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ORIGINAL ARTICLE

Colistin resistance in carbapenem non-susceptible *Acinetobacter baumanii* in a tertiary care hospital in India: clinical characteristics, antibiotic susceptibility and molecular characterization

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Abstract

Introduction *Acinetobacter baumanii* (AB) is a bacterium of concern in the hospital setup due to its ability to thrive in unfavorable conditions and the rapid emergence of antibiotic resistance. Carbapenem resistance in this organism is disheartening, further clouded by the emergence of colistin resistance.

Aim The present prospective study aims to note the epidemiology, molecular profile, and clinical outcome of patients with colistin resistance AB infections in a multispecialty tertiary care setup in Odisha, Eastern India.

Methods All AB strains received from March 2021 to February 2022, identified by Vitek2 (Biomerieux) and confirmed by oxa-51 genes, were included. Carbapenem and colistin resistance were identified as per CLSI guidelines. Known mutations for blaOXA-23-like, blaIMP, blaVIM, blaKP, lpxA, lpxC, pmrA, pmrB, and plasmid mediated mcr (mcr1-5) were screened by conventional PCR techniques. The clinical outcome was noted retrospectively from case sheets. Data was entered in MS Excel and tabulated using SPSS software.

Results In the study period, 350 AB were obtained, of which 317(90.5%) were carbapenem resistant (CRAB). Among the CRAB isolates, 19 (5.9%) were colistin resistant (ABCoR). The most valuable antibiotics in the study were tigecycline (65.4% in ABCoI; 31.6% in ABCoR) and minocycline (44.3% in CI; 36.8% in CR). There was a significant difference in mortality among ABCoI and ABCoR infections. bla OXA was the predominant carbapenem resistance genotype, while pmrA was the predominant colistin resistant genotype. There were no plasmid mediated mcr genes detected in the present study.

Keywords Carbapenem resistant *Acinetobacter baumanii* (CRAB) · Colistin resistant *Acinetobacter baumanii* (ABCoR) · bla OXA · pmrA · mcr gene

Introduction

Genus *Acinetobacter* is a Gram-negative, non-fermenting, catalase-positive, and oxidase-negative bacterium. The remarkable capability of this organism to thrive in dry conditions has caused it to colonize hospital environments and is increasingly associated with hospital acquired infections, particularly in ICUs.Community-acquired *A. baumannii* (AB) infections have been described chiefly in individuals with co-morbidities [1].

The bacteria have become a primary concern for clinicians worldwide due to their ability to acquire resistance to a wide range of antibacterial agents rapidly. Most AB strains isolated in ICUs are resistant to beta lactams, fluroquinolones, carbapenems, and aminoglycosides [2]. As per ICMR- AMRN data *Acinetobacter* spp is the predominant non-fermenting GNB isolated, consisting of 12.9% of

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organisms [3]. Carbapenem resistant AB (CRAB) is also steadily increasing and is 87.5% in 2021 Indian setup [3]. XDR–AB is now recognized as one of the most challenging hospital pathogens to treat and control and is considered a global threat in the healthcare setting [4].

With the crisis of antibiotics and smarter AB bacteria, colistin has become an important therapeutic option either singly or combined with other antibiotics like tigecycline, ampicillin sulbactam, rifampin, and carbapenems [5]. Moreover, the newer BLBLIs like ceftazidime avibactam and ceftalozane tazobactam remain ineffective for this difficult bug. However, a grim situation of resistance to colistin among AB has been reported in recent studies [6–8].

Genetic mechanisms of colistin resistance in AB have been demonstrated by complete loss of LPS production mediated by mutations in LPS producing genes (lpxA, lpxC, lpxD, and lpxB) [9] or by modification of lipid A components of LPS through mutations in pmrA and pmrB genes [6, 9]. There is an emergence of a plasmid-mediated mobile colistin resistance mediated by mcr genes in many members of Enterobacterales, [10] but has been scant clinical data in *Acinetobacter* spp in this regard [11]. Plasmids tend to disseminate rapidly across different species, making the already extensive drug resistant strains into pan-drug-resistant ones.

