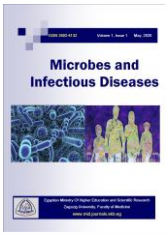
Microbes and Infectious Diseases 2022; 3(3): 693-702



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Detection of carbapenem-resistant *Pseudomonas aeruginosa* in a tertiary care hospital in Saudi Arabia

***Enas Sh. Khater*[*1,2], *Khalid H.M. Abdo*[3]**

1 *Department of Microbiology and Immunology, Faculty of Medicine, Benha University, Egypt.*
2 *Microbiology Laboratory, Al-quwayiyah General Hospital, Riyadh, KSA.*
3 *Department of Chest, Al-quwayiyah General Hospital, Riyadh, KSA.*

ARTICLE INFO

Article history:
Received 2 June2022
Received in revised form 15 June2022
Accepted 21 June2022

Keywords:
P. aeruginosa
Carbapenems
Metallo-beta-lactamases

Background:*Pseudomonas aeruginosa* is one of the common emerging multidrug-resistant causative bacteria which causes healthcare associated infections that leads to increased morbidity and mortality rates. **Aim:** Detection of resistance pattern of studied isolates to different antipseudomonal drugs and to determine the prevalence of carbapenem resistant *Pseudomonas aeruginosa* and to detect the involved carpabenemasesgenes among resistant isolates. **Methods:**The study was done from November 2021 to April, 2022 in Al-Quwayiyah general hospital. Eighty six *Pseudomonas aeruginosa* isolates were collected. Identification of isolates and antimicrobial susceptibility testing were done using vitek-II machine. Carbapenem resistance was detected by modified Hodge Test then confirmed using multiplex PCR for the detection of *blaVIM, blaKPC, blaIMP, blaNDM-1, blaOXA-48, blaGIM, blaSPM* and *blaSIM* genes. **Results:** Thirty two (37.20%) strains were carbapenem-resistant *Pseudomonas aeruginosa*(CRPA) as detected by multiplex PCR. Among these 32 strains the resistance was 100% to imipenem, meropenem and ciprofloxacin. The isolates had least resistance to aztreonam, 21.88% and colistin, 31.25%. Among 32 CRPA isolates 23 (71.88. %) were multi-drug resistant, 19 (59.38%) were Extensively-drug resistant. PCR identified the presence of blaOXA-48 in 15 (46.88%) isolates, *blaVIM* gene in 10 (31.25%) isolates and *blaNDM* in 12 (37. 5%) isolates. On the other hand, *blaGIM, blaIMP* and *blaSIM* were only detected in 2 isolates for each and blaKPC detected in one isolate only. **Conclusion:** The prevalence of CRPA was high (37.2%). The appropriate study of the antimicrobial resistance molecular mechanisms will help in management of CRPA patients and implementation of infection control procedures.

Introduction

Pseudomonas aeruginosa (P. aeruginosa) is a major opportunistic bacterium that has been linked to a variety of illnesses in the health care facilitiesand community, includingbacteremia, wounds, respiratory tract infections, otitis media, eye infections and other hospital acquired infections [1].

For the management of multi-drug resistant (MDR) *P. aeruginosa* infection, carbapenems have been kept as a last choice. Carbapenem-resistant *P. aeruginosa* (CRPA), on the other hand, may be resistant to other antibiotic classes, and such infections are associated with restricted therapy options and increased rates of death and morbidity, particularly in immunosuppressed and hospitalized patients [2].

The Centers for Disease Control and Prev ention (CDC) has classified CRPA as an organism that leads to a serious consequences[3]. Carbapenem resistance in *P. aeruginosa* has been demonstrated to be multifactorial, it may be due to carbapenemase synthesis, efflux pump or gene overexpression[4].

DOI:10.21608/MID.2022.142569.1320
* *Corresponding author:*Enas SH Khater
E-mail address:drenaskhater@yahoo.com

Carbapenemases are classified into three categories: A, D (serine carbapenemases), and B (metallo—lactamases)[5]. Carbapenemases are classified into three categories: A, D (serine carbapenemases), and B (metallo—lactamases) [5]. Class A (*blaKPC*), class B (*blaVIM , blaIMP,blaNDM, blaSIM,blaSPM*and *blaGIM*), and class D (*blaOXA-48*) genes are examples of carbapenemase genes in *P. aeruginosa*

Carbapenemase genes are frequently found on mobile genetic elements that have the ability to spread quickly. Carbapenems are a type of beta-lactam that inhibits synthesis of cell wall of the bacteria by attaching to penicillin-binding proteins (PBPs). Imipenem, meropenem, ertapenem, and doripenem are some of the carbapenems that are available clinically. All B-lactams excluding monobactams, are resistant to metallo-beta lactamases (MBLs) [6].

