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Antimicrobial resistance and the distribution of carbapenemase (*bla*_{OXA-48}, *bla*_{NDM-1}, *bla*_{VIM}, *bla*_{KPC} and *bla*_{IMP}) and *OmpA* alleles in different bacterial species isolated from burn infection of Thi-Qar province of Iraq

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ABSTRACT

Background: Bacteria's remarkable resistance to antibiotics is caused by carbapenem resistance genes, which presented a significant obstacle to medication. **Aim:** This study aims to determine the prevalence of carbapenem-resistant genes and their genetic variations (alleles) in each type of carbapenem gene for various bacterial isolates. **Methods:** Using swabs from hospital burn patients, the doctors isolated 57 bacteria from 152 individuals. The bacterial species were identified using standard microbiological techniques, followed by antibiotic susceptibility tests and 16S *rDNA* sequencing. All isolates have their carbapenem genes molecularly detected using PCR. **Results:** Bacteria classified as Gram-negative (89.4%) versus Gram-positive (10.5%), comprising 13 distinct species found using 16S *rRNA* sequencing. FEP, DOR, CIP, CRO, and AMC were all 100% resistant in 57 bacterial isolates. The MEM, ATM, and AK (98%) and IMP (96%) were analyzed both genotypically and phenotypically for carbapenem resistance and *OmpA* genes. Of the 57 isolates, *bla*_{OXA-48} 47 (82.45%), *bla*_{NDM-1} 46 (80%), *bla*_{VIM} 8 (14%) and *bla*_{KPC} 1 (1.75%) were found; however, no bacterium exhibited the *bla*_{IMP} gene. They showed up for 33 (57%) of *OmpA*. As bacterium isolated, the *bla*_{OXA-48}, *bla*_{NDM-1}, and *OmpA* genes were more prevalent. At bacterium isolated, it exhibited resistance to the carbapenems DOR, IMP, and MEM, the frequency of the *bla*_{OXA-48} gene was higher (80.70%, 83.63%, and 82.14%, respectively), but the frequency of the *OmpA* gene was (57.89%, 60%, and 58.92%, respectively). The *bla*_{NDM-1} gene displayed four distinct alleles in several bacterial species, whereas the sequence of *bla*_{OXA-48} and *OmpA*. **Conclusion:** The presence of the *OmpA* gene alongside the carbapenem genes is one of the primary causes of the bacterial isolates' resistance to carbapenem antibiotics.

Introduction

When the body comes into contact with a heat source, burns happen [1]. Every year, between 7 and 12 million individuals worldwide suffer

serious burns that require hospitalization, making burns and burn-related injuries a significant public health concern [2]. Burns provide an ideal environment for the growth of bacteria, which can result in more abundant and long-lasting sources of

infection that result in surgical wounds. This is largely because the patient remains in the hospital for a longer amount of time and a larger area is affected. Given the circumstances, infection plays a major role in the morbidity and mortality of hospitalized burn patients [3, 4]. Bacteria are among the most significant issues that arise in burn care units [5-7]. The primary organisms that infect burn wounds are *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Additionally, when overall antimicrobial resistance rises, the antibiotic-resistant pattern of burn isolates is likewise local and rapidly evolving [8].

Recombination of exogenous DNA into the chromosome or mutations at other chromosomal loci might result in the horizontal transmission of resistance genes (delivered by plasmids or transposons) and antibiotic resistance [9]. As the strongest β -lactam antibiotics, Carbapenems are frequently used as a last resort to treat infections that are resistant to cephalosporins [10]. Due to the large number of resistance genes and the lack of effective therapies, the rise of carbapenem-resistant Enterobacteriaceae is a serious public health concern. One of the few drugs believed to be beneficial in treating infections caused by multi-resistant Gram-negative bacteria is carbapenem [11]. Carbapenem resistance is caused by mutations or other alterations that alter the quantity of penicillin-binding proteins (PBPs) generated or their binding affinity. Mutations in the PBP protein and/or decreases in PBP transcription also result in carbapenem-resistant phenotypes [12,13]. Mutations can occasionally occur as silent mutations (degeneracy) that do not alter the amino acid, or they can occur to alter the amino acid within a gene but in an inactive region, which will not affect the cell in any noticeable way. On the other hand, if it happens in the active gene, it can affect the gene product and cause an obvious shift in the organism's phenotypic response [14]. Because bacteria have haploid DNA for the majority of their genes and a short generation turnover, phenotypic variation due to gene or point mutations can occur relatively quickly. Spontaneous mutations, which result from errors in DNA replication, can happen without the use of an inducer [15,16].

