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Phenotypic and Genotypic Evaluation of Antibiotic Resistance of *Acinetobacter baumannii* Bacteria Isolated from Surgical Intensive Care Unit Patients in Pakistan

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Abstract

Background: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a significant nosocomial pathogen, causing serious threats concerning community-wide outbreaks globally, as well as in Pakistan. Antimicrobial resistance in *A. baumannii* is increasing day by day.
Objectives: The study aimed to find out the antibiotic resistance (AMR) patterns and evaluate the AMR genes in clinical isolates from patients admitted to the surgical Intensive Care units (ICUs) at different hospitals in Lahore, Pakistan.
Methods: A total of 593 clinical specimens were collected from patients admitted to the surgical ICUs of three different local hospitals in Lahore, Pakistan. From these samples, a total of 90 *A. baumannii* isolates were identified and further investigated to observe phenotypic resistance patterns and detect carbapenemases resistance genes.
Results: The results showed that phenotypic resistance against amikacin was 27.2%, ceftriaxone 100%, ceftazidime 27.2%, cefepime 63.3%, ciprofloxacin and co-trimoxazole 100%, gentamicin 40%, imipenem 22.2%, meropenem 21.1%, piperacillin-tazobactam 27.2%, tigecycline 27.2%, and tetracycline 63.3%. All *A. baumannii* isolates were found to be sensitive to colistin (CT), polymixin-B (PB), and tobramycin (TOB). The PCR amplification of carbapenemases genes revealed the prevalence of *blaOXA-23*, *blaOXA-51*, and *blaOXA-40* in 73, 90, and 64.4% of the isolates, respectively, along with *blaNDM1* (92.2%), *blaVIM* (40%), *blaIMP* (90%), *ISAba1* (85.5%), *sul1* (16.6%), *sul2* (20%), *armA* (32.2%), and *PER-1* (12%) while the *blaOXA-24* and *blaOXA-58* genes were not detected in the isolates. The sequence analysis of the *blaOXA-23* and *blaOXA-51* genes showed 98% and 95% similarity with previously reported sequences in the GenBank database.
Conclusions: The present study indicated that the emergence of high carbapenem resistance in CRAB isolates has increased, which may pose serious limitations in the choice of drugs for nosocomial infections.

Keywords: Nosocomial Infections, Carbapenem-Resistant *Acinetobacter baumannii*, Hospital-Acquired Infections, *blaOXA-51*

1. Background

Antimicrobial resistance (AMR) due to ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli*) is posing a serious threat to public health globally (1, 2). According to the WHO, carbapenem-resistant *A. baumannii* (CRAB) has been one of the main concerns for the last 10 years due to the risk of antibiotic resistance. *Acinetobacter baumannii* is considered a "red alert" pathogen by the Infectious Diseases Society of America due to its resistance against antibiotics. It is associated with major health concerns in different regions of the world. These infections are responsible for about 1.5 million cases yearly (3, 4).

Acinetobacter baumannii is one of the most prevalent pathogens causing nosocomial infections, especially in people admitted to the intensive care units (ICUs) (5, 6). It has been demonstrated that such resistance in these pathogens is due to plasmids, transposons, and integrons, particularly class I and class II. The nosocomial propagation of isolates has been illustrated in both environmental and clinical specimens (7, 8). The development of resistance in CRAB pathogens has been increasing due to the emergence of class B, C, and D carbapenemase, which

declines membrane permeability, modifies PBP, and increases efflux pump expression. Carbapenem resistance in *A. baumannii* is mainly mediated by intrinsic (*OXA-51*) or acquired (*OXA-23*) oxacillinases (9, 10). It is considered a health care pathogen mostly encountered in several serious medical problems such as septicemia, meningitis, bacteremia, ventilator-associated pneumonia, endocarditis, and urinary tract infection (4, 11). Epidemiologically reported data provide evidence of *A. baumannii* infectious globally, e.g., in Korea, Iran, Brazil, America, Europe, China, Iraq, Hong Kong, Taiwan, and Argentina. In several regions of the world, due to climate change, community-acquired pneumonia is due to this infection, as reported in the literature (12).

