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Resistance Mechanisms of *Acinetobacter baumannii* in North Indian Clinical Isolates: A Molecular Study of β-Lactam, Carbapenem, Cephalosporin, Fluoroquinolone, and Aminoglycoside Genes

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Abstract:

Background- The WHO recently added to priority list Carbapenem-resistant *Acinetobacter baumannii* (CRAB) major infection cause death throughout the developing world. With time the bacteria have become smarter so antimicrobial resistance is major problem so treatment of infection.

Aim-This study explored the relationship between antimicrobial resistance patterns and identified the resistance genes responsible for β-lactam, fluoroquinolone, and aminoglycosides. *A. baumannii* isolates susceptibility patterns of antimicrobial agent and conventional PCR was used to detection of β-lactamase leading gene to carbapenem- resistant class (bla_{SHV}, bla_{TEM}, bla_{VEB}, bla_{PER}, bla_{GES}, bla_{KPC}, $bla_{CTX\text{-}M\text{-}1}$), (MBL) genes ($bla_{IMP}$, bla_{VIM}, $bla_{NDM\text{-}1}$), Oxacillinase genes ($bla_{OXA\text{-}48}$, $bla_{OXA\text{-}23}$, $bla_{OXA\text{-}24/40}$, $bla_{OXA\text{-}58}$), Aminoglycoside-modifying enzyme (AME) genes (*aac(3)-Ia, aac(3)-IIa, aph(3)-Ia, ant(2)-Ia),* Plasmid-mediated quinolone resistance genes *(qnrA, qnrB, qnrS),* and DNA gyrase genes *(parC, gyrA)*.

Results- Resistance genes were detected in 62.09% of $bla_{OXA\text{-}23}$, 15.3% of bla_{PER}, 24.18% of bla_{NDM}, 15.6% of bla_{SHV}*-1*, 36.6% of *parC*, 20.26% of *aph (3')-VIa*, 23.5% of *ant(1a)*, and 9.1% of *aac(1a)*, predominantly in extensively drug-resistant (*XDR-AB*) isolates. Additionally, 2.6% of *blaOXA-58*-like genes were found in multidrug-resistant (MDR-AB) isolates. A high prevalence of *blaOXA-23*-like and specific ESBL genes in extensively drug-resistant (XDR) strains suggests the co-evolution of resistance mechanisms, contributing to enhanced resistance profiles. The co-occurrence of carbapenem resistance genes with aminoglycoside-modifying enzymes or ESBL genes shows *A. baumannii* versatility and flexibility to acquire several resistance mechanisms; co-resistance is more common in XDR isolates. The high prevalence of $bla_{OXA\text{-}23}$, bla_{NDM}, *and parC* among resistant isolates means that carbapenem and fluoroquinolone resistance has a major part in the continuing existence of *A. baumannii* outbreaks.

1. INTRODUCTION

Acinetobacter baumannii is a gram-negative the *Moraxellaceae* family. It is basically a nosocomial, opportunistic human bacterium that causes several kinds of illnesses, including hospital-acquired and ventilator-associated pneumonia (HAP, VAP); central line-associated, *A. baumannii* is characterized by catalase positivity, oxidase negativity, and non-fermentation *A. baumannii* is characterized by catalase positivity, oxidase negativity, and non-fermentation **(**Ayenew Z et al. 2021)

The rapid development and variation in drug tolerance among common gram-negative microbes are a major cause of alarm, particularly in India and across the world, due to high death and morbidity rates. The World Health Organization (WHO) designated carbapenem-resistant A. baumannii as a key group on its list of bacteria posing the greatest danger to human health in 2017, focusing on research and development activities for novel antimicrobial therapies. Considering this, the Centre for Disease Control preventative (CDC) classified as a significant danger, there by encouraging and sustaining public health monitoring and preventative actions (Harding CM et al. 2018). Carbapenem multidrug resistance must be treated urgently. MDR resistance if they were at least three antimicrobials (including carbapenem, piperacillin, tazobactam, and fluroquinolone resistance), extensively drug-resistant XDR, if three or more antimicrobials (Penicillin and cephalosporins, including

fluroquinolones, aminoglycosides, carbapenem resistance), PDR, if, XDR plus polymyxin resistance. (Harding CM et al. 2006 and Han H et al.2021).

Antimicrobials are classified into four molecular types. Class A, C, and D enzymes have a serine functioning site, but class B metalloenzymes have one or two zinc ions where they work. Several *Acinetobacter species* include intrinsic β-lactamase genes, including Amp C, ADC, and OXA enzymes. penicillin and cephalosporins are usually hydrolysed by class C enzymes, whereas carbapenems are primarily hydrolysed by class D enzymes. (Gordon NC and Wareham DW 2010).

Group of aminoglycosides (AG) substrates are chemically modified by three types of AMEs: AG N-acetyltransferases (AACs), AG O- Nucleotidyl-transferases (ANTs), and AGO-Phosphotransferases (APHs). The link between aminoglycoside resistance and clinical *A. baumannii* AMEs is considerable, with *aph (3)-Ia* amikacin resistance and aac1a co-relate with tobramycin. In recent years, clinical isolates of *A. baumannii* have shown increasing detection of class C β-lactamase, which causes cephalosporine resistance and aminoglycoside resistance (*aphA6)* (Zhang Y et al. 2023).

Quinolones suppress DNA gyrase activity, which is a topoisomerase enzyme that transforms circular DNA into negative supercoils. It consists of two components. A subunit, coadded by the *gyr A* gene, that responsible for reconnecting double-stranded breaks in DNA, whereas B subunit, encoded by *gyr B*, facilitated energy transfer and ATP hydrolysis during topological transformation. Mutation in ser-83 leads to high ciprofloxacin resistance (Vila J et al.1995).