As carbapenem resistance is difficult to be detected using the standard disc diffusion method, The Clinical and Laboratory Standards Institute (CLSI), 2022 [7] has recommended inhibition-based tests like the double-disk synergy assay, modified Hodge technique (MHT), and combination disk test as comprehensive phenotypic methods for carbapenemase detection. In addition, polymerase chain reaction (PCR) is recommended as a reliable approach for identifying the most common genes in carbapenemase positive clinical samples [8,9].

Aim of this study is the detection of resistance pattern of studied *P. aeruginosa* isolates to different antipseudomonal drugs and to determine the prevalence of carbapenem resistant *P. aeruginosa* among Al-Quwayiyah general hospital admitted patients as well as to detect the prevalence of carbapenemase genes; *blaKPC, blaNDM1, blaIMP, blaVIM, blaSIM, blaSPM, blaGIM,* and *blaOXA-48* among *P. aeruginosa*

Materials and Methods

This work was done in Al-Quwayiyah general hospital which is a general hospital in Riyadh, Saudi Arabia which has 250 beds capacity in the period from November 2021 to April, 2022in the microbiology department. *P. aeruginosa* isolates were collected from ICU, medical,surgical and pediatrics departments from various clinical specimens (blood, urine, sputum, sterile body fluids, pus and others), after receiving ethical approval from the Institutional Ethics Committee. The study included all admitted patients who had isolates of *P. aeruginosa* infections 48 hours after admission.

Bacterial strains

Eighty six *P.areuginosa*strains were isolated and identified by colony shape on MacConkey's and blood agar plates (oxoid, UK), gram stainand using the VITEK II machine (bioMérieux, Marcy l'Etoile, France) *P. aeruginosa*quality control ATCC strain 27853 was used.

Antimicrobial susceptibility testing

The VITEK 2 system was used to evaluate antimicrobial susceptibility to 10 antimicrobial drugs, including imipenem (IPM), meropenem (MEM), amikacin (AN), tobramycin (TM), ceftazidime (CAZ), cefepim(FEP), ciprofloxacin (CIP), colistin (CS), aztreonam (ATM) and piperacillin-tazobactam (TZP)was doneusing 291 AST card by VITEK II machine, and the interpretation of data was done according to the CLSI guidelines, 2022 [7].

Both imipenem and meropenem minimum inhibitory concentration (MIC) weredone for each strainby the use of an E-test (BioMérieux, France), that were carried out and interpreted asper the manufacturer's guidelines. *Pseudomonas aeroginosa* carbapenem resistance was defined whenimipenem or meropenem MIC was ≥2 mg/L as recommended by CLSI, 2022 guidelines [7]. Moreover, MDR was identified by the presence of resistance to minimally one antimicrobial drug in more than three different groups.*Pseudomonas aeruginosa* strains nonsusceptible to at least one agent in all antibiotic categories but only susceptible to two or less antimicrobial groups was defined as XDR[10,11].

MBL screening

All strains were tested for MBL production by a double-disk synergy test using10 μg of IPM and 2.5 μM ethylenediaminetetraacetic acid as described in CLSI, 2022 guidelines [7]. A positive result was interpreted if there is asynergistic inhibition zone seen between both disks.

Carbapenem Inactivation Method (CIM)

Carbapenem Inactivation Method was used to determine carbapenem resistance in *P. aeruginosa* isolates. *Escherichia coli* ATCC 25922 diluted culture (0.5 McFarland standard) was streakedin three different directions on the Mueller-Hinton agar plates, thenthe Meropenem disk (10 μg) (Oxoid,

Basingstoke, UK) was placed at each plate center, From the edge of the meropenem disk the tested strainwas streaked as a thin line to the plate edge . All plates were incubated for 18 hours at 37 °C. A positive MHT was interpreted if there was an indentation in the *E. coli* inhibition zone or clove growth of *E. coli* around the meropenem disk [7,12].

Molecular detection of carbapenemase gene

Multiplex polymerase chain reaction (PCR) was done for the detection of *blaVIM, blaKPC, blaIMP, blaNDM-1, blaOXA-48, blaGIM, blaSPM* and *blaSIM* was done as described in the techniquecarried out by **Weiß et al.** [13] and **Hamid et al.** [14].

