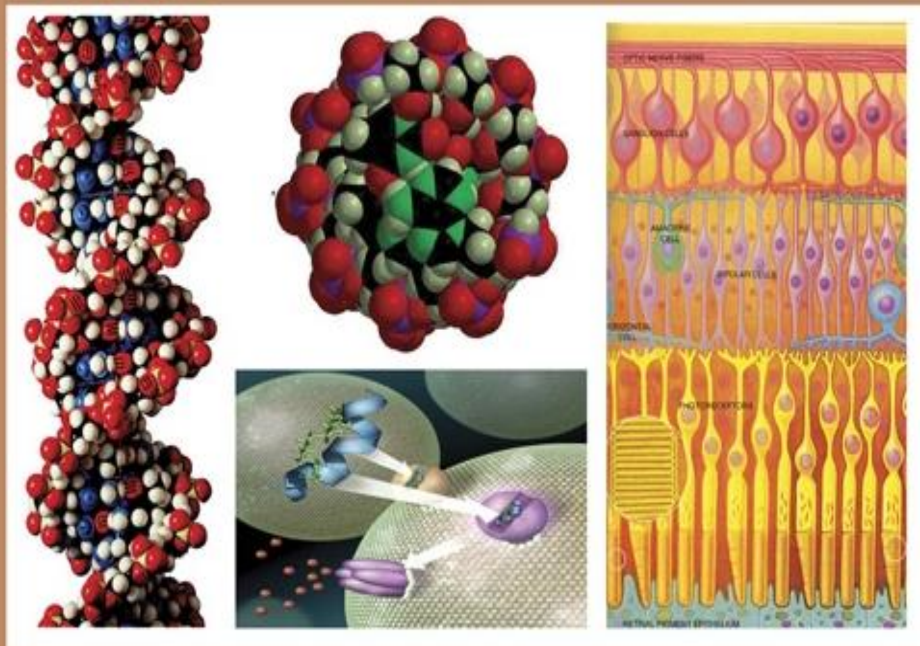
EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

C



ISSN 2090-0767

WWW.EAJBS.EG.NET

Vol. 15 No. 1 (2023)

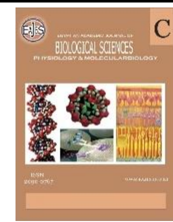
.

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(1) pp259-265 (2023)*

DOI: 10.21608/EAJBSC.2023.290749

Egypt. Acad. J. Biolog. Sci., 15(1):259-265 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Urinary Tract Infection Caused by Carbapenemase-Producing *K. pneumoniae* and *E. coli* at the Institute of Kidney Disease Peshawar, Pakistan

Sumyya H. Hariri[1] and Suleman Khan[2]

1. Department of Microbiology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia
2. Department of Agricultural Sciences, Food, Natural Resources and Engineering, Università degli studi di Foggia, Italy

***E-mail:** sulemankhanazmat333@gmail.com

ARTICLE INFO

Article History
Received:16/1/2023
Accepted:15/3/2023
Available:20/3/2023

Keywords:
Urinary tract infection;
Carbapenemase-producing;
Escherichia coli, Klebsiella pneumoniae.

ABSTRACT

Aims: Carbapenemase-producing bacteria make infections of the urinary tract (UTIs) challenging to cure with last-resort treatment like carbapenem. Carbapenemase-producing *E. coli* and *K. pneumoniae* implicated in UTI must be detected molecularly since their ability to spread broadly among patients is rising (Nomeh *et al.,* 2022). **Methodology**: Ten non-repeated clinical isolates of *Escherichia coli* (Ecoli1, Ecoli2, Ecoli3, Ecoli4, and Ecoli5) and *Klebsiella pneumoniae* (Kp6, Kp7, Kp8, Kp9, Kp10) were selected from urinary tract infection patients at Institute of Kidney Disease Peshawar, Pakistan, based on their in vitro phenotypic carbapenem antibiotic resistance. These isolates were confirmed using standard routine microbiological techniques. PCR-specific primers screened *E. coli* and *K. pneumoniae* strains for Carbapenemase-producing genes. **Result**: Molecular Detection of Carbapenemase-producing Gene in UTI Patients with Uropathogenic *Escherichia coli* and *Klebsiella pneumoniae.* The higher proportion of Carbapenemase-producing genes in all the bacterial isolates in this study was blaKPC 15(100 %), followed by blaNDM 12.3(90.1 %), blaIMP 6(60.2 %) and blaVIM 3(30.6 %). The most common Carbapenemase gene in *Escherichia coli* 8 (80%) was blaKPC, followed by blaNDM 7 (70%) and blaOXA 45 (4.5%), which was the least common. *Klebsiella pneumoniae* had more blaNDM and blaKPC than blaOXA. Both had a percentage of 4.5 (40.9%). **Conclusion**: These results are consistent with the rapid spread of genes responsible for generating Carbapenemases in *E. coli* and *Klebsiella pneumoniae* that cause urinary tract infections. Despite the lack of blaVIM in *K. pneumoniae,* the pathogenic function of Carbapenemase-producing genes in UTI in this study should not be underestimated because of the potential they have to cause treatment failure and the subsequent persistence of UTI in patients.

