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Molecular Detection Of Carbapenemases and ESBLs in Gram Negative Bacilli Isolated From Burn Wound Infections In Mosul City, Iraq

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ABSTRACT

The presence of multidrug-resistant bacteria containing beta-lactamase enzymes (carbapenemase and Extended-Spectrum β-Lactamases ESBLs) that cause burn wound infections is a serious threat to patient's health with the risk of their transmission to the blood stream and causing sepsis. This study seeks to identify antibiotic resistance patterns and ascertain the existence of β-lactamase enzymes (Carbapenemases and ESBLs) in Gram-negative bacilli isolated from burn wound infections. Following culture on MacConkey agar, 127 isolates were recovered from 150 samples, with Pseudomonas aeruginosa being the predominant bacterial species isolated (43.3%), succeeded by Escherichia coli (18.89%), while the isolation percentages of other species exhibited significant variability. Antimicrobial susceptibility testing results showed absolute resistance to Cefotaxime, Amoxicillin and Ceftazidime, and low resistance was against to Meropenem with rates of 58.2%. Furthermore the results showed that 0.79% of the isolates were Multiple Drug-Resistant (MDR) and 68.5% of them were Extensively Drug-Resistant (XDR), while Thirty-nine isolates (30.71%) were Pan Drug-Resistant (PDR). Phenotypic and molecular detection of carbapenemase and ESBL enzymes were performed for twenty-six antibiotic-resistant isolates, 96.2% of the isolates were ESBL producers and 80.8% were carbapenemase producers. Those results were confirmed at molecular level using 14 primers for carbapenemase genes and ESBLs (IMP, NDM, KPC, GES, NAM-C, OXA23, OXA48, OXA58, VIM, SPM, SIM, TEM, SHV, and CTX-M), results indicated the presence of carbapenemase genes OXA23, NDM, VIM with rates of 11.5% ,61.5%, 73%respectively,while OXA48 , KPC 42.3% for each. SHV, TEM and CTX-M ESBL genes detection rates were 69.2%, 84.6%, 88.4% respectively, the rest genes were not found.

Keywords: *carbapenemases, bacilli, Mosul city*

1. INTRODUCTION

Bacaterial Infection in patients with burn is a significant cause of morbidity and mortality (Church *et a*l., 2006). The larg rate of acquisition of these infection is largely in hospital-acquired due to various factors such as burn injury itself, extent and depth of the injury, the age of the patient, and the microbial agents and their number that matter becomes more complicated in the case of opportunistic bacteria to the internal and external origin (Pruitt *et al.*,1998; Ogunsola *et al*.,1998) . That may lead to the occurrence of sepsis in these patients(Mooney and Gamelli 1989).

The extensive and random use of antimicrobials in more than one field is one of the essential thing that helped in spreading resistance and lack the effect of antibiotics on pathogenic bacteria. The resistance of bacteria to antimicrobials is increasing day after day, which has led to an increase in the rates of disease and death, whether in infections acquired from the community or from hospitals(Raffelsberger *et al*., 2023). Microbial resistance to antibiotics can be divided into natural resistance resulting from the functional structure of the cell and resistance acquired from other bacteria (Forbes et al., 2007). One of the mechanisms of microbial resistance is enzyme production. These enzymes work to hydrolyze antibiotics or modify them and convert them into inactive forms against microbes. These enzymes are found in in the periplasmic space of Gram-negative bacteria (Garica 2013).

Beta-lactamase enzymes are the most important enzymes that hydrolyze the beta-lactam ring and include carbapenemase, cephalosporinase, and penicillinase which have the ability to hydrolyz carbapenems, cephalosporins, and penicillins

respectivily (Sawa et al., 2020). Mutations in genes encoding these enzymes lead to increase of substrates on which they work, and thus the emergence and development of new enzyme variants with higher and broader analytical capabilities (Primeau et al., 2022).

According on the mechanism used in degradiation of Beta-lactam antibiotics Beta-lactamases can be divided into two main groups: metalloenzymes that require zinc ions in active site , and serine β-lactamases that consume serine in active site (Jin et al., 2023). There are two main classifications of beta-lactamases:

1- Functional Classification: which is based on the substrate on which the enzyme works, substrate that inhibits it, isoelectric for using , and other characteristics. (Bush and Jacoby 2010)

2- Molecular Classification or Ambler Classification: which is based on similarity in the sequence of amino acids. This classification includes four classesas follow serine enzymes are placed in classes A and D, AmpC enzymes are placed in class C, while metalloenzymes are placed in class B (Ambler 1980).