QIAamp DNA Mini kit (QIAGEN, Hilden, Germany) was used to extractDNA from *P. aeruginosa*strainsaccording to manufacturer's guidelines. Multiplex PCR were carried outin 2 groups using KAPA2G Fast Multiplex PCR Kit (2X) according to manufacturer's guidelines (Kapa Biosystems- Roche diagnostics, Switzerland). The first set of multiplex PCR wasdone to detect *blaVIM, blaIMP,blaSIM, blaSPM,* and*blaGIM* and the second set of multiplex PCR tested the presence of *blaKPC,blaOXA*-48, and *blaNDM*-1. A final reaction volume of 25 µl was prepared in the PCR reactions which contained 12.5 µl of 2X KAPA2G Fast Multiplex Master Mix, 0.5 µl of every oligonucleotide primer andfive µl ofDNA template. Amplification was carried outby initial denaturation step for 3 min at 95°C, then denaturation for 15 sat 95°C, annealing for 30s at 60°C, extension for 30 sat 72°C, and finaly extension step for 7 minat 72°C. The products wereseenusing 1.5% agarose gel electrophoresis which was ethidium bromidestained, and was seenusing ultraviolet illumination under automated gel system (Syngene G: Box, Syngene, Cambridge, U.K.). primers were shown in **table (1)** as carried out by **Verma et al.** [15]

Statistical analysis

Analysis of the study data was carried outby the use of SPSS version 16 software. Data interpretation was done as percentages and numbers. _ Z ″ test was used to compare 2 variables and ″ χ2 _(Chi square) test was used to compare between more than two variables. Presence of *p* value <0.05 was interpreted as statistical significance.

Results

Eighty six *P. aeruginosa* strains were obtained from 61 (70.93%) males and 25 (29.07%) females. The strains were collected from different clinical samples that included 32urine samples(37.2%), followed by 26 sputum (30.2%), 22 pus sample (25.6%), 4 blood (4.66%), and 2 other samples (2.33%), Out of these 86 *P. areuginosa* strains, 32 (37.20%) were CRPA strains as detected by multiplex PCR Of these 32CRPA, 16 were collected from ICUs, 10 were from medical departments, 4 from surgical departments and 2 from pediatric department were from various admitted patients.Of 32 CRPA isolates, MHT was positive in 30 (93.75%).Out of the total 86 isolates there was 39 isolates were resistant to at least one carpabenem either imipenem or meropenem.

Table 2 showed that the antimicrobial susceptibility testing revealed that all 32 CRPAstrainsshowed resistance to imipenem and meropenem, the resistance was also 100% toCiprofloxacin,followed byamikacin and tobramycin 29 (90.63%),ceftazidime 28 (87.5%),piperacillin–tazobactam in 26(81.25%). The isolates was most susceptible to aztreonam, 21.88% and colistin,31.25%.Among 32 CRPA isolates 23 (71.88.%) were MDR, 19 (59.38%) were XDR.

Table 3the PCR detectedcarbapenemases encoding genes in 32 strains of the 39 proved imipnem or meropenem resistant strains. PCRidentified the presence of bla OXA-48 in 15 (46.88%), blaVIM gene in 10 isolates (31.25%), blaNDMcarbapenemases in 12 (37. 5%) isolates, 5 (15.63) *P. aeruginosa* isolates had blaNDM-1. On the other hand, blaGIM, blaIMP and blaSIMwere only detected in 2 isolates for each and blaKPC detected in one isolate only. Some strains had more than one gene as shown.

Figure 1 showed the Multiplexpolymerase chain reaction, in Lane 1 100 bp ladder was seen, Lane 5 showed *blaGIM*, 477 bp, *blaVIM*, 390 bp and *blaSPM* ,271 bp and Lanes 4, 6, and 8 had *blaVIM* .

Table 1. Primers used for different carbapenemase genes in the multiplex polymerase chain reaction.

Primers	Sequence	Productsize
VIM family-F	GAT GGT GTT TGG TCG CATA	390
VIMfamily-R	CGA ATG CGC AGC ACCAG	
IMP family-F	GGA ATA GAG TGG CTT AAY TCTC	188
IMP family-R	CCA AAC YAC TAS GTT ATCT	
GIM-1-F	TCG ACA CAC CTT GGT CTG AA	477
GIM-1-R	AAC TTC CAA CTT TGC CAT GC	
NDM-1 F	ACC GCC TGG ACC GAT GAC CA	264
NDM-1 R	GCC AAA GTT GGG CGC GGT TG	
OXA-48-F	TTGGTGGCATCGATTATCGG	744
OXA-48-R	GAGCACTTCTTTTGTGATGGC	
KPC-F	ATGTCACTGTATCGCCGTCT	893
KPC-R	TTTTCAGAGCCTTACTGCCC	
SPM-1A-F	AAA ATC TGG GTA CGC AAA CG	271
SPM-1A-R	ACA TTATCC GCT GGA ACA GG	
SIM-1-F	TAC AAG GGA TTC GGC ATC G	571
SIM-1-R	TAA TGG CCT GTT CCC ATG TG	

