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Original Article

CHARACTERIZATION OF CARBAPENEM RESISTANT *ACINETOBACTER BAUMANNII* ISOLATED IN A TERTIARY CARE HOSPITAL: EPIDEMIOLOGY AND TREATMENT OUTCOME

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ABSTRACT

Objective: Carbapenem resistant *Acinetobacter baumannii* (CR-*Ab*) has emerged as a major nosocomial pathogen, but optimal treatment regimens are unknown. Our objectives were to determine the epidemiology and outcome of CR-*Ab* infections at a tertiary care hospital.

Methods: CR-*Ab* isolates were collected from January to April 2013. MICs were determined and isolates were subjected to screening for carbapenemase production by Modified Hodge test (MHT), metallo-β-lactamase (MBLs) by EDTA disk synergy test and AmpC β-lactamase by AmpC disk test. 15 isolates were subjected to PCR for detection of resistant genes, $bla_{\text{OXA-23}}$, bla_{VIM} and bla_{NDM}. Treatment outcomes of infections were evaluated.

Results: 51 CR-*Ab* isolates from tracheal aspirate (21), blood (15); tissue/wound/drainage (13) and urine samples (2) were collected. Colistin appeared to be the most effective agent with 98% *in vitro* activity. MHT showed 98% positivity, MBLs production was detected in 94.1% isolates and 64.7% were positive for AmpC β-lactamase production. All 15 isolates carried $bla_{\text{OXA-23}}$ and bla_{VIM}, of these 3 also carried bla_{NDM} gene. Colistin containing combinations were more commonly used (68.3%). Colistin-noncarbapenem combination showed improved clinical response compared to colistin-carbapenem combination against *Acinetobacter* isolates carrying $bla_{\text{OXA-23}}$ and bla_{VIM}.

Conclusion: A stringent infection control practice along with antimicrobial stewardship is needed to prevent emergence of *Acinetobacter* carrying multiple carbapenemase genes along with bla_{NDM}. Various colistin combinations are preferentially used to treat CR-*Ab* infections. Identification of antimicrobial combinations with proven *in vitro* activity that encompass local susceptibility patterns as well as molecular mechanisms of resistance is needed to provide better outcome.

Keywords: *Acinetobacter baumannii*, Carbapenem resistance, Carbapenemases, Colistin combination, metallo-β-lactamase, NDM

INTRODUCTION

Acinetobacter baumannii has emerged as one of the most troublesome group of pathogens in the healthcare settings both globally and locally. Its remarkable ability to develop or acquire multiple antibiotic resistance and tendency to survive for prolonged periods under varied environmental conditions, make it a frequent cause of hospital outbreaks and an endemic healthcare associated pathogen [1].

For many years, the carbapenem class has been considered as the drug of choice to treat serious *Acinetobacter* infections. With the rapid escalating prevalence of carbapenem resistant *Acinetobacter baumannii* (CR-*Ab*) isolates in many parts of the world in past few years has undermined the reliability of the carbapenem class [2]. It significantly limits treatment options to tigecyclin, aminoglycosides, colistin and addition of sulbactam. Clinical experience on the value of each of these agents is limited [3]. Despite treatment of infections by CR-*Ab,* it has still been associated with poor outcomes [3]. Carbapenem resistance is predominantly conferred by carbapenemases, such as oxacillinase (OXA)-type enzymes and metallo-β-lactamases (MBLs) [1]. Of these, acquired OXA-type enzymes, belonging to families OXA-23,-24 and-58, are more prevalent [2]. Some are plasmid-mediated, explaining their rapid dissemination. MBLs [mostly Imipenemase (IMP) and Verona Italy metallo-β-lactamases (VIM) type and, more recently, of New Delhi metallo-β-lactamases (NDM) type] have also been found in *A. baumannii* [4]. Non-enzymatic carbapenem resistance mechanisms include membrane porin changes and multi-drug efflux pumps [1, 2].