Therefore, *A. baumannii*, particularly MDR strains, has steadily received recognition as a human pathogen in hospital settings. Recent research has documented the biofilm forming capacity to link the primary virulence factors, boosting bacterial persistence and chronicity in a particular way. (Al-Kadmy IM et al.2018).

This study examines the frequency and genetic factors that determine β-lactamase releasing and fluroquinolone resistance variants of *A. baumannii* from respiratory samples of those admitted to hospitals.

2. MATERIAL AND METHODOLOGY

2.1 Bacterial strains and antimicrobial susceptibility testing:

Out of 180 total isolates collected, 27 were non-viable ,10 identified as other *Acinetobacter* species, 7 were contaminated and 9 failed to regrow under standard condition. Thus,153 *A. baumannii* isolates were analysed in this study and revived isolates of *A. baumannii* obtained from respiratory samples (sputum, endotracheal aspirates, bronchoalveolar lavage) of equal number of patients admitted to various wards of Gandhi Memorial and associated hospitals, King George's Medical University, Lucknow and were isolated in the Bacteriology Laboratory, Department of Microbiology of the same hospital. Of these, 58 isolates were obtained from patients admitted in intensive care units (ICUs), 39 in adult general medicine wards, 24 in neonatal intensive care units (NICUs), 17 in general surgical wards and 15 in emergency and trauma centre. In the laboratory *A. baumannii* isolates were identified based on conventional microbiological procedures including Gram staining, colony characteristics and biochemical reactions such as citrate, catalase, oxidase, indole test, methyl red, Voges Prausker, triple sugar iron agar and urease test. Their identities were validated using matrix-assisted laser desorption ionization time-of-flight mass spectrometry **(MALDI-TOF-MS).** Antibiotic susceptibility test (AST) for these revived isolates was repeated for 12 different antibiotics using Kirby Bauer disk diffusion method in accordance with the clinical laboratory standards institute (CLSI) guideline 2022.

The antibiotics tested were Amikacin (30µg), Gentamicin (30 µg), Tobramycin (30 µg), Cefepime (30 µg), Ceftriaxone (30µg), Co-trimoxazole (25 µg), Piperacillin-Tazobactam (100/10µg), Levofloxacin (5µg), Ciprofloxacin (5µg), Imipenem (10 µg), Meropenem (10 µg), and Tetracycline (10 µg) (Hi-media, India). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control.

2.2 Broth Microdilution Method for Colistin Susceptibility Testing

The Broth Microdilution (BMD) method is considered the gold standard for colistin susceptibility testing and is recommended by both EUCAST and CLSI, especially for *Acinetobacter* species. Minimum inhibitory concentration (MIC) testing was performed using Cation-Adjusted Mueller-Hinton Broth (CA-MHB). Colistin sulfate (Hi-media), with a potency of 19,000 IU/mg, was used for antibiotic preparation. Quality control (QC) strains included *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

To prepare the stock solution, 5 mg of colistin (equivalent to 19,000 IU) was dissolved in 5 ml of sterile distilled water to achieve a final concentration of 1024 µg/ml. Following antibiotic preparation, the bacterial inoculum was prepared by selecting 2–3 morphologically similar colonies from a 24-hour culture grown on nutrient agar. These were inoculated into 1.5 ml of nutrient broth and incubated at 37°C for 2 hours. The culture was then adjusted to a 0.5 McFarland standard. A volume of 100 µl of this adjusted inoculum was diluted in 2 ml of normal saline to obtain the final inoculum. Test tubes were labelled from the highest to the lowest colistin concentration. Each tube received 1 ml of CA-MHB. Then, 1 ml of the 1024 µg/ml stock solution was added to the tube labelled 512 µg/ml. Serial dilutions were performed to achieve final concentrations ranging from 512 µg/ml to 0.12 µg/ml.

Next, 100 µl of each colistin dilution was dispensed into the corresponding wells of a 96-well microtiter plate. Subsequently, 10 µl of the final bacterial inoculum was added to each well. For quality control, negative control wells contained only CA-MHB, while positive control wells contained CA-MHB with the inoculum. The microtiter plates and nutrient agar plates were incubated at 37°C for 16–18 hours. (Diana Albertos Torres et al. 2021)

2.3 Molecular characterization of antibiotic resistance determinants

Conventional PCR was performed to identify the gene including β-lactamase leading gene to carbapenem- resistant class bla_{SHV}, bla_{TEM}, bla_{VEB}, bla_{PER}, bla_{GES}, bla_{KPC}, $bla_{CTX-M-1}$) (Alyamani EJ et al. 2015). Furthermore, the carbapenem group includes *blaKPC, blaVIM, blaIMP,* bla_{NDM}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-58}, and bla_{OXA-48} (Avila-Novoa MG et al. 2019). Amp-C β-lactamase production *MOXM, CITM, FOXM, DHAM, EBCM and ACCM* Aminoglycoside modifying enzyme (AME) group include *aac-1a, aac-2a and aph3-1a* gene (Abo-State MA et al. 2018), for quinolones resistance gene produced

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topoisomerase mutation efflux pump include *par C* and Plasmid-mediated Quinolones resistance gene like *qnrA, qnrB* and *qnr S* (Kar B et al. 2021).

