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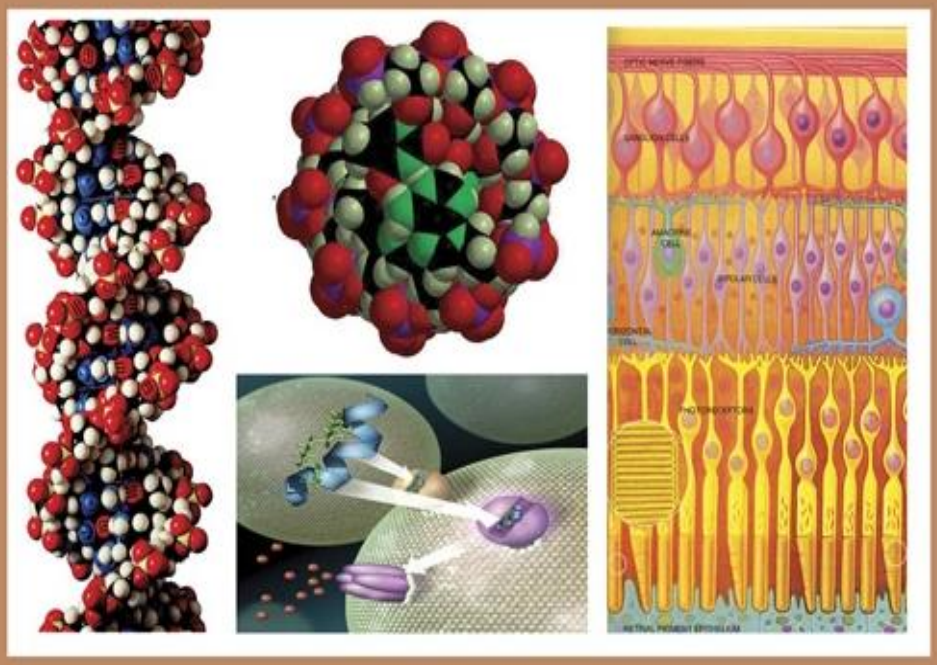

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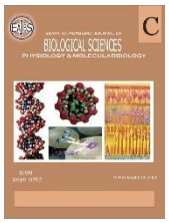

Phenotypic Detection of Metallo-B Lactamase Producing Carbapenem-Resistant *Pseudomonas Aeruginosa*

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ABSTRACT

The opportunistic bacteria Pseudomonas aeruginosa is commonly linked to skin infections. When dealing with P. aeruginosa, carbapenem is your best bet. It is of international concern because beta-lactamase synthesis might lead to carbapenem resistance. Among all the beta-lactamases, Metallo-Beta lactamases are the most versatile. The purpose of this study was to isolate metallo—lactamase-producing P. aeruginosa from wound infections. One hundred and twenty samples from patients with burn wound infections were collected and tested using conventional microbiological methods for the presence of P. aeruginosa. Using species-specific primers against the oprL and Oprl genes of P. aeruginosa, a polymerase chain reaction was used to conduct a molecular characterisation of P. aeruginosa. Antimicrobial susceptibility testing was conducted using the Kirby Bauer disc diffusion technique. Certain primers were used in a polymerase chain reaction to detect P. aeruginosa carrying the carbapenemase gene. Using the modified carbapenem inactivation technique and the EDTA carbapenem inactivation approach, we were able to phenotypically identify metallo-beta-lactamase-expressing genes. Carbapenemase-encoding genes' sensitivities and specificities were determined. Forty-six out of a possible one hundred P. aeruginosa (38%) were successfully recovered, with their identity validated by a polymerase chain reaction (PCR) experiment. The highest level of resistance was discovered against Cefepime (87%) and the lowest level of resistance was recorded against colistin (33%). A total of 25 (54%) were multidrug-resistant isolates. Out of 46, 35(76%) were confirmed for carbapenemase production by performing PCR. The prevalence of carbapenemase encoding genes was as follows: blaSPM (14%), blaVIM (25.7%), blaNDM (40%), blaKPC (2.85%) and blaIMP (17%). The modified carbapenem inactivation method showed 91.42% positive results and eCIM showed 90.62% positive results. Phenotypic detection showed more sensitivity and less specificity. Results concluded that mCIM and eCIM were considered a less expensive, more sensitive and suitable method to distinguish class A and class B of carbapenemase-producing *P. aeruginosa.*

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, aerobic bacillus, pigment-producing bacterium (Aabed and Mohammed, 2021). *Pseudomonas aeruginosa* is an opportunistic as well as nosocomial pathogen (Abbas *et al*., 2019).

It is a non-lactose fermenter, that has flat, large greenish colonies, which have a pearlescent appearance and fruity smell (Birla *et al*., 2003). It has a circular chromosome, which is quite large comprising of 5.5 to 6.8 million base pairs approximately. Its G + C content is 65 to 67%. *Pseudomonas aeruginosa* can thrive in extreme environments because of its ability to withstand moisture (Abdolhosseini *et al*., 2019; Bose and Chatterjee, 2016). A wide spectrum of infections caused by *P. aeruginosa* in a clinical environment and community including bacteremia, ear infection, lung infection, eyes infection, pneumonia, UTI and surgical site infection, and it causes significant mortality and morbidity (Habash *et al*., 2017). It also causes wound-associated infection, infection of joints, and gastrointestinal tract infection. Patients with cystic fibrosis are at great risk (Habash *et al*., 2014; Abdulhaq *et al*., 2020). Carbapenem has emerged as an important antibacterial agent for the clinical treatment of extreme infections affected by *P. aeruginosa*. *P. aeruginosa* resistant to carbapenem is a developing global issue (Ibraheem *et al*., 2019). It is a great challenge to treat infection caused by *P. aeruginosa*, as it recognizes as a "Superbug" because of its increasing tendency of developing resistance to almost every drug used (Tasleem *et al*., 2022). *P. aeruginosa* is listed as a priority pathogen and an Urgent Threat Pathogen according to World health organization, as well as a center for disease control and Prevention (Abdullah *et al*., 2021).

Intrinsic resistance pathways are significantly more common, and carbapenem-resistant *P. aeruginosa* frequently exhibits the following: Overexpression of chromosomal cephalosporins, diminished expression or deletion of OprD outer membrane protein, by losing prions i.e. OprD as well as by producing enzymes that inactivate antibiotics e.g β-lactamase, extended-spectrum β-lactamase AmpC β-lactamase and Metallo-β-lactamase and hyperexpression of efflux pump systems, which quickly pumps antibiotics outside of the cell (Abo-Shama *et al*., 2020; Ali *et al*., 2020). The resistance mechanism also includes the production of serine and metallo-β-lactamase which are the classes of β-lactamase (Dupuy *et al*., 2018). Beta lactamases trigger the hydrolysis of antibiotics by cleaving an amide bond present in their β-lactam ring i.e. penicillin, carbapenem, cephalosporins, etc. (Aabed and Mohammed, 2021). For the development of acquired resistance, horizontal gene transfer or mutational mechanisms are used (Ijaz *et al*., 2019; Jaiswal and Mishra, 2018).