Because a significant portion of burns have the potential to be fatal. Determining the frequency of carbapenem-resistant genes in burn settings as well as the genetic variations (alleles) in each type

of carbapenem gene for various bacterium isolates is the goal of this study.

Materials and Methods

Bacterial Isolates

Using swabs from burn infection patients at hospitals in several provinces (Nasiriyah, Al-Muthanna, Maysan, and Basrah), the physicians obtained fifty-seven samples from 152 burn patients between February 2023 to October 2023. For testing, these swabs (Citotest) were cultivated on nutritional agar (TM media) for 20 hours at 37° C after being inserted in tubes of brain-heart infusion broth (BHIB) as a transport medium.

Before the samples were collected, the patients' verbal agreement was acquired. Participants' safety was guaranteed by taking the appropriate safety measures during sample collection. The Iraqi Ministry of Health's Ethics Committee also conducted this work, adhering to all applicable national laws. The research protocol was approved by the Ethics Committee of the Institution (Protocol No.99 dated 2023 \2\14).

Bacterial Species Identification

In accordance with the Presto™ Mini g DNA bacterium kit methodology, a single colony of the bacterial isolate was cultured in 5 ml of sterilized BHIB and incubated at 37°C for 24 hours in order to extract DNA. Using the primers 27F 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R 5'-GGTTACCTTGTTACGACTT-3' (Alpha, USA), the bacterial species was identified by 16S rDNA sequencing in accordance with [17]. 2 µl of each forward and reverse primer, 25 µl of Go Taq green master mix (Promega, USA), 19 µl of nuclease-free water (Bioneer, Korea), and 2 µl of DNA template made up the amplification reaction's composition. The thermocycler (Bioneer) condition includes 35 cycles of denaturation (95° C for 30 seconds), annealing (55° C for 30 seconds), and extension (72° C for 1 minute). Last but not least, the extension was 72° C for 2 minutes with a modification [18]. Agarose gel electrophoresis was performed using 1.5% agarose gel in order to identify the bands that were 1500 base pairs in length. The MacroGen company received 20 µl of the PCR product for 57 isolates in order to purify and sequence them. The bacterial species were identified using the Basic Local Alignment search tool (BLAST) and the National Centre for Biotechnology Information [19]. Following the concatenation of all of the bacterial species' nucleotide sequences together at

1166 bp using the Clustal Omega program [18], the bacterial species and their reference strain built the phylogenetic tree using MAFFT (Multiple Alignment Program for Nucleotide Sequences).

Antibiotic Susceptibility Test

The Kirby-Bauer disc diffusion method was used to assess the antibiotic resistance of fifty-seven isolation. Ten antibiotics from the "Mast group" were evaluated (Amikacin (AK) 10 µg, Amoxicillin-calavulanic AMC) acid 30 µg, Azithromycin (ATM) 30 µg, Cefepime (FEP) 10 µg, Ceftriaxone (CRO) 10 µg, Ciprofloxacin (CIP) 10 µg, Doripenem (DOR) 10 µg, Imipenem (IMP) 10 µg, Meropenem (MEM) 10 µg, and Levofloxacin (LEV) 5 µg. Cultivation was carried out on Muller Hinton agar (Biomark) in compliance with the guidelines provided by the Clinical and Laboratory Standard Institute [20].

Detection of Carbapenem Genes

The carbapenem genes including *bla_{OXA-48}*, *bla_{NDM-1}*, *bla_{VIM}*, *bla_{KPC}*, *bla_{IMP}* and *OmpA* were amplified utilising the primers specified in Table (1).

Twelve microliters of Go Taq green master mix, produced by Promega in the United States, two microliters of each forward and reverse primer, ten microliters of nuclease-free water, produced by Bioneer in Korea, and one microliter of DNA template were also included in the combination. The thermocycler (Bioneer) condition includes 35 cycles of denaturation (94° C for 30 seconds), annealing (56° C *bla_{OXA-48}*, *bla_{NDM-1}*, *bla_{KPC}*, and *bla_{IMP}*, 54.9° C *bla_{VIM}* and 51° C *OmpA* for 30 seconds), and extension (72° C for 1 minute). The samples were sent to Macrogen for sequencing in order to determine the carbapenem and *OmpA* alleles. The results were analyzed using BLAST and Multiple Sequence Alignment (MSA) via the Clustal Omega tool to compare the carbapenem and *OmpA* gene sequences.