INTRODUCTION

A Carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* is a bacterium that carries a Carbapenemase gene or is resistant to carbapenem antibiotics in susceptibility tests. The World Health Organization categorized Carbapenemase-producing *E. coli* and *K. pneumoniae as* "critical" and "high priority" pathogens in 2017. As a result of the rapid development and spread of Carbapenemase-generating genotypes, the prevalence of carbapenem-resistant strains of *E. coli* and *K. pneumoniae* has increased worldwide (Nomeh *et al.,* 2022).

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(1) pp259-265 (2023)*
DOI: 10.21608/EAJBSC.2023.290749

K. pneumoniae Carbapenemase (blaKPC), Verona integron metallo-beta-lactamase (vim), imipenemases (blaIMP), oxacillinase (blaOXA), and the Delhi Metallo—lactamase (blaNDM) are all examples of such resistant strains (Nasir *et al.,* 2021). They are increasingly being reported among healthcare-connected severe Urinary infections as a result of their capacity to truncate the action of carbapenem and other beta-lactam medicines. Urinary tract infections are contagious disorders that are typically carried upon with Enterobacteriaceae. These bacteria include *E. coli* and *K. pneumoniae.* These bacteria can infiltrate and colonize any part of the urinary tract (Tenney *et al.,* 2018) causing a variety of symptoms such as fever, a burning sensation while urinating, lower abdominal pain (LAP), itching, blister and ulcer formation in the genital area, genital and suprapubic pain, and pyuria. Pyuria is an inflammation of the urinary tract that can be caused by bacteria (Al Yousef *et al.,* 2016)). The age of the person who is infected and the location of the infection in the urinary system are the two primary factors that influence these. Urinary tract infections (UTIs) are prevalent bacterial illnesses that affect roughly 150 million people every year all over the world. This places a huge financial burden on the community as a whole as well as the health care system (Udeme Peter *et al.,* 2022). It is difficult to treat a urinary tract infection (UTI) with a drug of last resort such as carbapenem due to the prevalence of the Carbapenemase gene, which is carried by *E. coli* and *K. pneumoniae,* as well as the rapid evolution of this gene. This reduces the number of treatment options available. The bacteria that have these resistance genes are known as superbugs (Peter *et al.,* 2022). They are also known as Carbapenemase-producing *E. coli* and *K. pneumoniae,* and they present a challenge to the empirical treatment of urinary tract infections all over the world. Due to the global proliferation of the Carbapenemase gene in clinical isolates of *E. coli* and *K. pneumoniae,* molecular research of these bacteria implicated in urinary tract infections is needed. These strains of bacteria are widely prevalent among patients (Edemekong *et al.,* 2022).

It is hypothesized that Carbapenemase-producing bacteria cause infections of the (UTIs) challenging to cure with last-resort treatment like carbapenem. Therefore this study was envisaged to molecularly detect the implications of Carbapenemase-producing *E. coli* and *K. pneumoniae*.