Metallo-β-lactamases are extracellular or periplasmic enzymes generated by bacteria. Metal binding sites are preserved in all known representatives and zinc ions are essential as enzyme cofactors. These enzymes can break down all β-lactam antibiotics except monobactames and have a particular carbapenemase activity that is continuous and efficient. Furthermore, therapeutic β-lactamase inhibitors have little effect on metallo-β-lactamases (Bayroodi and Jalal, 2016). Metallo-β-lactamases catalyze the same chemical process as serine enzymes but with one or two divalent cations (Zn^{2+}) coordinated to water molecules as the reactive nucleophiles.

Carbapenemase has been identified using a variety of phenotypic and genotypic methods (Danjuma and Abdullahi, 2020). Phenotypic tests detect carbapenemase activity allowing for the detection of previously unknown carbapenemase. mCIM and eCIM techniques are such phenotypic assays (Brink, 2010). For detecting carbapenemase synthesis, the mCIM exhibits higher sensitivity than the CIM in *Enterobacteriaceae* and glucose-nonterminating Gram-negative bacteria, the test is the simplest procedure, especially in a laboratory with low resources. The mCIMs employed to recognize carbapenemase synthesis in Gram-negative bacteria, on the other hand, can be enhanced (Boyanova, 2018).

The capacity of EDTA to inhibit metallo-β-lactamase activity has been validated for the identification of *Enterobacteriaceae* that produce MBL (Hari *et al*., 2014). To distinguish metallo-β-lactamase-producing *enterobacteriaceae*, the eCIM is simple to use and can be applied easily in any medical laboratory (Haghi *et al*., 2018). The primary goal of the current investigation was isolation, identification, molecular characterization and Antibiogram analysis of *P. aeruginosa* as well as phenotypic detection of carbapenem-resistant *P. aeruginosa* that has the ability to produce metallo beta-lactamase by utilizing mCIM and eCIM.

MATERIALS AND METHODS

Ethical Permission:

Before beginning the study project, approval from the University's ethical review committee was obtained and prior to collecting samples, a formal authorization from the hospital was also acquired.

Sample Collection:

One hundred and twenty samples were taken from patients at Allied Hospital Faisalabad who had an underlying burn wound infection. Specimens were collected by employing a sterile cotton swab (El-Deeb *et al*., 2020).

Pseudomonas aeruginosa Isolation And Identification:

Swabs samples were streaked via the streak plate method on Pseudomonas cetrimide and incubated at 37^{o}C for 24 hours for the isolation of *P. aeruginosa* (Duran *et al*., 2010). colony morphology, Pigment production, gram staining, and biochemical tests such as the indole test, citrate utilisation test, oxidase, catalase, and the triple sugar iron test were used to determine the identities of the isolates (Ahmed and Dablool, 2017). Biochemical tests were also done for the identification of *Pseudomonas aerugniosa*.

DNA Extraction and Molecular Characterization of *Pseudomonas aeruginosa:*

All isolates were subjected to DNA extraction by boiling method (Farva and Bhutta, 2021). The isolated DNA was polymerized using species-specific primers (Table 1) targeting the oprL and oprl genes. The conditions used in the polymerase chain reaction (PCR) consisted of an initial denaturation at 94 degrees Celsius for 4 minutes, followed by 30 cycles of denaturation for 45 seconds, annealing at 57 degrees Celsius for 1 minute, and polymerizing at 72 degrees Celsius for 1 minute, and finally an extension cycle at 72 degrees Celsius for 5 minutes. The gel doc system was used on an ethidium bromide-stained 1% agarose gel to see PCR amplification results (Boyanova, 2018; Khan and Hariri, 2023).

Table 1: Primer Sequence Of Specie Specific Genes.

Targeted gene	Forward primer	Reverse primer	Product size (bp)	Reference
Oprl	5'-ATGAACAACGTTCTGAAATT CTCTGCT-3'	5'-CTTGCGGCTGGCTTTT TCCAG-3'	249bp	(Fadwa *et al*., 2021)
oprL	5'-ATG GAAATGCTGAAATTCGGC-3'	5'-CTTCTTCAGCTCGACG CGACG-3'	504bp	[40]
blaSPM	5-CCTACAATCTAACGGCGACC-3	5-TCGCCGTGTCCAGGTA TAAC-3	674	(Momenah *et al*., 2023)
blaVIM	5'-GTT TGG TCG CAT ATC GCA AC-3'	5'-AAT GCG CAG CAC CAG GAT AG-3'	382	(Jalal *et al*., 2023)
blaIMP	5'-GAA GGA GTT TAT GTT CAT AC-3'	5'-GTA CGT TTC AAG AGT GAT GC-3'	587	
blaKPC	5'-CGT CTA GTT CTG CTG TCT TG-3'	5'-CTT GTC ATC CTT GTT AGG CG-3'	798	
blaNDM	5'-GGT TTG GCG ATC TGG TTT TC-3'	5'-CGG AAT GGC TCA TCA CGA TC-3'	621	

Antimicrobial Susceptibility Test:

All isolates were subjected to detect antibiogram analysis by utilization of Kirby-Bauer disc diffusion assay in accordance with CLSI 2020 recommendations (Almalah *et al*., 2019). Mueller Hinton agar was used for an antimicrobial profile of different antibiotics given in Table 2.

Table 2: Interpretive Criteria For Zone Of Inhibition Of Different Antibiotics Against *P.Aeruginosa.*

Class of antimicrobial drugs	Drug name	Disc content (µg)	Disc diameter		
			Sensitive	Resistant	Intermediate
Polymyxin	Colistin	10	≥15	≤12	13-14
Aminoglycoside	Tobramycin	30	≥15	≤12	13-14
	Amikacin	30	≥17	≤14	15-16
	Gentamicin	10	≥15	≤12	13-14
Carbapenemase	Meropenem	10	≥19	≤15	16-18
	Imipenem	10	≥19	≤15	16-18
Cephalosporins	Cefoxitin	30	≥18	≤14	15-17
	Cefepime	30	≥18	≤14	15-17
	Ceftazidime	30	≥18	≤14	15-17
	Ceftriaxone	30	≥18	≤14	15-17
Fluoroquinolones	Ciprofloxacin	5	≥25	≤18	19-24
Penicillin	Ampicillin	25	≥17	≤13	12-16
	Piperacillin	100	≥21	≤14	15-20

Molecular Detection Of Carbapenemase in *Pseudomonas aeruginosa:*

For the molecular characterization of carbapenemase-producing *P. aeruginosa* PCR was performed. Carbapenemase-encoding genes blaIMP, blaSPM, blaNDM, blaVIM and blaKPC were identified by utilizing specific primers (Table 1). Initial denaturation was carried out at 94 degrees Celsius for 4 minutes, annealing was carried out at 52.3 degrees Celsius for 45 seconds, at 94 degrees Celsius for 35 cycles of 1 minute, and extension was carried out at 72 degrees Celsius for 4 minutes (Jalal *et al*., 2023).