Because of the lesser pool of data regarding the prevalence, clinical outcome, and molecular epidemiology of colistin-resistant *Acinetobacter baumanii* (ABCoR), particularly in our area, it is pertinent to generate data this drug bug combination. The present study aims to bridge the gap in the molecular epidemiology of ABCoR in our area. It is a prospective study describing the epidemiology, molecular profile, and clinical outcome for patients with ABCoR infection in a multispecialty tertiary care setup in Odisha in Eastern India.

Material and method

Study setting

This study was conducted prospectively over one year from March 2021 to February 2022 in the Department of Microbiology at the Institute of Medical Sciences and SUM Hospital in Bhubaneswar, India. It is a premier 1400 bedded tertiary care teaching hospital catering to lower and middle-income patients. All the samples growing AB during the study period from various samples from different wards and ICUs of the hospital were considered. Clinical significance was ascertained before inclusion. Repeated AB isolates obtained from the same patient from the same site and clinically insignificant isolates were excluded from the analysis.

Identification of carbapenem resistant *Acinetobacter baumanii* and antimicrobial susceptibility testing

The AB was identified using Vitek 2 systems (bioMérieux, Durham, North Carolina, US) using GN cards and susceptibility using AST cards. All interpretations were done using breakpoints of the Clinical and Laboratory Standards Institute guidelines [12] except tigecycline, where breakpoints by US Food and Drug Administration were used. An isolate was defined as Carbapenem-resistant *Acinetobacter baumannii* (CRAB) if it showed resistance to any of the carbapenems. (MIC ≥ 8 µg/mL for imipenem/doripenem/meropenem).

Detection of MIC of Colistin

MIC of Colistin was detected by broth microdilution method as per CLSI guidelines [12]. The result was interpreted as intermediate if MIC ≤ 2 µg/ml and resistant if MIC ≥ 4 µg/ml. *Escherichia coli* ATCC 25,922 and *Pseudomonas aeruginosa* ATCC 27,853 were used as quality control (QC) strains. Also, mcr-1 positive *E. coli* was used as an internal control.

Clinical characteristics of the patients and the outcome

The patient demographics, clinical history, and outcome of the ABCoR were noted retrospectively from the patient case sheet obtained from the medical records department. For each strain of ABCoR isolated, three successive strains of ABCoI were taken as controls. Isolates were termed hospital acquired infection (HAI) if cultured from specimens collected after 3 days of admission and the patient was admitted for a reason other than the infection in context. In the case of ventilated patients, microbiological culture, Gram stain findings, suggestive clinical picture, and radiological signs were considered while diagnosing Ventilator associated pneumonia (VAP) [13]. Patient demographics, underlying medical conditions, types of infection (HAI/ VAP/ Community acquired), antimicrobial agents given before and after isolation of colistin-resistant *A. baumannii* isolates, intensive care unit (ICU) admission, and clinical outcomes in terms of discharge or death were extracted from the case sheets.

PCR analysis of genetic modifications of known genes

DNA extraction was performed using hot cold technique as described earlier [14], and the resulting DNA obtained was standardized using Thermo Scientific Multiskan Sky Microplate Nanodot. Common genetic mutations conferring carbapenemase production were screened - blaOXA-23-like, blaIMP, blaVIM, blaKPC. Mutations conferring colistin resistance i.e., lpxA, lpxC, pmrA, pmrB, and plasmid-mediated mcr genes (mcr1-5), were also noted.PCR. Table 1 lists the various primers used in the process. All the PCR were performed using Thermofisher Dream Taq master mix 2X in 'Veriti'ProFlex thermal cycler from Applied Biosystems (Foster City, California, USA).

For each single reaction mixture (25 µL), we used 12µL Taq DNA master mix,1 µL of each primer (10 picomoles; Eurofins Scientific), and 5 mL of template DNA (50 ng/mL) and 6µL of nuclease-free water to maintain volume. The reaction conditions for pmr A, and pmrB gene amplification were as follows: initial denaturation at 95 °C for 1 min, 30 cycles at 95 °C for 15 s, 48 °C for 40 s, 72 °C for 30 s, and final extension at 72 °C for 7 min. For lpxA and lpxC reaction, the conditions were initial denaturation at 95 °C for 3 min, 30 cycles at 95 °C for 30 s, 57 °C for 45 s, 72 °C for 1 min, and final extension at 72 °C for 7 min. The amplified product was identified by agarose gel electrophoresis using 1.5% agarose gel with 80–150 V, with the run typically around 1–1.5 h.