Extended-spectrum β-Lactamases (ESBLs) are the most significant of these enzymes. These are categorized into two classes, A and D, and functionally belong to the second category. The significance of these enzymes arises from the genes encoding them being situated on plasmids, facilitating their transfer to various bacterial species. (van Hout et al., 2020). ESBLs can be inhibited by tazobactam, sulbactam and clavulanic acid, their encoding genes are interchangeable between bacteria. The most common enzyme variants in ESBLs today is CTX-M. Together, these enzymes represent an important resistance mechanism facing bacterial infections therapy, especially those caused by members of the Enterobacteriaceae family. (Al-Hasso and Mohialdeen.2023.;Primeau *et al.*, 2022) Gram-negative bacteria that produce ESBLs are the primary culprits behind the development of resistance to beta-lactam antibiotics with a broad spectrum of activity. Since the discovery of these enzymes in the early 1980s, it has spread all over the world and is now endemic in members of the Enterobacteriaceae that have been identified from bacterial illnesses that have been obtained in hospitals as well as that have been acquired in the community (Castanheira et al., 2024)

Chromosomes, plasmids, and other jumping elements are responsible for the encoding of carbapenemases, which are classified as belonging to the Ambler classes (A, B, and D). *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter spp*, *Serratia spp*, and *Proteus spp* are some of the bacteria that are resistant to carbapenems. These enzymes are the most powerful β-lactamases that are currently known. They are capable of degrading a wide variety of β-lactam antibiotics, including carbapenems, which are the final line of treatment for the bacteria that are resistant to their antibiotics (Aurilio *et al*., 2022 ; Aslam et al., 2018). There is a growing awareness and interest in the role of non-fermenting bacteria, including *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, as well as other Gram-negative bacteria, particularly Enterobacteriaceae, that create carbapenemases. These enzymes have been identified in bacteria obtained from environmental and animal samples, heightening worries regarding their dissemination and the necessity for stringent health measures to mitigate the repercussions of their proliferation (Halat and Moubareck 2020). Carbapenem-resistant bacteria represent a serious threat to public health and they occur mainly among Gram-negative bacteria and may be self-originating or horizontally transmitted via transferable genes encoding for carbapenemase. In specific regions of the globe, such as Europe, Asia, and South America, this specific form of resistance gene is already widespread. Conversely, the situation in other regions, particularly sub-Saharan Africa and Africa, is inadequately documented and necessitates further investigation (Codjoe and Donkor 2018).

2. MATERIALS AND METHODS

2.1 Sample collection

One hundred and fifty swabs were taken from patients Burns attending the Hospital in Mosul who had an underlying burn wound infection, for the period from March to setamper 2023. Samples were transferred to the laboratory as soon as possible for microbiological processing.

2.2 Isolation and Identification

The samples were inoculated onto MacConkey agar plates and incubated at 37°C for 24-48 hours (Baron et al 1994). Monthly re-culturing and testing were conducted on the pure bacterial isolates in the refrigerator, where they were preserved on nutrient agar medium.

Bacterial isolates were primarily identified by observing the cultural characteristics of the colonies on MacConkey agar. Thin smears of pure colonies were prepared and stained with Gram stain to observe the shape, colore and arrangment of the cells (Prescot *et al* 2002). Identification of the of the isolates was conducted using Analytic Profile Index (API 20E) strips (Biomerieux Co., France).

2.3 Antimicrobial Susceptibility Testing

In accordance with the Clinical Laboratory Standards Institute's (2012) guidelines, the disc diffusion method was implemented to evaluate the isolates' susceptibility. In order to achieve a turbidity equivalent to 0.5 McFarland standard

containers (1.5 x 10^8 CFU/ml), nutritional broth was used to produce fresh bacterial suspensions ((Baron *et al* 1994). After inoculating Mueller-Hinton agar plates with the bacterial suspensions using sterile cotton swabs, the plates were allowed for fifteen minutes to allow for absorption to take place. Following this, antimicrobial discs were included in the experiment (Bioanalyse Co., Turkey) had been dispersed out. Plates were incubated at a temperature of 35 degrees Celsius for sixteen to eighteen hours. The diameter of the inhibitory zone, which included the diameter of the disk, was measured, and the isolates were classified into three categories: susceptible, moderately susceptible, and resistant, according to the recommendations of the CLSI. In addition to that, MDR, XDR, and PDR isolates were discovered (CLSI 2022).

2.4 Phenotypic Detection of ESBLs

Bacterial suspensions that met the 0.5 McFarland standard were generated and inoculated onto Mueller-Hinton agar in accordance with CLSI guidelines. The agar surface was incubated at 35 ˚C for 16-18 hours, and cefotaxime, ceftazidime, and aztreonam discs were deposited on this surface. The inhibitory zone diameters were quantified and compared to the CLSI table for this assay after the incubation period (Prescot *et al.*, 2002; CLSI 2022).

2.5 Phenotypic Detection of Carbapenemases

A suspension of fresh bacterial culture that was diluted to a ratio of 1:10 was used to inoculate a Mueller-Hinton Agar plate. The turbidity of the suspension was determined to be equivalent to that of a 0.5 McFarland standard tube. Two meropenem discs were positioned, one of which contained 10 µl of (0.5 M) EDTA and the other of which was devoid of EDTA. A positive result for the detection test was obtained after incubation when the diameter of the inhibition zone for the EDTA-containing disc increased by 7 mm or more in comparison to the other disc (Franklin *et al.*, 2006, 30).

2.6 Molecular Study

The most twenty six resistant isolates were selected for the molecular study. DNA extraction was carried out using Geneaid kit/ Taiwan and according to manufacturer's instructions (www.geneaid.com). Concentration and purity of DNA were determined by using nanodrop, and DNA sample were kept at -20°C for further study.