A single isolated colony of *A. baumannii* was inoculated into 3 ml of Mueller Hinton broth and incubated at 37 °C Overnight, shaking and then 150 rpm and adjust turbidity 0.5 McFarland .then 1 ml of culture was centrifuged at 1200 rpm for 10 minutes, supernatants discard and palate wash three time with TE (Tris-EDTA) buffer, then pallet was lysed with 50µl of lysozyme (10 mg/mL) and incubated at 37 for 1 h. to the cell lysate, 70ul of 10 % sodium dodecyl Sulphate (SDS), 6µl of proteinase K (20mg /mL) was added and incubated at 50 C for 1 h. 100 µL of 5M NaCl and 600 of phenol: chloroform: isoamyl alcohol (25:24:1) were added to the lysate and vortexed gently for 10 seconds. The was centrifuged at 10000 rpm for 10 min. supernatant precepted was the DNA was adding 450 µL of chilled isopropanol and centrifuged at 12000 rpm for 10 min then palate was slightly washed with 70% ethanol and dissolved in 100µL Tris-EDTA buffer. The DNA was visually checked in 0.8% agarose gel electrophoresis after staining with ethidium bromide.

PCR amplification was performed with 25µl reaction volume using 5µl of genomic DNA, 12µl of PCR Master Mix, 6µl of nuclease water, and 1 µL of each primer. The cycling conditions included in Table 1. The PCR result was electrophoresed on a 1.2% agarose gel using 3µL of dye and 7µL of PCR product. The size was determined using a 100bp DNA ladder (Zeighami H et al. 2019)

Table 1: Details about primers, amplicon size and amplification condition of the polymerase chain reaction

Sl. no.	Mechanism	Primer	Sequence (5' → 3')	Amplicon Size(bp)	Amplification condition
1.	**Extended spectrum β-lactamase (ESBL)**	***blaSHV***	F: CCTTTAAAGTAGTGCTCTGC R: TTCGCTGACCGGCGAGTAAGT	119	Initial denaturation at 95 °C for 15 min, denaturation at 94 °C for 30 s, followed by 30 cycles, annealing temperature at 59 °C for 1.5min and extension at 72 °C for 1.5 s, and a final elongation at 72 °C for 10 min
2.		***blaTEM***	F: CATTTCCGTGTCGCCCTTATTC R: CGTTCATCCATAGTTGCCTGAC	800	
3.		***blaVEB***	F: CATTTCCCGATGCAAAGCGT R: CGAAGTTTCTTTGGACTCTG	648	
4.		***blaPER***	F: GCTCCGATAATGAAAGCGT R: TTCGGCTTGACTCGGCTGA	520	
5.		***blaGES***	F: AGTCGGCTAGACCGGAAAG R: TTTGTCCGTGCTCAGGAT	399	

6.		***blaCTXM-1***	F: AAAAATCACTGCGCCAGTTC R: AGCTTATTCATCGCCACGTT	415	Initial denaturation at 95 °C for15 min, followed by 1 cycle, denaturation at 94 °C for 30 s followed by 30 cycles, annealing temperature at 59 °C for 1.5 min. and extension 72 °C for 1.5 min. and a final elongation at 72 °C for 10 min
7.	**Carbapenemase**	***blaIMP***	F: GGAATAGAGTGGCTTAAYTCTC R: GGTTTAAYAAAACAACCACC	232	
8.		***blaVIM***	F: GATGGTGTTTGGTCGCATA R: CGAATGCGCAGCACCAG	390	
9.		***blaKPC***	F: TGTCACTGTATCGCCGTC R: CTCAGTGCTCTACAGAAAACC	1011	
10.		***blaOXA-48***	F: TATATTGCATTAAGCAAGGG R: CACACAAATACGCGCTAACC	800	
11.		***blaNDM***	F: CACCTCATGTTTGAATTCGCC R: CTCTGTCACATCGAAATCGC	984	
12.		***blaOXA-23***	F: GATCGGATTGGAGAACCAGA R: ATTTCTGACCGCATTTCCAT	501	Initial denaturation at 95 °C for15 min, followed by 1 cycle, denaturation at 94 °C for 30s, followed by 30 cycles, annealing temperature at 52 °C for 1.30 min. and extension 72 °C for 1.30 s, and a final elongation at 72 °C for 5 min.
13.		***blaOXA-24/40 like***	F: GGTTAGTTGGCCCCCTTAAA R: AGTTGAGCGAAAAGGGGATT	246	
14.		***blaOXA-58***	F: AAGTATTGGGGCTTGTGCTG R: CCCCTCTGCGCTCTACATAC	599	

13.	**AmpC β-lactamase**	***MOX***	F: GCTGCTCAAGGAGCACAGGAT R: CACATTGACATAGGTGTGGTGC	520	Initial denaturation at 95°C for 15 min, followed by 35 cycles of 94 °C for 30s, annealing temperature at 65°C for 1.5 min. and extension 72 °C for 1.5min. and a final elongation at 72 °C for 10 min
14.		***CIT***	F: TGGCCAGAACTGACAGGCAAA R: TTTCTCCTGAACGTGGCTGGC	462	
15.		***DHA***	F: AACTTTCACAGGTGTGCTGGGT R: CCGTACGCATACTGGCTTTGC	405	
16.		***ACC***	F: AACAGCCTCAGCAGCCGGTTA R: TTCGCCGCAATCATCCCTAGC	346	
17.		***EBC***	F: TCGGTAAAGCCGATGTTGCGG R: CTTCCACTGCGGCTGCCAGTT	302	
18.		***FOX***	F: AACATGGGGTATCAGGGAGATG R: CAAAGCGCGTAACCGGATTGG	190	
19	**Aminoglycosides-modifying enzymes genes**	***aac (3)-Ia***	F: GACATAAGCCTGTTCGGTT R: TCCGAACTCACGACCGA	372	Initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, annealing temperature 55 °C for 30 s and extension at 72 °C for 40 s, and a final elongation at 72 °C for 10 min
20		***aac (3)-IIa***	F: ATGCATACGCGGAAGGC R: TGCTGGCACGATCGGAG	822	
21		***aph (3)-Ia***	F: CGAGCATCAAATGAAACTGC R: GCGTTGCCAATGATGTTACAG	623	
22		***ant (2)-Ia***	F: ATCTGCCGCTCTGGAT	404	Initial denaturation at 95 °C for 5