Statistical Analysis

Using SPSS version 17.0, a one-way ANOVA was performed to evaluate the differences between the tested tests, with $P < 0.05$ being statistically significant.

Results

Fifty-seven (37.5%) bacterial isolates were identified from 152 samples of burn patients. Six (10.53%) gram positive bacteria and 51 (89.47%)

gram negative bacteria were found to vary significantly at $P < 0.05$.

Bacterial Species

Approximately 1500 base pairs away from the conventional molecular DNA ladder, the 16S *rRNA* gene of each of the 57 bacterial isolates was evident on agar gel electrophoresis as a separate band per isolate. The effective sequencing of 57 isolates' 16S *rRNA* gene allowed for the identification of bacterial species. *Pseudomonas aeruginosa* (n=28 / 49.12%) was the most common ($P < 0.05$) among the 13 species. *Enterococcus faecalis* (n=1/1.75%), *Pseudomonas azotoformans* (n=2/3.5%), *Escherichia coli* (n=3/5.26%), *Acinetobacter baumannii* (n=4/7.01%), and *Klebsiella pneumoniae* (n=12/21.05%). *Enterobacter hormaechei* (n = 1 / 1.75%), *Streptococcus infantis* (n = 1 / 1.75%), *Streptococcus mitis* (n = 1 / 1.75%), *Streptococcus pneumonia* (n = 1 / 1.75%), *Desemzia incerta* (n = 1 / 1.75%), and *Providencia rettgeri* (n = 1 / 1.75%).

Phylogenetic tree of 16S rRNA gene of bacterial species

Figure (1) illustrates The distribution and evolutionary relationships among twelve unique bacterial species identified from burn illnesses, together with their respective type strains.

The National Center for Biotechnology Information (NCBI) has registered five bacterial isolates as new worldwide strains under the designations IRQNAS215, IRQNAS216, IRQNAS217, IRQNAS218 and IRQNAS219.

Antimicrobial Susceptibility Test

Table (2) shows the antimicrobial susceptibility test findings for 57 isolates. The 100% resistant bacteria FEP, DOR, CIP, CRO, and AMC that were isolated from burn infections did not respond to the medicines. The percentages of resistance to various antibiotics were as follows: IMP (96%) and MEM, ATM, and AK (98%) with no significant differences at ($P < 0.05$).

Carbapenem Resistant Genes

There were five carbapenem and *OmpA* genes found. When compared to other genes, the *bla_{OXA-48}* gene was found in 47 out of 57 (82.45%) bacterial isolates, with significant differences at $P \leq 0.05$. When compared to other genes, the *bla_{NDM-1}* gene was found in 46 out of 57 (80%) with significant differences at $P < 0.05$. There were eight (14%) *bla_{VIM}* gene detections with no discernible variations. Only 1 out of 57 (1.75%) had the *bla_{KPC}*

gene, and there were no discernible changes. All isolated bacteria lacked the *bla_{IMP}* gene. *P. aeruginosa* had an overall gene frequency of 49 (85%), *K. pneumoniae* 24 (42%), *A. baumannii* 8 (14%), *E. coli* 5 (8%), *P. azotoformans* 4 (7.01%), *E. aquaticum* 3 (5%), *P. rettgeri*, *S. infantis* 2 (3% each), *S. pneumoniae*, *E. hormacchei* 1 (1% each), *D. incerta* 3 (5.26%), and no results for *E. facials* and *S. mitis*. In Figure (2) and Table 3, the *OmpA* gene was found in 33 (57%) of the separated species, with significant differences at $P \leq 0.05$.

Frequency of Resistance Genes in the Isolates

Of the isolates, 17 (29.82%) have both the *bla_{OXA-48}* and *bla_{NDM-1}* genes, 16 (28.07%) have the *bla_{OXA-48}*, *bla_{NDM-1}*, and *OmpA* genes, and 7 (12.28%) have the *bla_{OXA-48}* and *OmpA* genes. The *bla_{OXA-48}*, *bla_{NDM-1}*, *bla_{VIM}*, and *OmpA* genes are present in 5 (8.77%) of the isolates. Three (5.25%) were required to have solely the *bla_{NDM-1}* gene. Only one (1.75%) isolate out of four seemed to have either the *bla_{NDM-1}*, *bla_{KPC}*, *OmpA*, *bla_{OXA-48}*, *bla_{NDM-1}*, and *bla_{VIM}* genes, whereas two (3.50%) isolates have the *bla_{NDM-1}* and *OmpA* genes. Only one isolate, however, tested positive for *bla_{OXA-48}*, and only one tested negative for every gene. Overall, there was a significant difference ($p \leq 0.05$) in the frequency of the *bla_{OXA-48}*, *bla_{NDM-1}*, and *OmpA* genes among bacterial isolates.