Resistance in ESKAPE pathogens *E. faecium* (13), *S. aureus* (14), *K. pneumonia* (15), *A. baumannii* (16), *P. aeruginosa* (17), and *E. coli* (18) is caused by the enzymatic degradation of antibiotics, target site mutations/modifications, decreased porin expression, and overexpression of multidrug efflux pumps. However, lactamases, such as carbapenem hydrolyzing class D-lactamases (CHDLs) and Metallo-lactamases, are frequently involved in carbapenem resistance. Resistance to CHDLs, also known as oxacillinases, is primarily achieved by the generation of carbapenemase enzymes encoded by the genes of *blaOXA-23*, *blaOXA-40*, and *blaOXA-58* lineages; however, *blaOXA-23* is the most widespread one worldwide (19). In *A. baumannii*, transposable elements such as insertion sequences (*ISAba1*) play a key role in carbapenem resistance, since they are found upstream in the promoter regions of the *blaOXA-23*, *blaOXA-40*, *blaOXA-58*, and *blaOXA-51* genes, inducing the overexpression of these resistance genes (20, 21).

2. Objectives

The main objectives of this cross-sectional study were to find out the AMR patterns in *A. baumannii* isolates and evaluate the AMR genes (*blaOXA-23*, *blaOXA-24*, *blaOXA-40*, *blaOXA-51*, *blaOXA-58*, *blaNDM1*, *blaIMP*, *blaVIM*, *ISAba1*, *ssul1*, *sul2*, *armA*, and *PER-1*) in these isolates from patients admitted to the surgical ICUs at different hospitals in Lahore, Pakistan.

3. Methods

3.1. Sample Collection, Isolation, and Identification of Acinetobacter baumannii

A total of 593 clinical specimens of wound, blood, burn, and pus was collected from different local hospitals from June 2017 to March 2019 from patients admitted to the surgical ICUs in Lahore General Hospital (n-396), Mayo Hospital (n-103), and Jinnah Hospital (n-94), Lahore, Pakistan, using a simple random sampling technique. After collection, the samples were inoculated on blood and MacConkey agar using a disposable wire loop as primary, secondary, and tertiary streaking, and then the Petri plates were incubated at 37°C for 24 h. After the incubation period, the plates were observed for the appearance of bacterial colonies. *Acinetobacter baumannii* showed non-lactose fermenter colonies on MacConkey agar. The isolated bacterial colonies were then processed for biochemical identification of bacteria using standard biochemical tests (API) 10S kit (Biomeurix) (5).

3.2. Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility testing (AST) was performed by the Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines 2020 (3). To standardize the inoculum density for susceptibility tests, McFarland standards were used (10). They were prepared by using different concentrations of barium chloride ($BaCl_2$) and sulfuric acid (H_2SO_4) to make 0.5, 1, and 2% standards for visual differences. Different concentrations of $BaCl_2$ and H_2SO_4 were used and stored at 4°C for further use. A fresh colony was picked by a sterile inoculating loop and suspended in 2 mL of normal saline optically equal to 0.5 McFarland standards and streaked by a swab over the entire surface in three to four planes by rotating the plate at 60 °C each time to ensure the even distribution of inoculum. In the end, the rim of agar was swabbed, and the plate was left undisturbed for 15 min to absorb the excess inoculum over it. Finally, antibiotic disks were placed on the inoculated agar plates and incubated at 37°C for 24 h.

Tested isolates were used for AST to a panel of 15 different antibiotics as suggested by CLSI guidelines 2020. The antibiotics were amikacin (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), colistin (10 μg), co-trimoxazole (23.75 μg), gentamicin (10 μg), imipenem (10 μg), meropenem (10 μg), piperacillin-tazobactam (10 μg), tigecycline (15 μg), polymixin-B (300 units), tobramycin (10 μg), and tetracycline (30 μg).

3.3. Amplification and Detection of Resistance Genes

All of the *A. baumannii* isolates were processed for DNA extraction using a DNA extraction kit (WizPrepTM). The quality of DNA was checked by agarose gel electrophoresis. Then, 1% agarose gel was prepared in TAE buffer. After mixing and boiling, 0.5 mg/mL of ethidium bromide was

added. The mixture was then poured into a casting tray, and a comb was inserted into it. The casting tray was left at room temperature until getting solidified. The seal and the comb were removed carefully, and the gel was placed in an electrophoresis chamber containing TAE buffer. Then, 1 μL of Thermo scientific 6X loading dye was mixed with 6 μL of sample, and a volume of 5 μL was loaded in each of the wells. The first well was loaded with Thermo scientific DNA ladder, and the remaining wells were loaded with the DNA of our interest. In the end, the lid was placed on the gel box, and electrodes were connected with it, and the gel was run at 70 volts for 30 min. The lid of the gel box was removed, and the gel was picked out from the tray using sterile gloves and placed in a gel documentation system (Bio-Rad, Germany).