			R: CGAGCCTGTAGGACT		min, followed by 35 cycles of 95 °C for 30 s, annealing temperature 53 °C for 30 s and extension at 72 °C for 40 s, and a final elongation at 72 °C for 10 min
23	**Fluoroquinolones**	***par C***	F: GTCTGAACTGGGCCTGAATGC R: AGCAGCTCGGAATATTTCGACAA	249	Initial denaturation at 95 °C for 15 min, followed by 35 cycles of 92 °C for 1min. annealing temperature at 64 °C for 1min. and extension at 74 °C for 2min. and a final elongation at 72 °C for 10 min
24		***gyr A***	5: TAC ACC GGT CAA CAT TGA GG 3: TTA ATG ATT GCC GCC GTC GG	648	Initial denaturation at 95 °C for 15 min, followed by 35 cycles of 92 °C for 1min. annealing temperature at 64 °C for 1min. and 74 °C for 2min. and a final elongation at 72 °C for 10 min
25	**Plasmid-mediated Quinolones resistance**	***qnr A***	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	516	Initial denaturation at 95 °C for 15 min, followed by 30 cycles of 94 °C for 1min. annealing temperature at 53 °C for 1min. and 72 °C for 1min. and a final elongation at 72 °C for 7 min
26		***qnr B***	F: GTTGGCGAAAAAATTGACAGAA R: ACTCCGAATTGGTCAGATCG	526	

27		***qnr S***	F: GTTGGCGAAAAAATTG ACAGAA R: ACTCCGAATTGGTCAGA TCG	417	Initial denaturation at 95 °C for 15 min, followed by 30 cycles at 94 °C for 1min. annealing temperature at 51 °C for 1min. and 72 °C for 1min. and a final elongation at 72 °C for 7 min

3. RESULTS

3.1 The isolates characteristics:

Of the 153 clinical isolates of *A. baumannii,* 90 (58.8%) were obtained from male patients and remaining 63 (41.2%) from female patients. The mean age of patients was 49.2±26.31 years and ranged from 10 days and 75 years. Majority of the isolates were obtained from endotracheal tube samples 91 (59.4%), followed by tracheostomy tube 29 (18.9%), sputum 19 (12.4%) and bronchial aspirate 14 (9.1%).

Based on the CLSI interpretive criteria, a very high level of resistance was found with meropenem (152, 99%), ceftriaxone (148, 96.7%), ciprofloxacin (148, 96.7%), imipenem (146, 95.4%), cefepime (145, 94.7%), piperacillin- tazobactam (141, 92.15%) and amikacin (139, 90.8%), while lowest level was observed with tetracycline (110, 71.8%) (table 3). As per the definitions, 62 (40.5%) of the *A. baumannii* isolates were interpreted as MDR and 87 (56.9%) as XDR.

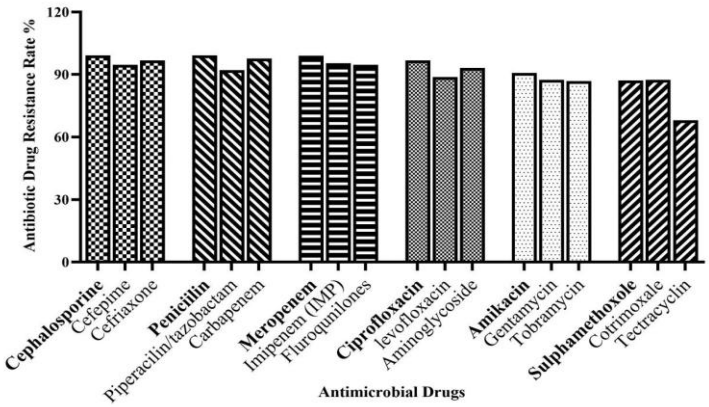

Fig 1: Antibiotic resistance pattern of Acinetobacter baumannii

3.2 Minimum Inhibition Concentration (MIC) for colistin

Read the MIC as the lowest colistin concentration that completely inhibited visible growth of the organism. If a single well appeared skipped, the highest MIC was recorded. Results were not reported if more than one well appeared skipped.

3.3 Molecular typing

The findings regarding the presence of β-lactamase genes in *A. baumannii* isolates highlight critical resistance mechanisms contributing to the challenge of treating infections caused by this pathogen, bla_{OXA-23} like Gene detected in 95(62.09%) of the isolates and more predominant in XDR strains (55/95, 57%) than in MDR strains (36/95, 37%). Statical analysis based on chi square test significantly contributes the gene of carbapenem resistance (P=0.04) and Gene of bla_{NDM-1} in 37(24.18%) isolates, whereas 23(15.03%) of XDR and 13(23.5%) of MDR.

bla_{OXA-58}-like gene Identified in only 4 (2.6%) ,100% of MDR isolates. Though less common, its presence signals additional mechanisms of carbapenem resistance in MDR strains. due to its ability to hydrolyse a wide range of β-lactams, including carbapenems. bla*IMP-1 a*nd bla*VIM-1* Each detected in 6(3.9%) of isolates.