MATERIALS AND METHODS

So this study aims to characterize the CR-*Ab* isolates at a tertiary care center with emphasis on their mechanism of resistance, epidemiology and treatment outcome of the infections caused by CR-*Ab* isolates. It was a prospective observational study conducted in a 750 bedded tertiary care hospital in Mumbai from January 2013 to April 2013 (4 mo). This study was approved by the Institutional Scientific and Ethics Board (ISEB) of the hospital.

Non-repeat, consecutive, clinically significant CR-*Ab* isolates obtained from clinical specimens received in microbiology laboratory for culture studies that exhibited an imipenem minimal inhibitory concentration (MIC)>4 µg/ml, according to Clinical and Laboratory Standard Institute (CLSI) guidelines, were considered [5]. All samples were processed as per standard microbiology protocol.

Identification and susceptibility testing of clinical isolates were performed by standard techniques using the automated broth microdilution system (Vitek 2; BioMerieux Ltd). Sensitivity testing against meropenem and amikacin was done using Kirby Baur disk diffusion method (HIMEDIA, Mumbai, India) as per CLSI guidelines, due to the limitation of sensitivity testing card used in Vitek 2 analyzer. Carbapenem resistance was rechecked by Kirby Baur disk diffusion method and interpreted as per CLSI standards [5]. For tigecyclin, interpretive criteria approved by the US Food and Drug Administration (US-FDA)[6] for *Enterobacteriaceae* was used for *Acinetobacter* spp. For colistin, break points proposed by European Committee on Antimicrobial Susceptibility Testing (EUCAST) [7] were used because relevant break points were not available from CLSI. MIC of colistin resistant isolates was rechecked by E-test method as per manufacturer's guidelines (BioMerieux Ltd).

To characterize the carbapenem resistance mechanism phenotypic tests like, Modified Hodge's test (MHT) for carbapenemase production, ethylene-diamine-tetra-acetic acid (EDTA) disk synergy test for MBLs production and AmpC disk test for AmpC β-lactamase production was performed, as described [8].

Due to limited consumables for PCR, 15 strains were selected at random and DNA was extracted from these strains by heat boil method. The resistant genes *bla*$_{OXA-23}$, *bla*$_{VIM}$ and *bla*$_{NDM}$ were amplified by single target PCR using previously published primers, OXA-23, Oxa-23-F 5'-GATCGGATTGGAGAACCAGA-3'/Oxa-23-R 5'-ATTTCTGACCGCATTTCCAT-3'; VIM, Vim-F 5'-GATGGTGTTTGGTC GCATA-3'/Vim-R 5'-CGAATGCGCAGCACCAG-3'; NDM-F 5'-GGTTTG GCGATCTGGTTTTC-3'/NDM-R 5'-CGGAATGGC TCATCACGATC-3' [9-11]. Amplified products were visualized under UV light on 3% Agarose Gel Electrophoresis. In house strains of *A. baumannii* and *Klebsiella pneumonia* harboring *bla*$_{OXA-23}$, *bla*$_{VIM}$ and *bla*$_{NDM}$ gene, identified by PCR and gene sequencing were included as positive controls.

Data, including patient demographics, co-morbid conditions, source of infection, and treatment were collected from the electronic medical records, laboratory data and medication administration records from the date of admission till discharge. Only single clinically significant isolate from the patient was included. Outcome at the end of treatment was defined as successful (partial or complete improvement of signs/symptoms of infection or positive microbial response in terms of sterile culture results post or during the treatment), and failure (no improvement or deterioration of signs/symptoms of infection or negative microbial response in terms of persistent positive culture results with the same organism 3 d after initiation of antibiotic therapy). Final disposition was defined as death, discharged during illness or transferred to ward or discharged.

