

## In Vitro Susceptibility of Colistin Against Multidrug Resistant *Klebsiella Pneumoniae* Clinical Isolates

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### ABSTRACT

**Objectives:** To determine in-vitro efficacy of colistin against resistant *Klebsiella Pneumoniae* clinical isolates.

**Study Design:** Cross-sectional study.

**Place and Duration of Study:** Microbiology Department of Pathology Lab, Armed Forces Institute of Cardiology & National Institute of Heart Diseases, Rawalpindi, Pakistan, from Jan 2021 to Dec 2021.

**Methodology:** Sampling was done by nonprobability consecutive technique. Antimicrobial susceptibility testing was done for all clinical isolates of KP.

**Results:** A total of 3066 culture and sensitivity requests were received, out of these 663(21.6%) specimens revealed growth of different microorganisms. Amongst 663 culture positive isolates, 150(22.6%) were identified as KP. Antibiotic susceptibility of KP showed >90% isolates resistant to 3rd & 4th generation cephalosporins, cotrimoxazole, ampicillin+salbactam and coamoxiclav. Aminoglycosides, quinolones, meropenem, aztreonam and tazobactam+pipracillin were found resistant in >80% isolates and doxycycline and imipenem in >70% isolates. Resistance to minocycline was 58%, chloramphenicol 40.7% and Tigecycline 38.7%. The least resistance was noted in Colistin 16%.

**Conclusion:** A very high antimicrobial resistance was observed in KP isolates against penicillins, cephalosporins, ampicillin beta lactamase inhibitor combinations, quinolones, aminoglycosides, carbapenems and cotrimoxazole. Comparatively tigecyclines and chloramphenicol were found to be less resistant than other antimicrobials to manage MDR and XDR cases. Colistin has excellent efficacy against MDR and XDR KP isolates.

**Keywords:** Colistin, Drug resistance, *Klebsiella pneumoniae*, Multidrug resistant, Susceptibility, Tigecycline.

**How to Cite This Article:** Fayyaz M, Zafar H, Mirza IA, Meral U, Hussain A, Khalil H. In Vitro Susceptibility of Colistin Against Multidrug Resistant *Klebsiella Pneumoniae* Clinical Isolates. *Pak Armed Forces Med J* 2022; 72(Suppl-3): S668-672. DOI: <https://doi.org/10.51253/pafmj.v72iSUPPL-3.9580>

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### INTRODUCTION

*Klebsiella Pneumoniae* (KP) belongs to a group of Gram-negative enteric rods. It is considered to have an opportunistic approach and is included in the list of ESKAPE pathogens. They include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*. All of them are becoming challenging to treat by clinicians due to increased resistant pattern around the Globe.<sup>1</sup> Amongst the whole list, multidrug-resistant (MDR)-KP infections, are gaining popularity due to high morbidity and mortality rates i.e. 22-72%. This rise in morbidity and mortality due to KP infection leads to prolong hospital stay, thus causing extra burden on health care system along with enhancing the treatment cost and suffering of the patients. Production of carbapenemases are the virulent factors imparting the worsening scenario especially in hospitalized and immunocompromised patients.<sup>2</sup> The added

factors include the presence of capsule, and production of biofilm, which helps its escape from opsonization and phagocytosis by macrophages. As a result, modulation of inflammatory cascade favors the emergence of severe invasive and chronic opportunistic infections.<sup>3</sup>

The emergence of antimicrobial resistance (AMR) along with MDR is pushing for the availability of new drugs with good sensitivity. Till the time of their discovery, the clinicians and researchers are devoted to search for better management options. Either usage of combination therapy or last line anti microbials seems the only way out.<sup>4</sup> Unfortunately, worsening of scenario is there due to emergence of MDR, extensively drug-resistant (XDR), and pan-drug-resistant (PDR) strains. MDR is defined as “non-susceptibility to at least one agent in three or more antimicrobial categories”, XDR is defined as “non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories)” and PDR is defined as “non-susceptibility to all agents in all antimicrobial groups”.<sup>5</sup> Such

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resistant pattern is getting great attention for *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* infections. It is because these organisms are the most commonly isolated pathogens from the clinical samples. Clinicians around the globe are much concerned about the growth of these pathogens owing to their potential to cause difficult to treat situations in clinical practice.<sup>6</sup>