Comparison Between DOR,IMP and MEM and *bla_{OXA-48}*, *bla_{NDM-1}*, *bla_{VIM}*, *bla_{KPC}*, *bla_{IMP}*, and *OmpA* genes

By comparing resistance genes with beta-lactam antibiotics from the carbapenem family, it was discovered that the majority of isolates exhibited resistance to these drugs, as well as the frequency of the genes causing this resistance. Because the *bla_{OXA-48}* gene is more frequently

associated with DOR, IMP, and MEM carbapenem antibiotics (82.45%, 85.45%, and 83.92%, respectively), it is followed by the *bla_{NDM-1}* gene (80.70%, 83.63%, and 82.14%, respectively) and the *OmpA* gene (57.89%, 60%, and 58.92%, respectively). However, Table (4) shows that the other genes were less common with carbapenem antibiotics, with a significant difference at $p \leq 0.05$.

Comparison Among Alleles of *bla_{OXA-48}*, *bla_{NDM-1}* and *OmpA* Genes

Thirteen bacterial isolates, including *D. incerta*, 5,19- *P. aeruginosa*, 16,17- *A. baumannii*, 40,41- *E. coli*, 3,6- *K. pneumoniae*, 22,23- *P. azotoformans*, 15- *S. pneumoniae*, and 38- *S. infantis*, have been shown to have a single allele of the *bla_{OXA-48}* gene. 15 bacterial isolates have four distinct alleles of the *bla_{NDM-1}* gene: 19-*P. aeruginosa* has the first allele, 23-*P. azotoformans* has the second, 16-*A. baumannii* has the third, and the other 12 bacterial isolates, which include 5, 18, 41, 38, 33, 17, 15, 6, 1, 3, 22, and 40, have the fourth allele. The *OmpA* gene, which is present in 30 bacterial isolates, has only one allele, according to the findings. These isolates include: NO.4,5,14,19,20,21,25,26,29,30,36,37,43,50,51,53 - *P. aeruginosa*, 40- *E. coli*, 3,6,9,45,46,47,48,49,55- *K. pneumoniae*, 18- *E. aquaticum*, 11- *P. rettgeri*, 38- *S. infantis*, and 57- *E. facials*. Conversely, *bla_{NDM-1}* and *OmpA* alleles were more prevalent than *bla_{OXA-48}*, with a very significant difference at $p \leq 0.05$. Figure 3.

Recording of Carbapenem Genes in Various Bacterial Species

This study is the first to document *bla_{OXA-48}* and *bla_{NDM-1}* carbapenemase genes in both Gram-positive and Gram-negative bacterial species, with sequences deposited in GenBank (Table 5).

Table 1. The primers, sequence and size to amplify carbapenem genes.

No.	Primers	Primer sequence	Length (bp)	Product size (bp)
1	F- <i>bla</i> _{KPC}	5- CGTCTAGTTCTGCTGTCTTG-3	20	798 ^[21]
	R- <i>bla</i> _{KPC}	5-CTTGTCATCCTTGTAGGCG-3	20	
2	F- <i>bla</i> _{VIM}	5-GATGGTGTGTTGGTCGCATA-3	19	390 ^[21]
	R- <i>bla</i> _{VIM}	5-CGAATGCCGAGCACCAG -3	17	
3	F- <i>bla</i> _{IMP}	5-GGAATAGAGTGGCTTAAYTCT-3	21	232 ^[21]
	R- <i>bla</i> _{IMP}	5-CGGTTTAAAYAAAACAACCACC-3	21	
4	F- <i>bla</i> _{OXA-48}	5-GCGTGGTTAAGGATGAACAC -3	20	438 ^[21]
	R- <i>bla</i> _{OXA-48}	5-CATCAAGTTCAACCCAACCG -3	20	
5	F- <i>bla</i> _{NDM-1}	5-GGTTTGGCGATCTGGTTTC -3	20	621 ^[21]
	R- <i>bla</i> _{NDM-1}	5-CGGAATGGCTCATCACGATC -3	20	
6	F – <i>OmpA</i>	5-TCTTGGTGGTCACTTGAAGC-3	20	100 ^[22]
	R – <i>OmpA</i>	5-ACTCTTGTGGTTGTGGAGCA-3	20	

Table 2. The antibiotic resistance of bacterial species isolates against ten antibiotic