RESULTS

A total of 51 clinically significant CR-*Ab* were isolated from tracheal aspirate (21), blood (15), tissue/wound/drainage (13) and urine samples (2) during the study period of 4 mo. Susceptibility profile of all the isolates is been given in table 1. Kirby Baur disk diffusion method confirmed the carbapenem resistance amongst the isolates and did not show any discrepancy in results of susceptibility for imipenem. One of the isolate was found to be resistant to colistin with an MIC of 4 µg/ml and it was reconfirmed by E strip method. This isolate was termed as pandrug resistant (PDR) as it was found to be resistant to all other group of drugs tested along with colistin [12]. MHT detected carbapenemase production in 50/51 (98%) isolates, whereas EDTA-disk synergy test detected MBLs production in 48/51 (94.1%) isolates. AmpC β-lactamase was found in 33 (64.7%) isolates. All 15 isolates considered for PCR analysis were found to be carrying *bla*$_{OXA-23}$ and *bla*$_{VIM}$ genes, whereas three of these also carried *bla*$_{NDM}$ gene.

Table 1: Susceptibility profile of 51 isolates of *Acinetobacter baumannii* to various antimicrobials

Acinetobacter baumannii				
Antimicrobial agent	**MIC range µg/ml**	**MIC$_{50}$ µg/ml**	**MIC$_{90}$ µg/ml**	**% Sensitivity**
Imipenem*	4-16	16	16	0
Meropenem*	ND	-	-	0
Amikacin*	ND	-	-	0
Tobramycin*	1-16	16	16	22
Gentamycin*	1-16	16	16	8
Colistin**	0.5-4	0.5	0.5	98
Tigecyclin***	0.5-8	2	4	57
Ciprofloxacin*	4	4	4	0
Ampicillin sulbactam*	4-32	32	32	6
Cefoperazone sulbactam*	16-64	64	64	10
Cotrimoxazole*	20-320	320	320	8

Note: MIC$_{50}$, MIC (µg/ml) required to inhibit the growth of 50% isolates of this study, MIC$_{90}$, MIC (µg/ml) required to inhibit 90% isolates of this study, MICs (µg/ml) were determined by broth dilution method, *MICs were interpreted in accordance with the CLSI, **MICs were interpreted in accordance with EUCAST, ***MICs were interpreted in accordance with US-FDA, ND-Not done

Medical records of 51 patients were reviewed. Table 2 describes the demographic and clinical features, including co-morbidity, of the study cohort (51 patients). Except one neonate, all patients had received other antimicrobial agents prior to acquiring infection by CR-*Ab*. Specifically, 78.4% of patients received β-lactam/β-lactamase inhibitor (BL/BLI, β-lactamase inhibitor co-formulated with β-lactam antibiotic) antibiotics, 47.1% of patients received carbapenems, 9.8% received aminoglycosides, and 5.9% received fluoroquinolones (The total number is more than 100%, since most of the patients received more than one antimicrobial agent).

Table 2: Demographic and clinical features of patients with infections caused by carbapenem resistant *Acinetobacter* spp. (n = 51)

Characterization of patient	***n* (%)**
Demographics	
Age group	
Pediatric patients	8 (15.7)
Adult patients	43 (84.3)
Sex (male)	27 (52.9)
Co-morbidity	
Heart dysfunction	18 (35.3)
Malignancy	4 (7.8)
Diabetes Mellitus	18 (35.3)
Hyper tension	23 (45.1)
Chronic renal failure	8 (15.7)
Admission to ICU	42 (82.4)
Prior surgery	8 (15.7)
Prior antibiotic use	50 (98)
BL/BLI	40 (78.4)
Carbapenem	24 (47.1)
Aminoglycosides	5 (9.8)
Fluoroquinolones	3 (5.9)
Prior hospitalization	19 (37.3)
Time to develop infection with CR-*Ab*(days) [median (range)]	7 (0 to 42)
Duration of hospitalization (days) [median (range)]	27 (2 to 209)

Note: BL/BLI: β-lactam/β-lactamase inhibitor

The in-hospital mortality in this study of 51 patients was 49% (25/51). Eight patients died because of the reasons unrelated to infection and attributed mortality of the infection was found to be 33.3% (17/51). Ten out of 17 patients who died had not received any effective therapy or received it for less than 48 h. Of these 10 patients seven had bacteremia, one had pneumonia, one had wound infection and one had a urinary tract infection. These were not included in further analysis.

