.0068-2022. PMID: 36261159; PMCID: PMC9724833.
2. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268-81.
3. Boucher HW, Talbot GH, Bradley JS. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis.* 2009;48:1–12.
4. Giamarellou H, Poulakou G. Multidrug-resistant Gram-negative infections: what are the treatment options? Drugs 2009;69:1879-901.
5. Marchaim D, Chopra T, Pogue JM, et al. Outbreak of colistin-resistant, carbapenem-resistant Klebsiella pneumoniae in metropolitan Detroit, Michigan. Antimicrob Agents Chemother 2011;55:593-9.
6. World Health Organization 2018. Global Antimicrobial Resistance Surveillance System (GLASS): the detection and reporting of colistin resistance (No. WHO/WSI/AMR/2018.4). World Health Organization.
7. Turlej-Rogacka A, Xavier B, Janssens L, et al. Evaluation of colistin stability in agar and comparison of four methods for MIC testing of colistin. European journal of clinical microbiology & infectious diseases 2018; official publication of the European Society of Clinical Microbiology, 37(2), 345–353. https://doi.org/10.1007/s10096-017-3140-3
8. Stoesser N, Mathers AJ, Moore CE et al. Colistin resistance gene mcr-1 and pHNSHP45 plasmid in human isolates of Escherichia coli and Klebsiella pneumoniae. The Lancet infectious diseases 2016; 16(3), 285-286.
9. Malchione MD, Torres LM, Hartley DM, Koch, et al. Carbapenem and colistin resistance in Enterobacteriaceae in Southeast Asia: review and mapping of emerging and overlapping challenges. International journal of antimicrobial agents 2015; 54(4), 381-399.
10. de Louvois J. Factors influencing the assay of antimicrobial drugs in clinical samples by the agar plate diffusion method. J Antimicrob Chemother 1982;9:253-65.
11. Dafopoulou K, Zarkotou O, Dimitroulia E, et al. Comparative evaluation of colistin susceptibility testing methods among carbapenem-nonsusceptible Klebsiella pneumoniae and Acinetobacter baumannii clinical isolates. Antimicrob Agents Chemother 2015;59:4625-30.
12. Bakthavatchalam YD and Veeraraghavan B. Challenges, issues and warnings from CLSI and EUCAST working group on Polymyxin susceptibility testing. J Clin Diagn Res 2017;11:DL03-DL4.
13. Clinical Lab. Standard Institute CLSI (2022). Performance Standards for Antimicrobial Susceptibility Tests. 32th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, USA.
14. Andrews JM. Determination of minimum inhibitory concentrations. Journal of antimicrobial Chemotherapy, 2001; 48(suppl_1), 5-16.
15. Chan YH. Biostatistics 103: Qualitative Data – Tests of Independence. Singapore Med J. 2003;44(10): 498-503.
16. Galen RS. Predictive values and efficiency of laboratory testing. Pediat J Clin North Am 1980; 27:861-69.
17. Saseedharan S, Sahu M, Chaddha R, et al. Epidemiology of diabetic foot infections in a reference tertiary hospital in India. brazilian journal of microbiology; 2018.
18. Ismail AA, Meheissen MA, Abd Elaaty TA, et al. Microbial profile, antimicrobial resistance, and molecular characterization of diabetic foot infections in a university hospital. Germs, 2021; 11(1), 39.
19. Kathirvel M, Jayarajan J, Sivakumar A, et al. Risk factors for the diabetic foot infection with multidrug-resistant microorganisms in South India. International Surgery Journal, 2018; 5(2), 675-682.
20. Sannathimmappa MB, Nambiar V, Aravindakshan R, et al. Diabetic foot infections: Profile and antibiotic susceptibility patterns of bacterial isolates

in a tertiary care hospital of Oman. Journal of Education and Health Promotion 2021; 10.

21. Gales AC, Reis AO, & Jones RN. Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines. Journal of clinical microbiology 2001; 39(1), 183-190.
22. Tan TY and Ng LSY. Comparison of three standardized disc susceptibility testing methods for colistin. Journal of Antimicrobial Chemotherapy, 2006; 58(4), 864-867.
23. Asser SL and Kholeif DA. Colistin Susceptibility Testing Methods for Carbapenem Resistant Acinetobacter baumannii, A Comparative Study. Egyptian Journal of Medical Microbiology, 2022; 31 (2), 63-67.
24. Elshaer M, Zekri L, Elewa A, et al. Rapid Screening for Colistin Resistant Bacteria by Chromogenic Agar. Egyptian Journal of Medical Microbiology, 2022; 31 (2), 103-107.