In such scenario, especially for well-known carbapenem resistant bugs, colistin and tigecycline have gained a good reputation.<sup>4</sup> Colistin belongs to a group of polymyxin compound i.e., *polymyxin E*. It is a secondary metabolite extracted from Gram-positive soil bacterium *Paenibacillus polymyxa* subspecies *Colistinus*. Besides desired outcome from its management few limitations are still reserving its use. The side effects like nephrotoxicity and neurotoxicity, are the mainly concerned ones. Besides these side effects, its overuse is also making their resistant pattern emergence.<sup>7</sup> The alteration in lipopolysaccharides of gram negative and mutation in *mgrB* gene are the responsible ones for its emerging reported resistant pattern.<sup>8</sup>

The tigecycline belongs to a group of first glycylicline, which was approved by the US Food and Drug Administration (FDA) in June 2005. It is well known for its worthwhile use to treat complicated skin and soft tissue infections (cSSTI), complicated intra-abdominal infections (cIAI), and community-acquired pneumonia (CAP).<sup>9,10</sup> Owing to its widened antimicrobial cover, especially for MDR, XDR and PDR Gram-negative bacteria, it is safely used for ventilator-associated pneumonia (VAP), hospital-acquired pneumonia (HAP) and blood stream infections (BSI).<sup>11</sup>

In view of this available literature including increased emergence of MDR, XDR and PDR *Klebsiella pneumoniae* infection. The current study has been planned to identify in vitro efficacy of colistin against resistant *Klebsiella pneumoniae* infections and to find out resistance pattern of different antimicrobial groups like, aminoglycosides, quinolones, cephalosporins and carbapenems against KP clinical isolates.

### METHODOLOGY

This cross-sectional study was carried out at the microbiology department of Pathology lab, Armed Forces institute of Cardiology & National Institute of Heart Diseases, Rawalpindi Pakistan through January 2021 to December 2021.

**Sample Size:** Prevalence of *Klebsiella Pneumoniae* in clinical samples is 15.8% so, the calculated sample size

was 19612. Sampling was done using non probability consecutive sampling technique.

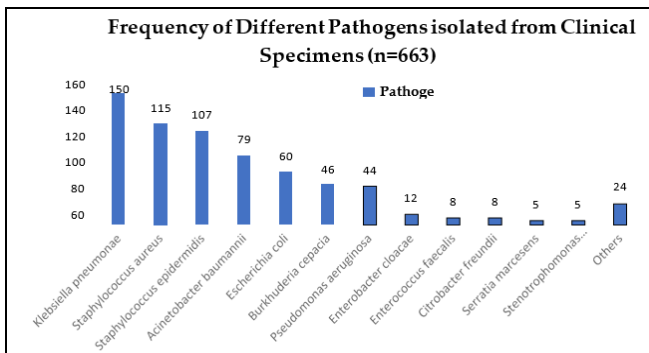
**Inclusion Criteria:** All Patients samples were dealt with standard microbiology lab practiced and only samples yeild growth of KP were included in this study. All requests for culture and sensitivity of clinical specimens from all wards of the hospital were incorporated in the study. No discrimination was made on basis of age and gender.

**Exclusion Criteria:** Culture of other microorganism and samples outdoor patients were excluded duplicate samples from same patient were not included in the data collection. Patient proforma was developed to note all demographic details like hospital registration, name, age, and gender of the patient.

Approval from the Institutional Ethical Review Board (IRB Itr no. 9/2/R&D/2022/188) along with departmental consent was granted and patient identity was kept confidential. Standard microbiological laboratory practices were followed starting from receiving of samples till finalization of results. Only specimens yielding the growth of KP were counted for this study. Identification of the organisms were carried out using colony morphology, Gram staining, catalase test and biochemical reactions on analytical profile index (API) 20E (Biomerieux, France). Mueller Hinton agar (Oxoid, UK) was used for antimicrobial susceptibility testing (AST) using modified Kirby-Bauer disk diffusion method in the light of guidelines of Clinical and Laboratory Standards Institute (CLSI) 2021.<sup>13</sup> Colistin agar test was used for colistin susceptibility as per CLSI guidelines. Colistin resistant isolates were further confirmed by VITEK-2 system (Biomerieux, France). European Committee for Antimicrobial Susceptibility Testing (EUCAST) breaking points were used for Tigecycline (TGC) and Colistin susceptibility.<sup>14</sup> AST was done using different antimicrobial disks including Ampicillin (AMP) 10 µg, Gentamicin (CN) 10 µg, Ciprofloxacin (CIP) 5 µg, Levofloxacin (LEV) 5 µg, Amikacin (AK) 30 µg, Cotrimoxazole (SXT) 25 µg, Ceftriaxone (CRO) 30 µg, Ceftazidime (CAZ) 30 µg, Cefipime (FEP) 30 µg, Imipenem (IMP)10 µg, Meropenem (MEM) 10 µg, Ampicillin+sulbactam (SAM) 20 µg, Piperacillin +Tazobactam (TZP) 110 µg, Coamoxiclav (AMC) 30 µg, Aztreonam (ATM) 30 µg, Minocycline (MIN) 30 µg, Doxycycline(DOX) 30 µg, Chloramphenicol (CAP) 30 µg, Tigecycline (TGC) 15 µg. ATCC (American Type Culture Collection, Rockville, MD) of *Escherichia coli* was used for quality control of AST.