MATERIALS AND METHODS

1. *E. coli* and *K. pneumoniae* Characterization:

In the Institute of Kidney Disease (IKDs) Hospital in Peshawar, clinical isolates of *E. coli* (Ecoli1, Ecoli2, Ecoli3, Ecoli4, Ecoli5) and *K. pneumoniae* (Kp6, Kp7, Kp8, Kp9, Kp10) were obtained from patients who had been diagnosed with urinary tract infection. KPK state Pakistan. Clinical isolates with in vitro carbapenem resistance were chosen. The World Medical Association (WMA) declaration required that all subject data be classified for confidentiality. Standard microbiological techniques were used to identify and characterize the 10 clinical isolates of *E. coli* and *K. pneumoniae* (Ogba *et al.,* 2022).

2. PCR-Based Carbapenemase Gene Screening (PCR):

2.1. DNA Extraction:

DNA *E. coli* and *K. pneumoniae* were extracted using ZR Fungal/Bacterial DNA MiniprepTM in the first lane of gel, and the samples were carefully put into the additional wells. The gel ran at 80-150 V for 1-1.5 h. After turning off the power and disconnecting the electrodes, the gel box was gently removed. After turning off the power and disconnecting the electrodes, the gel box was gently removed. 2 ml of bacterial cell broth and 750 µg lysis solutions were added to a ZR Bashing TM lysis tube. It was placed in a filament with a 2 ml tube holder assembly and processed at full speed for 5 minutes. ZR bashing bead Tm lysis tubes were centrifuged at >10,000 x g for 1 minute. Up to 400 µg of

supernatant was transferred to a Zygomo-Spin TM IV Filter in a collecting tube and centrifuged at 7,000 x g for 1 minute. The collecting tube filtrate received 1,200 µg of bacterial DNA binding buffer. Precisely 800 ul of the mixture from step 5 was transferred to a Zygomo-spinTM IIC column in a collecting tube and centrifuged at 10,000 x g for 1 minute. The collection tube flow-through was discarded. 200 ul DNA Pre-washed buffer was added to the Zymo-Spin TM IIC column in a fresh tube collection and centrifuged for 1 minute at 10,000 x g. The Zymo-Spin TM IIC column was centrifuged for 1 minute at 10,000 x g with 200 µg of bacterial DNA buffer. 100 µl (35 µl minimum) DNA was introduced directly to the column matrix of the Zymo-Spin TM IIC column in a clean 1.5 ml micro-centrifuge tube. To elute DNA, 10,000 x g was spun for 30 seconds (Ferreira *et al.,* 2022).

2.2. DNA/PCR Electrophoresis Product:

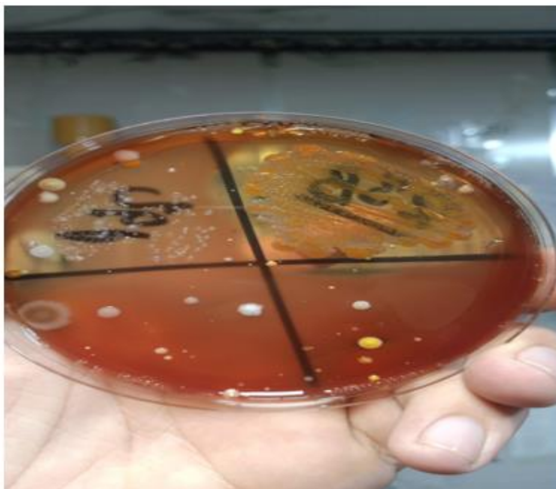
DNA was quantified at 1 g of agarose and PCR products at 2 g. A microwave flask combined 100 ml 1xTAE with agarose powder. Agarose was microwaved for 1-3 min to dissolve. The agarose solution was cooled to 50 ^{o}C. 10 µg EZ vision DNA stain followed. A gel tray with a good comb was filled with agarose. After pouring, the gel was kept at 4oC for 10-15 mins to solidify (Nomeh *et al.,* 2022).

2.3. Loading Samples And Running An Agarose Gel:

PCR product DNA samples received loading buffers. The gel box received the hardened agarose gel (electrophoresis unit). Filling the gel box with 1xTAE buffer covered it. A molecular weight ladder was carefully inserted into the first gel lane, and materials were carefully loaded into the other gel wells. The gel ran at 80-150 V for 1-1.5 h. The gel was carefully removed from the gel box after turning off the power and disconnecting the electrodes. DNA and PCR fragments were seen under a UV trans-illuminator (Yigit *et al.,* 2001).