Phenotypic Detection By Modified Carbapenem Inactivation Method:

With the help of a sterilized inoculated loop 10ul loopful of *Pseudomonas aeruginosa* colonies were streaked in 2ml of Trypticase soy broth. A 10-ug meropenem disc was inserted in the inoculated tube after the mixture was vortexed. Tubes were incubated for 4 hours. Just before the 4-h carbapenem inactivation step, an mCIM indicator bacteria mixture (Escherichia coli ATCC 25922, a carbapenem-susceptible strain) with turbidity approximating the 0.5 McFarland standard was prepared, and the surface of an MHA plate was inoculated following the procedure for standard disc diffusion susceptibility testing. The meropenem (MEM) disc was taken from the TSB bacterial solution using a 10l inoculating loop and dragged down the tube's edge to remove surplus liquid before being put on the infected MHA plate and incubated inverted for 18 to 24 hours at 35°C in the ambient atmosphere (Haghi *et al*., 2018). The Outcome Was Seen.

EDTA Carbapenem Inactivation Method:

EDTA-modified carbapenem inactivation method test was carried out with each isolate (10-ul loopful of *P. aeruginosa*) and homogenized in a 2-ml aliquot of TSB-EDTA (EDTA concentration, 5 mM). The procedure was the same as in mCIM (Hari *et al*., 2014). Results were observed

Data Analysis:

The sensitivity and specificity of mCIM and eCIM were calculated by utilizing [https://www.medcalc.org/calc/diagnostic_te

st.php], an internet-based calculator.

RESULTS

Isolation and Identification of *Pseudomonas aeruginosa:*

Out of 120 burn wound samples, 46(38%) were positive for *P. aeruginosa* by examining the results of gram staining that showed *P. aeruginosa* is gram-negative bacteria and results of different biochemical tests were also observed.

Molecular Characterization of *Pseudomonas aeruginosa*:

A total of 46 isolates were subjected to molecular characterization of *P. aeruginosa*. All isolates were confirmed positive for *P. aeruginosa* by observing the results of PCR (Fig. 1).

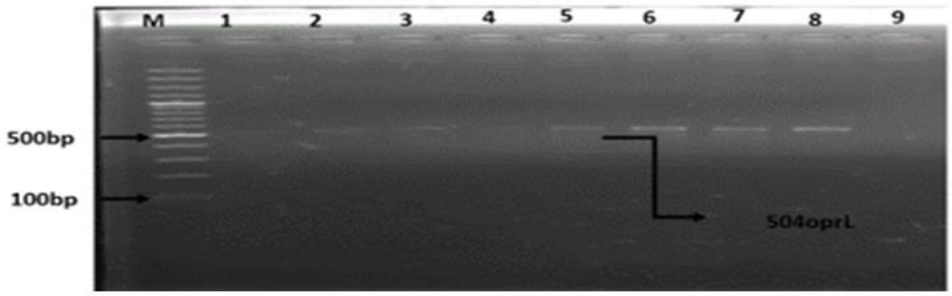

Fig. 1: molecular characterization of Pseudomonas aeruginosa: Polymerase chain reaction based detection of P.aeruginosa oprL (504bp); ladder size 100bp, lane 2-8: positive samples

Antimicrobial Susceptibility Test:

In the current study, different antibiotics were used. The highest resistance was observed against Cefepime (87%) and the least resistance was observed against colistin (33%). The resistance pattern of isolates against different antibiotics is shown in detail in Table 3. Prevalence of MDR *P. aeruginosa* was found 54%.

Table 3: Antibiogram analysis of different antibiotics against *P. aeruginosa.*

Class of antimicrobial drugs	Antibiotics	Sensitive (%)	Resistant (%)	Intermediate (%)
Polymyxin	Colistin	26 (56%)	15 (33%)	5 (11%)
Aminoglycoside	Tobramycin	5 (11%)	39 (85%)	2 (4%)
	Amikacin	11 (24%)	28 (61%)	7 (15%)
	Gentamicin	5 (11%)	38 (83%)	3 (6%)
Carbapenemase	Meropenem	11 (24%)	26(57%)	9 (19%)
	Imipenem	7 (15%)	35 (76%)	4 (9%)
Cephalosporin	Cefoxitin	10 (22%)	30 (65%)	6 (13%)
	Ceftriaxone	8 (17%)	32 (70%)	6 (13%)
	Cefepime	4 (9%)	40 (87%)	2 (4%)
	Ceftazidime	6 (13%)	36 (78%)	4 (9%)
Fluoroquinolones	Ciprofloxacin	10 (22%)	30 (65%)	6 (13%)
Penicillin	Ampicillin	9 (20%)	31 (67%)	6 (13%)
	Piperacillin	12 (26%)	27 (59%)	7 (15%)

Molecular Identification of Carbapenemase in *P.aeruginosa*:

All 46 isolates were subjected to the identification of carbapenemase-producing *P. aeruginosa* by utilizing PCR (Fig. 2 a, b, c, d, e). Out of 46, 35(76%) prevalence of carbapenemase encoding genes were noted as follows: blaOXA 5(14%), blaVIM 9(25.7%), blaNDM 14 (40%), blaKPC 1(2.85%) and blaIMP 6 (17%) (Table 4).

Table 4: Prevalence of carbapenemase-producing *P. aeruginosa*.

Name of gene	Number of positive samples	Percentage
blaSPM	5	14%
blaVIM	9	25.7%
blaNDM	14	40%
blaKPC	1	2.85%
blaIMP	6	17%