Extended-Spectrum β-Lactamase bla_{SHV-1} Gene Found in 24(15.6%) of isolates, more common in XDR strains (15/24, 62.5%) compared to MDR strains (9/24, 37.5%), detected predominantly in XDR strains. bla_{PER} gene present in 23(15.3%) of isolates. $bla_{CTX-M-1}$ Gene Identified in 14(9.1%) of isolates. bla_{TEM-1} Gene detected in only 3(1.9%) of isolates.

Aminoglycoside Resistance *ant (2)-Ia* gene found in 36(23.5%) of isolates detected in (17/36, 11.11%) XDR and MDR strains each encodes an aminoglycoside-modifying enzyme, conferring resistance by inactivating the drug. *aph (3)- Ia* gene present in 31(20.26%) of isolates and *aac (3)-Ia* gene found in 14 (9.1%) of isolates.

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Quinolone Resistance *parC* Gene Detected in 56(36.6%) of isolates. Higher prevalence in XDR strains (33/56, 58.9%) compared to MDR strains (22/56, 39.2%). (Table 3) (Figure3)

Table:2: Pattern of antibiotic resistance gene in *Acinetobacter baumannii* isolates

Enzyme	Gene	No. of isolates with resistance gene n=153(%)	XDR (n=87)	MDR (n=62)	P-Value
ESBL	***bla*$_{SHV-1}$**	24(15.6%)	15(9.8)	9(5.8%)	0.22
	***bla*$_{TEM-1}$**	3(1.9%)	1(0.6%)	2(1.3%)	0.5
	***bla*$_{VEB}$**	2(1.3%)	2(1.3%)	-	-
	***bla*$_{PER}$**	23(15.03%)	9(5.8%)	14(9.1%)	0.2
	***bla*$_{CTXM-1}$**	14(9.1%)	10(6.5%)	4(2.6%)	1.00
Carbapenem	***bla*$_{IMP}$**	5(3.2%)	2(1.3%)	3(1.9%)	0.6
	***bla*$_{VIM}$**	6(3.9%)	3(1.9%)	3(1.9%)	1.00
	***bla*$_{NDM-1}$**	37(24.18%)	23(15.03%)	13(23.5%)	0.09
	***bla*$_{OXA-23}$**	95(62.09%)	55(35.9%)	36(23.5%)	0.04
	***bla*$_{OXA-58}$**	4(2.6%)	0	4(2.6%)	-
Aminoglycoside	***aac (3')-IA***	14(9.1%)	9(5.8%)	5(3.2%)	0.2
	aph (3')-IA	31(20.26%)	16(10.4%)	13(23.5%)	0.5
	ant (2")-IA	36(23.5%)	17(11.11%)	17(11.11%)	1.0
Quinolones	***par C***	56(36.6%)	33(21.5%)	22(14.3%)	0.13

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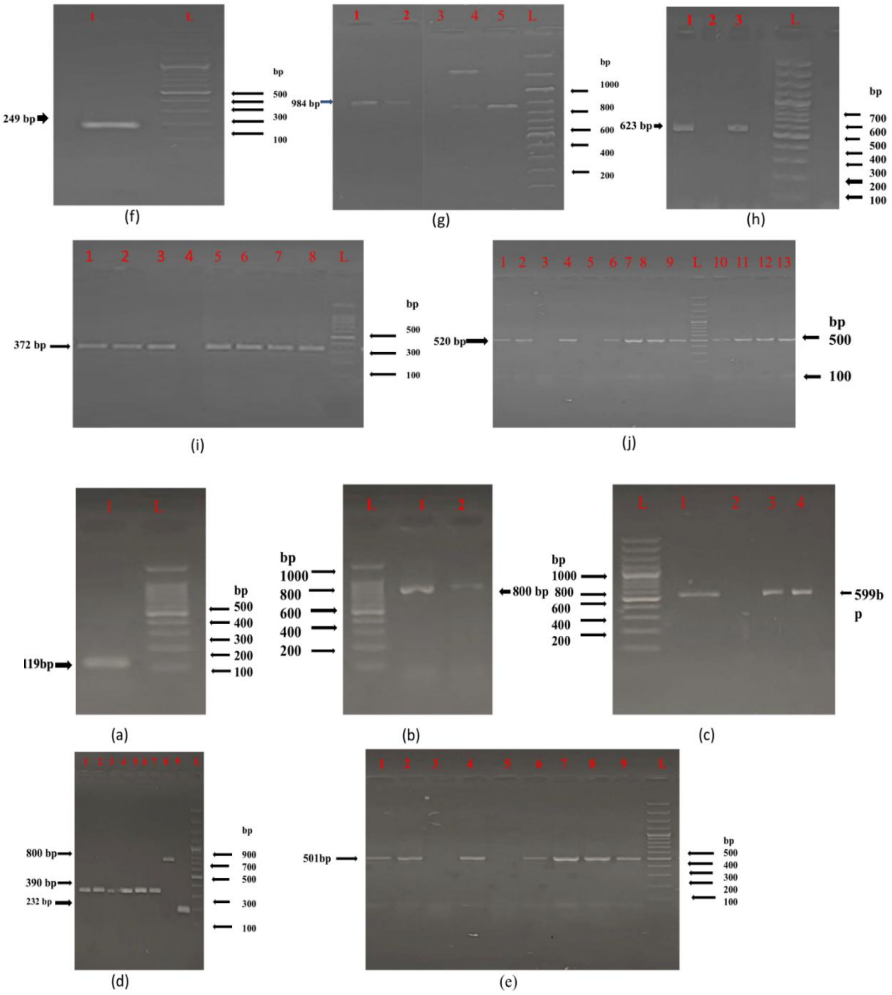