The resistance genes (*blaOXA-23*, *blaOXA-24*, *blaOXA-40*, *blaOXA-51*, *blaOXA-58*, *blaNDM1*, *blaIMP*, *blaVIM*, *ISAba1*, *ssul1*, *sul2*, *armA*, and *PER-1*) were amplified using gene-specific primers as given in Table 1 and amplification conditions listed in Table 2. The PCR products were loaded on an agarose gel to visualize the amplified genes using 2% agarose gel. To amplify each gene, a PCR was carried out in a final volume of 25 μL containing 1$\times$ PCR buffer, 1U Taq polymerase, 1.5 mM $MgCl_2$, 200 μM of dNTP, 10 pmol of each primer, and 1 μL of extracted DNA. The conditions of amplification were programmed in Master-cycler Eppendorf as follows: Initial denaturation at 94°C for 3 min, 35 cycles of 94°C for 45 s, annealing varying according to the individual gene for 45 s, extension at 72°C for 1 min, and final extension at 72°C for 5 min. The PCR products were separated on the 1.5% agarose gel by electrophoresis, stained with ethidium bromide, and then visualized under a UV gel documentation system (Sigma-Aldrich) (Table 1).

3.4. Sequencing and Phylogenetic Analysis

The carbapenemase resistance genes, *blaOXA-23* (extrinsic) and *blaOXA-51* (intrinsic), were subjected to sequencing and phylogenetic analysis. Sequencing was performed using commercial sequencing services from Macrogen (Korea). We used NCBI-Nblast to determine the similarity index of the obtained sequences with already submitted sequences. The direct sequenced positive isolates were aligned to the reference sequences using phylogeny.fr software for phylogenetic tree construction.

3.5. Statistical Analysis

A chi-square test with SPSS version 21.0 software was used to determine the correlation between phenotypic and genotypic resistance patterns. A P-value of < 0.05 was considered significant.

4. Results

4.1. Characteristics of Specimens and Isolates

Out of 593 samples, 90 were found positive for *A. baumannii*. The gender-wise prevalence of *A. baumannii* isolates was 56 (62.2%) in males and 34 (37.8%) was in females. The prevalence of *A. baumannii* was 17.7% in wound samples (n = 16), 21.1% in blood samples (n = 19), 30% in pus samples (n = 27,) and 31.1% in burn samples (n = 28). The sites of infection were the respiratory tract of hospital intensive-care patients (n = 46, 51.1%), blood intensive-care patients (n = 20, 22.2%), urinary tract (n = 14, 15.5%), surgical soft tissue (n = 6, 6.6%), bone and joint (n = 2, 2.2%), and central nervous system lesion (n = 2, 2.2%).

4.2. Antimicrobial Susceptibility Pattern

The antibiotic resistance patterns against amikacin (AK), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), ciprofloxacin (CIP), colistin (CT), co-trimoxazole (SXT), gentamicin (CN), imipenem (IPM), meropenem (MEM), piperacillin-tazobactam (TZP), tigecycline (TGC), polymixin-B (PB), tobramycin (TOB), and tetracycline (TE) are shown in Table 2. All isolates (100%) demonstrated resistance to a minimum of three classes of antibiotics and thus met the MDR criteria. In *A. baumannii* isolates, CT, PB, TOB, AK, CAZ, TZP, TGC, IPM, and MEM were most sensitive, while CIP, CRO, FEP, SXT, TE, and CN were most resistant. Multi-drug resistant *A. baumannii* was highly resistant to CIP, CRO, FEP, SXT, TE, AK, CAZ, and TE. A gradual increase in carbapenem resistance up to 20 (23%) was noted in the present bacterial isolates (Table 2).

