Research Article

Prevalence of Mobile Colistin Resistance (*mcr*) Genes in *Acinetobacter baumannii*: Isolates of Clinical Infection from an Iranian University Hospital

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Abstract

Background: The emergence of colistin resistance is a global issue that threatens the effectiveness of colistin treatment against multidrug-resistant Gram-negative bacteria.

Objectives: The present study reports on the prevalence of colistin resistance and *mcr*-encoding genes in *Acinetobacter baumannii* strains isolated from patients referred to Shahid Rahimi Hospital, Khorramabad, Iran, from January to August 2019.

Methods: A total of 47 non-duplicate *A. baumannii* isolates were obtained from clinical specimens of patients. Colistin susceptibility of the isolates was assessed using the broth microdilution method, and the presence of *mcr*-encoding genes was determined through the use of PCR.

Results: Out of the total 47 isolates belonging to *A. baumannii*, 14 isolates (29.8%) were resistant to colistin. The minimum inhibitory concentration (MIC) of colistin ranged from 2 to 256 µg/mL, and the MIC50 and MIC90 values for colistin were 0.25 µg/mL and 16 µg/mL, respectively. The presence of *mcr-1* and *mcr-2* was detected in 6 (12.7%) and 2 (4%) isolates, respectively. However, no isolates harbored the *mcr-3* gene. Except for one isolate, none of the *mcr*-positive isolates exhibited phenotypic resistance to colistin.

Conclusions: The higher phenotypic prevalence of colistin resistance compared to genotypic characterization, along with the discrepancy with the presence of *mcr* genes, suggests the need to monitor and consider all molecular mechanisms of colistin resistance as a last-resort treatment option.

Keywords: Acinetobacter baumannii, *mcr* Gene, Nosocomial Infection, Colistin

1. Background

Acinetobacter baumannii is an opportunistic, Gram-negative pathogen known to cause a variety of healthcare-associated infections in immunocompromised patients, including bacteremia, meningitis, pneumonia, urinary tract infections, and wound infections (1). Patients who contract these infections, particularly ventilator-associated pneumonia and bloodstream infections, face elevated risks of significant mortality rates, ranging from 5% in general hospital wards to 54% in the intensive care units (ICU). Additionally, there is an increasing incidence of community-acquired *A. baumannii* infections being reported (2).

The rapid emergence of *A. baumannii* strains resistant to nearly all available antibiotics, including β-lactams, carbapenems, fluoroquinolones, macrolides, and aminoglycosides, has further necessitated the use of colistin (also known as polymyxin E) as the only remaining treatment option (3-5). Although colistin is considered the last resort for treating drug-resistant *A. baumannii*, reports of clinical isolates resistant to this antibiotic have risen (6, 7). Resistance to colistin is attributed to both chromosomally-encoded resistance mechanisms and plasmid-borne *mcr* genes (*mcr-1* to *mcr-*

10) in various bacterial species. However, only four mobile colistin resistance genes (*mcr-1* to *mcr-4*) have been identified in *A. baumannii* isolates (2, 8, 9).

Considering that the dissemination of plasmid-borne colistin resistance jeopardizes this treatment option, epidemiological surveillance assumes a crucial role in controlling and preventing the spread of the resistance mechanism (10). Up to this point, most studies on *A. baumannii* clinical isolates have primarily focused on the prevalence of the *mcr-1* gene, with limited attention given to other *mcr* genes (9, 11-14).

2. Objectives

This knowledge gap could impact the development of effective treatments for *A. baumannii* infections. Therefore, this study aims to evaluate the prevalence of *mcr* genes (*mcr-1* to *mcr-3*) in *A. baumannii* strains recovered from Shahid Rahimi Hospital.

3. Methods

3.1. Clinical Specimens, Bacterial Isolates, and Identification

The present cross-sectional study examined *A. baumannii* isolates obtained from various specimens, including blood, wound, sputum, urine, and cerebrospinal fluid (CSF) samples. These samples were collected from patients referred to Shahid Rahimi Hospital in Khorramabad, Iran, during a seven-month period, from January to August 2019. In this research, hospitalized patients with laboratory-confirmed *A. baumannii* infections during the specified study period were included, while those without a confirmed *A. baumannii* infection or whose infections were attributed to microorganisms other than *A. baumannii* species were excluded.