Molecular detection of beta lactamase was carried out using thermocycler (SensoQuest GmbH, Germany). The reaction mixture was prepared as follows: (12.5 µl) master mix (Promega Co.), (2 µl) DNA template, (2 µl) forward and reverse primers, and (6.5 µl) distilled water (free of ions and Nuclease enzymes). The amplification product size and primer sequences employed in the investigation are illustrated in Table 1. The PCR products were subjected to agarose gel electrophoresis in 1x (TAE) buffer with 2% agarose. The UV transilluminator was employed to observe and validate gene bands.

Table 1: Primer sequences and amplification product size used in the study

Genes	Primer sequences	Product size	Reference
bla$_{NDM}$	F-GGGCAGTCGCTTCCAAGGT R-GTAGTGCTCAGTGTCGGCAT	375	Deshpande et al., 2010
bla$_{OXA48}$	F-TTGGTGGCATCGATTATCGG R-GAGCACTTCTTTTGTGATGGC	744	Poirel et al., 2012
bla$_{OXA23}$	F-GATCGGATTGGAGAACCAGA R-ATTTCTGACCGCATTTCCA	501	Ranjbar et al., 2020
bla$_{NAM-C}$	F-TGCGGTCGATTGGAGATAAA R-CGATTCTTGAAGCTTCTGCG	399	Hong et al., 2012
bla$_{GES}$	F-GCTTCATTCACGCACTATT R-CGATGCTAGAAACCGCTC	323	Hong et al., 2012
bla $_{VIM}$	F-GATGGTGTTTGGTCGCATA R-CGAATGCGCAGCACCAG	390	Ellington et al., 2007

bla oxA58	F-CGATCAGAATGTTCAAGCGC R-ACGATTCTCCCCTCTGCGC	529	Poirel et al., 2006
bla KPC	F- TGTCACTGTATCGCCGTC R-CTCAGTGCTCTACAGAAAACC	882	Scavuzzi et al., 2019
bla SIM	F-TACAAGGGATTCGGCATCG R-TAATGGCCTGTTCCCATGTG	570	Ellington et al., 2007
bla SPM	F-AAAATCTGGGTACGCAAACG R-ACATTATCCGCTGGAACAGG	271	Ellington et al., 2007
bla IMP	F-GGAATAGAGTGGCTTAAYTCTC R-CCAAACYACTASGTTATCT	188	Ellington et al., 2007
blaTEM	F: CATTTCCGTGTCGCCCTTATTC R: CGTTCATCCATAGTTGCCTGAC	800	Yazdansetad et al., 2019
bla SHV	F:CGCCTGTGTATTATCTCCCTGTTAGCC R- TTGCCAGTGCTCGATCAGCG	843	Yazdansetad et al., 2019
blaCTX-M	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	550	Yazdansetad et al., 2019

3. RESULT

one hundred and twenty seven Gram-negative isolates were recovered from the collected samples in this study. *Pseudomonas aeruginosa* was the most isolated bateria 43.3%, followed *Klebsiella pneumoniae*, *Escherichia coli*, *Klebsiella sp*, *Proteus mirabilis*, *Acinitobacter baumannii\ calcoaceti*, *Serratia odrifera*, 18.89%, 15.7%, 6.29%, 1.57%, 2.36%, respectively, While the species of *Serratia macescense*, *Serratia liquefuecience*, *Serratia finticola*, *Enterobacter sakazakii Morganella morganii*, *Klebsiella terriginae Raoultella terriginae* were isolated with a percentage of 8% for each, as showen in Table 2.

Table 2: Bacterial species isolated in the study.

Species	**N**	%
Pseudomonas aeruginosa	55	43.3
Escherichia coli	24	18.89
Klebsiella pneumoniae	20	15.7
Klebsiella sp	8	6.29
Proteus mirabilis	8	6.29
Acinitobacter baumannii\ calcoacetia	3	2.36
Serratia odrifera	2	1.57
Serratia macescense	1	0.8
Serratia liquefuecience	1	0.8
Serratia finticola	1	0.8
Enterobacter sakazakii	1	0.8
Morganella morganii	1	0.8

Klebsiella terriginae	1	0.8
Raoultella terriginae	1	0.8
total	127	100%

The isolates showed absolute resistance to Amoxicillin, Ceftazidime and Cefotaxime (100%), and their resistance was high to Amoxicillin-clavulanic acid Tetracycline and Ceftriaxone (99.2%, 98.4% ,96.8% respectively), the least resistance was against Meropenem Norfloxacin and Imipenem at a rate of 58.2% 64.6% 68.5% respectively as shown in Table 3 .

