Original Article

Detection of Plasmid-Mediated Colistin Resistance Genes in Clinical Isolates of *Klebsiella pneumoniae* and *Escherichia coli* from Some Hospitals in Khartoum

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ABSTRACT

Background: Currently, a final-resort therapy for MDR *Klebsiella pneumoniae* and *Escherichia coli* is the antibiotic colistin (polymyxin E). Plasmid-mediated colistin-resistance genes (*mcr*-9, *mcr*-8, *mcr*-5, *mcr*-3, *mcr*-2 and *mcr*-1) is one of the mechanisms by which these bacteria resist colistin. The aim of the study is to evaluate the presence of these genes in isolates of *Klebsiella pneumoniae* and *Escherichia coli* that are phenotypically colistin-resistant in a number of hospitals in Khartoum State. **Materials and Methods:** One hundred eighty-five bacterial strains (gram-negative rods) were identified from clinical specimens of male and female hospitalized patients. Various patients' urine, wounds, tissues, and blood samples were obtained. Isolates were identified using conventional methods of identification. Antimicrobial susceptibility testing for colistin was conducted using the disc diffusion method. Colistin-resistant *K. pneumoniae* and *E. coli* isolates were isolated and conventional PCR was employed to identify plasmid-mediated *mcr*-9, *mcr*-8, *mcr*-5, *mcr*-3, *mcr*-2 and *mcr*-1genes. The Chi-square test was administered, and p-values of 0.05 were deemed statistically significant. **Results:** The disc diffusion method revealed that around 35 (18%) of 185 isolates were resistant to colistin. 21 (60%) of the 35 colistin-resistant isolates were *E. coli*, and 14 (40%) were *K. pneumoniae*. All 35 colistin-resistant isolates and 13 colistin-susceptible isolates were examined for colistin-resistant genes. In 21 of 48 tested isolates, colistin-resistant genes were found. 14 (66%) of 21 isolates were resistant according to both the disc diffusion and PCR tests. There were determined to be *mcr*-3, *mcr*-2, and *mcr*-1 gene. *mcr*-1 accounted for 18 (85%), 5% *mcr*-1 combined with *mcr*-2 , 5% *mcr*-2 , and 5% *mcr*-2 in combination with *mcr*-3. **Conclusion:** The frequency of colistin-resistant *E. coli* and *K. pneumoniae* in Sudanese hospitals was found to be worrisome, thereby reducing the range of available treatment alternatives. The emergence of the colistin-resistant genes *mcr*-2 and *mcr*-1 in some Sudanese hospitals is caused by plasmids, and this is a problem for hospitals because horizontal transfer of this plasmid could cause the resistance to spread to many isolates there.

Keywords: Colistin, Antibiotics, Drug-resistant, Resistance, *E. coli*, *K. pneumoniae*, Sudan.

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INTRODUCTION

Antibiotics have saved millions of lives from deadly bacterial infections and relieved human pain and suffering for decades since their discovery in the 1950s. On the other hand, scientists neglected the fact that bacteria have great metabolic ability and have lasted millions of years in our changing world.[1] Self-medication, genetic variations, or bacterial mutations often cause resistance in bacterial pathogens.[2] Additionally, modifications to bacteria's antibiotic targets, changes in membrane permeability, and the passing of resistant genes to succeeding generations all contribute to antibiotic resistance.[3] A recent worldwide issue for health systems was the emergence of the multidrug-resistant (MDR) phenotype as a result of the overuse of antibiotics in the treatment of human and animal illnesses. The medication of choice for treating these infections is carbapenems. However, due to the rising prevalence of carbapenem resistance worldwide, colistin or other similar drugs are one of the few treatments available for life-threatening infections brought on by MDR bacteria (polymyxin-E).[2,3]

Most studies reported that resistance to carbapenems is rising, and because tigecycline has not been approved in many nations, colistin is often the sole antibiotic that is effective against multidrug-resistant organisms.[4] Increasing usage of colistin to control MDR-gram-negative pathogens has resulted in the establishment of resistance of colistin in *Klebsiella pneumoniae* in a number of countries throughout the globe, including Europe, and colistin resistance rates continue to rise.[5] Recent strategies for combining colistin with other antibiotics offer promise for enhancing its antibacterial activity. Colistin is probably going to be the "last-resort" treatment for Gram-negative infections that are resistant to multiple drugs in the 21st century.[6]