Figure 2: A representative PCR gel (1.5%) agarose showing the biofilm producing gene in this study, each band represents the amplicon of one of the positive isolates for given with 100bp/1000bp ladder (L). (a) lane 1 shows bla_{SHV} (119bp). (b) lane 1,2 bla_{TEM} (800bp). (c) lane 1,3 and 4 shows bla_{OXA-58} (599). (d) lane 1,2,3,4,5,6,7 shows bla_{VIM} (390bp) and lane 8 bla_{TEM} (800bp) and lane 9 bla_{IMP} (232bp). (e) lane 1,2,4,6,7,8,9 shows bla_{OXA-23}(501bp). (f) lane 1; *par C* (249bp). (g) lane 1,2,4 and 5 shows bla_{NDM} (984bp). (h) lane 1,3 shows *aph* (623bp) (i) lane 1,2,3,5,6,7,8 shows *aac1a*(372bp). (j) lane 1,2,4,6,7,8,9,10,11,12,13 shows bla_{PER}(520bp), L-1000 bp ladder.

3.3.1 Represents the correlation of phenotypically and genotypically showed that β-lactam carbapenem-resistant gene

In our study, the carbapenem-resistant group of (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{OXA-23}, bla_{OXA-58}) that considered meropenem and imipenem were 95 (73%) and 124 (96%), respectively. Based on the Chi- square test, and the presence of resistance genes was shown to be strongly associated with phenotypic resistance to carbapenems *(P=0.05)* (Table 4).

Whereas the ESBL group of antimicrobial resistant genes (bla_{SHV}, bla_{TEM}, bla_{VEB}, bla_{PER}, bla_{CTXM-1}) considered the Piperacillin-Tazobactam, Cefepime, and Ceftriaxone was 50 (38%) and 128 (99%), Based on the Chi- square test, and the presence there was an extremely strong association between the presence of the ESBL gene and resistance to these antibiotics *(P=0.0001)*.

A group of aminoglycosides of resistant *gene (aac-1a, aph-1a, ant-1a*) detected in 48 (37% of isolates) is associated with Amikacin, Gentamycin, and Tobramycin in 117 (90% of isolates). Based on the Chi- square test, and the presence in our study, an extremely strong association was observed within the existence of aminoglycoside resistance genes and patterns of resistance *(P=0.0001)*.

Fluoroquinolone resistance gene of *par C* detected in 46(35%) of isolates, correlate with levofloxacin and ciprofloxacin 117(90%) of isolates, statistical correlation, based on the Chi- square test, and the presence of the *parC* gene and phenotypic resistance to fluoroquinolones (*P=0.0001).* (Table 4)

Table:3: Co-relation between disk diffusion method and PCR based test

Test group	**Antimicrobial agent**	**Resistance gene no (%) of isolates**	**Antibiogram pattern [n=129(%)]**	**p-value**
ESBL *(bla$_{SHV}$, bla$_{TEM}$, bla$_{VEB}$, bla$_{PER}$ bla$_{CTXM-1}$)*	Piperacillin-Tazobactam Cefepime Ceftriaxone	50(38%)	128(99%)	**0.0001**
Aminoglycoside *(aac-1a, aph-1a, ant-1a)*	Amikacin Gentamycin Tobramycin	48(37%)	117(90%)	**0.0001**
Carbapenem *(bla$_{IMP}$, bla$_{VIM}$, bla$_{NDM}$, bla$_{OXA-23}$, bla$_{OXA-58}$)*	Meropenem Imipenem	95(73%)	124(96%)	0.05
Fluoroquinolones *(par C)*	Levofloxacin ciprofloxacin	46(35%)	117(90%)	**0.0001**

3.3.2 Association of resistance mechanisms in *Acinetobacter baumannii* isolates

Association of carbapenem resistance genes and aminoglycoside-modifying enzyme genes was identified in 32.8% (n=42) of isolates, predominantly found in 64% (n=27) XDR isolates, indicating a strong linkage between these resistance mechanisms in extensively drug-resistant strains. Co-association of carbapenem-resistant and ESBL genes was observed in 24.19%(n=31) of isolates, Significant distribution among 67%(n=21) XDR isolates, 32.2%(n=10) MDR isolates. A significant correlation was identified for the co-association with resistance phenotypes ***(P=0.04)***. (Table 5)

Table:4: Association of antimicrobial resistance gene in *Acinetobacter baumannii*-

Combination of 2 groups of resistance gene				
Resistance group	**No of isolates (%)**	**XDR**	**MDR**	**p- value**
Carbapenems+ aminoglycosides	42(32.8%)	27(64%)	15(35.7%)	0.064
Carbapenems + fluoroquinolones	35(27.13%)	23(65%)	12(34.2%)	0.06
Carbapenems +ESBL	31(24.19%)	21(67%)	10(32.2%)	**0.04**
ESBL +aminoglycosides	18(13.95%)	11(61%)	7(38.8%)	0.3
ESBL+ fluoroquinolones	15(11.62%)	9(60%)	6(40%)	0.4
Aminoglycosides + fluoroquinolones	16(12.40%)	8(50%)	8(50%)	1.00
Combination of 3 groups of resistance gene				
Carbapenems + Aminoglycosides	15(11%)	7(46%)	8(53.3%)	0.7

+fluoroquinolones				
Carbapenems + Aminoglycosides +ESBL	12(9.3%)	7(58%)	5(41.6%)	0.5
Aminoglycosides + fluoroquinolones +ESBL	4(3.1%)	1(25%)	3(75%)	0.3
Combination of 4 groups of resistance gene				
Carbapenem + Aminoglycosides +fluoroquinolones +ESBL	3(2.3%)	1(33.3%)	2(66%)	0.5

4. DISCUSSION

Acinetobacter baumannii is an opportunistic pathogen and an important contributor to of nosocomial diseases. Treatment of such infections can be extremely challenging due to widespread resistance to antimicrobial drugs (El-Kholy et al. 2021). The goal of this study was to identify antimicrobial resistance patterns phenotypically and genetically, to identify XDR and MDR *A. baumannii* clinical strains, and to look in to the profile of genes involved in antimicrobial resistance in MDR-AB isolates clinically recovered from hospitalized patients' respiratory samples.