2.4. PCR Mix Components And Cycling Conditions:

12.5 µl of New England Biolabs' Taq 2 x Master Mix (M0270), 1 µl each of 10µm forward and reverse primer (Invitrogen, U.S.ATM) (Table 1), 2 µl of DNA template, and 8.5 µL nuclease-free water made up the PCR mix. PCR was performed. Previously documented cycling settings for Carbapenemase resistance gene amplification were used (Chen *et al.,* 2012). The bacterial growth of *E. coli* and *K. pneumoniae* are shown in Figure.1.



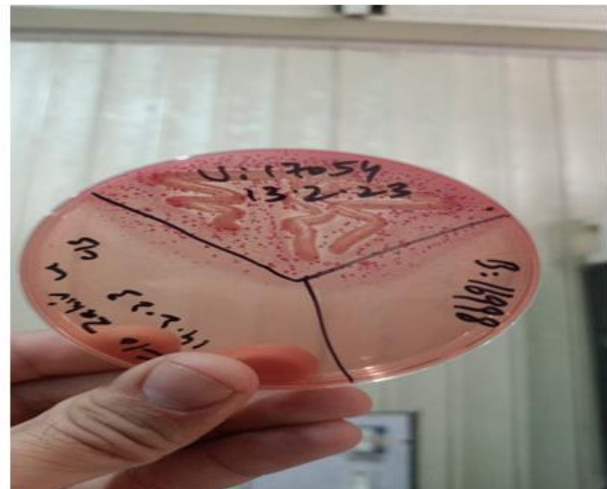


Fig. 1: Bacterial growth of mentioned Bacteria *Escherichia coli* and *Klebsiella pneumoniae*

.

Table 1. Primer Sequences use for the detection of Carbapenemase-producing resistance genes.

Primers	Sequence (5'–3'),	Amplicon size (bp)
bla_{KPC}	F: 5AATATTAGCCTGCGCGCAA3	530
	R:3 TTATAATCGGACGCGCGTT3	
bla_{OXA}	F:5AAGGCCAATTAGCGTATAAG3	570
	R:3TTCCGGTTAATCGCATATTC3	
bla_{IMP}	F:5AAAATAGCGCGGGCCCATA3	345
	R:3TTTTATCGCGCCCGGGTAT3	
bla_{NDM}	F:5GCCTTAACCGGATTATTTTT3	700
	R:3CGGAATTGGCCTAATAAAA3	

RESULTS

Molecular Detection of Carbapenemase-producing Gene in UTI Patients with Uropathogenic *E. coli* and *K. pneumoniae.* The higher proportion of Carbapenemase-producing genes in all the bacterial isolates in this study was blaKPC 15(100 %), followed by blaNDM 12.3(90.1 %), blaIMP 6(60.2 %) and blaVIM 3(30.6 %). The most common Carbapenemase gene in *E. coli* 8 (80%) was blaKPC, followed by blaNDM 7 (70%) and blaOXA 45 (4.5%), which was the least common. As shown in Table 2, *K. pneumoniae* had more blaNDM and blaKPC than blaOXA. Both had a percentage of 4.5 (40.9%) (Wang *et al.,* 2018).

Table 2. Molecular detection of Carbapenemase-producing gene in Uropathogenic *Escherichia coli and Klebsiella pneumoniae*

Carbapenemase class	Genes	Uropathogenic (n=10)		
		Ecoli1-7 (%)	KP8-12 (%)	Frequency (%)
A	bla_{KPC}	8(80%)	2(2.5)	10(100)
B	Bla_{IMP}	6(60.2)	1 (10.0)	7(70.2)
C	bla_{NDM}	3.3(70)	2 (20)	5.3 (50.70)
D	bla_{OXA}	4.5(4.50)	3(30)	7.3(70.3)

Key: n-Number of isolate, blaKPC-Klebsiella pneumonia carbapenemase, blaIMP-Imipeeneemases integron blaNDM-New Delhi Metaallobeta-lactamase, blaOXA-Oxaacillinase