Statistical analysis

All consecutively isolated CRAB strains during the study period were processed for phenotypic detection of colistin resistance. The patients from whom the ABCoR was isolated were designated as cases. Controls were the ABCoI patients among the CRAB. For each case, three successive ABCoI patients were taken as controls. Data collected was entered in an excel sheet and interpreted for routine statistics. Clinical data between cases and controls were compared

Table 1 Various primers used

Genes	Primer used (5′−3′)	Annealing Temp Time Cycle	Primer basepair	References
*bla*OXA23 like	ATGGCAATCCGAATCTTC- F TTATCGCGCAGCGTCCGAG- R	55 °C	828 bp	[15]
*bla*VIM	GTTTGGTCGCATATCGCAAC-F AATGCGCAGCACCAGGATAG-R	55 °C	382 bp	[15]
*bla*IMP	GAATAGRRTGGCTTAAYTCTC-F CCAAACYACTASGTTATC-R	55 °C	188 bp	[15]
*bla*NDM	GGGCAGTCGCTTCCAACGGT-F GTAGTGCTCAGTGTCGGCAT-R	53.60 °C	743 bp	[16]
*bla*KPC	GCT CAG GCG CAA CTG TAA G-F AGCACAGCGGCAGCAAGAAAG-R	59 °C	151 bp	[16]
pmrA	CGCAGGATAATCTGTTCTCCA- F GGTCCAGGTTTCAGTTGCAA- R	52.5 °C	808 bp	[20]
pmrB	GCGAAAAGATTGGCAAATCG- F GGAAATGCTGGTGGTCATCTGA- R	52.75 °C	659 bp	[21]
lpxA	ATTAGCGCACAATTCCACGCT- F ATGACCGAGTCTACTACACCT- R	57 °C	1283 bp	[22]
lpxC	CAATGACTTATGTCACACTCAC- F GTGAGTGTGACATAAGTCATTG- R	57 °C	913 bp	[22]
mcr1	CGGTCAGTCCGTTTGTTC-F CTTGGTCGGTCTGTAGGG-R	58 °C	309 bp	[17]
mcr2	TGGTACAGCCCCTTTATT-F GCTTGAGATTGGGTTATGA-R	49 °C	1747 bp	[17]
mcr3	TTGGCACTGTATTTTGCATTT-F TTAACGAAATTGGCTGGAACA-R	50 °C	542 bp	[18]
mcr4	ATTGGGATAGTCGCCTTTTT-F TTACAGCCAGAATCATTATCA-R	52.5 °C	487 bp	[19]
mcr5	ATGCGGTTGTCTGCATTTATC-F TCATTGTGGTTGTCCTTTTCTG-R	51 °C	1644 bp	[19]

by chi-square and Fisher's exact test as appropriate. Shapiro-Wilk test was used to classify the continuous variables. Normally distributed parameters were analyzed using the Student's t-test, while non-normally distributed variables were analyzed using the Mann–Whitney U test. The univariate logistic regression model was used to calculate odds ratios (OR) for the association of risk factors for colistin resistance. P values ≤ 0.05 were considered significant. Statistical analysis was performed with SPSS version 26.0(IBM® SPSS® Statistics).

Ethical consideration

The study had received approval from the Institute's ethics committee via- no. IEC/IMS.SH/SOA/2O22/290. All the processes done were within patient care standards. Written informed consent was obtained from all the patients or the immediate caregiver (whichever is applicable) during admission for all the procedures and sample collection as necessitated for therapeutic purposes. No patient data was disclosed during the study, and diagnostic or therapeutic activity was not hampered.

Results

Isolates of AB

During the study period of the 350 non-repetitive clinically significant isolates of AB obtained, 317(90.5%) were carbapenem resistant (CRAB). CRAB isolates were obtained from various samples like - tracheal aspirates (159, 50.2%), blood (66, 20.8%), wound swab (56, 17.7%), pus (20, 6.3%), tissue (11, 3.5%) CSF (3, 0.9%) and urine (2, 0.6%). There were only 69, 21.8% CRAB isolates from different wards, while rest were from ICUs. Most of the CRAB samples in the present study were from Burn ICUs (76; 23.9%), which also had the highest percentage of ABCoR (8; 42.1%). Colistin resistant AB (ABCoR) was detected in 19 (5.9%) strains, of which 4 (21.1%) were from different wards and the rest from various ICUs. (Table 2)