Table 3: antibacterial Susceptibility testing results

Antimicrobia Agent	symbol	**Concentration µg /disc**	susceptible		intermediate		resistant	
			N	**%**	**N**	**%**	**N**	**%**
Amoxicillin-clavulanic acid	AMC	30	-	-	1	0.8	26	99.2
Ceftazidime	CAZ	30	-	-	-	-	127	100
Cefotaxime	CTX	30	-	-	-	-	127	100
Norfloxacin	NOR	30	35	27.6	10	7.9	82	64.6
Meropenem	MEM	10	43	33.9	10	7.9	74	58.2
Cefoxitin	FOX	30	8	6.3	8	6.3	111	87.4
Ceftriaxone	CRO	30	1	0.8	3	2.4	123	96.8
Nitrofurantoin	F	300	4	3.1	12	9.4	111	87.4
Trimethoprim-Sulfamethoxazole	SXT	25	16	12.6	4	3.1	107	84.3
Aztreonam	ATM	30	16	12.6	7	5.5	104	81.9
Imipenem	IPM	10	26	20.5	14	11	87	68.5
Gentamicin	CN	10	24	18.9	5	3.9	98	77.2
Amoxicillin	AX	10	-	-	-	-	127	100
Ampicillin	AM	10	-	-	1	0.8	126	99.2
Ciprofloxacin	CIP	10	16	12.6	9	7.1	102	80.3
Nalidixic acid	NA	30	8	6.3	15	11.8	104	81.9
kanamycin	K	30	4	3.1	8	6.3	115	90.6
Chloramphenicol	C	30	25	19.7	12	9.4	90	70.9
Tetracycline	TE	30	2	1.6	-	-	125	98.4

All bacterial species showed absolute resistance to amoxicillin, cefotaxime, Ceftazidime , results showed that only one isolate of *Pseudomonas aeruginosa* was sensitive to Amoxicillin-clavulanic acid while the all other isolates were resistance. ,concered to Tetracycline, the result showed that all bacterial species were absolute resistance except *Escherichia coli*. other species including ,*Serratia macescense Serratia liquefuecience Acinitobacter baumannii\ calcoacetia , Enterobacter sakazakii klebsiella terriginae Raoultella terriginae*, were absolute resistance to imipenem and meropenem as shown in Table(4)

Table 4: The sensitivity of isolates to antibiotics according to bacterial type.

ISOLATES	ANTIBIOTIC																	
	AMC		CAZ		CTX		NOR		MEM		FOX		CRO		F		SXT	
	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R
Pseudomonas aeruginos(55)	54	98.18	55	100	55	100	42	76.36	35	63.6	55	100	55	100	55	100	55	100
Escherichia coli (24)	24	100	24	100	24	100	17	70.83	15	62.5	17	70.83	23	95.83	21	87.5	17	70.83
Klebsiella pneumoniae (20)	20	100	20	100	20	100	11	55	10	50	16	80	19	95	12	60	13	65
Klebsiella sp(8)	8	100	8	100	8	100	2	25	3	37.5	7	87.5	8	100	4	50	7	87.5
Proteus mirabilis(8)	8	100	8	100	8	100	2	25	3	37.5	6	75	7	87.5	8	100	5	62.5
Acinitobacter bumanii calcoacetia(3)	3	100	3	100	3	100	3	100	3	100	3	100	3	100	3	100	3	100
Serratia macescense(1)	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	-	
Serratia odrifera(2)	2	100	2	100	2	100	-	-	-	-	1	50	2	100	2	100	2	100
Serratia finticola(1)	1	100	1	100	1	100	1	100	-	-	-	-	1	100	-	-	-	-
Serratia liquefuecience(1)	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100
Enterobacter sakazakii (1)	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100
Morganella morganii (1)	1	100	1	100	1	100	-	-	-	-	1	100	-	-	1	100	1	100
Raoultella Terriginae(1)	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100
Klebsiella Terriginae(1)	1	100	1	100	1	100	-	-	1	100	1	100	1	100	1	100	1	100
127	126		127		127		82		74		111		123		111		107	

ISOLATES	ANTIBIOTIC																	
	ATM		IPM		CN		AX		AM		CIP		NA		K		C		TE	
	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R	العدد	%R
Pseudomonas aeruginos (55)	44	80	38	69.1	44	8o	55	100	55	100	42	76.36	55	100	55	100	49	89.1	55	100
Escherichia coli (24)	18	75	17	70.83	16	66.66	24	100	24	100	19	79.16	18	75	20	83.33	18	75	22	91.6
Klebsiella pneumoniae (20)	19	95	15	70	15	75	20	100	20	100	17	85	12	60	17	85	7	35	20	100
Klebsiella sp (8)	7	87.5	4	50	7	87.5	8	100	8	100	8	100	4	50	7	87.5	7	-	8	100
Proteus mirabilis(8)	5	62.5	5	62.5	6	75	8	100	8	100	6	75	7	87.5	7	87.5	3	37.5	8	100

Acinitobacter baumannii calcoacetia(3)	3	100	3	100	3	100	3	100	3	100	3	100	3	100	3	100	2	66.66	3	100
Serratia macescense(1)	1	100	1	100	-	-	1	100	1	100	1	100	1	100	-	-	-	-	1	100
Serratia odrifera(2)	2	100	-	-	2	100	2	100	2	100	1	50	-	-	-	-	-	-	2	100
Serratia finticola(1)	1	100	-	-	-	-	1	100	-	-	-	-	-	-	1	100	-	-	1	100
Serratia liquefuecience(1)	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100
Enterobacter sakazakii (1)	1	100	1	100	1	100	1	100	1	100	1	100	-	-	1	100	1	100	1	100
Morganella morganii(1)	–		-		1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100
Raoultella Terriginae(1)	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100
Klebsiella Terriginae(1)	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	-		1	100
127	104		87		98		127		126		102		104		115		90		125	

Phenotypic and molecular detection of ESBLs and carbapenemase enzymes showed that 96.2% of the isolates were ESBL producers, with detection rates of SHV, TEM and CTX-M being 69.2%, 84.6% and 88.4% respectively (Tables 5, 6, Figures 1).