Bacterial species	no.	FEP n (%)	DOR n (%)	IMP n (%)	MEM n (%)	ATM n (%)	CIP n (%)	AK n (%)	CRO n (%)	AMC n (%)	LEV n (%)
<i>Desemzia incerta</i>	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
<i>Pseudomonas aeruginosa</i>	28	28(100)	28 (100)	27(96)	28(100)	28(100)	28(100)	28(100)	28(100)	28(100)	28(100)
<i>Pseudomonas azotoformans</i>	2	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)
<i>Klebsiella pneumoniae</i>	12	12(100)	12(100)	11 (91)	11 (91)	11 (91)	12(100)	11 (91)	12(100)	12(100)	11 (91)
<i>A.baumannii</i>	4	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)
<i>E.coli</i>	3	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)
<i>Providencia rettgeri</i>	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
<i>Enterobacter hormacchei</i>	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
<i>Exiguobacterium aquaticum</i>	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
<i>Streptococcus pneumoniae</i>	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
<i>streptococcus infantis</i>	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
<i>Enterococcus facials</i>	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
<i>Streptococcus mitis</i>	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
Total	57	57 (100)	57 (100)	55 (96)	56(98)	56(98)	57 (100)	56(98)	57 (100)	57 (100)	56 (98)

* p≤0.05: Levofloxacin (LEV), Ciprofloxacin (CIP), Cefepime(FEP), Doripenem(DOR), Meropenem(MEM), Imipenem(IPM), Azthromycin(ATM), Amikacin(AK), Ceftriaxone(CRO) and Amoxicillin-calavulanic acid (AMC)

Table 3. Occurrence of carbapenem resistance and *OmpA* genes in bacterial species

Bacterial species	no. of isolate	Carbapenem genes					Total n (%)	<i>OmpA</i> n (%)
		<i>bla</i> _{OXA-48} n (%)	<i>bla</i> _{NDM-1} n (%)	<i>bla</i> _{VIM} n (%)	<i>bla</i> _{KPC} n (%)	<i>bla</i> _{IMP} n (%)		
<i>Pseudomonas aeruginosa</i>	28	*24(85)	*22(78)	3(10)	0(0.00)	0(0.00)	49(85)	18(64)
<i>Klebsiella pneumoniae</i>	12	*11(91)	10(83)	3(25)	0(0.00)	0(0.00)	24(42)	9(75)
<i>A.baumannii</i>	4	4(100)	4(100)	0(0.00)	0(0.00)	0(0.00)	8(14)	1(25)
<i>E.coli</i>	3	2(66)	3(100)	0(0.00)	0(0.00)	0(0.00)	5(8)	1(33)
<i>pseudomonas azotoformans</i>	2	2(100)	2(100)	0(0.00)	0(0.00)	0(0.00)	4(7)	0(0.00)
<i>Desemzia incerta</i>	1	1(100)	1(100)	1(100)	0(0.00)	0(0.00)	3(5)	0(0.00)
<i>Exiguobacterium aquaticum</i>	1	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	3(5)	1(100)
<i>Providencia rettgeri</i>	1	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(3)	1(100)
<i>Streptococcus pneumoniae</i>	1	1(100)	1(100)	0(0.00)	0(0.00)	0(0.00)	2(3)	0(0.00)
<i>streptococcus infantis</i>	1	1(100)	1(100)	0(0.00)	0(0.00)	0(0.00)	2(3)	1(100)
<i>Enterobacter hormacchei</i>	1	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(1)	0(0.00)
<i>Streptococcus mitis</i>	1	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0)	0(0.00)
<i>Enterococcus facials</i>	1	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0)	1(100)
Total	57	47(82)	46(80)	8 (14)	1(1.75)	0(0.00)	103	33(57)

* p≤0.05

Table 4. Comparison between carbapenem genes presence in DOR, IMP and MEM resistant strains.