Sensitivity pattern of AB (Fig. 1)

Of the 19 ABCoR isolates, 2 isolate had MIC = 64 µg/ml, 8 isolates each had MIC = 32 µg/ml and 16 µg/ml and one isolate had MIC of 8 µg/ml. The results of the antimicrobial susceptibility testing of both colistin intermediate and

Table 2. Characteristics of the 19 colistin resistant *Acinetobacter baumanii* isolated

ID	MIC (in µg/ml)	Age	Sex	Sample	Site	S	MS	Outcome	Resistance genes
1	16	70	F	Blood	NSICU	Tgc	Le	Discharge	pmrA, oxa48
4	16	58	F	Sputum	BICU	–	–	Death	pmrA, lpxA, ndm
7	32	52	F	ET	MICU	Cfs, Ak, Cot, Mi, Tgc	Caz	Discharge	pmrB, oxa48
8	64	65	M	ET	MICU	Ak, Cot	Tgc	Discharge	pmrA, oxa48
13	16	25	M	Tissue	BICU	–	Mi	Death	lpxA, ndm, oxa48
14	32	29	M	Pus	MICU	Mi, Tgc	Caz	Discharge	pmrA, oxa48
23	16	55	F	ET	NSICU	Cot	Caz, Tgc	Discharge	pmrA, pmrB, oxa48
24	32	67	M	Wound swab	SICU	Caz, Mi, Tgc	–	Discharge	pmrA, pmrB, lpxA, lpxC, kpc, vim, oxa48
25	8	85	M	Blood	Ward	Caz, Mi, Le, Mi, Tgc	–	Discharge	pmrB, oxa48
26	32	25	M	ET	NSICU	Caz, Ak, Mi, Tgc	Cfs	Discharge	lpxA, vim, oxa 48
28	16	45	M	Pus	BICU	–	–	Death	pmrA, oxa48
31	32	25	M	Wound swab	BICU	Caz, Cpm, Tic, Cfs, Mi	Gen, Cip, Tgc	Discharge	pmrA, pmrB, oxa48, lpxA
35	32	75	F	Wound swab	BICU	–	–	Death	pmrA, pmrB, oxa48
40	16	36	M	Wound swab	BICU	–	–	Death	pmrB, oxa48, ndm
41	64	65	F	Blood	BICU	Cfs,Ak, Mi, Tgc	–	Death	pmrA, pmrB, vim, oxa48
45	16	53	M	Wound swab	Ward	Cot	Tgc	Death	pmrA, ndm, pmrB,oxa48,
52	32	62	M	Pus	Ward	–	–	Death	pmrA, oxa48, ndm
62	32	41	M	Sputum	Ward	–	–	Death	pmrA, pmrB, lpxA, lpxC, vim, imp, ndm, oxa48
67	16	57	F	Tissue	BICU	Caz	Tgc, Tic	Death	pmrA, kpc, oxa48, ndm

Caz Ceftazidime, *Cpm* Cefepime, *Pit* Piperacillin tazobactam, *Tic* Ticarcillin clavulanic acid, *Cfs* Cefoperzone sulbactam, *Ak* amikacin, *Gen* Gentamicin, *Cip* Ciprofloxacin, *Le* Levofloxacin, *Mi* Minocycline, *Tgc* Tigecycline, *Cot* Cotrimoxazole