The specimens were inoculated onto blood agar and MacConkey agar plates and then incubated overnight at 37°C. The primary identification of the *A. baumannii* isolates was performed using conventional microbiological and biochemical methods, as previously described (15). Subsequent confirmation of *A. baumannii* was carried out through PCR for the *bla* $_{oxa-51-like}$ gene (15).

This study was approved by the local ethics committee of Lorestan University of Medical Sciences (IR.LUMS.REC.1399.340).

3.2. Antimicrobial Susceptibility Testing

The minimum inhibitory concentrations (MICs) of colistin (Colistin Sulfate salt powder, Sigma-Aldrich, St. Louis, MO, USA) were determined using the broth microdilution method, following CLSI guidelines (CLSI, 2023). Stock solutions were prepared from colistin in distilled water to provide concentrations ranging from 0.25 µg/mL to 256 µg/mL. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were used as quality control strains.

3.3. DNA Extraction

DNA extraction was performed using the boiling method, and the DNA samples were subsequently stored at -70°C until further use (16).

3.4. Detection of Plasmid-Mediated Colistin-Resistant Genes

Multiplex PCR with specific primers, as described in Table 1, was used to screen for the presence of *mcr-1* to *mcr-3* genes. The Multiplex PCR reaction was performed in a total volume of 25 µL containing 2X Taq PCR Master Mix, 10 µM of each primer, and 5 µL of template DNA. Following amplification, the resulting product was analyzed by electrophoresis on a 1.5% agarose gel. The strain provided by Prof. C. Giske from Karolinska Institute, Sweden, was used as a positive control, and sterile water was used as a negative control.

3.5. Statistical Analysis

Data analysis was conducted using SPSS version 26 (SPSS, Inc., Chicago, IL, USA) with Fisher's exact tests employed, and P-values less than 0.05 were considered statistically significant.

4. Results

4.1. Characteristics of Patient Demographics and Clinical Isolates

A total of 47 *A. baumannii* clinical isolates were collected and studied during the aforementioned period to identify underlying resistance mechanisms. Of these, 32 *A. baumannii* isolates (68.1%) were obtained from male patients, while 15 (31.9%) were from female patients. The age range of the patients varied from 20 to 90 years. The isolates primarily originated from patients admitted to the ICU, accounting for 34 (72.4%), followed by other hospital wards with 13 (27.6%).

In total, 14 (29.8%) strains exhibited resistance to colistin, with MICs ranging from 2 to 256 µg/mL (Table 2). Notably, within this group, 3 isolates demonstrated intermediate resistance, while 11 isolates exhibited full resistance to colistin. The MIC50 and MIC90 values for colistin were calculated as 0.25 µg/mL and 16 µg/mL,

Table 1. Primer Sequences Used for Multiplex PCR for Detection of *mcr-1* to *mcr-3* in Clinical Isolates of *Acinetobacter baumannii*

Target Gene and Primer Name	Sequence (5'-3')	Size (bp)	Reference
mcr-1		320	(6)
F	AGTCCGTTTGTTCTTGTGGC		
R	AGATCCTTGGTCTCGGCTTG		
mcr-2		715	(6)
F	CAAGTGTGTTGGTCGCAGTT		
R	TCTAGCCCGACAAGCATACC		
mcr-3		929	(6)
F	AAATAAAAATTGTTCCGCTTATG		
R	AATGGAGATCCCCGTTTTT		

Table 2. The Minimum Inhibitory Concentration Results of *Acinetobacter baumannii* Isolates Non-susceptible to Colistin

MIC Range (µg/mL)	2	4	8	16	32	256
The number of isolates	3	2	3	1	3	2

Abbreviation: MIC, minimum inhibitory concentration.

respectively. Among the *A. baumannii* isolates, 20 out of 47 (42.6%) were classified as multidrug-resistant (MDR), while 23 out of 47 (48.9%) demonstrated extensive drug resistance (XDR). Multidrug-resistant and XDR phenotypes were determined using the standardized definitions outlined in a previously published article, based on the disk diffusion results (17).

4.2. Molecular Characterization of the mcr Genes

A total of 47 *A. baumannii* isolates underwent multiplex PCR screening for the presence of *mcr-1* to *mcr-3* genes.