4.3. Distribution of Antibiotic Resistance Genes

The PCR amplification of all the 13 resistance genes showed that the prevalence of *blaOXA-23*, *blaOXA-51*, *blaOXA-40*, *blaNDM1*, *blaIMP*, *blaVIM*, *ISAba1*, *ssul1*, *sul2*, *armA*, and *PER-1* was 73% (63/90), 90% (81/90), 64.4% (58/90), 92.2% (83/90), 90% (81/90), 40% (36/90), 85.5% (77/90), 16.6% (15/90), 20% (18/90), 32.2% (29/90), and 12.2% (11/90), respectively. The *blaOXA-24* and *blaOXA-58* markers of class D carbapenemases were not detected. A p-value of < 0.05 was obtained for the relationship between genotypic and phenotypic resistance patterns of *A. baumannii* isolates. The overall statistical resistance rate between drugs and genes was 22.22% (20/90), and its prevalence was illustrated individually (Tables 3 and 4).

Table 1. Primers Used in This Study for Amplification of Genes in *Acinetobacter baumannii* Clinical Isolates

Gene	Sequence (5' - 3')	Amplicon Size, bp	Annealing temperature, °C	Reference
blaOXA-51	F-TAATGCTTTGATCGGCCTTG	353	52	(22)
	R-TGGATTGCACTTCATCTTGG			
blaOXA-23	F-GATCGGATTGGAGAACCAGA	501	52	(22)
	R-ATTTCTGACCGCATTTCCAT			
blaOXA-24	F-CAAGAGCTTGCAAGACGGACT	420	Not detected	(23)
	R-TCCAAGATTTTCTAGCTTATA			
blaOXA-58	F- AAGTATTGGGGCTTGTGCTG	599	Not detected	(24)
	R- CCCCTCTGCGCTCTACATAC			
blaOXA-40	F-GGTTAGTTGGCCCCCTTAAA	246	52	(25)
	R-AGTTGAGCGAAAAGGGGATT			
blaNDM1	F- GGTTTGGCGATCTGGTTTTC	621	52	(25)
	R- CGGAATGGCTCATCACGATC			
blaIMP	F- GTTTATGTTCATACWTCG	432	48	(24)
	R- GGTTTAAYAAAACAACCAC			
blaVIM	F- TTTGGTCGCATATCGCAACG	500	66	(25)
	R- CCATTCAGCCAGATCGGCAT			
ISAba1	F- ATGCAGCGCTTCTTTGCAGG	393	50	(24)
	R- AATGATTGGTGACAATGAAG			
sul1	F- CGGCGTGGGCTACCTGAACG	433	58	(21)
	R- GCCGATCGCGTGAAGTTCCG			
sul2	F- GCGCTCAAGGCAGATGGCATT	293	58	(21)
	R- GCGTTTGATACCGGCACCCGT			
armA	F- ATTCTGCCTATCCTAATTGG	315	56	(21)
	R- ACCTATACTTTATCGTCGTC			
PER-1	F-ATGAATGTCATTATAAAAG	920	45	(25)
	R-TTGGGCTTAGGGCAG			

4.4. Sequencing and Phylogenetic Analysis

Different genotypes of CRAB isolates circulating in Pakistan, based on the NCBI data bank, were explained. The results disclosed that the isolates could be clustered into cardiographs, mostly represented by clade1 to clade4. The phylogenetic tree analysis indicated that *blaOXA-51* was clustered in clade1 with its closely related species together. The strains were phylogenetically distinct from others and still not reported in Pakistani isolates. Similarly, *blaOXA-23*-like gene analysis was found in clade 2 after clade 1 and was reported for the first time in a Pakistani isolate. The relation of these resistance gene sequences with the closely related species represented 96% - 99% similarities to already submitted sequences in the NCBI databank. The phylogenetic tree represented the correlation of the strain with closely related species. The tree was generated following the neighbor-joining method (Figure 1 and Table 5).

5. Discussion

For the past 60 years, β-lactam antibiotics have been amongst the most successful drugs used for the treatment of bacterial infections in humans. *Acinetobacter* has emerged as a significant class of pathogens, presenting continuous threats and challenges to the health care system throughout the world (20, 26, 27). The CRAB isolates created major therapeutic problems in the hospitals examined (1, 3, 28). In the current study, we investigated the occurrence of β-lactamase and carbapenemase-producing *A. baumannii* in patients admitted to the ICUs of a tertiary

Table 2. Phenotypic Antimicrobial Sensitivity Pattern of *Acinetobacter baumannii* Clinical Isolates