**RESULTS**

A total of 3066 culture and sensitivity requests were made from different indoor patients of a tertiary care hospital, out of these 663(21.6%) specimens revealed growth of different microorganisms. Amongst 663 culture positive isolates, 150(22.6%) were KP. The most abundant pathogen yielded was KP followed by *Staphylococcus aureus* 115(17.3%), *Staphylococcus epidermidis* 107(16.1%), *Acinetobacter baumannii* 79 (11.9%), *Escherichia coli* 60(09%), *Burkholderia cepacia* 46(6.9%), *Pseudomonas aeruginosa* 44(6.6%) and rest of the organisms ranged between 1-2%. Breakdown of the culture positive pathogens is shown in Figure-1.



**Figure-1: Breakdown of Different Pathogens Isolated from Different Clinical Specimens.**

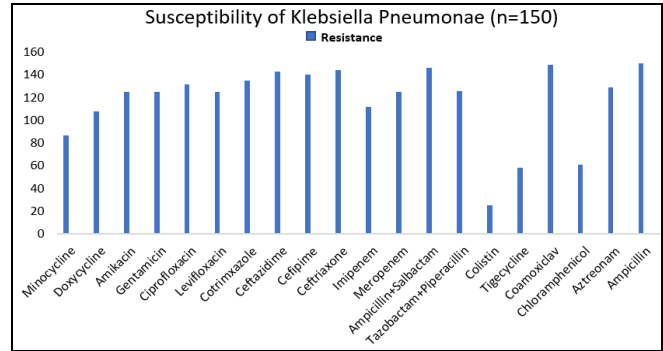
Specimens from male patients were 113(75.3%), while those of female patients were 37(24.7%). Age of Patient ranges from 1 month to 94 years with mean age 46.68±27, 20% of patients were below the age of 5 years and 60% were between the age of 30-70 years. Sources of specimen for culture and sensitivity are shown in Table-I. The most common were 38(25.3%) from wound sites, then 36(24%) respiratory specimens followed by blood culture 28(18.7%) and CVP tips 23(15.3%).

**Table-I: Sources of Specimens for Culture and Sensitivity.**

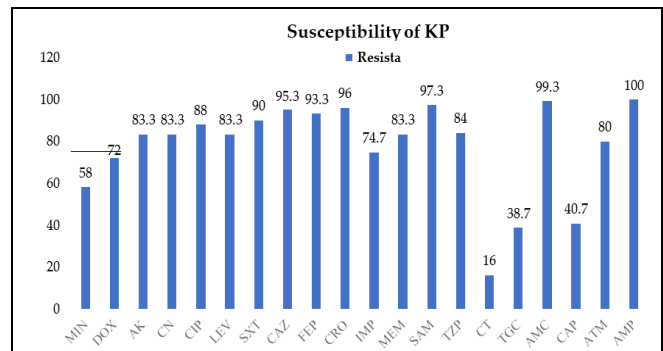
Specimen Source	Number of Specimens (n)	Percentage (%)
Respiratory Samples	36	24
Blood	28	18.7
CVP Tip	23	15.3
Pus & Pus Swab	38	25.3
Urine	15	10
Others	10	6.7
Total	150	100

Antibiotic susceptibility of KP showed >90% resistance to 3<sup>rd</sup> & 4<sup>th</sup> generation cephalosporins, cotrimoxazole, ampicillin+salbactam and coamoxiclav. Resistance to aminoglycosides, quinolones, meropenem, aztreonam, tazobactam+piperacillin was >80%

and >70% to doxycycline and imipenem. Resistance to minocycline was 87(58%), chloramphenicol 61(40.7%) and Tigecycline 58(38.7%). The least resistance was noted in Colistin 25(16%). The detail of drugs along with resistance pattern is shown graphically Figure-2 & percentage of resistance is given in Figure-3 below.