To assess the role of plasmid-encoded resistance genes (*mcr-1*, *mcr-2*, and *mcr-3*), PCR was performed using bacterial genomic DNA extracted from the patients.

Molecular analysis of mobile colistin resistance (*mcr*) genes associated with *A. baumannii* isolates revealed that 6 (12.7%) and 2 (4.2%) of the clinical isolates were positive for *mcr-1* and *mcr-2*, respectively. However, no isolate was positive for the *mcr-3* resistance gene. Figure 1 displays the results of the PCR analysis for the detection of *mcr* genes in *A. baumannii* isolates.

It should be noted that, except for one isolate, the genes responsible for colistin resistance, specifically *mcr-1* and *mcr-2*, were detected in susceptible *A. baumannii* isolates with a MIC value < 2 µg/mL.

5. Discussion

Patients in ICUs face a higher risk of contracting hospital-acquired infections, including *A. baumannii*, compared to those in general wards. This heightened risk is primarily attributed to the frequent use of invasive devices in ICUs and increased exposure to antibiotic-resistant pathogens (18). Consequently, this study demonstrates that *A. baumannii* is predominantly isolated from ICU settings. Given the significant prevalence of resistance to current treatments, colistin has regained attention as a potential solution for combating severe infections caused by carbapenem-resistant *A. baumannii* (19, 20). However, the increased use of colistin as a treatment option has led to the emergence of colistin-resistant strains on a global scale (7).

In this study, 14 isolates (29.7%) displayed intermediate resistance or full resistance to colistin. Moreover, the emergence of colistin resistance has been reported not only in Iran but also in various parts of the world (7, 9, 21-23). Consistent with findings from recent research and similar studies conducted globally, a failure to address this issue promptly may result in frequent outbreaks of pan-drug-resistant *A. baumannii* strains that also exhibit resistance to colistin in the near future.

Colistin susceptibility testing is performed using broth microdilution, which is the gold standard phenotypic method, but it requires an overnight incubation to obtain results. Accordingly, the adoption of faster and more accurate techniques, such as molecular detection, is anticipated for identifying the
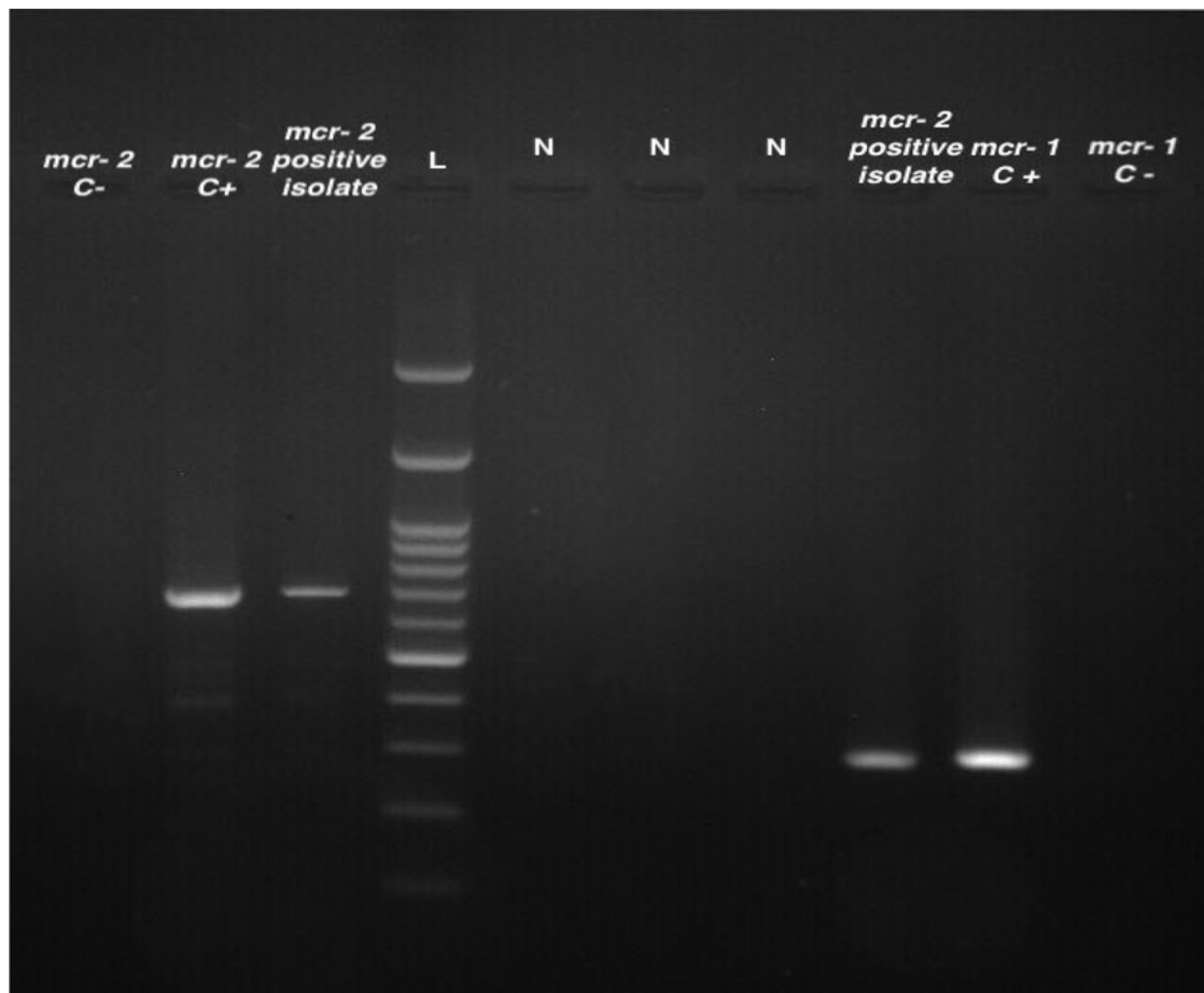
Figure 1. Multiplex PCR products of *mcr* genes in *A. baumannii* isolates. Lane 1, *mcr-2* negative control; lane 2, *mcr-2* positive control; lane 3; *mcr-2* positive isolate; lane 4, molecular size markers (100-bp DNA ladder; SinaClon); lane 5 to 7, negative isolates; lane 8, *mcr-2* positve isolate; lane 9, *mcr-1* positive control; lane 10, *mcr-1* negative control.