Patients who have been exposed to highly resistant bacteria such as *Klebsiella pneumoniae* carbapenemase (KPC-2) and New Delhi Metallo beta-lactamase are treated with colistin (NDM-1).[7] The Enterobacteriaceae's colistin resistance may result through the mechanisms of bacterial chromosome and plasmid-borne mobile colistin-resistant genes, appreciated as *mcr*.[8] A few more *mcr* genes, including *mcr-9, mcr-8, mcr-5, mcr-3,* and *mcr-2*, have been reported since the discovery of the first *mcr* gene (*mcr*-1), in 2016.[9-15]

This is a worrying situation that requires an urgent solution to the global problem of antibiotic vulnerability to bacteria, particularly Colistin. *Acinetobacter* spp., along with other pathogens like *Streptococcus pneumoniae*, *Salmonella* spp., *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*, have remarkable levels of multi-drug resistance (MDR) worldwide, according to a recent study by the WHO. World Health Organization's Global antimicrobial resistance surveillance programme.[16] Regretfully, dose-increasing is not recommended as antibiotics having a risk for toxicity should only be provided and examined by clinical pharmacists if the infectious condition is life-threatening and there is no alternative therapeutic option.[17] The current study aimed to evaluate the presence of *mcr-9, mcr-8, mcr-5, mcr-3, mcr-2* and *mcr-1* genes in colistin-resistant and some susceptible *E. coli,* and *K. pneumoniae* from clinical isolates in some Hospitals of Khartoum State.

MATERIALS AND METHODS

Collection and Identification of bacterial isolates

One hundred eighty-five aerobic gram-negative rods were isolated from different clinical specimens, including pus swab, urine, blood, and tissue, at Soba University Hospital, Omdurman Military Hospital, Al-Aml National Hospital, and the microbiology laboratory of the University of Khartoum-Faculty Sudan's of Medical Laboratory Science. Bacterial strains were identified, sub-cultured on MacConkey agar for purity and then standard biochemical assays such as the oxidase test, indole, citrate, urease, Kligler's iron agar, and motility were used to determine the purity of the isolates.[18,19]

Antimicrobial susceptibility test for colistin

Using the Kirby-Bauer disc diffusion method as suggested by CLSI.[20] A pure saline solution of isolated colonies with a turbidity of 0.5 McFarland standard was used to inoculate Muller-Hinton agar medium. After 18 hr of incubation at 37°C, the antibiotic disc colistin 10.0 mcg was placed on the agar surface. Results were explained by measuring the diameter of the zone of inhibition around each of the loaded discs. The *E. coli* (ATCC@ 25922) strain served as the control strain.

DNA extraction and PCR amplification

PCR was conducted utilizing spastic primers for *mcr*-9, *mcr*-8, *mcr*-5, *mcr*-3, *mcr*-2 and *mcr*-1 in a PCR equipment (Prime Technee, England), 0.5 μL of each of the twelve primers solutions (10 μM), DNA lysate (5 μL), and nuclease-free water (5.5 μL) were added. One cycle of denaturation at 94°C for 15 min was followed by twenty-five cycles of denaturation of DNA (at 94°C for 30 sec), annealing for 90 sec at 58°C, and elongation at 72°C for 60 sec, with a final elongation cycle at 72°C for 10 min. The amplification was seen by gel-electrophoresis on 1.5% agarose gel (at 130 Volts), followed by ethidium-bromide staining.[21,22]

Ethical consideration

The Ethical Review Committee (ERC) of the National University biomedical research institution, Khartoum State, Sudan, gave its approval to the project.

Statistical analysis

Statistical package was used for data monitoring and control so that the analysis and interpretation of descriptive data could be done with enough accuracy. The data for this study were analyzed with "IBM SPSS Statistics" (version number: 22; Chicago, Illinois, USA).