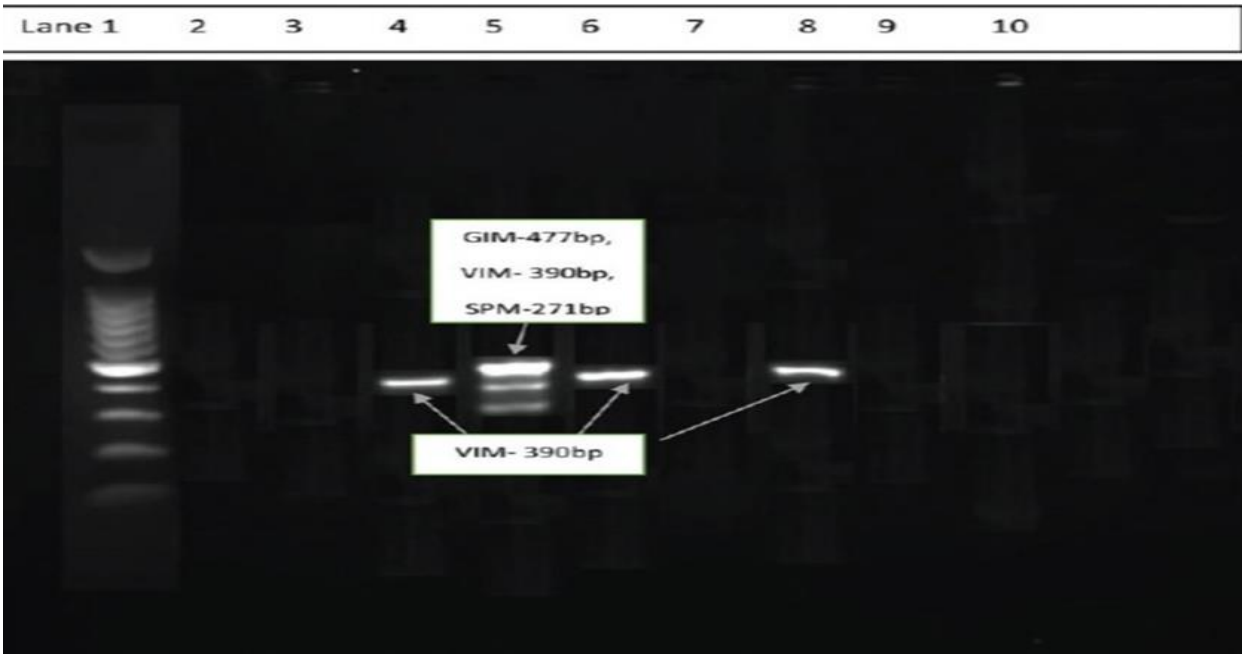
Table 2. Antimicrobial resistance rate among CRPA strains.

Antimicrobial agent	CRPA =32	
	No	%
Piperacillin-Tazobactam	26	81.25%
tobramycin	29	90.63%
Imipenem	32	100%
Meropenem	32	100%
Ceftazidime	28	87.5%
Cefepime	20	62.5%
Amikacin	29	90.63%
Ciprofloxacin	32	100%
Colistin	10	31.25%
Aztreonam	7	21.88%

Table 3. Carbapenem-resistant genes distribution in *P.aeroginosa* strains among studied groups

Genes identified	No of isolates = 32	Percentage
bla OXA-48	15	46.88%
blaVIM	10	31.25
blaNDM	5	15.63
blaKPC	1	3.13%
blaGIM	2	6.26%
blaSIM	2	6.26%
blaIMIP	2	6.26%
blaSPM	1	3.13%
blaVIM+blaNDM-1	2	6.26%
blaVIM+blaGIM	1	3.13%
bla OXA +blaNDM-1	1	3.13%
blaVIM+blaGIM+blaNDM-1	1	3.13%
bla OXA+blaSPM+blaNDM-1	1	3.13%

Figure 1. Multiplex chain reaction showing *blaVIM*, blaGIM, blaSPIM**.**



Discussion

The increase in the use of carbapenems in management of health care associated infections is believed to be associated to the presence of increased carbapenem resistancein *P. aeruginosa* [16].