Successful clinical outcome of the infection was observed in 32 out of 41 patients (78.0%). Seven out of nine patients whose infections were unresponsive to therapy died and two were discharged during illness. Table 3 presents the clinical response associated with the site of the infection and the type of treatment regimen followed. Colistin was most commonly used 78.0% (32/41 cases) either as monotherapy (4 cases) or combination therapy (28 cases) along with another antibacterial agent. 160 to 240 mg (2 to 3 MIU) of Colistimethate sodium (CMS) per 8 or 12 h was administered and doses were adjusted according to the renal functions.

The PDR isolate of *Acinetobacter baumannii* was isolated from pleural effusion; the patient was successfully treated with Colistin, rifampin and sulbactam combination along with adjunctive procedure (drainage of pleural effusion).

Particular attention was paid to the successful clinical outcome observed with carbapenem therapy. In all the cases patients were administered with 1 g dosing every 8 h with prolonged infusion over a period of 3 h. In all four cases patient had received antibiotics with an adjunctive procedure (eg. catheter removal, drainage, or debridement) for the removal of focus of infection.

Table 3: Clinical response associated with the type of the infection and type of treatment regimen followed

Treatment regimen	Total (n)	Success (%)	Type of infection (%)			
			Respiratory tract infection	Blood stream Infection	Tissue Infection	Urinary tract infection
Monotherapy						
COL	4	4 (100)	2/2 (100)	2/2 (100)	-	-
CAR	4	4 (100)	2/2 (100)	-	2/2 (100)	-
Tg	1	1 (100)	-	-	1/1 (100)	-
Combination therapy						
COL+CAR	18	11 (61.1)	3/9 (33.3)	4/4 (100)	4/5 (80.0)	-
COL+non CAR combination	10	8 (80)	3/5 (60)	2/2 (100)	3/3 (100)	-
1. COL+Tg	7	5 (71.4)	2/4 (50)	1/1 (100)	2/2 (100)	
2. COL+Rif+sul	3	3 (100)	1/1 (100)	1/1 (100)	1/1 (100)	
Non COL combination	4	4 (100)	2/2 (100)	-	1/1 (100)	1/1 (100)
1. CAR+Tg+sul	1	1 (100)	1/1 (100)	-	-	-
2. CAR+TOB	1	1 (100)	1/1 (100)	-	-	-
3. CAR+Ak	1	1 (100)	-	-	1/1 (100)	-
4. COT+NF	1	1 (100)	-	-	-	1/1 (100)
Total	41	32 (76.2)	12/20 (60)	8/8 (100)	11/12 (91.7)	1/1 (100)

Note: COL: Colistin, CAR: Carbapenem, Tg: Tigecyclin, Rif: Rifampin, sul: sulbactam, TOB: Tobramycin, Ak: Amikacin, NF: Nitrofurantoin.

DISCUSSION

The remarkable ability of *Acinetobacter* to upregulate or acquire resistance determinants makes it one of the organisms threatening the current antibiotic era. It is an opportunistic pathogen which commonly targets the critically ill patients [1]. Carbapenem resistance in this species is now observed increasingly worldwide, and constitutes a sentinel event for emerging antimicrobial resistance [2]. This is a cause of concern due to poor outcome associated with infection caused by CR-*Ab* and limited treatment option available [3]. Aim of the study was to characterize CR-*Ab* isolates in our institute. Maximum (41.1%) isolates were obtained from respiratory secretions. Similar studies have also reported respiratory tract infection as a predominant infection caused by *A. baumannii* [13, 14]. The susceptibility profile of isolates recovered during the present study underscores the extremely limited therapeutic choices available for treatment of patients infected with CR-*Ab*. Colistin had maximum *in vitro* activity, with 98% followed by tigecyclin retaining sensitivity against 57% isolates. Furthermore, with reported PDR isolate of *Acinetobacter baumannii* from the study cohort adds to the rapidly escalating reports worldwide, for which there is no active agent [15, 16].