RESULTS

As shown from Table 1, the investigation revealed that, Among the 185 isolates, 61 (33%) of them were *K. pneumoniae* and 124 (67%) of them were *E. coli*. A urine sample yielded 102 pathogens, 73 pathogens from swabs, 9 pathogens from blood, and 1 pathogen from tissue. Only 35 (or 18%) of the 185 isolates were resistant to colistin. Of the 35 isolates that were resistant to colistin, 14 (40%) were *K. pneumoniae* and 21 (60%) were *E. coli*. Colistin-resistance genes were examined in all 35 colistin-resistant isolates as well as 13 colistin-susceptible isolates. Genes for colistin resistance were found in 21 of the 48 examined isolates.

Table 2 represented the results of biochemical tests carried out on order to identify *E. coli* and *K. pneumoniae* form the collected samples. Table 3 shows the Sequences of primers used for detection of *mcr* genes. The results of the multiplex PCR assay revealed that out of 21, only 14 (66%) were resistant to disc diffusion and PCR. We discovered the mcr-1, *mcr*-2, and *mcr*-3 genes mcr-1 represented 18 (85%), *mcr*-1 and *mcr*-2 combined (5%), *mcr*-2 alone (5%), and mcr-2 alone and mcr-3 mixed (5%).

Table 1: Frequency of isolated *E. coli* and *K. pneumoniae* among gram-negative rods, distribution of clinical specimen and percentage of isolates resistant to colistin.

Category	Result	Frequency (n)	Percentage (%)
Bacteria	*K. p.*	61.0	33.0
	E. c.	124.0	67.0
Specimen	Urine	102.0	55.1
	Swab	73.0	39.5
	Blood	9.0	4.9
	Tissues	1.0	0.5
Colistin-resistant	*K. p.*	14.0	40.0
	E. c.	21.0	60.0

%: Percentage, n: number, *K.p.* : *Klebsiella pneumoniae* , *E.c.*: *Escherichia coli*

DISCUSSION

Colistin is employed as a "last-line" medication to treat bacterial pathogens brought on by MDR gram-negative germs. The expansion of the colistin resistance genes *mcr-9, mcr-8, mcr-5, mcr-3, mcr-2* and *mcr-1* genes throughout the *Enterobacteriaceae* presents a serious danger to world health. Worldwide reports on the frequency of hospital acquired illnesses caused by *K. pneumoniae* and *E. coli* were made. In total, 124 *E. coli* and 61 *K. pneumoniae* were isolated from Sudanese hospitals in the present investigation. The development of antibiotic-resistant germs and the spread of drug-resistant strains among these organisms have become critical global public health concerns. The detection and treatment of such infections has substantial consequences for patient health. A previous study showed that the gene in charge of particular colistin resistance has been identified as the mobilized colistin resistance (*mcr*) gene. In fact, the issue is becoming worse and worse every day despite the fact that several studies have shown there is a wide variety in the occurrence of that gene, not only for the *mcr*-genes major group but also related to its numerous subgroups.[23]

From several clinical specimens isolated by multiplex PCR, the study explored the frequency of *mcr-9, mcr-8, mcr-5, mcr-3, mcr-2* and *mcr-1* genes that are responsible for colistin resistance. Compared to previously published data,[24] which used multiplex PCR to determine the presence of the *mcr*-genes (mcr-1 to mcr-5), which responsible for colistin resistance. Compared to our investigation, there were 185 isolates found in the present investigation. *K. pnemoniae* came in second at 33% (61%), with *E. coli* coming in first at 67% (124). Of them, 35 were discovered to be colistin resistant. Multiplex PCR revealed that some *mcr*-genes (*mcr*-1, *mcr*-2, and *mcr*-3 genes) were

Table 2: Result of biochemical Reactions of the isolates *E. coli* and *K. pneumoniae*.

Identified organisms	Lactose fermentation	Oxidase test	Motility test	Citrate test	Indole test	Urease test	[1]KIA				Appendix
							[2]G	[3]L	[4]H_2S	[5]Gas	
E. coli	LF	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	1
K. pneumoniae	LF	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	2

LF: Lactose ferment; G: Glucose; L: Lactose; H_2S: Hydrogen Sulfide.

Table 3: Sequences of primers used for detection of mcr genes multiplex PCR assay.