In our study 58.13% were XDR and 38.7% of MDR isolates in *A. baumannii.* Various study showed that 91%, 89%, 78.5% of XDR and 5%, 6.7% and 85.8% of MDR from Greece, 2019 and Iran 2024 respectively (Falagas ME, Zeighami H et al. 2019 &. Rostamani M et al. 2024).

The prevalence rate of cefepime, ceftriaxone resistance rate was 100% in our study with compared to other slightly lower rate in other studies: 86% (Iran, 2022), 92% (South Africa, 2020), and 93.93% by Irina Gheorghe (Gheorghe I et al. 2021 and Madaha EL et al. 2020).

In our investigation, we found disturbingly high resistance rates to several antimicrobial drugs, particularly ciprofloxacin (98.4%) and piperacillin-tazobactam (96%). These statistics show a considerable rise over earlier research. Ciprofloxacin resistance rates in Mexico have previously been reported to range from 46% to 73%. (2019), 74% in India (2008) and varying rates across South Asia (2020) (Avila-Novoa MG et al.2019, Banerjee T et al. 2018 and Hsu LY et al. 2017). Regarding piperacillin-tazobactam, a 2020 study from South Africa reported a 100% resistance rate, while studies from Iran in 2016 showed resistance rates of 42% and 66%, respectively (Madaha EL et al. 2020 and Khoshnood S et al. 2023). In our study, we observed a 91% resistance rate to levofloxacin, which is notably higher than rates reported in previous studies. For instance, Estell Longla Madaha reported a 74% resistance rate, and Irina Gheorghe reported an 87% resistance rate (Madaha EL et al. 2020 and Gheorghe I et al. 2021).

In our study, we observed high resistance rates to aminoglycosides: Amikacin (90%), Tobramycin 86.9%), and Gentamicin (87.5%). These rates are significantly higher compared to previous studies In 2019 study from Mexico reported a 60% resistance rate for Amikacin (Avila-Novoa MG et al.2019) Notably, Irina Gheorghe's research indicated a 90% resistance rate for Gentamicin, which is slightly lower than our findings Additionally For instance, a 2020 study from South Africa reported resistance rates of 55% for Amikacin, 68% for Gentamicin, and 81% for Tobramycin((Gheorghe I et al. 2021 and Madaha EL et al. 2020).

The present study showed significant resistance to Cefepime, Ceftriaxone, Meropenem Imipenem, Amikacin, Tobramycin to comparatively lower resistance rate to Piperacillin tazobactam, Tigecycline, and Colistin. Similarly, a study in middle east, Bahrain in 2023 reported a high resistance rate of Cephalosporine and Carbapenems and lower resistance to Amikacin (56%), Gentamycin (64%), Tigecycline (8.5%) (Al-Rashed N et al .2023). In 2009, resistance to ceftriaxone (83.6%), piperacillin-tazobactam (82.0%), and ceftazidime (80.3%) was highest in the Middle East Ceftazidime resistance increased by 19.1% in the Asia-Pacific region, whereas Levofloxacin resistance increased by 38.9%. Resistance to piperacillin-tazobactam, ceftriaxone, cefepime, amikacin, meropenem, and levofloxacin has also grown in Africa and Europe (Wang Y et al. 2020 and Elbehiry A et al. 2023).

To date, we believe that no study has determined the prevalence of carbapenemase, aminoglycoside, fluroquinolones and ESBL gene from north region. In our study, highly resistant group Cephalosporine, Penicillin (99.2%) each, Carbapenem (97.7%), Fluroquinolone (94.7%), Aminoglycosides (93.2%) and Tetracycline (69%), whereas previous study showed similar result of Carbapenem resistance reported from Karachi and from Bahrain 89% in 2023 Al-Rashed N et al 2023) and in Contrast, 33% and 55.8% of Carbapenem resistance show the previous research from India and Korea respectively. Vijayakumar S et al 2019, Lee CM et al.2022.

In current study, $bla_{OXA\text{-}23}$, bla_{NDM}, $bla_{CTXM\text{-}1}$, $bla_{OXA\text{-}58}$, bla_{IMP} and bla_{VIM} were the most Commonly-detected carbapenemase-producing gene, occurring at frequencies of 62.09%, 24.1%, 9.3%, 2.6% and 3.2% each respectively. In our study $bla_{OXA\text{-}24}$ and bla_{KPC} marker of carbapenem-resistant in *A. baumannii*, was not found in any isolates.

These *OXA* genes, which are found on plasmids, integrons, and transposons and its role in the resistance of antibiotic and mostly Carbapenemase. In the current investigation, as in other studies done in China (Tian GB et al. 2011). Our analysis did not find a combination of *blaOXA-58/blaOXA-23-like* -like genes, but two isolates in Iran reported. In Iran, the prevalence of *OXA-23, OXA-24*, and *OXA-58* was reported to be 73.7%, 21.9%, and 6.2%, respectively. However, our study results were

consistent with the results of previous research that did not detect the *blaOXA-24-like* gene in isolates respectively, while another study a high frequency rate of *blaOXA-23* (95.4%) in 81 strains by Laís Lisboa Corrêaa et al (2012) reported from Brazil, and also According to Abouelfetouh et al. (2019)100% of the *blaoxa-23* resistance gene was greater than our findings from Egypt .We also discovered a *blaoxa-58-like* gene, although at lower frequencies, which corresponds to comparable results by Mohamed H. Al-Agamy et al. (2013) from Egypt (Rostamani M et al 2024).