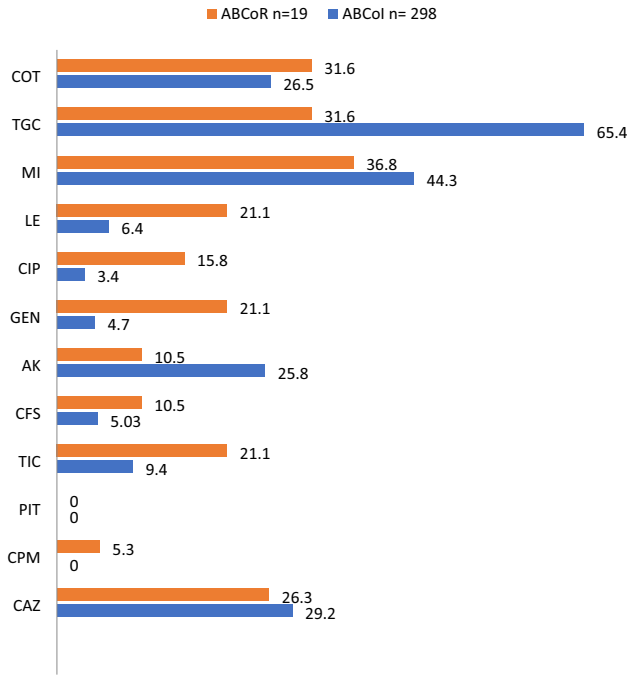

Fig. 1 Susceptibility pattern of CRAB isolates (in %). *Caz* Ceftazidime, *Cpm* Cefepime, *Pit* Piperacillin tazobactam, *Tic* Ticarcillin clavulanic acid, *Cfs* Cefoperzone sulbactam, *Ak* amikacin, *Gen* Gentamicin, *Cip* Ciprofloxacin, *Le* Levofloxacin, *Mi* Minocycline, *Tgc* Tigecycline, *Cot* Cotrimoxazole

resistant isolates are showed in Table 3. The strains were found to be highly resistant to almost all the drugs tested. The antibiotics which were most susceptible were tigecycline (65.4% in ABCoI; 31.6% in ABCoR) and minocycline (44.3% in ABCoI; 36.8% in ABCoR). Among the BL agents ceftazidime had the highest susceptibility (29.2%). Cefepime (94.7% Resistant in ABCoR vs. 100% in ABCoI), TIC (78.9% resistant in ABCoR vs. 85.9% in ABCoI), CFS (89.5% resistant in ABCoR vs. 94.97% in ABCoI) had lower rate of resistance in colistin resistant isolates than the colistin intermediate ones. Similarly levofloxacin and ciprofloxacin had better sensitivity in ABCoR than ABCoI strains.

Clinical characteristics

The 19 ABCoR were taken as cases to determine the clinical status, and for analysis of various risk factors, three times the cases, i.e., 57 controls were taken. There was no significant difference in age and gender characteristics of the cases and controls. 73.7% of cases and 78.9% of controls were admitted to ICU respectively. When prior ICU days were considered, the mean ICU stay of the patients harboring ABCoR was lesser than those of the controls. The mean duration of getting the colistin-resistant organisms was 5 ± 6.3days of ICU stay. Only 57.9%of ABCoR were hospital-acquired in contrast to 78.9% of controls. There was no significant difference in the development of ABCoR or ABCoI due to nosocomial infections or ventilator-associated pneumonia. When preexisting illnesses were considered, there was a significant difference between cases and controls regarding Type 2 diabetes mellitus, chronic kidney diseases, and COPD. Patients having hypothyroidism, Chronic obstructive pulmonary disease (COPD), Chronic Kidney disease (CKD), Pneumonia, post-coronavirus disease 2019 (COVID) pneumonia, and post surgery patients have higher odds of developing ABCoR infections. Pneumonia patients have the highest likelihood of developing ABCoR infections. Prior colistin regimen was offered to 89.5% of cases and 33.5% of controls. Among the ABCoR harboring cases, 10 (52.6%) died. There is a significant difference in mortality of the cases in ABCoR and ABCoI infections. Similarly, the ICU stay duration was longer in cases (16.3 ± 13.3 days) than in controls (6.8 ± 7.3 days).

The ABCoR patients were treated variously as per the treating physician's discretion and patient's clinical profile. Out of the 19 cases, 13 patients received combination therapy of tigecycline and carbapenem, 2 patients received tigecycline with cefoperazone sulbactam and 1 patient received tigecycline with a carbapenem and cefoperazone sulbactam. 3 other patients received combination of colistin with tigecycline. There was no significant difference in the colistin added or sparing therapy when outcome in terms of death or discharge was considered.