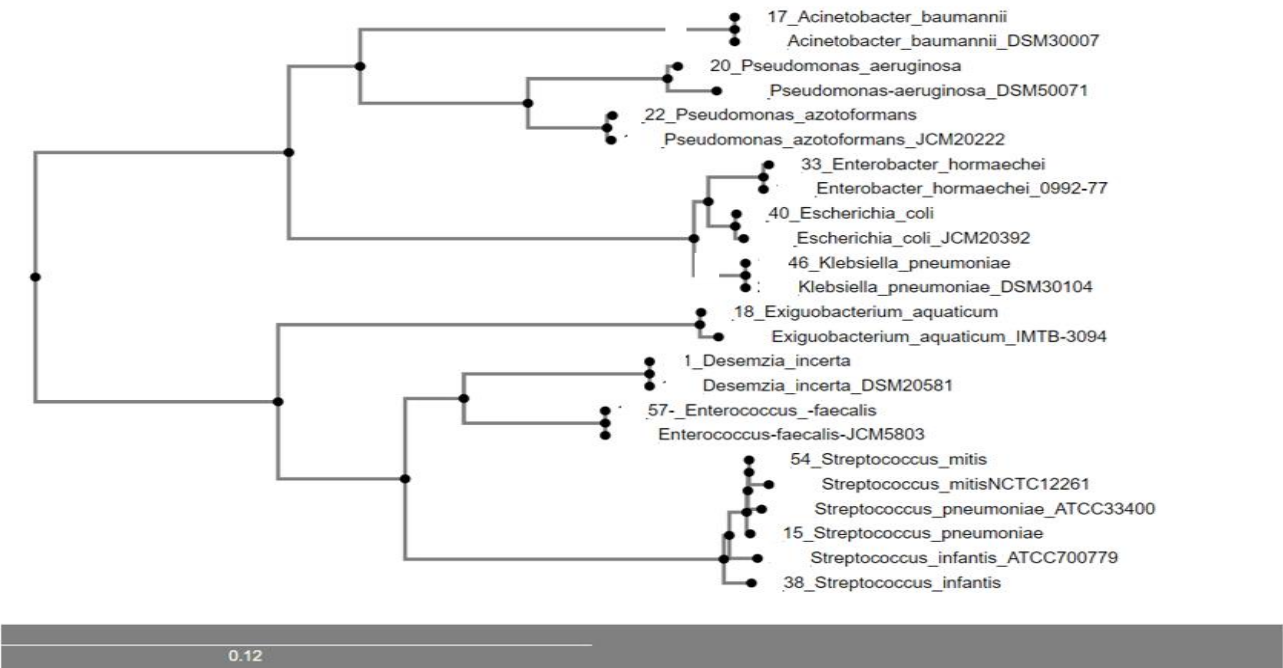
Disc Genes	DOR n=57	IMP n= 55	MEM n= 56
<i>bla</i> _{OXA-48} n= 47	*82.45%	*85.45%	*83.92%
<i>bla</i> _{NDM-1} n= 46	*80.70%	*83.63%	*82.14%
<i>bla</i> _{VIM} n= 8	14.03%	14.54%	14.28%
<i>bla</i> _{KPC} n= 1	1.75%	1.81%	1.78%
<i>bla</i> _{IMP} n= 0	0.00%	0.00%	0.00%
<i>OmpA</i> n= 33	*57.89%	*60.00%	*58.92%

*p≤0.05

Table 5. First recording of carbapenem gene in different bacterial species.

The gene	Bacterial species	Recording code
<i>bla</i> _{OXA-48} gene	1- <i>D. incerta</i>	<i>bla</i> _{OXA-48} -IRQNAS221-G
	15- <i>S. pneumoniae</i>	<i>bla</i> _{OXA-48} -IRQNAS222-G
	23- <i>P. azotoformans</i>	<i>bla</i> _{OXA-48} -IRQNAS223-G
	38- <i>S. infantis</i>	<i>bla</i> _{OXA-48} -IRQNAS224-G
<i>bla</i> _{NDM-1} gene	1- <i>D. incerta</i>	<i>bla</i> _{NDM-1} -IRQNAS225-G
	15- <i>S. pneumoniae</i>	<i>bla</i> _{NDM-1} -IRQNAS226-G
	18- <i>E. aquaticum</i>	<i>bla</i> _{NDM-1} -IRQNAS227-G
	23- <i>P. azotoformans</i>	<i>bla</i> _{NDM-1} -IRQNAS228-G
	38- <i>S. infantis</i>	<i>bla</i> _{NDM-1} -IRQNAS229-G

Figure 1. Inseparable Neighbor Joining Concatenated sequences of 1310 base pairs for every strain were used to create the phylogenetic tree. An alignment of 16S rRNA sequences produced these sequences. After that, a MAFFT alignment was used to create the tree, and Forester version 1046 was used to visualize it. This tree shows the range and evolutionary relationships of the twelve species that have been isolated from burn diseases with their corresponding type strains (ATCC, NCTC, DCM, JCM, or IMTB). Each horizontal branch's length was represented to scale. The bootstrap values are shown after 1000 iterations.