Target gene	Primers sequence (5→3)	Size (bp)
mcr-1 fw	AGTCCGTTTGTTCTTGTGGC	320
mcr-1 rev	AGATCCTTGGTCTCGGCTTG	
mcr-2 fw	CAAGTGTGTTGGTCGCAGTT	700
mcr-2 rev	TCTAGCCCGACAAGCATACC	
mcr-3 fw	AAATAAAAATTGTTCCGCTTATG	900
mcr-3 rev	AATGGAGATCCCCGTTTTT	
mcr-5 fw	ATGCGGTTGTCTGCATTTATC	1,644
mcr-5 rev	TCATTGTGGTTGTCCTTTTCTG	
mcr-8 fw	CCCAAGCTTTTGATTGTCCCTGTCGCCAT	2631
mcr-8 rev	CACCGATAAGAGGAACCAGTGAATTCCGG	
mcr-9 fw	TTCCCTTTGTTCTGGTTG	1011
mcr-9 rev	GCAGGTAATAAGTCGGTC	

found in 44% (21) isolates, 95% (20) *E. coli*, and 5% (1) *K. pneumoniae*. mcr-1 (85%), *mcr*-1 and *mcr*-2 combined (5%), *mcr*-2 alone (5%), and *mcr*-2 alone and *mcr*-3 combined (5%). This outcome is higher than that of previous articles, which found the *mcr*-1 gene in some clinical isolates of bacteria belongs to *Enterobacteriaceae*.[25,26]

Additionally, our outputs showed that the *mcr*-1 gene is more common (85%) than the mcr-3 and mcr-2 genes. Moreover, *mcr*-1 was shown to be most prevalent in *E. coli*. This result is higher than a previously reported finding.[27] Only one isolate, *K. pneumoniae*, displayed mcr-3, and it combined with *mcr*-2. Similar to a study conducted by another researcher, mcr-5, *mcr*-8, and *mcr*-9 were not discovered using multiplex PCR in this investigation.[28] Seven of the 21 isolates in this investigation with positive *mcr*-3, *mcr*-2, and *mcr*-1 gene results were colistin sensitive (33%), which was consistent with a previous study that reported that the percentage was 25%.[29]

Our study's finding of a colistin-susceptible/mcr-1-positive isolate suggests that this gene may widely spread. The high frequency of *mcr*-genes seen in this study necessitated relation to hospital policies on the choice of antibiotic treatment and empirical use of antibiotics. For the purpose of choosing an antibiotic medication for an illness caused by a multidrug-resistant strain, routine molecular method identification of resistance genes has become very crucial.

Finally, the current study supports repeated calls from previous researchers that urgent international development of fast and inexpensive tests to detect colistin-resistant genes is badly needed. In order to further prevent the spread of colistin-resistant and mcr-producing bacteria, regular monitoring of hospitals, farms, foods, and the environment should be carried out.[30-32]

CONCLUSION

The emergence and horizontal spread of colistin resistance spotlights the need for increased stewardship actions for this final-resort antibiotic as well as for all antibiotics being used in humans to prevent serious outbreaks in developing communities that are already struggling with poverty and shaky healthcare systems. The current study is a clear example of this tragedy. We suggest using a combination of different antibiotic medications with other agents to produce synergistic interactions until new antibiotics against *mcr*-positive bacteria are developed or recommended.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ATCC: American Type Culture Collection; ***mcr*:** Mobilized Colistin Resistance; ***E. coli*:** *Escherechia coli;* ***K. pneumoniae*:** *Klebsiella pneumoniae;* **PCR:** Polymerase Chain Reaction; **CLSI:** Clinical Laboratory Standard Institute; **PEA:** Phospho Ethanol Amine; **MDR:** Multi Drug Resistant.

SUMMARY

Antibiotic resistance rates are rising as a result of the extensive use of antibiotics in people. Bacterial infections that could formerly be treated now need to be treated with medicines that are the last resort. Colistin is often used as a last-resort antibiotic for gram-negative bacterial infections that are resistant to carbapenem. This paper discusses evidence indicating a changing pattern of colistin resistance in several hospitals in Khartoum, the capital of Sudan and the targeted bacteria were *Klebsiella pneumoniae* and *Escherichia coli*. As a result, 18% of 185 isolates were resistant to colistin. 60% of the 35 colistin-resistant isolates were *E. coli*, and 40% were *K. pneumoniae*, and carrying multiple *mcr* genes. The national and international community should take immediate action to address the increasing incidence of colistin-resistant germs.