PCR results of carbapenemase and colistin resistant genes (Fig. 2)

Among the CRAB strains, blaOXA-23 gene was the most common genetic pattern observed in 147(49.3%) strains and in combination with other genes in 54(17.03%) strains. Metallo-β-lactamase genes were present in 46.7% of strains of CRAB. Among these, 24.2% of strains carried blaNDM-1, followed by 17.4% of strains with blaVIM and 17.7% of CRABs with bla IMP. Many strains,i.e.,17.02%, co-harbored MBL and non-MBL genes in this study. Among the 19 ABCoR strains, 52.6% had blaOXA genes alone, serving as the most common pattern, while 42.2% of the stains had multiple genes, including Class A, C, and D beta-lactamase genes.

pmrA was the commonest 14(73.8%) colistin-resistant gene present in our isolates; 6(31.6%) alone and rest along with other genes. Other genes were pmr B (47.4%), lpxA

Table 3 Comparison of the various clinical characteristics of the cases and controls

Characteristics	Cases (n = 19)	Controls (n = 57)	p-value	OR	95% C.I.	
					Lower	Upper
Age (Mean ± SD)	53.16 ± 16.85	53.33 ± 16.68	0.972	0.978	0.949	1.008
Gender						
Male	12(63.2%)	34(59.6%)	0.801			
Female	7(36.8%)	23(40.4%)				
ICU admission	14 (73.7%)	45 (78.9%)	0.227			
Prior ICU days	5 ± 6.3d	8.7 ± 15.5 d	0.316			
Hospital acquired infection	11(57.9)	45(78.9)	0.071	1.333	0.381	4.661
Ventilator associated pneumonia	2(10.8)	15(26.3)	0.153	0.230	0.071	0.740
Preexisting illness						
Type II Diabetes mellitus	11(57.9)	16(28.1)	**0.019**	0.450	0.153	1.323
Hypertension	5(26.3)	12(21.1)	0.634	0.230	0.071	0.740
Hypothyroidism	5(26.3)	3(5.3)	**0.010**	**3.857**	0.856	17.380
Malignancy	1(5.3)	2(3.5)	0.734	0.000	0.000	
Chronic Kidney disease	1(5.3)	17(29.8)	**0.029**	**1.114**	0.315	3.940
Chronic obstructive pulmonary disease	6(31.6)	5(8.8)	**0.014**	**2.333**	0.267	20.354
Cerebro vascular accident	3(15.8)	5(8.8)	0.388	0.300	0.079	1.138
Pneumonia	3(15.8)	4(7)	0.252	**10.769**	1.876	61.807
Post-Corona virus disease pneumonia	3(15.8)	11(19.3)	0.733	**4.636**	1.350	15.921
Surgery within 30 days	11(57.9)	28(49.1)	0.508	**1.071**	0.372	3.086
Antibiotic use						
Prior colistin (iv/ intranasal) use	17 (89.5)	19 (33.5%)	**< 0.001**	**17**	3.553	81.327
Outcome						
Death	10(52.6)	8(14.1)	**< 0.001**			
Discharge	9 (47.4%)	49 (85.9)				
Days of ICU stay prior to discharge	16.3 ± 13.3 d	6.8 ± 7.3 d				

It represents the odd ratio and p-value.

(31.6%), and lpx C (10.5%). None of the strains possessed any plasmid-mediated mcr 1–5 genes (Table 4).

Discussion

A.baumanii is a member of the dreaded group of ESKAPE pathogens as they are often XDR and PDR organisms. In the present study, carbapenem-resistant AB accounted for 90.5% of cases, similar to other previous Indian studies [3]. A worldwide surge in carbapenem resistance has been observed recently, primarily driven by the spread of several international clones [5].

Colistin, or its prodrug CMS, is a key therapeutic option for the treatment of carbapenem-resistant *A. baumannii*, alone or in combination with other agents such as tigecycline, ampicillin sulbactam, rifampin, and carbapenems [5]. In the present study, the prevalence of colistin resistance in CRAB infections is 5.9% of cases. Over all colistin resistance in a study in northern India was 1.7% in AB [23]. While in a study from South India ABCoR was noted in 8–11% between 2016 and 2019 [24]. The prevalence of ABCoR is estimated to be around 5.3% in the USA [25]. Colistin resistance in CRAB has also been noted in other studies across the globe [6, 7].