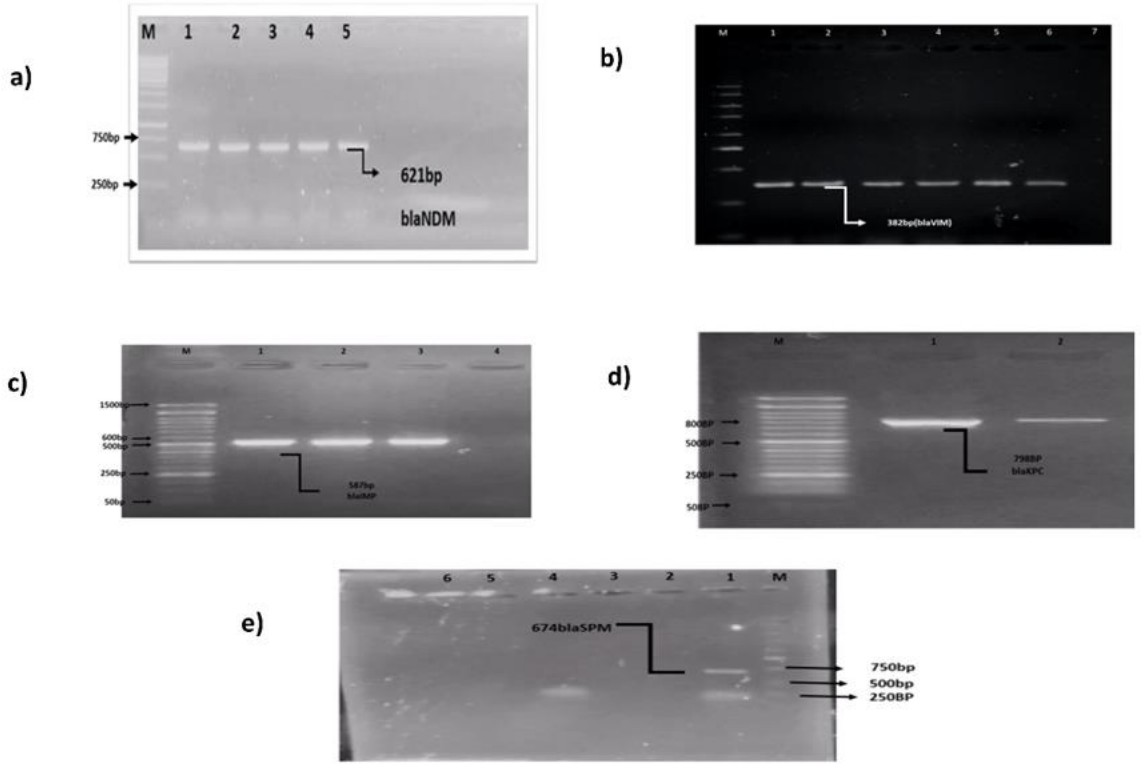

Fig. 2. Genotypic characterization of carbapenemase producing *Pseudomonas aeruginosa:* a) Gel electrophoresis analysis of carbapenemase producing *P. aeruginosa* encoding blaNDM (621bp), ladder size: 250bp, lane 1-5: positive samples. b) Molecular identification carbapenemase producing *P. aeruginosa* encoding blaVIM (621bp), ladder size: 100bp, lane 1-6: positive samples. c) PCR based analysis of carbapenemase producing *P. aeruginosa* encoding blaIMP (587bp), ladder size: 50bp, lane 1-3: positive samples. d) Gel electrophoresis analysis of carbapenemase producing *P. aeruginosa* encoding blaKPC (798bp), ladder size: 50bp, lane 1-2: positive samples. e) PCR based analysis of carbapenemase producing *P. aeruginosa* encoding blaSPM (674bp), ladder size: 250bp lane 1: positive samples.

Phenotypic Detection of Metallo-B-Lactamase Producing *Pseudomonas aeruginosa*:

Phenotypic detection of Metallo-β-lactamase was carried out for all 35 carbapenemase-producing *Pseudomonas aeruginosa* and was performed by the following method.

Modified Carbapenem Inactivation Method:

To find Pseudomonas aeruginosa MBL producers, a carbapenem inactivation procedure with certain modifications was used. The disc was infected with TSB, and the results were examined by measuring the zone of inhibition surrounding the inoculated

area. Positive findings were reported in the zone of inhibition measured between 5 and 15 millimetres in size without the presence of pinpoint colonies, or between 16 and 18 millimetres in size with the presence of pinpoint colonies. Results were considered intermediate if the zone of inhibition was 16-18mm without pinpoint colonies and 19mm with pinpoint colonies present in the zone. If the zone was more than 19mm, the results were considered negative. Out of 35, 32 showed positive results, 1 sample was considered intermediate and 2 samples were negative. Results were shown (Fig. 3 and Table 5). The prevalence of carbapenemase encoding genes by mCIM is shown in (Table 6).

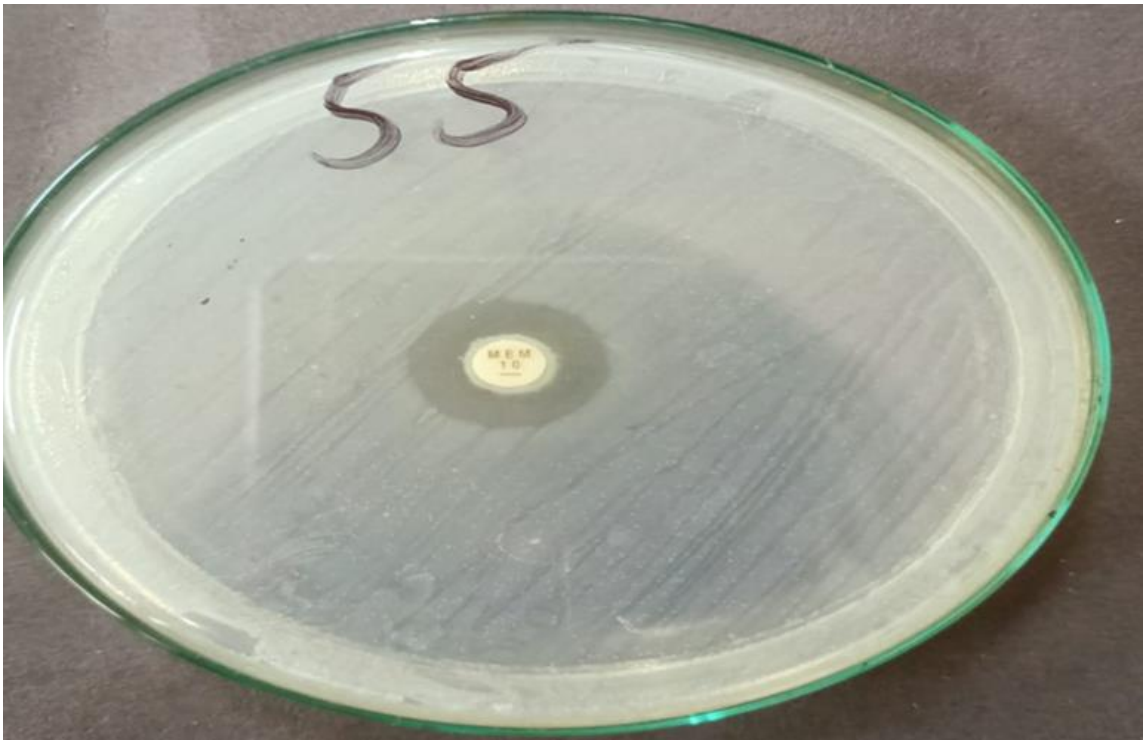

Fig. 3: modified carbapenem inactivation method: representation of zone of inhibition (19mm) with pinpoints colonies around the disc, positive modified carbapenem inactivation method.