Eighty six *P. aeruginosa* strains were isolated.Out of these 86 *P. areuginosa* strains, 32 (37.20%) were CRPA isolates as detected bymultiplex PCR. In Saudi Arabia, **Al-Agamy et al.** [17] noted that the prevalence of carbapenem-resistant *P. aeruginosa* was 34%. **Abd alhamid et al.** [18] also studied the prevalence of CRPA in two

hospitals among the ICU patients and it was also high as he reported the prevalence of CRPA rate 45.1%. In Egyptian study, which found that 32.3%) of strains were MBL-positive [19]. **Haji et al.** [20] reported 18% in their study and the incidence of prevalence in India varies from 14-69%. The difference in the prevalence of CRPA isolates was mostly due tothe number of antibiotics used, infection control practices and infrastructure of hospitals [21].

Multidrug resistantand XDR *P. aeruginosa* infection is a great medical concern because it is a significant reason of life-threatening healthcare-associated infections such as urinary tract infections,bacteremia, ventilator-associated pneumonia and, as well as tissue and wound soft-infections, which cause high fatality[22].

The strains were collected from various clinical samples that included32 urine samples (37.2%), followed by 26 sputum (30.2%), 22 pus sample (25.6%), 4 blood (4.66%), and 2 other samples (2.33%). **Rabani et al.** [23]also reported that *P. aeruginosa* is a major cause of urinary tract infections,bacteremia, respiratoryinfections, surgic al site infections and many other systemic diseases,he noted in his study that the most common type of specimen from which strains were obtained was urine, that represented about 67%. In Saudi Arabia it was isolated from 88.9% urine samples, thus describing the difficulty in treating urinary tract infections caused by MDR *P. aeruginosa*[24].

In this study the antimicrobial susceptibility testing revealed that all 32 CRPA strains were resistant to imipenem and meropenem, the resistance was also 100% in Ciprofloxacin, followed by amikacin and tobramycin 29 (90.63%), ceftazidime 28 (87.5%), piperacillin–tazobactam in 26 (81.25%). Similar results were obtained from near countries;in Bahrain, therate of resistance 72–100% was reported to ceftazidime, carbapenems, Amikacin,piperacillintazobactamandciprofloxacin[25]. In an Egyptian research by **El Far et al.** [26], he showed that the resistance rates of CRPA isolates to fluoroquinolones were from 91–94%, while ceftazidimeresistance was 69.7%, cefepime resistance was 88%, carbapenems resistance was 81.8% and tazobactam–piperacillin resistance was 63.3%. The increased rate of the resistance to anti-pseudomonal drugs, mostlycarbapenems, fluoroquinolones and aminoglycosides has been attributed to the emergence of *P. aeruginosa* MDR/XDR strains, leading toa dangerous and life-threatening medical conditions. [27,28]

In this study the CRPA isolates was most susceptible to aztreonam, 21.88% and colistin, 31.25%. **Basha et al.** [29] showed also similar results that aztreonam was the most effective antibiotic and Al Far et al,2021 [26] reported 21% resistance toaztreonam in Saudi Arabia, although 100% susceptibility to colistin in *P. aeruginosa* strains was revealedby **Saeed et al.** [30] **Ramadan et al.** [31] and **Azim et al**. [32] revealed that 8% Pseudomonas aeruginosa strains showed resistance to colistin. Moreover, **Ibrahim et al.** [33] noted that colistin resistance was 30% among *P. aeruginosa* isolates

The bacterial phenotypes XDR and MDR *P. aeruginosa* have led to a lot of attention all over the world; this bacterial phenotype might be the cause of diseases with a high death rate, making treatment challenging [34]. In the current study among 32 CRPA isolates 23 (71.88. %) were MDR, 19 (59.38%) were XDR. **El Far et al.** [26] reported approximately 79 %% of the *P. aeroginosa* strains were MDR and revealed that XDR among carbapenem-resistant isolates was 55%.high MDR detection also found by **El-Sokkary et al.** [35] in Egypt, he reported that 65.2% of the studied *P. aeruginosa* strains were multidrug resistant.A study from Greece reported high rates of MDR and XDR isolation, that 88.9% and 79% of the *P. aeruginosa* strains were MDR and XDR respectively, whilelessstrains from Spain (33.3%) and Italy (43.5%) showed antibiotic resistance[36]. The increased rate of MDR and XDR *P. aeroginosa* strainsreported in this study may be caused by the improper use of antibiotics that urge the need for strict monitoring and applying proper antibiotic use strategies[37].