Antibiotics	Drug Content	Zone of Inhibition, mm		Antibiotic Susceptibility Pattern		Resistance, %
		S (>)	R (<)	S (>)	R (<)	
Amikacin, μg	30	$\geq$ 17	$\leq$ 14	65	25	27.27
Ceftriaxone, μg	30	$\geq$ 21	$\leq$ 13	00	90	100
Ceftazidime, μg	30	$\geq$ 18	$\leq$ 14	65	25	27.27
Cefepime, μg	30	$\geq$ 18	$\leq$ 14	33	57	63.33
Ciprofloxacin, μg	5	$\geq$ 21	$\leq$ 15	00	90	100
Colistin, μg	10	MIC	90	00	00	
Co-trimoxazole, μg	23.75	$\geq$ 16	$\leq$ 10	00	90	100
Gentamicin, μg	10	$\geq$ 15	$\leq$ 12	54	36	40.0
Imipenem, μg	10	$\geq$ 16	$\leq$ 13	70	20	22.2
Meropenem, μg	10	$\geq$ 16	$\leq$ 13	71	19	21.1
Piperacillin-tazobactam, μg	10	$\geq$ 21	$\leq$ 17	65	25	27.27
Tigecycline, μg	15	$\geq$ 16	$\leq$ 12	65	25	27.27
Polymixin, units	300	MIC	90	00	00	
Tobramycin, μg	10	$\geq$ 15	$\leq$ 12	90	00	00
Tetracycline, μg	30	$\geq$ 15	$\leq$ 11	33	57	63.33

Abbreviations: R, resistant; S, sensitive.

care hospital in Lahore, Pakistan. The results of the present study showed that of a total of 457 samples for bacterial culture, 90 (19.6%) were positive for *A. baumannii*, 82% for *E. coli*, 89% for *Klebsiella* (89%), and 63% for *Pseudomonas* spp. A similar study was conducted in Iraq on a total of 112 samples, in which most samples were positive for *A. baumannii*, while the other organisms were *Candida albicans*, *Staphylococcus* sp., *P. aeruginosa*, *E. coli*, and *K. pneumoniae* (29).

A previous study reported the appearance of carbapenem-resistant *A. baumannii* in Pakistan and showed increased resistance to cephalosporin, sulfamethoxazole, and beta-lactam antibiotics (30). They reported that the most sensitive antibiotics were tigecycline (80%) and colistin (50%). However, the results of the current study showed that CRAB strains were 100% resistant to CIP, CRO, and SXT. Tetracycline was found moderately effective against *A. baumannii*, indicated by the antibiograms and minimum inhibitory concentrations (MICs). Biglari et al. (31) reported that the isolates were most resistant to carbapenems and cephalosporin (70%) with high MIC values. Except for colistin, tetracycline, and rifampicin, the difference in resistance between the ICUs and other units was statistically significant ($P < 0.05$). Similar results were also reported in China (32) and Iraq (29). In the present study, the isolates were 100% resistant to ceftriaxone, ciprofloxacin, and co-trimoxazole, while moderate resistance was noted against gentamycin, piperacillin-tazobactam, and tigecycline. No resistance was noted against colistin, polymixin, and tobramycin. The reason behind the variations in antibiotic susceptibility patterns of *A. baumannii* could be due to the prolonged hospitalization because all the samples were collected from patients who were admitted to ICUs.

Carbapenemases represent the most versatile family of β-lactamases. These enzymes with catalytic efficiencies for carbapenem hydrolysis, resulting in elevated carbapenem MICs, include enzymes from classes A, B, and D (17). Investigations of the present study included genes from class B (*blaIMP*, *blaNDM*, and *blaVIM*), class D (*blaOXA 23*, *blaOXA 24*, *blaOXA 40*, *blaOXA 51*, and *blaOXA 58*), sulfonamide resistance genes (*sul1* and *sul2*), aminoglycoside resistance methyltransferase gene (*armA*), an enzyme associated with *blaOXA* (*ISAba1*), and the PER1 gene. The most prevalent genes in the current study were *blaNDM1* (92.2%), *blaIMP* (90%), *blaOXA 51* (90%), *ISAba1* (85%), *blaOXA 23* (70%), and *blaOXA 40* (64%). Besides, *blaVIM* was detected in 40% of total isolates, while the prevalence of *armA*, *sul2*, *sul1*, and *PER1* was 32%, 20%, 16.6%, and 12%, respectively. In a previous study from Pakistan, the prevalence of the *blaOXA-23* gene was 23.7% (26), 51.8% (14/27) in Switzerland (1), and 75.4% in Tehran (9).