genetic mechanisms responsible for antimicrobial resistance (24, 25). Resistance transmitted by plasmids can confer resistance to multiple antibiotics. Furthermore, plasmids can disseminate resistance among bacteria at a higher rate, potentially leading to rapid and widespread distribution in clinical settings (26, 27). As with other plasmids, plasmid-mediated *mcr* genes can spread horizontally among humans, animals, and the environment, posing a significant risk to human health (28).

In the current study, out of the 47 *A. baumannii* isolates, 6 (12.7%) and 2 (4.2%) isolates harbored *mcr-1* and *mcr-2* genes, respectively. However, no isolate carried the

mcr-3 gene. Higher incidence rates were reported in Iraq. Al-Kadmy et al. found that out of 121 clinical isolates, 89 (73.5%), 78 (64.5%), and 82 (67.8%) of isolates were positive for the *mcr-1*, *mcr-2*, and *mcr-3* genes, respectively (9). Moreover, a study by Hafudh et al. revealed that among 13 isolates with reduced sensitivity to colistin, 5 isolates (38.5%) were positive for the *mcr-1* gene, and 4 isolates were positive for both *mcr-2* and *mcr-3* genes, respectively (29). In a study conducted by Kareem, the *mcr-1* gene was detected in 22 isolates (11%), while no isolates carried the *mcr-2* or *mcr-3* genes (30). However, other studies in Iran and South Africa reported that none of the identified *A. baumannii* isolates carried the

mcr resistance genes (14). For instance, studies conducted by Babaei et al. and Tehrani et al. in Tehran and Ghazvin cities revealed no detection of the *mcr-1* gene among the examined *A. baumannii* isolates, indicating a potential regional absence of this specific colistin resistance mechanism (31, 32).

It is important to note that, with the exception of one isolate, none of the *mcr*-positive isolates were phenotypically resistant to colistin. This lack of resistance may be associated with the non-expression of resistance genes due to various factors (33, 34). Moreover, the prevalence of the resistance genes did not align with the number of resistant isolates detected by the phenotypic test. The lower detection rate of *mcr* genes compared to the phenotypically observed resistance suggests that phenotypically resistant isolates may be associated with other resistance mechanisms (7).