REFERENCES

1. Abdallah EM. Medicinal plants as an alternative drug against methicillin-resistant *Staphylococcus aureus*. Int J Microbiol. 2016;3(1):35-42.
2. Elamin AEH, Saleh HAM, Saadeldin W, Abualgasim E, Ali L, Abdallah EM. Irrational use of three antibiotics in Khartoum, Sudan: A cross-sectional study. Nov appro drug. Des Dev. 2022;6(2):555685.

3. Elamin AEH, Saleh HAM, Mohammed AAH, Khair RMM, Alawad S, Alhag ZMA, *et al*. A cross-sectional study of cephalosporin prescriptions for the treatment of respiratory and urinary tract infections in two Sudanese hospitals. Nov appro drug. Des Dev. 2022;6(3):555687.
4. Bialvaei AZ, Samadi Kafil H. Colistin, mechanisms and prevalence of resistance. Curr Med Res Opin. 2015;31(4):707-21. doi: 10.1185/03007995.2015.1018989.
5. Ah YM, Kim AJ, Lee JY. Colistin resistance in *Klebsiella pneumoniae*. Int J Antimicrob Agents. 2014;44(1):8-15. doi: 10.1016/j.ijantimicag.2014.02.016, PMID 24794735.
6. Biswas S, Brunel JM, Dubus JC, Reynaud-Gaubert M, Rolain JM. Colistin: An update on the antibiotic of the 21st century. Expert Rev Anti Infect Ther. 2012;10(8):917-34. doi: 10.1586/eri.12.78, PMID 23030331.
7. Yao X, Doi Y, Zeng L, Lv L, Liu JH. Carbapenem-resistant and colistin-resistant *Escherichia coli* co-producing NDM-9 and MCR-1. Lancet Infect Dis. 2016;16(3):288-9. doi: 10.1016/S1473-3099(16)00057-8, PMID 26842777.
8. Al Agha AGM. Extraction and purification of lipopolysaccharide of *Klebsiella pneumoniae* isolates. Int J Curr Microbiol Appl Sci. 2017;6(8):90-100. doi: 10.20546/ijcmas.2017.608.012.
9. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, *et al*. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. Lancet Infect Dis. 2016;16(2):161-8. doi: 10.1016/S1473-3099(15)00424-7, PMID 26603172.
10. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr*-2, in Escherichia coli, Belgium, June 2016. Eurosurveillance. 2016;21(27):30280.
11. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, *et al*. Novel plasmid-mediated colistin resistance gene mcr-3 in *Escherichia coli*. mBio. 2017;8(3):e00543-17. doi: 10.1128/mBio.00543-17, PMID 28655818.
12. Carattoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppi A, *et al*. Novel plasmidmediated colistin resistance mcr-4 gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Euro Surveill. 2017;22(31):30589. doi: 10.2807/1560-7917.ES.2017.22.31.30589, PMID 28797329.
13. Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. Identification of a novel transposon-associated phosphoethanolamine transferase gene, mcr-5, conferring colistin resistance in D-tartrate fermenting *Salmonella enterica* subsp. enterica serovar Paratyphi B. J Antimicrob Chemother. 2017;72(12):3317-24. doi: 10.1093/jac/dkx327, PMID 28962028.
14. AbuOun M, Stubberfield EJ, Duggett NA, Kirchner M, Dormer L, Nunez-Garcia J, *et al*. mcr-1 and mcr-2 variant genes identified in Moraxella species isolated from pigs in Great Britain from 2014 to 2015. J Antimicrob Chemother. 2017;72(10):2745-9. doi: 10.1093/jac/dkx286, PMID 29091227.
15. Yang YQ, Li YX, Lei CW, Zhang AY, Wang HN. Novel plasmidmediated colistin resistance gene mcr-7.1 in *Klebsiella pneumoniae*. J Antimicrob Chemother. 2018;73(7):1791-5. doi: 10.1093/jac/dky111, PMID 29912417.
16. Abdallah EM, Abdalla RM. *Acinetobacter baumannii*, a global health-threatening bacterium: A short review. J Microbiol Experiment;2021(6):181-4.