A previous study from Iraq reported that genotypi-

Table 3. Resistance Patterns of Imipenem and Meropenem According to the Individual Genes Among Clinical Isolates

Genes	Imipenem		Meropenem	
	Sensitive (N = 70)	Resistance (N = 20)	Sensitive (N = 71)	Resistance (N = 19)
blaOXA-23				
Positive (n = 63)	49	14	60	11
Negative	21	06	11	08
blaOXA-51				
Positive (n = 81)	61	20	62	19
Negative	09	00	09	00
blaOXA-40				
Positive (n = 58)	40	18	42	16
Negative	30	02	29	03
blaNDM1				
Positive (n = 83)	63	20	64	19
Negative	07	00	07	00
blaIMP				
Positive (n = 81)	61	20	62	19
Negative	09	00	09	00
blaVIM				
Positive (n = 36)	18	18	18	18
Negative	52	02	53	01
ISAba1				
Positive (n = 77)	59	18	61	16
Negative	11	02	10	03
sul1				
Positive (n = 15)	12	03	10	05
Negative	58	17	61	14
sul2				
Positive (n = 18)	14	04	14	04
Negative	56	16	57	15
armA				
Positive (n = 29)	20	09	22	07
Negative	50	11	49	12
PER-1				
Positive (n = 11)	10	01	10	00
Negative	60	19	61	19

cally identified *A. baumannii* represented resistance to all of the investigated β-lactam antibiotics. Besides, *blaOXA-51*, *blaIMP*, *blaNDM*, and *blaOXA-23* were seen in 100%, 87.5%, 62.5%, and 59.4% of isolates (19). A similar study from Pakistan reported that in CRAB isolates, *blaOXA-24*, *blaOXA-58*, *blaIMP*, *blaVIM*, and *blaSIM* were completely absent (30). A similar result of *blaOXA-24*-like and *blaOXA-58*-like genes was also seen in the present study, as in the present study, neither of the genes was detected.

5.1. Conclusions

This study provides information about treating drug-resistant *A. baumannii* and the relationship of β-lactamases