The result of Phenotypic detection of carbapenemase enzyme, showed that they were presence in (8.80%) of asolates .wherase, the Molecular detection of genes encoding these enzymes showed to the only (5) genes were detected including OXA23, NDM and VIM with rates of 11.5%, 61.5% and 73% respectively while OXA48 and KPC were 42.3% each (Figures 1,2,3,4,5,6,7). as shown in Tables 5 and 6.

Table 5: Phenotypic detection of carbapenemase and ESBLs enzymes.

isolates	**N**	**ESBL (%)**	**carbapenemase(%)**
Pseudomonas aeruginosa	(7)	%)85.7.(6	%)57.1(4
Klebsiella pneumoniae	(7)	%)100(7	%)85.7(6
Escherichia coli	(3)	%)100(3	%)66.6(2
Acinitobacter baumannii	(3)	%)100(3	%)100(3
Klebsiellae sp	(1)	%)100(1	%)100(1
Proteus mirabilis	(1)	%)100(1	%)100(1
Serratia liquefuecience	(1)	%)100(1	%)0(0
Raoultella Terriginae	(1)	%)100(1	%)100(1
Klebsiella Terriginae	(1)	%)100(1	%)100(1
Enterobacter sakazakii	(1)	%)100(1	%)100(1
	26	%)96.2(25	%)80.8 (21

Table 6: Distribution of carbapenemase and extended-spectrum b-lactamases (ESBL)genes among bacterial isolates

isolates	sours	genes													
		NDM	*OXA48*	*OXA23*	*NAM-C*	*GES*	VIM	*OXA58*	KPC	SIM	SPM	IMP	TEM	*SHV*	*CTX-M*
Klebsiella pneumoniae 1	burn	+	+	-	-	-	+	-	+			-	+	+	+
*Klebsiella pneumoniae*2	burn	+	+	-	-	-	-	-	-			-	-	-	+
Klebsiella pneumoniae 3	burn	+	-	-	-	-	+	-	-			-	+	+	+
Klebsiella pneumoniae 4	burn	+	-	-	-	-	+	-	+			-	+	+	+
Klebsiella pneumoniae 5	burn	+	+	-	-	-	+	-	+			-	+	+	+
Klebsiella pneumoniae 6	burn	+	+	-	-	-	+	-	+			-	+	+	+
Klebsiella pneumoniae 7	burn	+	+	-	-	-	+	-	+			-	+	+	+
Escherichia coli 1	burn	-	-	-	-	-	-	-	-			-	+	-	+
Escherichia coli 2	burn	+	-	-	-	-	-	-	+			-	+	+	+
Escherichia coli 3	burn	+	+	-	-	-	+	-	+			-	+	+	+
Enterobacter sakazakii	burn	-	+	-	-	-	-	-	-			-	+	-	+
Raoultella terriginae	burn	+	+	-	-	-	+	-	+			-	+	+	+
Acinitobacter baumannii 1	burn	-	-	+	-	-	+	-	-			-	+	+	+
Acinitobacter baumannii 2	burn	-	-	+	-	-	+	-	-			-	+	+	-
Acinitobacter baumannii 3	burn	+	-	-	-	-	+	-	+			-	+	+	+
Serratia liquefuecience	burn	+	-	-			-	-	-			-	+	+	+
Klebsiella terriginae	burn	+	+	-			+	-	-			-	+	+	+
Proteus mirabilis	burn	+	+	+			+	-	-			-	+	+	+
Klebsiellae sp	burn	+	-	-	-	-	+	-	+			-	+	+	+

Pseudomonas aeruginosa 1	burn	-	-	-			+	-	-			-	+	-	+
Pseudomonas aeruginosa 2	burn	-	+	-			+	-	+			-	-	-	+
Pseudomonas aeruginosa 3	burn	-	-	-			-	-	-			-	+	-	-
Pseudomonas aeruginosa 4	burn	-	-	-			+	-	-			-	-	-	+
Pseudomonas aeruginosa 5	burn	-	-	-			+	-	-		-	-	+	+	+
Pseuomonas aeruginosa 6	burn	-	-	-			-	-	-		-	-	-	+	-
Pseudomonas aeruginosa 7	burn	+	-	-			+	-	-		-	-	+	-	+
total	26	16 **61.5%**	11 42.3 %	3 11. 5 %	0 %	0%	19 73 %	0 0 %	11 42. 3%	0 %	0%	0 0%	22 84.6 %	18 69. 2 %	23 88.4 %