EDTA Carbapenem Inactivation Method:

EDTA-modified carbapenem inactivation method was performed only if the modified carbapenem inactivation method show positive results. Positive isolates confirmed by the Modified carbapenem inactivation method were 32. The zone from the disc with EDTA present minus the zone from the mCIM was taken into consideration when interpreting the results of the eCIM. Results were reported as positive if the zone of inhibition was ≥ 5mm. If the zone of inhibition was ≤ 4mm results were considered as negative. Out of 32, 29 were considered positive, 1 sample showed negative results and only 1 sample was considered intermediate. Results were shown in (Fig. 4 and Table 5). The prevalence of carbapenemase encoding genes by eCIM is shown in (Table 6).

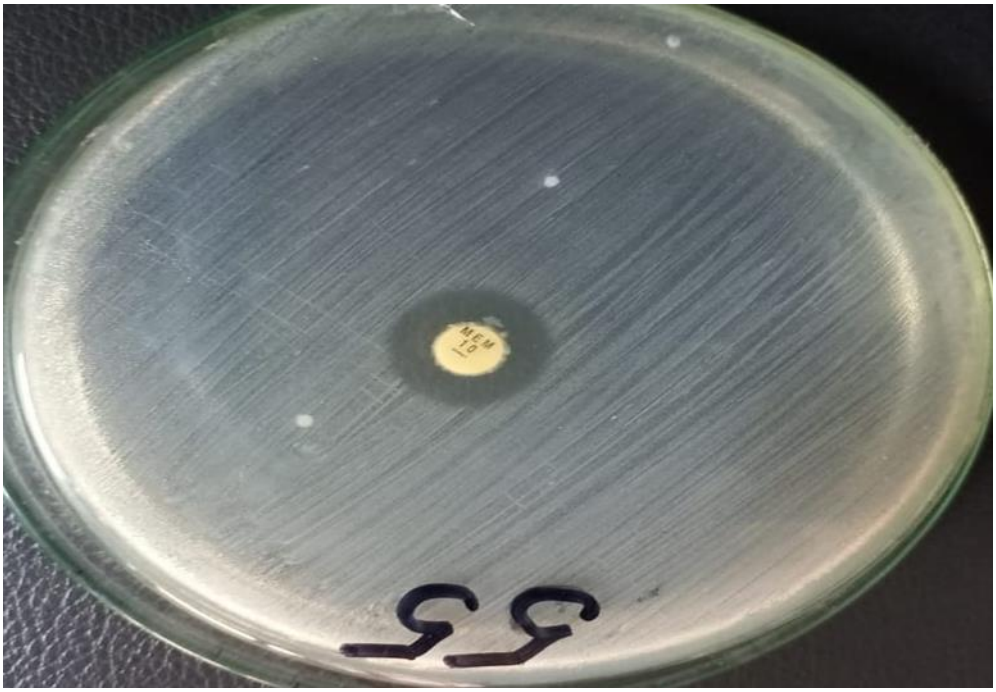

Fig. 4: EDTA carbapenem inactivation method representation of measured zone of inhibition (14mm) positive for EDTA modified carbapenem inactivation method.

Table 5 Phenotypic detection of carbapenemase.

No positive isolates	Test name	Positive %	Negative %	Intermediate %
35	Modified carbapenem inactivation method	32 (91.42%)	2 (5.7%)	1(2.85%)
32	EDTA carbapenem inactivation method	29 (90.62%)	1 (3.4)	2 (6.25%)

Table 6 :mCIM and eCIM results for carbapenemase-producing *P.aeruginosa.*

Beta-lactamase type	N	% (no. positive/total no. tested)	
		mCIM	eCIM
Class B, metallo-β-lactamases			
VIM	9	8/9	7/9
NDM	14	13/14	12/14
IMP	6	5/6	4/6
SPM	5	5/5	5/5
Class A, serine-carbapenemase			
KPC	1	1/1	1/1

Data Analysis:

The sensitivity and specificity of carbapenemase genes for mCIM and eCIM were shown in (Table 7).

Table 7: Sensitivity and specificity of carbapenemase genes for mCIM and eCIM**.**

Enzyme type	N	Sensitivity (95% CI)		Specificity (95% CI)	
		mCIM	eCIM	mCIM	eCIM
KPC	1	1/1 (2.50% to 100.00%)	1/1 (2.50% to 100.00%)	NC	NC
SPM	5	5/5 (47.82% to 100.00%)	5/5 (47.82% to 100.00%)	NC	NC
IMP	6	5/6 (47.82% to 100.00%)	4/6 (39.76% to 100.00%)	(47.82% to 100.00%)	(15.81% to 100.00%)
NDM	14	13/14 (75.29% to 100.00%)	12/14 (73.54% to 100.00%)	(2.50% to 100.00%)	(15.81% to 100.00%)
VIM	9	8/9 (63.06% to 100.00%)	7/9 (59.04% to 100.00%)	(2.50% to 100.00%)	(15.81% to 100.00%)

Biochemical Tests For Identification:

The biochemical tests were conducted to test the enzymatic and other activities of *Pseudomonas aeruginosa* that are presented in Table 8.

Table 8: Biochemical tests for identification of *Pseudomonas aeruginosa***.**

Tests	Results
Catalase	Positive
Oxidase	Positive
Simon citrate	Positive
Voges Proskauer	Negative
Methyl Red	Negative
Triple sugar iron	Non-Fermenter
Urease	Negative
Indole	Negative

DISCUSSION

Pseudomonas aeruginosa is an opportunistic organism that is most concerned about infections in various types of wounds, immunocompromised individuals and patients in intensive care units (Bazighandi *et al*., 2020). Burn infections induced by *Pseudomonas aeruginosa* not only progress rapidly but also lead to systemic involvement and death within days or weeks (Aubaid and Dekil, 2017; Daphedar *et al*., 2020). Resistance against many antimicrobial drugs has become frequent in one of the most therapeutically important microorganisms, *Pseudomonas aeruginosa*, which is responsible for a high mortality rate and a high economic burden. This lethal disease possesses a diverse variety of resistance mechanisms, including adaptive as well as intrinsic resistance to structurally and functionally distinct drugs, eventually leading to multi-drug and even pan-drug resistance (Dastidar *et al*., 2021; de Lacerda Coriolano *et al*., 2021). Carbapenemase is one of three types of β-lactamase enzymes (BL). Ambler class A and D (serine carbapenemase) and class B (proline carbapenemase) (zinc-dependent). Metal chelators, such as EDTA and thiol-based chemicals, reduce the catalytic activity of these enzymes, which are known as Metallo-BLs (MBLs).