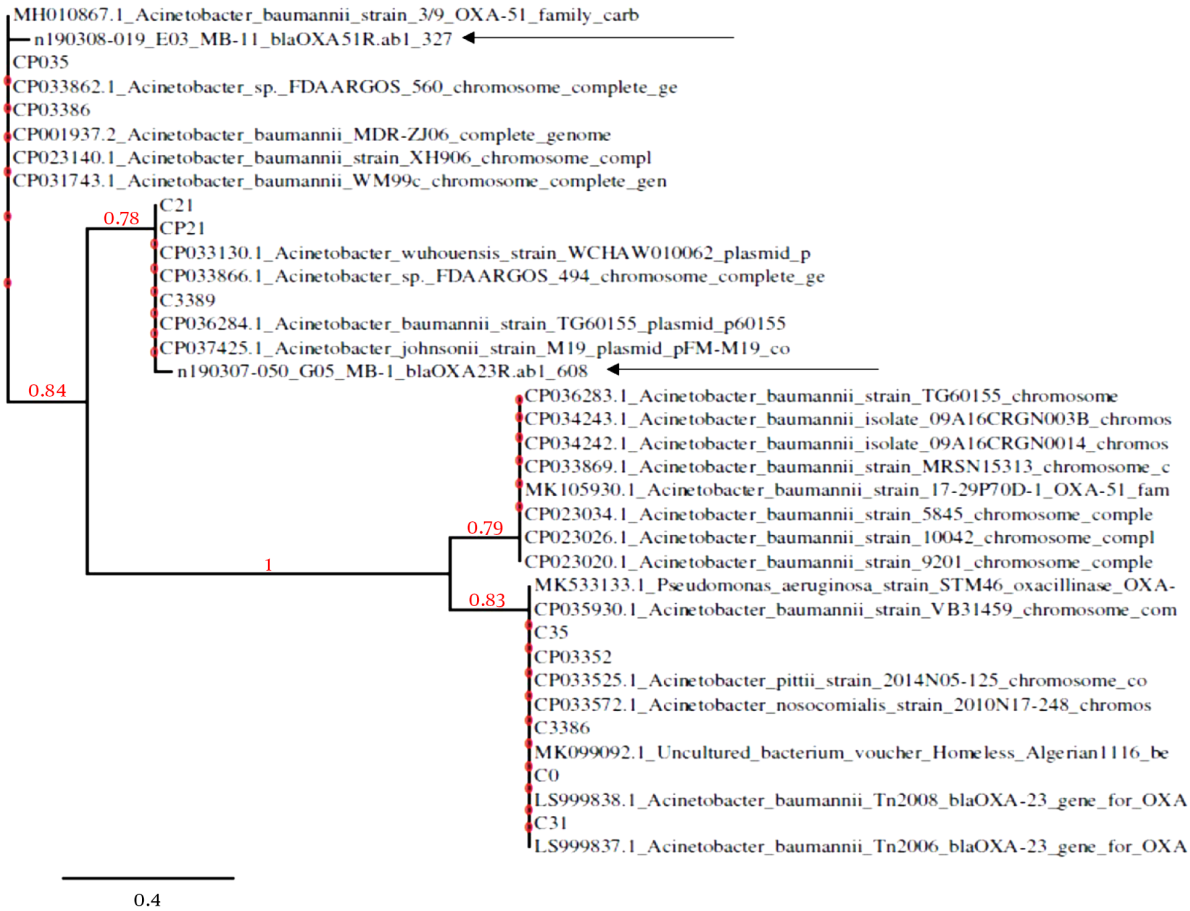

Figure 1. Phylogenetic tree displaying inter-relationship of genes with closely related species. The tree was developed using the neighbor-joining method. Bootstrap values (> 50%), expressed as the percentage of 1000 replications, and are shown at the nodes.

Table 4. Phenotypic and Genotypic Resistance Rate

Sr. Numbers	Gene	Imipenem resistance rate	Meropenem resistance rate
1	*blaOXA-23*	22.22222222	17.46031746
2	*blaOXA-51*	31.74603175	30.15873016
3	*blaOXA-40*	28.57142857	25.3968254
4	*blaNDM1*	31.74603175	30.15873016
5	*blaIMP*	31.74603175	30.15873016
6	*blaVIM*	28.57142857	28.57142857
7	*ISAba1*	28.57142857	25.3968254
8	*sul1*	4.761904762	7.936507937
9	*sul2*	6.349206349	6.349206349
10	*armA*	14.28571429	11.11111111
11	*PER-1*	1.587301587	0

with the phenotypic resistance patterns. The co-existence of multiple drug-resistant bodies and virulent genes has important implications for the treatment of patients. The genotypic resistance pattern was closely related to the phenotypic patterns by detecting the resistance genes using PCR and antimicrobial susceptibility testing by disk diffusion method. This study provides information about treating the drug-resistant *A. baumannii* and also the relationship of virulent genes with phenotypic resistance patterns.

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Table 5. Relationship Between Resistance Genes and Closely Related Taxa Described Using nBlast[a]

Gene	GenBank Accession Number	Closely Related Taxa Identified	Sequence Identity, %	Sequence Query Coverage, %
blaOXA-23	LC096090.1	*Acinetobacter baumannii* strain KKG5	98	25
blaOXA-23	LC096088.1	*A. baumannii* strain KKG3	97	26
blaOXA-23	LC096087.1	*A. baumannii* strain KKG2	98	25
blaOXA-23	LC096086.1	*A. baumannii* strain KKG1	98	25
blaOXA-51	MH010867.1	*A. baumannii* strain 3/9 *OXA-51*	95	84
blaOXA-51	CP036283.1	*A. baumannii* strain TG60155	95	84
blaOXA-51	CP035930.1	*A. baumannii* strain VB31459	95	84

[a]The mentioned GenBank Accession Numbers are of those species that showed resemblance with our sequences.

Footnotes

Authors' Contribution: Study concept and design: Basit Zeshan, Muhammad Afzal, and Musarat Ishaq. Analysis and interpretation of data: Nureen Zahra, Mian Mubeen Ali Qadri, and Naveed Ahmed. Drafting of the manuscript: Naveed Ahmed and Nureen Zahra. Critical revision of the manuscript for important intellectual content: Basit Zeshan, Musarat Ishaq, and Muhammad Afzal.

Conflict of Interests: Authors have declared no conflict of interest.

Ethical Approval: An ethical approval letter was obtained from the Institutional Review Board (IRB) and sent to each hospital for their acknowledgment. Once permission was granted from each of the hospitals, the collection of samples was started.

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