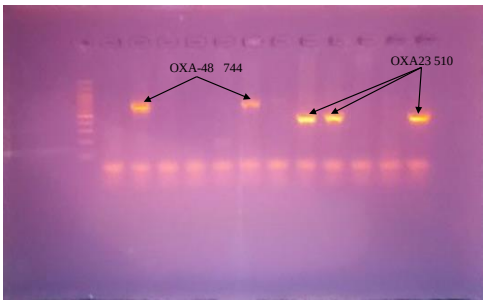

Fig 2. KPC gene (882bp)

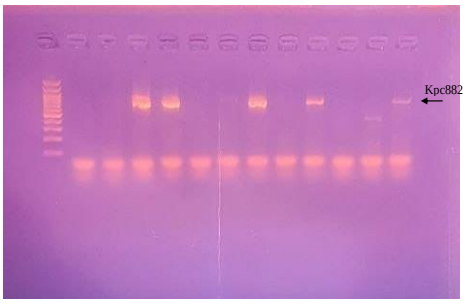

Fig 1. $_{OXA23}$ gene (501bp) and $_{OXA48}$(744)

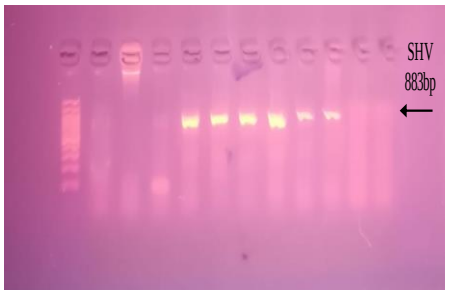

Fig 3. CTX-M *gene* (550 bp)

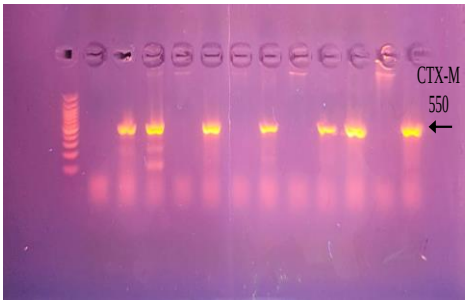

Fig 4..SHV *gene* (883 bp)

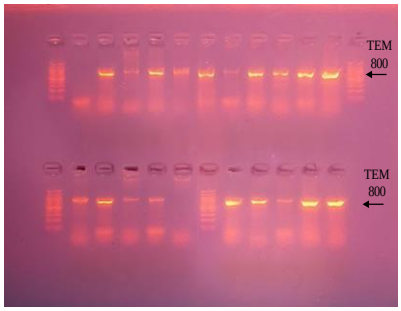

Fig 5. TEM *gene* (800 bp*)*

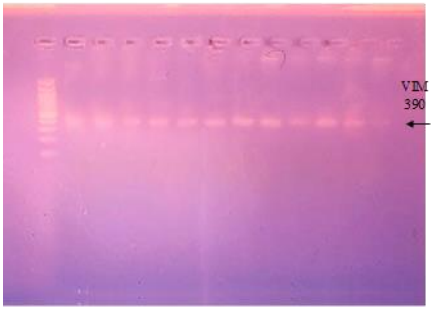

Fig 6. VIM *gene* (390 bp*)*

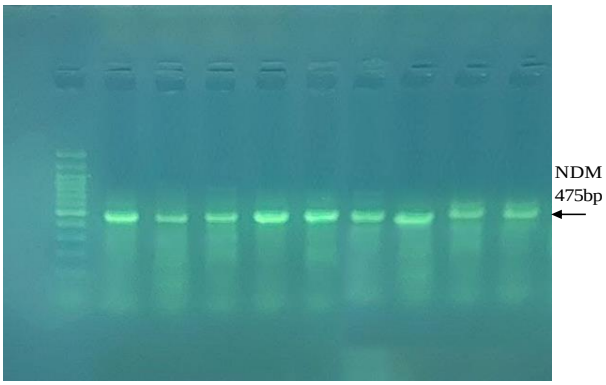

Fig 7. NDM *gene* (475bp*)*

4. DISCUSSION

The proportions and types of Gram-negative bacteria isolated from burns infection are discrepancy according to the studies and research. Researcher Rahim 2021 indicated that the Gram-negative bacterial species isolated from burns were *Pseudomonas aeruginosa Eenterobacter sp, Proteus vulgaris Escherichia coli, Klebsiella pneumoniae,* and *Aeromonas sorbia* at 20% 16.19, %, 13.33%, 10.47%, 7.6%, 6.6%, 4.7% respectively. While Ghafil and flieh (2021) indicated that the highest isolation rate was for Proteus mirabilis with 31.1%, followed by *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia haemolyticus, Acinitobacter spp* with 22.2%, 17.78%, 4.4%, respectively, while both of *Salmonella spp, Burkholderia cepacia* with 2.2% each of them . In addition, the study of conducted by Tchakal-Mesbahi et al. (2021) found that *Pseudomonas aeruginosa* are the predominant Gram-negative bacteria in burn injuries, with 25.71%, followed by *Klebsiella pneumoniae, Acinetobacter baumannii, Proteus spp, Escherichia coli, Enterobacter spp, Citrobacter freundii*, at 20.15%, 18.70%, 7.91%, 5.75%, 3.22%, 1.73%, respectively.