5.1. Conclusions

In conclusion, this study provides valuable insights into the prevalence of colistin resistance and the presence of *mcr-1* and *mcr-2* genes in *A. baumannii* clinical isolates. Given that phenotypic resistance was higher than the number of genotypically positive isolates and was largely unrelated to the presence of *mcr* genes, it is essential to investigate other colistin resistance mechanisms, considering its critical role as a last-resort treatment for drug-resistant *A. baumannii*.

Footnotes

Authors' Contribution: M. S. and N. Y. N. conducted the tests. M. S. also wrote the manuscript. S. D. and F. R. supervised the project and worked on concept and design of the study and critically revised the paper. N. Y. N. and I. P. analyzed and interpreted data. N. Y. N., I. P., and F. R. critically revised the paper. All authors read and approved the final version of manuscript and authorship list.

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Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: This study was approved by the local Ethics Committee of Lorestan University of Medical Sciences (IR.LUMS.REC.1399.340).

References

1. Morris FC, Dexter C, Kostoulias X, Uddin MI, Peleg AY. The Mechanisms of Disease Caused by Acinetobacter baumannii. *Front Microbiol.* 2019;**10**:1601. [PubMed ID: 31379771]. [PubMed Central ID: PMC6650576]. https://doi.org/10.3389/fmicb.2019.01601.

2. Ayoub Moubareck C, Hammoudi Halat D. Insights into Acinetobacter baumannii: A Review of Microbiological, Virulence, and Resistance Traits in a Threatening Nosocomial Pathogen. *Antibiotics (Basel).* 2020;**9**(3). [PubMed ID: 32178356]. [PubMed Central ID: PMC7148516]. https://doi.org/10.3390/antibiotics9030119.

3. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. *Clin Microbiol Rev.* 2008;**21**(3):538-82. [PubMed ID: 18625687]. [PubMed Central ID: PMC2493088]. https://doi.org/10.1128/CMR.00058-07.

4. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. *Nat Rev Microbiol.* 2007;**5**(12):939-51. [PubMed ID: 18007677]. https://doi.org/10.1038/nrmicro1789.

5. Snyman Y, Whitelaw AC, Reuter S, Dramowski A, Maloba MRB, Newton-Foot M. Clonal expansion of colistin-resistant Acinetobacter baumannii isolates in Cape Town, South Africa. *Int J Infect Dis.* 2020;**91**:94-100. [PubMed ID: 31765820]. https://doi.org/10.1016/j.ijid.2019.11.021.

6. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. *Euro Surveill.* 2018;**23**(6). [PubMed ID: 29439754]. [PubMed Central ID: PMC5824125]. https://doi.org/10.2807/1560-7917.ES.2018.23.6.17-00672.

7. Novovic K, Jovcic B. Colistin Resistance in Acinetobacter baumannii: Molecular Mechanisms and Epidemiology. *Antibiotics (Basel).* 2023;**12**(3). [PubMed ID: 36978383]. [PubMed Central ID: PMC10044110]. https://doi.org/10.3390/antibiotics12030516.

8. Snyman Y, Reuter S, Whitelaw AC, Stein L, Maloba MRB, Newton-Foot M. Characterisation of mcr-4.3 in a colistin-resistant Acinetobacter nosocomialis clinical isolate from Cape Town, South Africa. *J Glob Antimicrob Resist.* 2021;**25**:102-6. [PubMed ID: 33757821]. https://doi.org/10.1016/j.jgar.2021.03.002.

9. Al-Kadmy IMS, Ibrahim SA, Al-Saryi N, Aziz SN, Besinis A, Hetta HF. Prevalence of Genes Involved in Colistin Resistance in Acinetobacter baumannii: First Report from Iraq. *Microb Drug Resist.* 2020;**26**(6):616-22. [PubMed ID: 31816255]. https://doi.org/10.1089/mdr.2019.0243.

10. Center for Preparedness and Response. *Alert to U.S. Healthcare Facilities: First mcr-1 Gene in E. coli Bacteria found in a Human in the United States.* 2016, [cited 27 October 2022]. Available from: https://emergency.cdc.gov/han/han00390.asp.