In Algeria, the first descriptions of *blaNDM-1* in Algerian patient were reported in 2010 (Drissi M et al. 2010). According to El-Kholy et al., 2021. this study was showed high prevalence rate of *blaNDM-1 gene* 27.8% of isolates (El-Kholy et al. 2021). In our finding comparing this study showed lower prevalence rate bla_{NDM} gene 19.5% in 26 isolates

ESBLs are less commonly identified in our *A. baumannii strains* but their presence in MDR *A. baumannii* were very difficult to detect, In fact our study, 17% in 23 isolates harboured both *PER* and *GES* gene whereas previous studies of Nabila Benamrouche et al. and Raffaele Zarrilli et al. very low resistance reported of ESBLs- producing *A. baumannii* strains (Benamrouche N et al. 2020 and Zarrilli R et al 2013). We found that *blaPER* gene is the most prevalent ESBL in *A. baumannii* Khamaria et al. 2019, Jussimara Monteiro et al.2012.brazil, Maria del Mar Toma´s et al.2005 in Spain (Khamari B et al. 2020, Widen RH et al. 2012, and Ramirez MS et al 2020).

Fluoroquinolone resistance mechanisms divided into three categories: gyrase and topoisomerase IV, plasmid-mediated resistance, and chromosome-mediated resistance.

Bacterial DNA gyrase and DNA topoisomerase IV are enzymes essential for genetic material production in bacteria. DNA gyrase enzyme is made up of two *gyr A* and two *gyr B* subunits, and topoisomerase IV enzyme is made up of two *par C* and two *par E* subunits. The most common method of fluoroquinolone resistance is alteration in the genes that encode these subunits. The majority of these changes are the consequence of quinolone resistance mutations in the *gyrA and parC* genomes; the most frequently described mutations are at Ser-83 in *gyrA* and at Ser80 in *parC.* Genetic alterations in the *gyrB* (Ser83Leu) and *parE* (Ser80Leu) loci, on the other hand, are not involved in fluoroquinolone resistance (Mohammed MA et al. 2021)

Our study identified the *parC* resistance gene in 36% of 46 isolates, indicating its role in fluoroquinolone resistance. Notably, *gyrA,* as well as plasmid-mediated resistance genes *(qnrA, qnrB, qnrS),* were not found in any isolates, similar report showed in 2023 *parC* reported by Marwa S. Taha. A study from Egypt reported a very low prevalence of the *parC* gene but found *gyrA* mutations in 90% of isolates (Taha MS et al. 2023)

In our study highlights notable difference in the prevalence of aminoglycoside resistance gene in *A. baumannii* which were resistance to amikacin, gentamycin and tobramycin with *ant-1a, aph-1a and aac-1* in 31(25%), 22(17%), 6(4.6%)% isolates respectively, and *aac-2a* gene were not present in any isolates to compared other reports from Pakistan (2020) and Iran(2021),(2022) (Ramirez MS et al 2020, Mohammed MA et al. 2021, and Taha MS et al.2023). The rate of generally higher than in our study, like *aph, aacC1* with 74%,59%,16% and 11% respectively by Mohsin and Maryam Asadi Jouybari and Saba GHASEMI et al.77% of *aph* ,73% of *ant* and *aac6-1a* 33% and *aac3-1a 54*% and *aph* 51.9% respectively high to our study (Khurshid M et al. 2020, Ahanjan M et al. 2021, and Ghasemi S et al. 2022.)

This study highlights the extensive diversity and distribution of *β-lactamase genes* in *A. baumannii* isolate s and their significant association with resistance to critical antibiotics. A high prevalence of *blaOXA-23*-like and specific ESBL genes in extensively drug-resistant (XDR) strains suggests the co-evolution of resistance mechanisms, contributing to enhanced resistance profiles. The nearly universal resistance to cefepime and ceftriaxone among isolates carrying ESBL genes the limited efficacy of these antibiotics against ESBL-producing strains.

5. CONCLUSION

These findings the diversity of β-lactamase genes in *A. baumannii* and their differential distribution among XDR and MDR isolates. The higher prevalence of *blaOXA-23-like* and specific ESBL genes in XDR strains suggests a co-evolution of resistance mechanisms in more drug-resistant phenotypes.The presence of ESBL genes in *A. baumannii* is strongly associated with resistance to critical β-lactam antibiotics, highlighting the role of these genes in driving resistance. The co-association of carbapenem resistance genes with aminoglycoside-modifying enzymes or ESBL genes highlights the complexity and adaptability of *A. baumannii* in acquiring multiple resistance mechanisms. This co-resistance is more prominent in XDR isolates, which poses a significant clinical challenge in managing infections caused by these strains.

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7. COMPETING INTEREST

The author de declare that they have no competing interest.

8. AUTHOR CONTRIBUTION

Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by Vimala Venkatesh. Upma Singh conducted all experiment and wrote the manuscript (drafting, reviewed and data analysis). Parul Jain monitored the experiment and supervised the work. Rashmi manuscript review. Raj Kumar Kalyan helped in data management. Sheetal Verma revised the manuscript.

9. ETHICS APPROVAL

Ethical clearance was granted with Ref. code (**117thECM II B-Ph.D./P4**) dated 3rd June 2022 by the Institutional Ethics Committee (IEC) of King George's Medical University Lucknow, U.P., India.

Consent to participate

Not application

Consent for publication

Not applicable.

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