The disk diffusion method shows a preliminary and rapid view of the sensitivity and resistance of bacterial isolates to antibiotics, but it does not show the mechanism used by bacteria. In a study performed by AL-Azzawi and AL-Kalifawi (2023), they indicated that the high resistance of negative bacteria isolated from burns to Meropenem with 75%, followed by Imipenem at 35%, while, the resistance rate of Ciprofloxacinwas 25% and 20% for Gentamicin.

Pseudomonas aeruginosa isolates showed absolute resistance to most antibiotics except for Amoxicillin- clavulanic acid Chloramphenicol, Aztreonam, Ciprofloxacin, Imipenem, and Meropenem, 98.18%, 89.1%, 80%,76.36%,69.1%, 63.6% respectively.

A study carried out by Tchakal-Mesbahi et al. 2021 indicated that the *Pseudomonas aeruginosa* bacteria isolated from burns were resistant to Ciprofloxacin, Ceftazidime, and Imipenem by 50%, 63.79 %, and 70.64%, respectively. While Elguindy et al. 2021 indicated that *Pseudomonas aeruginosa* isolated from burns showed resistance to Ciprofloxacin Ceftriaxone Ceftazidime Imipenem andMeropenem at 76.7%, 80%,66.7%,53.3% ,31%respectively

Escherichia coli isolates were 100% resistance to Amoxicillin-clavulanic acid ,Amoxicillin ,Ceftazidime ,Cefotaxime, Ampicillin but showed less sensitivity to Imipenem,Gentamicin Meropenem70.83%,66.66%,62.5% respectively. In a study by Rahim in 2021, indicated that *Escherichia coli* was resistance to, Ciprofloxacin, Cefotaxime Gentamicin at rate of 85.7%,55.6%,44.4% respectively, and absolute sensitivite to Imipenem. In addition, Yavari et al.2024 indicated but resistance to the antibiotic Ceftazidime and Cefotaxime at rate 66.6%,33.3% respectively.

Klebsiella pneumoniae isolates showed absolute resistance to Amoxicillin-clavulanic acid ,Amoxicillin ,Ceftazidime

,Cefotaxime, Ampicillin, Tetracycline while their resistance to Imipenem and Meropenem was 70% and 50%, respectively, The study of Yavari et al., (2024) showed that *Klebsiella pneumoniae* isolated from burns was absolutely resistant to Ceftriaxone, then to Cefotaxime and Ciprofloxacin at a rate of 80% for each, while Imipenem and Meropenem gave a resistance rate of 40% for each.

The bacterial species *Proteus mirabi*lis gave absolute resistance to Ceftazidime Cefotaxime Tetracycline Nitrofurantoin and gave resistance to the antibiotics Norfloxacin, Meropenem, Imipenem at a rate of 25%, 37.5%, and 62.5%, respectively. In a study in Iraq, researcher Rahim (2021) stated that the bacterial species *Proteus mirabilis* isolated from burns showed absolute resistance to Cefotaxime, while it showed resistance to Gentamicin, Ciprofloxacin, and Imipenem antibiotics, at a rate of 85.7%, 66%, and 11.1%, respectively, while researcher Alharbi (2022), indicated that the bacterial species *Proteus mirabilis* isolated from wound infections showed resistance to Ceftazidime, Ciprofloxacin, and Gentamicin at a rate of 50% for each, while it showed absolute sensitivity to Imipenem and Meropenem antibiotics.

The bacterial species *Acinitobacter baumannii* showed absolute resistance to all antibiotics used in the study except for Chloramphenicol. Saadulla and Muhammad (2023) indicated that the clinical *Acinitobacter spp* isolates showed resistance to the antibiotics Ceftriaxone, Ciprofloxacin, Imipenem at a rate of 81%, 79%, 75% respectively. The researcher Yavari (2024) also indicated that the *Acinitobacter spp* isolates isolated from burns were resistant to all antibiotics used in that study. The bacterial species *Enterobacter sakazakii* showed absolute sensitivity to the antibiotic Naldixic acid, while it showed absolute resistance to the rest of the antibiotics. The researcher Temesgen et al., (2023) indicated that the bacterial species *Enterobacter spp* showed absolute sensitivity to Meropenem, Tetracycline and resistance to Ceftazidime Gentamicin at a rate of 67% and 17% respectively.