In our study, patients with hypothyroidism, COPD and chronic kidney diseases have significant odds of harboring ABCoR. AB from pneumonia cases has a very high likelihood of being colistin-resistant. Prior surgery requiring hospitalization is another risk factor, as described before [26, 27]. Wang et al. described the male sex as an additional risk factor, [27] though in our case we did not find any male preponderance. We had prior exposure to colistin in 89.5%

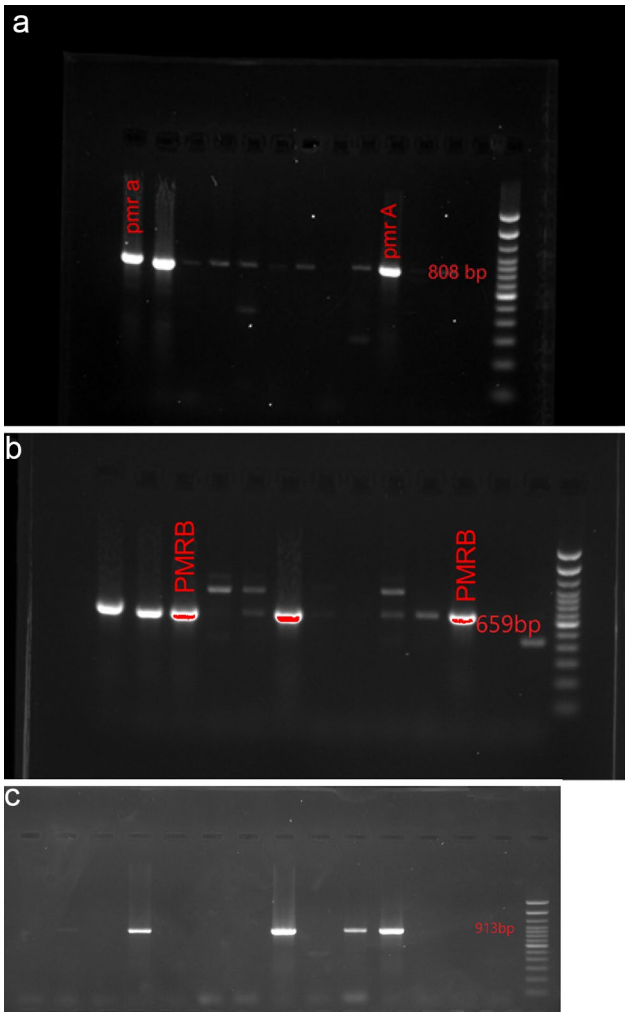

Fig. 2 **a** First 2 well demonstrate ABCoR (STRAIN-14.23) PMRA gene whereas 10th well demonstrate ABCol (STRAIN-44) PMRA gene. **b** Well 1-3 &6 depict ABCoR STRAIN(35, 41, 45, & 62) and well 11 depict ABCol STRAIN (77) PMRB gene. **c** All 4 well depict lpxA gene ABCoR STRAIN- (4, 13, 24 & 26)

of ABCoR, which was significantly higher than the control group. Similar finding of colistin exposure leading to colistin resistant AB was also seen in other studies [28, 29]. However, acquiring ABCoR strains without prior colistin treatment has also been reported in the literature [30]. In our study, 52.6% of ABCoR harboring cases died, much higher than controls, and the length of ICU stay of cases in cases was also higher than control population of CRAB with ABCoI. Other studies demonstrate that ABCoR infection, in general, has a high mortality rate and that colistin resistance is an independent risk factor for mortality [31, 32]. According to the extensive review by Wong et al., in the case of AB infection, antibiotic resistance drives the outcome of the patients [33].

In the present study, the betalactams quinolones, cefoperazone sulbactam, ticarcillin clavulanic acid, cefepime showed better sensitivity in ABCoR cases than ABCoI patients. Moffatt et al. [9,] in their study, have also demonstrated that loss of LPS production in ABCoR strains leads to a greater susceptibility to other antibiotics. Nevertheless, colistin therapy alone, probably because of the drug's toxicity and the critical nature of patients on treatment, leads to high mortality.

bla_{OXA-23} like was the most predominant type of carbapenem genes in CRAB isolates in our study, similar to previous studies [34]. Like other Indian studies, we also had very few (10%) bla_{KPC} strains [34]. About 17% of CRAB cases had bla_{IMP} and $bla_{VIM,}$ unlike other previous studies different from other studies [34]. NDM-1 genes were seen in 7/19 ,i.e., 36.8% ABCoR isolates in our study, which is a matter of concern. The presence of IMP and VIM variants together was seen in 12% of our isolates, and this confers a high level of carbapenem resistance in *A. baumannii* isolates, as well as resistance to all beta lactams except aztreonam, because of their strong hydrolytic efficiency against these antibiotics [35]. As per molecular epidemiology of CRAB this probably belongs to the most widespread IC2 clone known to harbor the acquired OXA-23 carbapenemase [36].