**Figure-2: Antibiogram of Klebsiella Pneumoniae Isolated from Different Clinical Specimens**



**Figure-3: Resistance Percentage of KP Isolated from Different Clinical Specimens.**

**DISCUSSION**

In view of increased incidence of KP infection {150 (22.6%) out of 663} along with emergence of MDR, XDR and PDR cases, the current study was planned to assess in-vitro efficacy of colistin. The results are comparable with the published study by Maltezou *et al.* Comparing the global statistics, he observed and concluded the high incidence for extended spectrum beta lactamase producing (ESBL) organisms. The reported incidence of ESBL for KP was 58.71% in Eastern Europe, 51.9% for South America, 28.2% for the Asia Pacific region, 24.41% for Southern Europe, 16.71% for Northern Europe and 12.3% for North America.<sup>15</sup> However, the current study results showed decreased incidence of ESBL producing KP when compared to a Pakistani study by Ejaz *et al.*<sup>16</sup> However, in another Iranian study, reported incidence was more i.e. 28%.

The identified resistant gene was bla TEM for ESBL isolates.<sup>17</sup>

The current study revealed 100% resistance to ampicillin, and 90% for 3<sup>rd</sup> & 4<sup>th</sup> generation cephalosporins, cotrimoxazole, ampicillin+salbactam and co-amoxiclav. For aminoglycosides, quinolones, meropenem, aztreonam and tazobactam+pipracillin, >80% resistance to KP was observed. An approximate 70% resistance was observed for doxycycline and imipenem. Resistance to minocycline was 58%, chloramphenicol 40.7% and tigecycline 38.7%. The least resistance was noted from Colistin 16%. The results are in line with the published study by Wangh *et al.* In view of high resistance pattern, he concluded that usage of combination therapy with colistin+ tigecycline, can be beneficial to manage MDR, XDR and PDR isolates of KP especially in nosocomial pneumonia.<sup>18</sup> Chloramphenicol an old drug has regained its efficacy against many multidrug resistant Gram positive and Gram-negative organisms because it is not in frequent clinical use from few years. This drug has very good tissue as well as fluid penetration including CSF. Like results of current study, one more study is in favour of using colistin as a drug of choice to manage resistant strains of KP.<sup>19</sup> The results of study are supporting the Japanese published data showing 60% clinical efficacy of colistin therapy against multidrug-resistant KP infection.<sup>20</sup> Another study revealed 70% imipenem resistance among KP isolates which is almost similar to our observation of 74% resistance to imipenem.<sup>21</sup> In the present era of increasing AMR perhaps colistin being the only choice left for treatment of such MDR and XDR pathogens. Unfortunately reports revealing resistance to this drug are also coming up in the literature as we saw in our study and the same has also been reported from our neighbouring countries like Iran, China, and India.<sup>22-24</sup>

Literature review has shown that some genetic modifications in chromosomal coding regions of some regulator genes have caused this phenotypic resistance to colistin. Regular genomic monitoring is required for all resistant isolates to see the colistin resistance.<sup>22</sup> Colistin resistance is predominantly mediated by mcr gene. Co-existence of multiple resistant genes for different drugs like colistin, carbapenem, and other drugs signifies to massive genomic malleability in KP with its capacity to arise as super-spreader of antimicrobial resistance.<sup>24</sup> AMR in KP is obvious and is confirmed by our study too. As the problem is uniformly present in the region and is documented in literature, the public

health authorities need to keep an eye and closely monitor the situation with reporting of the problem.<sup>25</sup>

### RECOMMENDATIONS

- Colistin may be considered as first line treatment option in suspected cases till the availability of culture reports in ICU patients.
- Tigecycline and chloramphenicol could be other choices of treatment for such infections.
- Effective implementation of infection control measures along with strict surveillance is required to control resistant bugs in hospital settings.
- Judicious use of antibiotics must be observed to avoid multi drug resistance.