The bacterial species *Serratia liquefuecience Raoultella terriginae* showed absolute resistance to all antibiotics used in the study, which makes them among the highly resistant isolates to antibiotics, if not among the completely resistant isolates (PDR)

The outcomes of the sensitivity and resistance assays exhibited by all bacterial isolates under examination can be classified into three categories: Multidrug-resistant (MDR) refers to acquired insensitivity to at least one agent in three or more antibiotic classes; extensively drug-resistant (XDR) denotes acquired resistance to at least one agent in all antibiotic classes except for two or fewer; and pandrug-resistant (PDR) signifies resistance to all antibiotic classes (Magiorakos *et al.*,2012). The results showed that 0.79% of the isolates were Multiple Drug-Resistant (MDR), 68.5% of them were Extensively Drug-Resistant (XDR), while Thirty-nine isolates (30.71%) were Pan Drug-Resistant (PDR). Tharia et al. (2024) indicated that a high level of multidrug resistance (MDR) represnted 76% of Gram-negative bacteria isolated from clinical isolates, distributed between XDR and MDR. Abdelhadi et al. (2024) also indicated that Gram-negative bacteria isolated from bloodstream infections were multidrug resistant. While ,Sobouti et al. (2020) indicated that *Acinetobacter baumannii* isolates isolated from burns were identified as PDR XDR MDR 14.5%, 27.5%, and 58%, respectively.

Multidrug resistance is a critical concern in public health. Previously, resistance was confined to hospital-acquired diseases; however, it has recently become prevalent in the population, environment (water and soil), and animals, owing to the indiscriminate use of antibiotics (Bharadwaji et al., 2022).

The current study showed that *Acintobacter baumannii* isolates have the OXA23, VIM, NDM genes and the SHV, CTX-M, TEM broad-spectrum beta-lactamase enzymes genes. Mahmood and Al-Brefkani (2022) explained that *Acintobacter baumannii* isolates possessed VIM, OXA23, OXA51 with 100%, 98%, and 71%, respectively, while NDM, KPC were not detected. The researcher Zarabadi-Pour et al. (2021) also indicated that clinical *Acintobacter baumannii* isolates had CTX-M and TEM enzymes at 20%, 7.9%, respectively, but SHV was not detect. A study conducted by Saaed and Sulaiman (2025) showed *Acinetobacter baumannii* isolates from clinical and environmental sources showed the presence of OXA23 in all isolates tested.

Klebsiella pneumoniae showed the presence of genes NDM, VIM, KPC, and OXA48, which encode carbapenemase enzymes. Hamad et al., (2022) confirmed in their study that *Klebsiella pneumoniae* isolated from urinary tract infections in Iraq possessed the NDM OXA48, VIM, IMP genes, ElAila et al., (2023) indicated that broad-spectrum beta-lactamase enzymes are present in pathological isolates from children, including TEM, CTX-M, SHV at a rate of 57%, 38.3%, 60% respectively, Jafari-sales et al., (2023) indicated that clinically isolated *Klebsiella pneumoniae* isolates possessed the KPC VIM genes at 18%, 3% respectively.

The results showed that *Escherichia coli* isolates possessed SHV, CTX-M, TEM genes as well as carbapenemase genes KPC, OXA48 NDM, VIM, which is close to what was found by researcher GatyaAlmuyhi et al. (2022), as *Escherichia coli* isolated from clinicl sources contained OXA48 genes at a rate of 57.8%. Researcher Hussaini et al., (2023) indicated that the strains of *Escherichia coli, Klebsiella pneumoniae*, isolated from clinical sources showed the presence of NDM, OXA genes at 42.8% and 57.14%, respectively.

Proteus mirabilis showed the presence of ESBLs genes and carbapenemase genes OXA23, OXA48, NDM, VIM. a study by AL-Nabhani and Shami (2023) indicated that *Proteus mirabilis* isolates showed the presence of the OXA23 gene, which is

consistent with our current study. The researcher ElTaweel et al., (2024) indicated that clinically isolated *Proteus mirabilis* isolates had SHV, CTX-M, TEM OXA48, NDM,

The study showed that *Pseudomonas aeruginosa* isolates contained SHV, CTX-M, TEM KPC, OXA48 NDM, VIM genes, but at a lower rate and frequency than the rest of the isolates. A study carried out by Ali et al., (2023) indicated the presence of VIM genes in *Pseudomonas aeruginosa* isolates isolated from pathological sources and the absence of GIM SPM. Cayci et al., (2022) indicated that *Pseudomonas aeruginosa* isolated from different pathological sources showed presence the NDM, VIM genes at a low frequency and while other genes including IMP, GES SIM, SPM, GIM are not found. *Pseudomonas aeruginosa* showed the presence of beta-lactam resistance genes, but not at the frequency found in other bacterial species under study, although these isolates were resistance to all antibiotics under study may be attributed to its reliance on other mechanisms such as transport systems such as the ABC transport system, which works to release the antibiotic to the outside. When a mutations occurs in genes coding to ABC transport, *Pseudomonas aeruginosa* becomes sensitive third genera tion of cephalosporin and this is what the researchers (Younis and Faisal 2024) confirmed in their study.

5. CONCLUSION

The current study recorded Pseudomonas aeruginosa was the most frequently isolated bacteria among gram negative bacteria isolated from burn infection and the most effective antibiotic on isolates was meropenem,The study also identified a significant number of MDR , XDR and PDR strains among the tested Gram-negative bacteria,and recorded the presence of carbapenemase and extended-spectrum β-lactamase (ESBLs) genes. this is a serious warning in the treatment of burn wound infections. It is suggested that new antibiotics be used to determine the sensitivity of clinical isolates of the burn patian to develop new treatment strategies.

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