In this study PCR identified the presence of bla*OXA*-48 in 15 (46.88%), *blaVIM* gene in 10 isolates (31.25%), *blaNDM*carbapenemases in 12 (37. 5%) isolates, 5 (15.63%) *P. aeruginosa* isolates had *blaNDM*-1. On the other hand, *blaGIM*, *blaIMP* and *blaSIM* were only detected in 2 (6.25%) isolates for each and blaKPC detected in one isolate only.**Jaffar et al.**2022[38] in Saudi Arabia reported similar results, he noted that *P. aeruginosa* oxacillinase-48 (OXA-48) was detected in 41.5%, *blaNDM* genes were found in22.5%The other carbapenemases were low detected, *blaIMIP* were

2%, and *blaKPC* were %. The current results also matched with **Zowawi et al.** [39]in Saudi Arabia who found that the major determined carbapenemases were *OXA*-48 (49%) and *NDM* (23%), while *KPC* or *VIM* or *IMP* not reported in any isolate.One studyin Meccabetween 2009 and 2010 conducted by **Asghar** [40] reported high*IMP* than *VIM* among MBL-producing *P. aeruginosa*, *IMP*genes were detected in 22.6 % while *VIM* genes were detected in 19.4 %. However, other reports from Riyadh suggest that *VIM* is the dominant carbapenemase in *P. aeruginosa* from that region, **AL-Tawfik** [41] identified *VIM* in 42.8 % of the isolates. *IMP*, *GIM, SIM, SPM* and *NDM* were not detected which coincoides with low incidence described in the current study for same genes. Another study which was done by **Tian et al.**[42] revealed that the highly detected gene was *blaOXA* (42%), then*blaNDM* (37%), followed by *blaKPC* (17%) and the least was *blaIMP* (1%). A study carried out in India on CRPA noted that the major detected carbapenemase was *blaVIM* (29%), followed *blaNDM* (28%) and the least were *blaSIM* and *blaGIM*, 5% for each [15].

Conclusion

The prevalence of resistance to carbapenems among *P. aeruginosa* was increasing (37.2%).The serious condition of decreased carbapenem susceptibility in *P. aeruginosa* has been recognized throughout the last years in Saudi Arabia and worldwide, indicating the need for properanalysis of the problem. An appropriate study of the antimicrobial resistance molecular mechanisms will help in management of CRPA patients and implementation of infection control procedures

Ethical approval

Ethical approval was obtained from institutional ethics committee of Al-Quwayiyh General Hospital

Acknowledgement

The authors wish to express their gratitude to the staff of the microbiology laboratory of Al Quwayiyah General Hospital for their cooperation during study.

Conflict of interest

The authors report no conflicts of interest**.**

Financial disclosures: Non declared.

Authorship

Both authors have worked together to complete this research. Author ESK planned and designed the study, prepared the protocol, gathered the samples, contributed in the interpretation and analysis of the findings, drafted and critically revised the paper. Author AAAF was involved in the study's planning and design, sample collection, clinical evaluation of cases and interpretation of the data. The final manuscript was reviewed and approved by both authors.

References

1- **Lister PD, Wolter DJ, Hanson ND** Antibacterial-resistant *Pseudomonas aeruginosa:* Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 2009; 22: 582-610.

2- **Hong DJ, Bae IK, Jang IH, Jeong SH, Kang HK, Lee K.** Epidemiology and characteristics of metallo-β-lactamase-producing *Pseudomonas aeruginosa.* Infect Chemother 2015;47: 81-97.

3- **Villegas MV, Lolans K, Correa A, Kattan JN, Lopez JA, Quinn JP.** First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing beta-lactamase. Antimicrob Agents Chemother 2007.;51: 1553-1555.

4- **Pragasam AK, Veeraraghavan B, Anandan S, Narasiman V, Sistla S, Kapil A, et al.** Dominance of international high risk clones in carbapenemase producing *Pseudomonas aeruginosa*: Multicentric molecular epidemiology report from India. Indian J Med Microbiol 2018;36:344 51

5- **Queenan AM, Bush K.** Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 2007;20(3):440–458.

6- **Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, et al.** Epidemiology of Carbapenem Resistant Enterobacteriaceae Infections: Report from the China CRE Network. Antimicrob. Agents Chemother 2017; 62.

7- **Clinical and Laboratory Standards Institute. Performance Standards for** Antimicrobial Susceptibility Testing. 32nd ed.CLSI supplement M100 Clinical and Laboratory Standards Institute, Wayne, PA. 2022

8- **Solanki R, Vanjari L, Subramanian S, Aparna B, Nagapriyanka N, Lakshmi V.** Comparative evaluation of multiplex PCR and routine laboratory phenotypic methods for detection of carbapenemases among gram negative bacilli. J Clin Diagn Res 2014;8:DC23–DC26.