### LIMITATIONS OF STUDY

Genomic characterization of the clinical isolates and detection of resistant mcr genes is not carried out in routine in our set up, neither it was done during this study due to financial constraints and non-availability of the electro-medical equipment in our lab settings.

### CONCLUSION

A very high antimicrobial resistance was observed in KP isolates against penicillins, cephalosporins, ampicillin & beta lactamase inhibitor combinations, quinolones, aminoglycosides, carbapenems and cotrimoxazole. Comparatively tigecyclines and chloramphenicol were found to be less resistant than other antimicrobials to manage MDR and XDR cases. Colistin has excellent in vitro efficacy against resistant KP isolates but resistance is coming up to this only rescuer drug too. So strict infection control measures and continuous surveillance are only means to avoid such MDR bugs in our hospital settings.

### ACKNOWLEDGMENT

I am deeply grateful to my supervisor for his guidance, patience and support who provided insight and expertise that greatly assisted my research project. I also want to share my gratitude for Comdt Exec Dir AFIC/NIHD & HOD R&D for their support and contribution in completion of the research paper.

**Conflict of Interest:** None.

### Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

MF: Idea, Manuscript writing, final approval  
HZ: Manuscript writing, referencing, editing  
IAM: Data management, analysis and Result compilation.  
UM: Manuscript writing, editing, intellectual contribution  
AH: Manuscript writing, editing, data collection  
HK: Editing, article review, referencing

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of

any part of the work are appropriately investigated and resolved.