In *A. baumannii*, two mechanisms of colistin resistance are- mutations affecting the pmrAB, which modifies the lipid A component of LPS. and in the genes lpxA, lpxC, lpxD that cause lack of LPS production. Recent studies have shown that the pmrAB efflux pump may also play a role in colistin resistance in *A. baumannii.* In our area mutations affecting pmr A superseded other mutations, unlike other studies where mutations in pmrB is the major contributor to colistin resistance in *A. baumannii* [37, 38]. Multiple mutations in pmrA and lpx A were seen in one isolate, and all the genes tested were mutated in another. Such combinations were also seen in 4 of ABCoI isolates. This observation indicates that mutations in lpxA or B alone and pmrB alone may not be sufficient to induce colistin resistance and support the synergistic activity of mutations within these genes in promoting colistin resistance [37]. A single case of mcr-1 has been detected in Indian set up in ABCoR on

Table 4 Various carbapenem and colistin resistant genes detected

Genes	Number of isolates from ABCoI n = 298 N (% total ABCoI)	Number of isolates ABCoR n = 19 N (% total ABCoR)	Total CRAB n = 317 N (% total CRAB)
Known Carbapenemase genes			
*bla*KPC alone	22 (7.4%)	0	22 (6.9%)
bla OXA-48 alone	137 (45.9%)	10 (52.6%)	147 (49.3%)
blaIMP alone	17 (5.7%)	0	17 (5.4%)
BlaNDM alone	46 (15.4%)	1 (5.2%)	47 (14.8%)
blaVIM alone	6 (2.01%)	0	6 (1.9%)
VIM + IMP	24 (8.1%)	0	24(7.6%)
VIM + IMP + OXA48	14 (4.7%)	0	14 (4.4%)
VIM + IMP + OXA48 + NDM	0 (0)	1 (5.2%)	1 (0.32%)
NDM + OXA-48	18 (6.04%)	3 (15.7%)	21 (6.6%)
VIM + OXA-48	4 (1.3%)	2 (10.4%)	6 (1.9%)
VIM + KPC + OXA48	3 (1.0%)	1 (5.2%)	4 (1.3%)
NDM + KPC + OXA48	7 (2.3%)	1 (5.2%)	8 (2.5%)
Colistin resistant genes			
lpx A alone	NA	2 (10.5%)	NA
lpxC alone	NA	0 (0)	NA
pmrA alone	2	6 (31.6%)	NA
pmr B alone	1	3 (15.8%)	NA
pmrA + pmrB	2	4 (21.1%)	NA
pmrA + lpxA	NA	1 (5.3%)	NA
pmrA + pmrB + lpxA	NA	1 (5.3%)	NA
lpx A + lpxC + pmrA + pmrB	NA	2 (10.5%)	NA
mcr 1–5	NA	0 (0)	NA

the chromosome. However, in the current study, none of the isolates had mcr1-5 genes.

In our study, OXA-23-like enzymes were the common mutations associated with CRAB isolates. Class B metallo-β-lactamases (MBLs) were seen in a lower frequency in *A. baumannii* like in previous Indian studies. pmrA is a common mutation resulting in ABCoR, and no transferable gene resulting in colistin resistance was noted.

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Author contributions We, the undersigned authors of this research article, hereby declare that we have contributed to this study as follows: BPR-Writing—Original , Methodology Data collection, Visualization. SO-Conceptualization, Data analysis, Writing—Review & Editing. SKD- Methodology Data collection Data analysis. BB-Methodology Data collection, Visualization. IP-Conceptualization, Writing—Review & Editing. KKS- Conceptualization, Writing—Review & Editing, Data analysis. We affirm that all authors have reviewed and approved the final version of this manuscript, and each author takes responsibility for the content and integrity of the work.

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Data availability Dataset Excel Sheet: The data is openly accessible and can be downloaded for research purposes.

Declarations

Conflict of interest The authors declare no conflicts of interest related to this study.

Ethical approval This research was conducted in full compliance with the ethical principles and guidelines set forth by the Institute of Medical Sciences (IMS) and SUM Hospital] IEC/IMS.SH/SOA/2022/290.

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