11. Hameed F, Khan MA, Muhammad H, Sarwar T, Bilal H, Rehman TU. Plasmid-mediated mcr-1 gene in Acinetobacter baumannii and Pseudomonas aeruginosa: first report from Pakistan. *Rev Soc Bras Med Trop.* 2019;**52**. e20190237. [PubMed ID: 31508785]. https://doi.org/10.1590/0037-8682-0237-2019.

12. Khoshnood S, Savari M, Abbasi Montazeri E, Farajzadeh Sheikh A. Survey on Genetic Diversity, Biofilm Formation, and Detection of Colistin Resistance Genes in Clinical Isolates of Acinetobacter baumannii. *Infect Drug Resist.* 2020;**13**:1547-58. [PubMed ID: 32547124]. [PubMed Central ID: PMC7266307]. https://doi.org/10.2147/IDR.S253440.

13. Rahman M, Ahmed S. Prevalence of colistin resistance gene mcr-1 in clinical Isolates Acinetobacter Baumannii from India. *Int J Infect Dis.* 2020;**101**:81. https://doi.org/10.1016/j.ijid.2020.09.238.

14. Lowe M, Singh-Moodley A, Ismail H, Thomas T, Chibabhai V, Nana T, et al. Molecular characterisation of Acinetobacter baumannii isolates from bloodstream infections in a tertiary-level hospital in South Africa. *Front Microbiol.* 2022;**13**:863129. [PubMed ID: 35992699]. [PubMed Central ID: PMC9391000]. https://doi.org/10.3389/fmicb.2022.863129.

15. Ghalavand Z, Eslami G, Hashemi A, Sadredinamin M, Yousefi N, Dehbanipour R. Characterization of Sequence Types and Mechanisms of Resistance to Tigecycline Among Acinetobacter baumannii Isolated from Children. *Curr Microbiol.* 2022;**79**(9):285. [PubMed ID: 35947200]. https://doi.org/10.1007/s00284-022-02976-5.

16. Chen L, Li H, Wen H, Zhao B, Niu Y, Mo Q, et al. Biofilm formation in Acinetobacter baumannii was inhibited by PAbetaN while it had no association with antibiotic resistance. *Microbiologyopen.* 2020;**9**(9). e1063. [PubMed ID: 32700454]. [PubMed Central ID: PMC7520992]. https://doi.org/10.1002/mbo3.1063.

17. Poladi I, Shakib P, Halimi S, Delfani S, Zadeh FE, Rezaei F. Investigation of EpsA, OmpA, and Bap Genes among MDR and XDR Acinetobacter baumannii Isolates in Khorramabad, Iran. *Recent Adv Antiinfect Drug Discov.* 2024;**19**(4):307-14. [PubMed ID: 38213149]. https://doi.org/10.2174/0127724344274260231220052526.

18. Meng X, Dong M, Wang DI, He J, Yang C, Zhu L, et al. Antimicrobial susceptibility patterns of clinical isolates of gram-negative bacteria obtained from intensive care units in a tertiary hospital in Beijing, China. *J Chemother.* 2011;**23**(4):207-10. [PubMed ID: 21803697]. https://doi.org/10.1179/joc.2011.23.4.207.

19. Bartal C, Rolston KVI, Nesher L. Carbapenem-resistant Acinetobacter baumannii: Colonization, Infection and Current Treatment Options. *Infect Dis Ther.* 2022;**11**(2):683-94. [PubMed ID: 35175509]. [PubMed Central ID: PMC8960525]. https://doi.org/10.1007/s40121-022-00597-w.

20. Aghapour Z, Gholizadeh P, Ganbarov K, Bialvaei AZ, Mahmood SS, Tanomand A, et al. Molecular mechanisms related to colistin resistance in Enterobacteriaceae. *Infect Drug Resist.* 2019;**12**:965-75. [PubMed ID: 31190901]. [PubMed Central ID: PMC6519339]. https://doi.org/10.2147/IDR.S199844.

21. Farajnia S, Lotfi F, Dehnad A, Shojaie M, Raisi R, Rahbarnia L, et al. The molecular characterization of colistin-resistant isolates of Acinetobacter baumannii from patients at intensive care units. *Iran J Microbiol.* 2022;**14**(3):319-27. [PubMed ID: 37124847]. [PubMed Central ID: PMC10132339]. https://doi.org/10.18502/ijm.v14i3.9768.