## REFERENCES

1. Bassetti M, Righi E, Carnelutti A, Graziano E, Russo A. Multi-drug-resistant *Klebsiella pneumoniae*: challenges for treatment, prevention and infection control. *Expert Rev Anti Infect Ther* 2018; 16(1): 749–761. doi: 10.1080/14787210.2018.1522249
2. Poerio N, Olimpieri T, Angelis LHD, Santis FD, Thaller MC, Andrea MMD, et al. Fighting MDR- *Klebsiella pneumoniae* infections by a combined Host- and pathogen-directed therapeutic approach. *Front Immunol* 2022; 13(1): 835417. doi: 10.3389/fimmu.2022.835417.
3. Piperaki ET, Syrogiannopoulos GA, Tzouveleki LS, Daikos GL. *Klebsiella pneumoniae*: Virulence, Biofilm and Antimicrobial Resistance. *Pediatr Infect Dis J* 2017; 36(1): 1002–1005. doi: 10.1097/INF.0000000000001675
4. Zafer MM, Mahallawy HAE, Abdulhak A, Amin MA, Agamy MHA, Radwan HH, et al. Emergence of colistin resistance in multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from cancer patients. *Ann Clin Microbiol Antimicrob* 2019; 18(1): 40. doi:10.1186/s12941-019-0339-4.
5. Khurshid M, Rashid A, Husnain M, Rasool MH, Waqas U, Saeed M, et al. In-Vitro assessment of the therapeutic potential of polymyxins and tigecycline against multidrug resistant *Acinetobacter* isolates from infected wounds. *J Ayub Med Coll Abbottabad* 2020; 32(4): 459–464.
6. Jeannot K, Bolard A, Plesiat P. Resistance to polymyxins in Gram-negative organisms. *Int J Antimicrob Agents* 2017; 49(5): 526–535.
7. Srinivas P, Rivard K. Polymyxin resistance in Gram-negative pathogens. *Curr Infect Dis Rep* 2017; 19(11): 38.
8. Gawad MAE, Ahmed ES, Zhong LL, Yang Y, Doi Y, Tian GB, et al. Colistin and its role in the Era of antibiotic resistance: an extended review (2000–2019). *Emerg Infect Microbe* 2020; 9(1): 868–885. doi.org/10.1080/22221751.2020.1754133
9. Zha L, Pan L, Guo J, French N, Villanueva EV. Effectiveness and safety of high dose tigecycline for the treatment of severe infections: a systematic review and meta-analysis. *Adv Ther* 2020; 37(1): 1049–1064. doi: 10.1007/s12325-020-01235-y
10. Kuti JL, Kim A, Cloutier DJ, Nicolau DP. Evaluation of plazomicin, tigecycline, and meropenem pharmacodynamic exposure against carbapenem-resistant Enterobacteriaceae in patients with bloodstream infection or hospital-acquired/ventilator-associated pneumonia from the CARE Study (ACHN-490-007). *Infect Dis Ther* 2019; 8(3): 383–396.
11. Wang J, Pan Y, Shen J. The efficacy and safety of tigecycline for the treatment of bloodstream infections: a systematic review and meta-analysis. *Ann Clin Microbiol Antimicrob* 2017; 16(1): 24.
12. Qureshi S, Maria N, Zeeshan M, Irfan S, Qamar FN. Prevalence & risk factors associated with MDRO carriage among pediatric patients at the time of admission in a tertiary care hospital of a developing country. A cross sectional study. *BMC Infect Dis* 2021; 21(1): 547–550.
13. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 8.0, 2018. Växjö, Sweden: EUCAST; 2018, [Internet] available at: [http://www.eucast.org/clinical\\_breakpoints/](http://www.eucast.org/clinical_breakpoints/) (accessed 19 August 2020).
14. Muzahed , Shaikh NS, Shaikh SS, Acharya S, Moosa SS, Shaikh MH, et al. Molecular epidemiological surveillance of CTX-M-15-producing *Klebsiella pneumoniae* from the patients of a teaching hospital in Sindh, Pakistan. *F1000 Res* 2021; 10(1): 444. doi: 10.12688/f1000research.53221.3.
15. Ejaz H, Wang N, Wilksch JJ, Page AJ, Cao H, Gujran S, et al. Phylogenetic analysis of *Klebsiella pneumoniae* from hospitalized children, Pakistan. *Emerg Infect Dis* 2017; 23(11): 1872–1875. doi: 10.3201/eid2311.170833
16. Zadeh BS, Shakib P, Zolfaghari M R, Sheikh FA. Prevalence of *Escherichia coli* and *Klebsiella pneumoniae*, producing extended-spectrum beta-lactamase (ESBLs) from clinical specimens in Khuzestan, Iran. *Gene Cell Tissue* 2021; 8(3): 112377. doi: 10.5812/gct.112377.
17. Wang SH, Yang KY, Sheu CC, Chen WC, Chan MC, Feng JY, et al. On behalf of the T-Care Taiwan critical care infection group . Efficacies of Colistin–Carbapenem versus Colistin–Tigecycline in critically ill patients with CR-GNB-associated pneumonia: A multicenter observational study. *Antibiotics (Basel)* 2021; 10(9): 1081. doi: 10.3390/antibiotics10091081.
18. Crémieux AC, Dinh AD, Nordmann P, Mouton W, Tattevin P, Ghout I, et al. Efficacy of colistin alone and in various combinations for the treatment of experimental osteomyelitis due to carbapenemase-producing *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2019; 74(9): 2666–2675. doi: 10.1093/jac/dkz257.
19. Percivalle E, Monzillo V, Pauletto A, Marone P. Colistin inhibits *E. coli* O157:H7 Shiga-like toxin release, binds endotoxins and protects Vero cells. *New Microbiol* 2016; 39(2): 119–123.
20. Tattevin P, Dinh A, Ghout I, Mouton W, Verdier MC, Laurent F, et al. Efficacy of generic meropenem products in combination with colistin in carbapenemase-producing *Klebsiella pneumoniae* experimental osteomyelitis. *Int J Antimicrob Agents* 2020; 56(5): 106152. doi: 10.1016/j.ijantimicag.2020.106152.
21. Bolourchi N, Shahcheraghi F, Giske CG, Nematzadeh S, Goodarzi NN. Comparative genome analysis of colistin-resistant OXA-48-producing *Klebsiella pneumoniae* clinical strains isolated from two Iranian hospitals. *Ann Clin Microbiol Antimicrob* 2021; 20(1): 74–78. doi: 10.1186/s12941-021-00479-y.
22. 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. doi: 10.1038/s41426-018-0124-z.
23. Singh S, Pathak A, Rahman M, Singh A. Genetic characterisation of Colistin resistant *Klebsiella pneumoniae* clinical isolates from North India. *Front Cell Infect Microbiol* 2021; 11(1): 666030. doi: 10.3389/fcimb.2021.666030. eCollection 2021.
24. Effah CY, Sun T, Liu S. *Klebsiella pneumoniae*: an increasing threat to public health. *Ann Clin Microbiol Antimicrob* 2020; 19(1): 1. doi: 10.1186/s12941-019-0343-8.