In the ongoing study, 120 burn wound samples were collected from persons diagnosed with skin infections from Allied Hospital Faisalabad. With the help of PCR, *P. aeruginosa* were molecularly identified and their prevalence rate was found to be 38% as compared to the other studies on the prevalence of *P.aeruginosa* in skin

infections. In a previous study, the prevalence rate was lower than in ongoing research, 11% in different wound infections by Ebrahimi *et al*. (2018), and 53 (39%) from 136 wound-infected patients by Esmaili and Hosseini Doust (2019). These findings suggest that *P. aeruginosa* is a common etiological cause of cutaneous infections, particularly in hospitalized patients. Indwelling and trauma, surgical methods, prolonged hospitalization, poor sanitization, and patient neglect all contributed to the high prevalence.

For the treatment of infections caused by *P. aeruginosa* various antibiotic classes have been used. Resistance pattern exhibited against antibacterial drugs was Colistin (33%), Tobramycin (85%), Amikacin (61%), Gentamicin (83%), Meropenem (57%), Imipenem (76%), Cefoxitin (65%), Ceftriaxone (70%), Cefepime (87%), Ceftazidime (78%), Ciprofloxacin (65%), Ampicillin (67%) and Piperacillin (57%) shown the highest level of efficiency against *P.aeruginosa*. Related studies carried out by Hassan and Elnasr (2019) observed resistance in *P.aeruginosa* isolates to be Cefepime (100%), Meropenem (81%), Imipenem (100%), Gentamicin (82%) and Ciprofloxacin (90%). As mentioned in a study led by Gopinath *et al*. (2014), *P.aeruginosa* resistance to Cefepime (71%), Ciprofloxacin (71%), Piperacillin (71%) and Ceftazidime (60%) as well as least level of resistance was observed in Amikacin (53%). The main cause of *P. aeruginosa's* high level of resistance is the irrational and inappropriate use of antibiotics in everyday clinical practice.

In the current study, the prevalence of MDR was 25(54%) out of 46 positive isolates of *P.aeruginosa* collected from wounded patients. The prevalence of MDR in *P.aeruginosa* in previous studies was 20(38.46%) out of 52 wound samples by Hassani Sangani *et al*. (2015), 31.42% from hospitalized patients by Ibraheem *et al* (2019), 46 isolates out of 162(28%) from clinical specimens by Hwang *et al*. (2012). Irrational antibiotic use, an unsanitary environment, and healthcare personnel's activities were identified as contributing factors that may enhance the occurrence of MDR *P. aeruginosa* in hospitalized patients.

In ongoing research prevalence of carbapenemase-producing *P. aeruginosa* was 35(76%) out of 46 confirmed isolates of *P. aeruginosa*. In previous study results were recorded as out of 123, 103 were carbapenemase-producing *P. aeruginosa* by Hemmati *et al*. (2020), 267 (72.3%) out of 369 confirmed *P.aeruginosa* by Gurunathan *et al*. (2014), out of 488, 36(7.4%) were considered positive for carbapenemase by Hudzicki (2009).

Out of 35 *P. aeruginosa* that have the ability to produce carbapenemase, the prevalence of carbapenemase encoding genes were 5(14%) for blaSPM, 9 (25.7%) for blaVIM, 14(40%) for blaNDM, 1(2.85%) for KPC and 6(17%) for blaIMP confirmed by PCR. A similar study was carried out by (Dupuy *et al*., 2018) which showed the results, out of 79 carbapenemase encoding genes, including 6 blaKPC, 2blaNDM, 68 blaSPM, and 3 blaVIM. Another study showed that out of 20, 2(10%) were positive for blaKPC, 7(35%) for blaVIM and 11(55%) for NDM by [39] out of 96 carbapenemase encoding genes prevalence were 27 for blaVIM, 22 blaIMP, 13 for blaNDM, 14 for blaSPM, 8 for blaKPC and 12 for GES by Hussein and Hami (2020).

Standard mCIM and eCIM were applied for the phenotypic detection of MBL. Out of 35 carbapenemase producing *P.aeruginosa* 32(91.42%) were positive, 2(5.7%) were negative and 1(2.85%) were intermediate by utilizing mCIM. In a previous study, the results were found that showed 27 positives, 16 intermediate and 57 negative results out of a total of 100 specimens by Ali *et al*. (2020), 29(54.71%) out of 53 were positive and 21(39.62%) were negative and 3(5.66%) were intermediate by Birla *et al*. (2003). The eCIM was applied only if mCIM showed positive results out of a total of 32, 29(90.62%) were positive, 1(3.4%) were negative and 2 (6.2%) were intermediate. Another study concluded that

out of 96, 75 were considered positive, 15 were negative and 6 were negative for EDTA-modified carbapenem inactivation method by 16, 26(100%) out 26 were considered positive by performing EDTA modified carbapenem inactivation method (Bayroodi and Jalal, 2016).

The diagnostic performance of the eCIM, a phenotypic technique to discriminate serine and MBL carbapenemase encoded by *Pseudomonas aeruginosa,* is described in the current study. In current research the sensitivity of mCIM for blaKPC (2.50- 100%), blaSPM (47.82- 100%), blaIMP (47.82-100%), blaNDM (75.29-100%) and blaVIM (63.06-100%) were observed with 95% confidence interval while the specificity of mCIM for blaKPC and blaSPM were not calculated while blaIMP (47.82-100%), blaNDM (2.50-100%) and blaVIM (2.50-100%) were recorded with 95% confidence interval. The sensitivity of eCIM for KPC, SPM, NDM, IMP and VIM were explained as (2.50-100%), (47.82-100%), (39.76-100%), (73.54-100%) and (59.04-100%) respectively while the specificity of KPC and SPM were not calculated and NDM, IMP and VIM were calculated as (15.81-100%), (15.81-100%) and (15.81-100%) respectively. A similar study was conducted by Gurunathan *et al*. (2014) which showed the sensitivity of VIM was (87-100%) and NDM (74-100%) with a 95% confidence interval while the sensitivity of IMP and SPM was not calculated.

CONCLUSION

In conclusion, the relatively highest resistance of *P. aeruginosa* towards cefepime gentamicin and tobramycin and the highest prevalence of blaNDM and blaVIM raised a concern regarding increasing, perhaps continuing to rise, carbapenem resistance. Our findings highlight the significance of using both genotypic and phenotypic MBL detection methods for early diagnosis of carbapenem resistance and to stop the spread of this resistant bacterium in addition to routine antibiotic susceptibility testing. Particularly in developing countries where molecular screening test is hardly available, a combination of eCIM and mCIM testing may be a helpful tool for the clinical laboratory. It might be a useful phenotypic technique for identifying *P. aeruginosa* strains that produce carbapenemase.

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REFERENCES

Aabed, K., & Mohammed, A. E. (2021). Synergistic and Antagonistic Effects of Biogenic Silver Nanoparticles in Combination With Antibiotics Against Some Pathogenic Microbes. *Frontiers in Bioengineering and Biotechnology*, *9*, 249.