22. Khoshbayan A, Shariati A, Shahmoradi S, Baseri Z, Mozafari H, Darban-Sarokhalil D. Prevalence and molecular mechanisms of colistin resistance in Acinetobacter baumannii clinical isolates in Tehran, Iran. *Acta Microbiol Immunol Hung.* 2021. [PubMed ID: 34370684]. https://doi.org/10.1556/030.2021.01420.

23. Asif M, Alvi IA, Rehman SU. Insight into Acinetobacter baumannii: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. *Infect Drug Resist.* 2018;**11**:1249-60. [PubMed ID: 30174448]. [PubMed Central ID: PMC6110297]. https://doi.org/10.2147/IDR.S166750.

24. 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). [PubMed ID: 31366692]. [PubMed Central ID: PMC6760966]. https://doi.org/10.1128/JCM.00501-19.

25. Anjum MF, Zankari E, Hasman H. Molecular Methods for Detection of Antimicrobial Resistance. *Microbiol Spectr.* 2017;**5**(6). [PubMed ID: 29219107]. https://doi.org/10.1128/microbiolspec.ARBA-0011-2017.

26. Seleim SM, Mostafa MS, Ouda NH, Shash RY. The role of pmrCAB genes in colistin-resistant Acinetobacter baumannii. *Sci Rep.* 2022;**12**(1):20951. [PubMed ID: 36470921]. [PubMed Central ID: PMC9722906]. https://doi.org/10.1038/s41598-022-25226-x.

27. Courvalin P. Transfer of antibiotic resistance genes between gram-positive and gram-negative bacteria. *Antimicrob Agents Chemother.* 1994;**38**(7):1447-51. [PubMed ID: 7979269]. [PubMed Central ID: PMC284573]. https://doi.org/10.1128/AAC.38.7.1447.

28. Hussein NH, Al-Kadmy IMS, Taha BM, Hussein JD. Mobilized colistin resistance (mcr) genes from 1 to 10: a comprehensive review. *Mol Biol Rep.* 2021;**48**(3):2897-907. [PubMed ID: 33839987]. https://doi.org/10.1007/s11033-021-06307-y.

29. Hafudh HH, Hassan JS, Mahdi QA. Detection of plasmid-mediated Colistin resistance genes (mcr-1 to mcr-5) in Acinetobacterbaumannii recovered from nosocomial versus community wound infection. *Ann Trop Med Public Health.* 2020;**23**(19):SP232106. https://doi.org/10.36295/asro.2020.232106.

30. Kareem SM. Emergence of mcr- and fosA3-mediated colistin and fosfomycin resistance among carbapenem-resistant Acinetobacter baumannii in Iraq. *Meta Gene.* 2020;**25**:100708. https://doi.org/10.1016/j.mgene.2020.100708.

31. Babaei S, Pourabdollah M, Aslanimehr M, Nikkhahi F, Mahmoodian S, Hasani Y, et al. Frequency of Multi-Drug Resistance and Molecular Characteristics of Resistance to Colistin in Acinetobacter baumannii Collected from Patients in Intensive Care Units with Ventilator-Associated Pneumonia. *Tanaffos.* 2021;**20**(4):345-52. [PubMed ID: 36267923]. [PubMed Central ID: PMC9577206].

32. Tehrani S, Samami H, Keyvanfar A, Hashemi A. Detection of Carbapenems and Colistin Resistance Genes in Pseudomonas aeruginosa and Acinetobacter baumannii: A Single-center Study in Iran. *Nov Biomed.* 2022;**10**(3):178-83.

33. Hughes D, Andersson DI. Environmental and genetic modulation of the phenotypic expression of antibiotic resistance. *FEMS Microbiol Rev.* 2017;**41**(3):374-91. [PubMed ID: 28333270]. [PubMed Central ID: PMC5435765]. https://doi.org/10.1093/femsre/fux004.

34. Nang SC, Li J, Velkov T. The rise and spread of mcr plasmid-mediated polymyxin resistance. *Crit Rev Microbiol.* 2019;**45**(2):131-61. [PubMed ID: 31122100]. [PubMed Central ID: PMC6625916]. https://doi.org/10.1080/1040841X.2018.1492902.