* 11-*Providencia rettgeri* was excluded for its short alignment(700bp)

Figure 2. A model of agarose gel electrophoresis (1.5%) demonstrated the amplification of the *bla*_{OXA-48}, *bla*_{NDM-1}, *bla*_{VIM}, *bla*_{KPC} and *OmpA* genes.

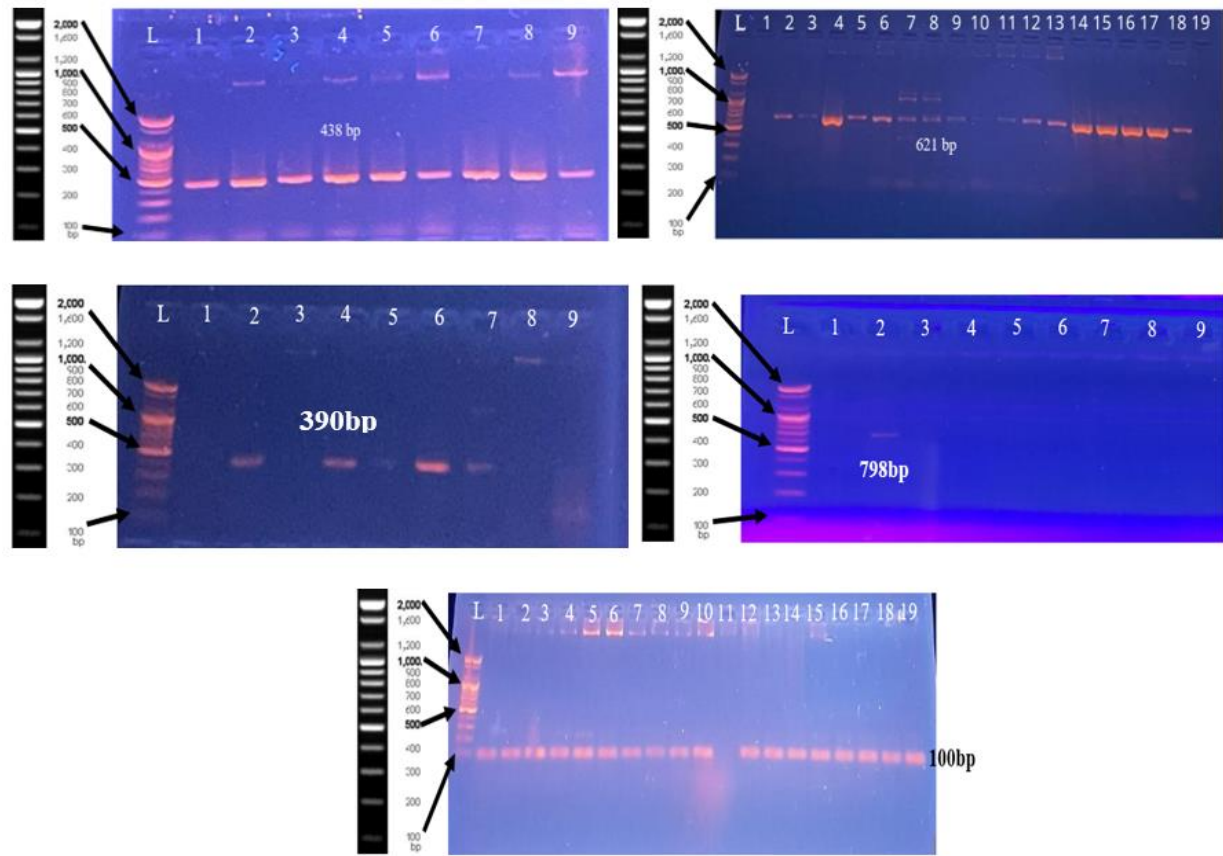


Figure 3. A model of multiple sequence alignments of 3 different alleles for *bla_{NDM-1}* gene. The arrows refer to the nucleotide mutations.



Discussion

The production of beta-lactamases, or carbapenemases, which hydrolyze carbapenems and the majority of other beta-lactams, is the most epidemiologically significant genetic mechanism for carbapenem resistance in Enterobacteriaceae [23]. All 57 isolates had high resistance to ten drugs, according to the antimicrobial susceptibility test, which is consistent with other research [24, 25]. In accordance with [26], the current study showed that the most commonly discovered carbapenemase, *bla_{OXA-48}*, was found in 47 out of 57 (82.45%) bacterial isolates, followed by the *bla_{NDM-1}* gene in 46 (80%). Because horizontal plasmid transfer is frequently used to transfer the *bla_{NDM-1}* gene [27].