9- **Yezli S, Shibl AM, Livermore DM, Memish ZA.** "Prevalence and antimicrobial resistance among Gram-negative pathogens in Saudi Arabia" . J Chemother 2014; 26:257-272.

10- **Mirzaei B, Bazgir ZN, Goli HR, Iranpour F, Mohammadi F, Babaei R.** Prevalence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) phenotypes of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated in clinical samples from Northeast of Iran. BMC Res Notes 2020;10:13(1):380.

11- **Kunz Coyne, A.J., El Ghali, A., Holger, D. et al.** Therapeutic Strategies for Emerging Multidrug-Resistant *Pseudomonas aeruginosa*. Infect Dis Ther 2022; 11: 661–682 .

12- **Tamma PD, Opene BN, Gluck A, Chambers KK, Carroll KC , Simner PJ.** Comparison of phenotypic assays for accurate detection of carbapenemase- producing Enterobacteriaceae. J Clin Microbiol 2017;55:1046–55.

13- **Weiß D, Engelmann I, Braun SD, Monecke S, Ehricht R.** A multiplex real-time PCR for the direct, fast, economic and simultaneous detection of the carbapenemase genes *blaKPC*, *blaNDM*, *blaVIM* and *blaOXA-48*. J Microbiol Methods 2017;142:20–6.

14- **Hamid OM, Omer MO, Magdi AB.** 'Prevalence of Carbapenemase Encoding Genes and Antimicrobial Resistance Pattern of Enterobacteriaceae Isolated from Hospitalized Patients in Khartoum State, Sudan.' American Journal of Infectious Diseases and Microbiology 2021; 9.2: 39-47.

15- **Verma N, Kumar AP, Mishra B, Behera B, Gupta K.** Detection ofcarbapenemase-producing *Pseudomonas aeruginosa* by phenotypic and genotypic methods in a tertiary care hospital of East India J Lab Physicians 2019; 11(4): 287–291.

16- **Sawa T, Kooguchi K, Moriyama K.** Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. Journal of intensive care 2020; 8(1):13.

17- **Al-Agay MH et al.** High prevalence of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* from Saudi Arabia. Journal of Chemotherapy (Florence, Italy) 2009; 21:461–462.

18- **Abdalhamid B, Elhadi N, Alabdulqader N, et al.** Rates of gastrointestinal tract colonization of carbapenem-resistant Enterobacteriaceae and *Pseudomonas aeruginosa i*n hospitals in Saudi Arabia. New microbes and new infections 2016;10:77-83.

19- **Fatma AA, et al**. Imipenem resistance and BLAIMP gene among hospital strains of *Pseudomonas aeruginosa* at university hospital

in Egypt. Journal of Infection in Developing Countries 2007; 1:42–47.

20-Haji SH,Aka STH, Ali FA. Prevalence and characterisation of carbapenemase encoding genes in multidrug-resistant Gram-negative bacilli. PLoS One 2021; 16: e0259005.

21-Mohammed MU, Manisha DR , Nagamani K. Clinical, phenotypic and genotypic profile of carbapenem resistant Gram negative infections in intensive care units. Indian J Microbiol Res 2021; 8: 28–34.

22-El-Mahdy R, El-Kannishy G. Virulence factors of carbapenem-resistant *pseudomonas aeruginosa* in hospital-acquired infections in Mansoura, Egypt. Infect Drug Resist 2019;12:3455–3461.

23-Rabani Z, Mardaneh J. Emergence of Multidrug-Resistant Pseudomonasaeruginosa: Detection of Isolates harboring blaCTX gene causing infections in hospital and determination of their susceptibility to antibiotics. Armaghanedanesh 2015; 20(8): 689-705.

24-Al Wutayd O, Al Nafeesah A, Adam I, Babikir IH. The antibiotic susceptibility patterns of uropathogens isolated in Qassim, Saudi Arabia. J Infect Dev Ctries 2018;12(11):946–952.

25-Balkhy HH, El-Saed A, El-Metwally A, Arabi YM, Aljohany SM, Al Zaibag M Antimicrobial consumption in five adult intensive care units: a 33-month surveillance study Antimicrob Resist Infect Control 2018;:" 156

26-El-Far A, Samir S, El-Gebaly E, Omar M, Dahroug H, El-Shenawy A, Soliman NS , Gamal D. High Rates of Aminoglycoside Methyltransferases Associated with Metallo-Beta-Lactamases in Multidrug-Resistant and Extensively Drug-Resistant Pseudomonas aeruginosa Clinical Isolates from a Tertiary Care Hospital in Egypt. Infect Drug Resist 2021; 19:14:4849-4858.