Abbas, W. S., Atwan, Z. W., Abdulhussein, Z. R., & Mahdi, M. (2019). Preparation of silver nanoparticles as antibacterial agents through DNA damage. *Materials Technology*, *34*(14), 867-879.

Abdolhosseini, M., Zamani, H., & Salehzadeh, A. (2019). Synergistic antimicrobial potential of ciprofloxacin with silver nanoparticles conjugated to thiosmicarbazide against ciprofloxacin resistant *Pseudomonas aeruginosa* by attenuation of MexA-B efflux pump genes. *Biologia, 74*(9), 1191-1196.

Abdulhaq, N., Nawaz, Z., Zahoor, M. A., & Siddique, A. B. (2020). Association of biofilm formation with multi drug resistance in clinical isolates of *Pseudomonas aeruginosa*. *EXCLI journal, 19*, 201.

Abdullah, S. R. A., Hasson, S. O., & Al-Yasari, A.-K. S. (2021). Antimicrobial effect of green synthesized silver nanoparticles (AgNPs) against multidrug resistant *Pseudomonas aeruginosa*. *Annals of the Romanian Society for Cell Biology*, 5033-5045.

Abo-Shama, U. H., El-Gendy, H., Mousa, W. S., Hamouda, R. A., Yousuf, W. E., Hetta, H. F., & Abdeen, E. E. (2020). Synergistic and antagonistic effects of metal nanoparticles in combination with antibiotics against some reference strains of pathogenic microorganisms. *Infection and drug resistance, 13*, 351.

Ahmed, O. B., & Dablool, A. (2017). Quality improvement of the DNA extracted by boiling method in gram negative bacteria. *International Journal of Bioassays, 6*(4), 53475349. Akinloye, A. O., Adefioye, J. O., Adekunle, C. O., Anomneze, B. U., Makanjuola, O. B., Onaolapo, O. J., . . . Olowe, O. A. (2021). Multidrug-Resistance Genes in *Pseudomonas aeruginosa* from Wound Infections in a Tertiary Health Institution in Osogbo, Nigeria. *Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders), 21*(1), 90-98.

Ali, S. G., Ansari, M. A., Alzohairy, M. A., Alomary, M. N., Jalal, M., AlYahya, S., . . . Khan, H. M. J. A. (2020). Effect of biosynthesized ZnO nanoparticles on multi-drug resistant *Pseudomonas aeruginosa*. *9*(5), 260.

Almalah, H. I., Alzahrani, H. A., & Abdelkader, H. S. (2019). Green synthesis of silver nanoparticles using cinnamomum zylinicum and their synergistic effect against multi- drug resistance bacteria. *Journal of Nanotechnology Research, 1*(3), 95-107.

Aubaid, A. H., & Dekhil, K. R. (2017). Synergistic Effect of Biosynthesized Silver Nanoparticles Combined with Antibiotics against *Pseudomonas aeruginosa* and Proteus mirabilis Isolated from Acute Otitis Externa. *Journal of Pharmaceutical Sciences and Research, 9*(12), 2644-2648.

Bayroodi, E., & Jalal, R. (2016). Modulation of antibiotic resistance in *Pseudomonas aeruginosa* by ZnO nanoparticles. *Iranian Journal of Microbiology, 8*(2), 85.

Bazghandi, S. A., Safarirad, S., Arzanlou, M., Peeri-Dogaheh, H., Ali-Mohammadi, H., & Khademi, F. (2020). Prevalence of multidrug-resistant *Pseudomonas aeruginosa* strains in Ardabil. *Journal of Ardabil University of Medical Sciences, 20*(2), 280-286.

Birla, S., Tiwari, V., Gade, A., Ingle, A., Yadav, A., & Rai, M. (2009). Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against Escherichia coli, *Pseudomonas aeruginosa* and Staphylococcus aureus. *Letters in Applied Microbiology, 48*(2), 173-179.

Bose, D., & Chatterjee, S. (2016). Biogenic synthesis of silver nanoparticles using guava (Psidium guajava) leaf extract and its antibacterial activity against *Pseudomonas aeruginosa*. *Applied Nanoscience, 6*(6), 895-901.

Boyanova, L. (2018). Direct Gram staining and its various benefits in the diagnosis of bacterial infections. *Postgraduate medicine, 130*(1), 105-110.

Brink, B. (2010). Urease test protocol. *Washington, DC: American Society for Microbiology*. D'Lima, L., Phadke, M., & Ashok, V. D. (2020). Biogenic silver and silver oxide hybrid nanoparticles: a potential antimicrobial against multi drug-resistant *Pseudomonas aeruginosa*. *New Journal of Chemistry, 44*(12), 4935-4941.

Danjuma, L., & Abdullahi, U. (2020). Antibacterial Efficacy Of Some Standard Antibiotics Discs Coated With Biologically Synthesized

Silver Nanoparticles From Cassia Occidentalis Leaves. *Journal of Medicine and Health Research*, 47-53.

Daphedar, A., Ritti, M., Patil, N., Patil, S., Kakkalameli, S., & Chandrashekharappa Taranath, T. (2020). Synthesis and characterization of Silver nanoparticles from fruit extract of Michelia Champaca L.: Their antioxidant and antibacterial activity. *International Journal of Nano Dimension, 11*(3), 267-276.

Dastidar, M. G., Nallal, V., & Razia, M. (2021). Synergistic antibacterial activity of mycosynthesized AgNPs with antibiotics against multidrug resistant *Pseudomonas aeruginosa* and investigation of protein profile. *Indian Journal of Experimental Biology (IJEB), 59*(12), 842-848.

de Lacerda Coriolano, D., de Souza, J. B., Bueno, E. V., Medeiros, S. M., Cavalcanti, I. D. L., & Cavalcanti, I. M. F. (2021). Antibacterial and antibiofilm potential of silver nanoparticles against antibiotic-sensitive and multidrug-resistant *Pseudomonas aeruginosa* strains. *Brazilian Journal of Microbiology, 52*(1), 267-278.

Dupuy, F. G., Pagano, I., Andenoro, K., Peralta, M. F., Elhady, Y., Heinrich, F., & Tristram- Nagle, S. (2018). Selective interaction of colistin with lipid model membranes. *Biophysical journal, 114*(4), 919-928.

Durán, N., Marcato, P. D., Conti, R. D., Alves, O. L., Costa, F., & Brocchi, M. (2010). Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action. *Journal of the Brazilian Chemical Society, 21*(6), 949-959.

Ebrahimi, A., Jafferi, H., Habibian, S., & Lotfalian, S. (2018). Evaluation of anti-biofilm and antibiotic potentiation activities of silver nanoparticles against some nosocomial pathogens. *Iranian Journal of Pharmaceutical Sciences, 14*(2), 7-14.