DISCUSSION

This study found that both *E. coli* and *K. pneumoniae* isolates had 10 (100%) blaKPC carbapenemase-producing genes. The KPC enzyme was first discovered in a *K. pneumoniae* sample from North Carolina, USA. Isolates that make KPC have spread to 38 states in the US since 2001 (Nomeh *et al.,* 2022). Even though *K. pneumoniae* C-producers are being found at an alarming rate in Europe, mostly through clonal propagation, we don't know of any reports of blaK, pneumoniae C-positive isolates from this area of study (kidney hospital (IKD), Peshawar, Pakistan).OXA-1 has been found most often in E. coli and K. pneumoniae, despite its global prevalence (Aminu *et al.,* 2021). The second most common carbapenem gene was blaOXA 7.3(70.7%). This research revealed that the common occurrence of a change in the primary Carbapenemase genotype may be related to the importation of strains from new geographical regions, animal or food sources, or the transformation of mobile elements that carried genes between species. Although the root reason is unclear, this phenomenon highlights the importance of active, long-term observation of CR-isolate resistance in both community and hospital settings (Nomeh *et al.,* 2022). Despite the widespread belief that India and Pakistan are the primary sources of NDM-producing isolates, population

exchange between Saudi Arabia and India suggests that the Middle East may actually be a secondary reservoir for the spread of blaNDM-1 isolates (Nasir *et al.,* 2021; Tacconelli *et al.,* 2017). This study hypothesises that population movement to endemic areas where blaKPC and blaNDM were originally detected may explain the high rate of isolates with blaKPC resistance gene in this scenario, however patient travel history data is lacking. blaIMP recorded 7 (70.0%) but was not the most frequently found gene in *K. pneumoniae, E. coli*, and other *Enterobacterales* as popularly believed (Nomeh *et al.,* 2022). Most bacteria's carbapenem resistance arises from their ability to produce. In contrast, numerous clones of *E. coli* have successfully spread around the globe, including the O15:K52:H1-D strain of sequence type 393 (ST393), the ST131 strain, and the ST38 strain, which is unique for its ability to produce the OXA - lactamase and is closely related to strains from the Mediterranean basin (Suwaiba *et al.,* 2021). Although blaOXA-48 has been associated with virulence in clinical *E. coli* and *K. pneumoniae* isolates, its impact on UTI in our investigation cannot be understated. The particular role of OXA-48 had not been addressed; nevertheless, several studies had reported on clinical isolates with unusually high lethality in murine infection models and the presence of genes linked with virulence or host colonisation (Nomeh *et al.,* 2022). It is known that the co-existence of Amber class A such as blaIPM with Amber class B and D confers resistance to oxyiiminocephalosporins (ceftriiaxone and cefaazidime), and cephaamycins (cefooxitin), and transcoonjugation has been established as the mechanism by which resistance is transmitted. In light of this, it is essential to acknowledge that Carbapenemase reservoirs in the healthcare professionals, patients, or the environment of the hospital could be a main mechanism of spread in nosocomial outbreaks (Nomeh *et al.,* 2022).

CONCLUSION

It is concluded from the findings that the rapid spread of genes responsible for generating Carbapenemases in *E. coli* and *K. pneumoniae* cause urinary tract infections. Despite the lack of blaVIM in *K. pneumoniae*, the pathogenic function of Carbapenemase-producing genes in UTI in this study should not be underestimated because of the potential they have to cause treatment failure and the subsequent persistence of UTI in patients. Antimicrobial susceptibility testing of the available antibiotic agent is vital, and urgent epidemiological surveillance is required in order to reduce the likelihood of the propagation of Carbapenemase-resistant genetic determinants**.**

REFERENCES

Al Yousef, S. A., Younis, S., Farrag, E., Moussa, H. S., Bayoumi, F. S., & Ali, A. M. (2016). Clinical and laboratory profile of urinary tract infections associated with extended spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*. *Annals of Clinical and Laboratory Science, 46*(4), 393-400.

Aminu, A., Daneji, I. M., Yusuf, M. A., Jalo, R. I., Tsiga-Ahmed, F. I., Yahaya, M., ... & Gadzama, G. B. (2021). Carbapenem- resistant Enterobacteriaceae infections among patients admitted to intensive care units in Kano, Nigeria. *Sahel Medical Journal*, *24*(1), 1.

Chen, L. F., Anderson, D. J., & Paterson, D. L. (2012). Overview of the epidemiology and the thr*et* of Klebsiella pneumoniae Carbapenemases (KPC) resistance. *Infection and drug resistance*, 133-141.