The transfer of a 62-kb IncL/M-type plasmid devoid of an extra resistance gene is the main cause of the present expansion of the *bla_{OXA-48}* gene [28]. On the same line as [29], however, the *bla_{VIM}* gene was found in 8 (14%) of the samples. Class 1 integron gene cassettes contain the *bla_{VIM}* gene. The gene's wide spread is explained by its frequent association with mobile genetic elements like plasmids and transposons. [30, 31]. Only 1 (1.75%) had the *bla_{KPC}* gene, which is consistent with [32,33]. Some carbapenem genes that cause antibiotic resistance were found to be highly prevalent in various bacterial species when the most common genes in the current study were analyzed. This high frequency may be the cause of the genes' quick transmission between species and between generations.

Given that 29.82% of the isolates exhibited resistance to both *bla_{OXA-48}* and *bla_{NDM-1}*, the data suggest that these resistance genes were quite common among the isolates. This implies that these genes contribute significantly to the isolates' resistance to antibiotics. Three resistance genes (*bla_{OXA-48}*, *bla_{NDM-1}*, and *OmpA*) were detected in

28.07% of the isolates, whereas four resistance genes (*bla_{OXA-48}*, *bla_{NDM-1}*, *bla_{VIM}*, and *OmpA*) were detected in a smaller number (8.77%). Although the *OmpA* gene was found in 33 cases (57%) of *P. aeruginosa*, it was more common in *P. aeruginosa* 18 cases (64%), and *K. pneumoniae* 9 cases (75%). *OmpA* is a well-known virulence factor that is essential for controlling adhesion, aggression, and biofilm formation.

The present study focused on the prevalence of resistance genes and their association with carbapenem antibiotic resistance; it was found that the genes *bla_{OXA-48}* and *bla_{NDM-1}* showed the highest association rate with the antibiotics used, indicating the main role of these genes overall the antibiotic resistance. The frequent occurrence of the *OmpA* gene in resistant isolates may indicate its role in antibiotic resistance, as it may be responsible for the change in cell membrane permeability and thus reduce the effectiveness of antibiotics, or it may work in tandem with other genes to enhance resistance [34].

The presence of the *OmpA* gene is very important because it is repeated in more than half of the isolates and is likely to increase antibiotic resistance and treatment difficulty, even though the *OmpA* gene showed a lower association rate with the disc of carbapenem antibiotics than *bla_{OXA-48}* and *bla_{NDM-1}*. The *bla_{OXA-48}* gene is found to have only one allele distributed among 13 bacterial isolates. The presence of a single *bla_{OXA-48}* gene in different bacterial isolates can pose a very high risk because this gene (single allele) can degrade carbapenems by transmission among these bacteria, making them resistant to conventional treatments [35, 36].

Fifteen bacterial isolates have four distinct alleles of the *bla_{NDM-1}* gene: 19-*P. aeruginosa* has the first allele, 23-*P. azotoformans* has the second, 16-*A. baumannii* has the third, and the remaining twelve isolates have the fourth allele. Due to extensive and

improper use of antibiotics, particularly carbapenems, the *bla_{NDM-1}* gene has been subjected to point mutations, insertions, or deletions in its genomic sequence, which has resulted in the diversity of alleles [37,38]. A universal primer may not be able to detect the several alleles, and in the future, multiple antibiotics may be required to treat the condition. Although there is only one allele of the *OmpA* gene spread across 30 bacterial isolates, the existence of a single allele may impact the degree of protein expression, resulting in compromised outer membrane integrity, particularly if the allele has mutations that impact its function.

It is important to recognize the limitations of this study. Even though PCR works well for genetic differentiation, it might not be as reproducible as whole-genome sequencing. The geographic reach and sample size could not accurately reflect the overall prevalence of carbapenem-resistant bacteria in burn settings. These results need to be confirmed and expanded upon in future research using bigger datasets and more sophisticated molecular techniques.

Conclusions

This study emphasizes how common carbapenem-resistant genes are in burn environments; bacterial isolates with carbapenem resistance are highly prevalent. One of the main reasons why the bacterial isolates are resistant to carbapenem antibiotics is because the *OmpA* gene coexists with the carbapenem genes. Confirmation of our findings would benefit from future research to explore *OmpA* gene expression. In order to slow the development of resistant strains, the results highlight the urgent need for improved infection control strategies and ongoing molecular surveillance. Furthermore, studies incorporating whole-genome sequencing may offer more profound understandings of epidemiological trends and resistance mechanisms.

Conflict of interest

Non declared

Financial disclosure

None declared

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