El-Deeb, N. M., Abo-Eleneen, M. A., Al-Madboly, L. A., Sharaf, M. M., Othman, S. S., Ibrahim, O. M., & Mubarak, M. S. (2020). Biogenically Synthesized Polysaccharides- Capped Silver Nanoparticles: Immunomodulatory and Antibacterial Potentialities Against Resistant *Pseudomonas aeruginosa*. *Frontiers in Bioengineering and Biotechnology, 8*, 643.

Esmaili, S., & Hosseini Doust, R. (2019). Synergistic effect of silver nanoparticles (AgNPs) and gentamicin against clinical isolates of Pseudomona aeruginosa. *Medical Science Journal of Islamic Azad Univesity-Tehran Medical Branch, 29*(1), 64-70.

Fadwa, A. O., Alkoblan, D. K., Mateen, A., & Albarag, A. M. (2021). Synergistic effects of zinc oxide nanoparticles and various antibiotics combination against *Pseudomonas aeruginosa* clinically isolated bacterial strains. *Saudi Journal of Biological Sciences, 28*(1), 928-935.

Farva, K., & Bhutta, G. A. (2021). Prevalence of gram-negative bacteria in infected burn wounds and antibiotic susceptibility pattern; A study of stable burn patients. *Professional Medical Journal, 28*(10).

Gopinath, P. M., Narchonai, G., Dhanasekaran, D., Ranjani, A., & Thajuddin, N. (2015). Mycosynthesis, characterization and antibacterial properties of AgNPs against multidrug resistant (MDR) bacterial pathogens of female infertility cases. *Asian Journal of Pharmaceutical Sciences, 10*(2),

138-145.

Gurunathan, S., Han, J. W., Kwon, D.-N., & Kim, J.-H. (2014). Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. *Nanoscale Research Letters, 9*(1), 1-17.

Habash, M. B., Goodyear, M. C., Park, A. J., Surette, M. D., Vis, E. C., Harris, R. J., & Khursigara, C. M. (2017). Potentiation of tobramycin by silver nanoparticles against *Pseudomonas aeruginosa* biofilms. *Antimicrobial Agents and Chemotherapy, 61*(11), e00415-00417.

Habash, M. B., Park, A. J., Vis, E. C., Harris, R. J., & Khursigara, C. M. (2014). Synergy of silver nanoparticles and aztreonam against *Pseudomonas aeruginosa* PAO1 biofilms. *Antimicrobial agents and chemotherapy, 58*(10), 5818-5830.

Haghi, F., Zeighami, H., Monazami, A., Toutouchi, F., Nazaralian, S., & Naderi, G. (2018). Diversity of virulence genes in multidrug resistant *Pseudomonas aeruginosa* isolated from burn wound infections. *Microbial pathogenesis, 115*, 251-256.

Hari, N., Thomas, T. K., & Nair, A. J. (2014). Comparative study on the synergistic action of differentially synthesized silver nanoparticles with β-cephem antibiotics and chloramphenicol. *Journal of Nanoscience, 2014*.

Hassan, A. M., & Elnasr, L. M. A. (2019). In vitro Study on the Efficacy of Silver Nanoparticles against Metallo-Beta-Lactamase and Biofilm Producing *Pseudomonas aeruginosa* Isolates. *Internatioal Journal of Current Microbiology and Applied Sciences, 8*(06), 1-12.

Hassani Sangani, M., Nakhaei Moghaddam, M., & Forghanifard, M. (2015). Inhibitory effect of zinc oxide nanoparticles on *Pseudomonas aeruginosa* biofilm formation.*Nanomedicine journal, 2*(2), 121-128.

Hemmati, F., Salehi, R., Ghotaslou, R., Kafil, H. S., Hasani, A., Gholizadeh, P., & Rezaee, M. M. (2020). The assessment of antibiofilm activity of chitosan-zinc oxide- gentamicin nanocomposite on *Pseudomonas aeruginosa* and *Staphylococcus aureus. International Journal of Biological Macromolecules*, 15 (163), 2248-2258. DOI: 10.1016/j. ijbiomac.2020.09.037

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology, 15*, 55-63.

Hussein, M. J. N., & Hami, S. K. (2020). Bio-creation of Ag-Nanoparticles via Burkholderiacepacia and Estimation of its Combined Ability with Ciprofloxacin against *Pseudomonas aeruginosa*. *International Journal of Pharmaceutical Research,12*(2).

Hwang, I.-s., Hwang, J. H., Choi, H., Kim, K.-J., & Lee, D. G. (2012). Synergistic effects between silver nanoparticles and antibiotics and the mechanisms involved. *Journal of medical microbiology, 61*(12), 1719-1726.

Ibraheem, S. A., Kadhem, H. A., Hadeethi, S., Jabir, M. S., Grigore, R., Popa, M., . . . Florin, M. D. (2019). Effects of silver nanoparticles on nosocomial *Pseudomonas aeruginosa* strains–an alternative approach for antimicrobial therapy. *Romanian Biotechnological Letters, 24*, 286-293.

Ijaz, M., Siddique, A. B., Rasool, M. H., & Shafique, M. (2019). Frequency of multi drug resistant *Pseudomonas aeruginosa* in different wound types of hospitalized patients. *Pakistan journal of pharmaceutical sciences, 32*.

Jaiswal, S., & Mishra, P. (2018).

Antimicrobial and antibiofilm activity of curcumin-silver nanoparticles with improved stability and selective toxicity to bacteria over mammalian cells. *Medical microbiology and immunology*, *207*(1), 39-53.

Jalal, N. A., Hariri, S. H., Momenah, A. M., Khan, S., & Bantun, F. (2023). Molecular detection of blaPER-1, blaVEB-1, and blaPSE-1 β-lactamase genes from P. aeruginosa Severe Urogenital UTI Infection. *Journal of Survey in Fisheries Sciences*, *10*(3S), 1628-1635.

Jattavathu, M., Dondapati, M., & pushpam Dara, L. (2021). Combating strategies of antibiotic resistance in *Pseudomonas aeruginosa* biofilm: A review. *Journal of Advanced Scientific Research, 12*(2).

Momenah, A. M., Hariri, S. H., Abdel-razik, N. E., Bantun, F., Khan, S., Alghamdi, S., & Tasleem, S. (2023). Aspergillus fumigatus-Mediated Biosynthesis of Silver Nanoparticles Efficiency, Characterization, and Antibacterial Activity Against Different Human Pathogens. *Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology*, *15*(1), 353-364.

Tasleem, S., Moon, S., Alghamdi, S., Suliman, R. S., Ateeq, M., Salman, M., ... & Bantun, F. (2022). Presence of BlaPER-1 and BlaVEB-1 Beta-Lactamase Genes among Isolates of Pseudomonas Aeruginosa from Burn and Trauma Hospital Peshawar, Pakistan. *Journal of Bioresource Management*, *9*(2), 15.