Edemekong, C. I., Uzoeto, H. O., Mbong, E. O., Ikusika, B. A., Didiugwu, C. M., Ngwu, J. N., ... & Peter, I. U. (2022). Molecular characterization and bioassay of soil Actinomycetes strains on multidrug resistant bacteria. *IOSR Journal of Biotechnology and Biochemistry, 1*, 6-11.

El Hussieny, M. S., Sahar, A., & El-Masry, E. (2020). Dirty renal sinus fat, a new radiological sign predicting simple urinary tract infection: sex prevalence. *Journal of The Arab Society for Medical Research* , *15*(2), 84.

Ferreira, R. L., Da Silva, B. C., Rezende, G. S., Nakamura-Silva, R., Pitondo-Silva, A., Campanini, E. B., ... & Pranchevicius, M. C. D. S. (2019). High prevalence of multidrug-resistant Klebsiella pneumoniae harboring several virulence and β-lactamase encoding genes in a Brazilian intensive care unit. *Frontiers in microbiology*, *9*, 3198.

Nasir, F., Khan, M. I., Kashif, S., Uddin, F., Naseer, A., & Masood, S. (2021). Prevalence of ESBLs secreting and carbapenem-resistant E. coli from urinary tract infection. *Rawal Medical Journal*, *46*, 518-521.

Nomeh, O. L., Chukwu, E. B., Ogba, R. C., Akpu, P. O., Peter, I. U., Nwuzo, A. C., & Iroha, I. R. (2022). Prevalence and Antibiogram Profile of Carbapenem-resistant Escherichia coli and Klebsiella pneumoniae among Patients with Urinary Tract Infection in Abakaliki, Nigeria. *International Journal of Pathogen Research, 11*(3), 14-28.

Ogba, R. C., Nomeh, O. L., Edemekong, C. I., Nwuzo, A. C., Akpu, P. O., Peter, I. U., & Iroha, I. R. (2022). Molecular Characterization of Carbapenemase Encoding Genes in Pseudomonas aeruginosa from Tertiary Healthcare in South Eastern Nigeria. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, *12*(4), 161-168.

Peter, I. U., Okolie, S. O., Okike, B. M., Nwuzo, A. C., Chukwu, E. B., Mohammed, I. D., ... & Edemekong, C. I. (2022). Phenotypic characterization and antibiogram of non-oral bacteria isolates from patients attending dental clinic at Federal College of Dental Technology and Therapy Medical Center Enugu. *International Journal of Pathogen Research*, *11*(2), 7-19.

Suwaiba, M., Dadah, A. J., & Sanusi, S. B. (2020). Prevalence of carbapenem resistant Escherichia coli and Klebsiella pneumoniae in urine samples of patients attending selected general hospitals within Kaduna Metropolis. *Science World Journal*, *15*(4), 99-107.

Tacconelli, E. (2017). Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development. Infection Control Africa Network. South Africa.

Tenney, J., Hudson, N., Alnifaidy, H., Li, J. T. C., & Fung, K. H. (2018). Risk factors for aquiring multidrug-resistant organisms in urinary tract infections: a systematic literature review. *Saudi pharmaceutical journal*, *26*(5), 678-684.

Udeme Peter, I., Chinenye Emelda, N., Blessing Chukwu, E., Nnenna Ngwu, J., Onyinye Uzoeto, H., Chinonyelum Moneth, E., ... & Romanus Iroha, I. (2022). Molecular detection of bone sialoprotein-binding protein (bbp) genes among clinical isolates of methicillin resistant *Staphylococcus aureus* from hospitalized orthopedic wound patients. *Asian Journal of Orthopaedic Research*, *8*(3), 1-9.

Wang, Q., Wang, X., Wang, J., Ouyang, P., Jin, C., Wang, R., ... & Wang, H. (2018). Phenotypic and genotypic characterization of carbapenem-resistant Enterobacteriaceae: data from a longitudinal large-scale CRE study in China (2012–2016). *Clinical Infectious Diseases* , *67*(suppl_2), S196-S205.

Yigit, H., Queenan, A. M., Anderson, G. J., Domenech-Sanchez, A., Biddle, J.

W., Steward, C. D., ... & Tenover, F. C. (2001). Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. *Antimicrobial agents and chemotherapy*, *45*(4), 1151-1161.