27-Pérez A, Gato E, Pérez-Llarena J, et al. High incidence of MDR and XDR *Pseudomonas aeruginosa* isolates obtained from patients with ventilator-associated pneumonia in Greece, Italy and Spain as part of the MagicBullet clinical trial. J AntimicrobChemother 2019;74(5):1244–1252.

28-Al-Orphaly M, Hadi HA, Eltayeb FK, et al. Epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in the Middle East and North Africa Region. mSphere 2021;6(3).

29-Basha AM, El-Sherbiny GM, Mabrouk MI. Phenotypic characterization of the Egyptian isolates "extensively drug-resistant *Pseudomonas aeruginosa*" and detection of their metallo-β-lactamases encoding genes. Bull National Res Centre 2020;44(1).

30-Saeed NK, Kambal AM, El-Khizzi NA. Antimicrobial-resistant bacteria in a general intensive care unit in Saudi Arabia Saudi Med J 2010; 1341-1349.

31-Ramadan RA, Gebriel MG, Kadry HM, Mosallem A. Carbapenem resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: characterization of carbapenemase genes and E-test evaluation of colistin-based combinations Infect Drug Resist 2018; 1261.

32-A. Azim NS, Al-Harbi MA, Al-Zaban MI, Nofal MY, Somily A. Prevalence and Antibiotic Susceptibility Among Gram Negative Bacteria Isolated from Intensive Care Units at a Tertiary Care Hospital in Riyadh Saudi Arabia. J Pure Appl Microbiol 2019; 201-208.

33-Ibrahim MA. High antimicrobial resistant rates among Gram-negative pathogens in intensive care units: A retrospective study at a tertiary care hospital in Southwest Saudi Arabia Saudi Med J (2018);(10):39: 1035-1043

34-De Rosa A, Mutters NT, Mastroianni CM, Kaiser SJ, Günther F. Distribution of carbapenem resistance mechanisms in clinical isolates of XDR *Pseudomonasaeruginosa*. Eur J Clin Microbiol Infect Dis 2019; 38:1547–1552.

35-El-Sokkary RH, Ramadan RA, El-Shabrawy M, El-Korashi LA, Elhawary A, Embarak S, et al. Community Acquired Pneumonia among Adult Patients at an Egyptian University Hospital: Bacterial Etiology, Susceptibility Profile and Evaluation of the Response to Initial Empiric Antibiotic Therapy. Infect. Drug Resist 2018; 11: 2141–2150.

36-ECDC. Surveillance of Antimicrobial Resistance in Europe 2016. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net)Stockholm, Sweden ECDC 2017; 1–88.

37-Ellappan K, Belgode Narasimha H, Kumar S. Coexistence of multidrug resistance mechanisms and virulence genes in carbapenem-resistant *Pseudomonas aeruginosa* strains from a tertiary care hospital in South India. J Glob Antimicrob Resist 2018;12:37–43.

38-Jaffar A Al-Tawfiq, Ali A Rabaan, Justin V Saunar, Ali M Bazzi. (Genotypes and prevalence of carbapenemase-producing Enterobacteriaceae and *Pseudomonas aeruginosa* in a hospital in Saudi Arabia.Trans R Soc Trop Med HygTransactions of the Royal Society of Tropical Medicine and Hygiene) 2022; 116(1):50-53.

39-Zowawi HM, Sartor AL, Balkhy HH, Walsh TR, Al Johani SM, AlJindan RY, et al. Molecular Characterization of Carbapenemase-Producing *Escherichia coli* and *Klebsiella Pneumoniae* in the Countries of the Gulf Cooperation Council: Dominance of OXA-48 and NDM Producers.Antimicrob. Agents Chemother 2014; 58:3085–3090.

40-Asghar A. Antimicrobial susceptibility and metallo-β-lactamase production among Pseudomonas aeruginosa isolated from Makkah hospitals, Pakistan Journal of Medical Sciences Online 2012.

41-Al-Tawfiq JA. Changes in the pattern of hospital intravenous antimicrobial use in Saudi Arabia, 2006–2008.Ann Saudi Med 2012; 32 (5) : 517-520.

42-Tian D, Pan F, Wang C, Sun Y, Zhang H. Resistance Phenotype and Clinical Molecular Epidemiology of Carbapenem-Resistant Klebsiella Pneumoniae among Pediatric Patients in Shanghai. Infect. Drug Resist 2018; 11: 1935–1943.