17. HajElamin AE, Mohammed RR, Iman AM, HajElamin OE, Abdallah EM. Assessment of Antibiotic Administration and its Interaction with other Biological Factors during Pregnancy among Pregnant Women in Sudan. Open acc J Gynec. 22;00023:7(1).
18. Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, *et al*. Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing *Klebsiella pneumoniae*. Emerg Microbes Infect. 2018;7(1):122.
19. Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO, Wiedmann M. Identification of novel mobilized colistin resistance gene mcr-9 in a multidrug-resistant, colistin-susceptible *Salmonella enterica* serotype Typhimurium isolate. mBio. 2019;10(3):00853-19. doi: 10.1128/mBio.00853-19.
20. Cheesbrough M. District Laboratory Practice in Tropical Countries, preparation of reagents and culturing media. 2nd ed. part 2; 2006. p. 383-407.
21. Khattab S, Sweify A, Ali M, Metwally L, Elazab S, Hashem A. Detection of plasmid-mediated colistin resistance in carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates in Suez Canal University Hospitals. Micro Infect Dise. 2021;2(3):497-507.
22. Rabie RA, Abdallah AL. Plasmid mediated colistin resistant genes mcr-1 and mcr-2 among *Escherichia coli* and *Klebsiella pneumoniae* isolates at Zagazig University hospitals, Egypt. Egypt J Microgr. 2020;29(1):61-6. doi: 10.21608/ejmm.2020.249858.
23. Gharaibeh MH, Shatnawi SQ. An overview of colistin resistance, mobilized colistin resistance genes dissemination, global responses, and the alternatives to colistin: A review. Vet World. 2019;12(11):1735-46. doi: 10.14202/vetworld.2019.1735-1746, PMID 32009752.
24. Lescat M, Poirel L, Nordmann P. Rapid multiplex polymerase chain reaction for detection of mcr-1 to mcr-5 genes. Diagn Microbiol Infect Dis. 2018;92(4):267-9. doi: 10.1016/j.diagmicrobio.2018.04.010, PMID 30220493.
25. Saavedra SY, Diaz L, Wiesner M, Correa A, Arévalo SA, Reyes J, *et al*. Genomic and molecular characterization of clinical isolates of Enterobacteriaceae harboring mcr-1. In: Colombia, 2002 to 2016. Antimicro Agents chemo. Vol. 61(12); 2017. p. e00841-17.
26. Mohammed HE. Molecular detection of plasmids mediated colistin resistance (MCR-1) gene in Enterobacteriaceae from clinical specimens in Khartoum State ([doctoral dissertation]. Sudan University of Science and Technology).
27. Dalmolin TV, De Lima-Morales D, Barth AL. Plasmid-mediated colistin resistance: What do we know? J Infectiology. 2018;1(2):16-22. doi: 10.29245/2689-9981/2018/2.1109.
28. Borowiak M, Baumann B, Fischer J, Thomas K, Deneke C, Hammerl JA, *et al*. Development of a novel mcr-6 to mcr-9 multiplex PCR and assessment of mcr-1 to mcr-9 occurrence in colistin-resistant *Salmonella enterica* isolates from environment, feed, animals and food (2011-2018) in Germany. Front Microbiol. 2020;11:80. doi: 10.3389/fmicb.2020.00080, PMID 32117115.
29. Moosavian M, Emam N. The first report of emerging mobilized colistin-resistance (mcr) genes and ERIC-PCR typing in *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates in southwest Iran Infec Drug Resis. 2019;12:1001-10.
30. Osei Sekyere, J. Mcr colistin resistance gene: A systematic review of current diagnostics and detection methods. Microbiologyopen. 2019;8(4):e00682. doi: 10.1002/mbo3.682
31. Falagas ME, Kasiakou SK, Saravolatz LD. Colistin: The revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infec Dis. 2005;40(9):1333-41. doi: 10.1086/429323
32. Bardet L, Rolain JM. Development of new tools to detect colistin-resistance among Enterobacteriaceae strains. Canadian J Infec Dis Med Micro. 2018;3095249. doi: 10.1155/2018/3095249