With 98% positivity of MHT and 94.1% positivity in EDTA disk synergy test, carbapenemases especially MBLs was found to be major resistance factor along with AmpC carbapenemase production. Our study is in accordance with previous reports from India on the occurrence of multiple carbapenemase encoding genes in *A. baumannii*, of which $bla_{\text{OXA-23 like}}$ and bla_{VIM} have been reported to be the most common type of carbapenemases contributing to carbapenem resistance in clinical isolates of *A. baumannii* [17, 19]. There are also reports of *Acinetobacter* harboring bla_{NDM} gene along with multiple other carbapenemase genes as demonstrated in the present study [17, 19]. It is possible that the spread of NDM carbapenemase may occur rapidly, mostly through *A. baumannii* rather than *Enterobacteriacae*, since *A. baumannii* may become much more difficult to eradicate. Thus, with emergence of the clinical isolates in which multiple carbapenem resistance mechanism coexists, show a very broad spectrum antibiotic resistance profile and it may seriously limit treatment options.

On studying the patient characteristics it was seen that 98% patients were exposed to antibiotics, indicating severity of underlying conditions and also problem of high antimicrobial consumption. With 78.4% of patients exposed to BL/BLI antibiotics and 47.1% of patients receiving carbapenems, the increasing prevalence of CR-*Ab* may be associated with previous exposure to BL/BLI and carbapenem as reported by Goel *et al.* [20]. With 82.4% patients admitted to ICU and other co-morbidities indicate the patients group in the present study to be critically ill, making it more vulnerable for an opportunistic pathogen like *Acinetobacter* [1, 2].

Colistin containing combinations were most often used, as it can be seen that only colistin showed the maximum *in vitro* activity against the isolates compared to other group of antibiotics. In terms of outcomes though less preferred, colistin monotherapy (100% successful outcome) was found to be non-inferior when compared with combination therapy (67.9% successful outcome). This supports the data from the small number of relevant human studies suggesting non-inferiority of colistin monotherapy compared with combination therapy [21]. However, in severe infections caused by CR-*Ab*, most clinicians had reservations about colistin mono-therapy as there are raised concerns regarding potential problem of heteroresistance among Gram negative bacterial population exposed to colistin alone. In that case combination therapy may be helpful in improving outcomes and preventing bacterial resistance [21]. The clinical success seen in case of carbapenem monotherapy may have

been due to the association of adjunctive therapy and prolonged infusion dosage regimen used. As prolonged infusion is known to improve pharmacokinetic/pharmacodynamics parameters and removal of focus of infection is been reported to be associated with patient survival [3, 22]. Thus the extent to which carbapenem therapy contributed to the outcome is difficult to ascertain. Tigecyclin can be used to treat tissue infection and respiratory tract infections considering excellent tissue penetration but given the low mean peak serum concentrations, its use in bacteremia caused by organisms with MIC value ≥1 µg/ml is warranted [23].

There have been *in vitro* studies that demonstrated synergy between colistin and carbapenem against high level CR-*Ab* isolates that do not produce MBLs or OXA-24 carbapenemases [24]. Whereas no marked synergy or borderline synergy of colistin in combination with carbapenem against CR-*Ab* isolates producing OXA-23 and MBLs carbapenemases was observed [24-26]. Colistin-rifampicin, colistin-tigecyclin, and rifampicin-sulbactam have been reported to exhibit promising bactericidal activity even against OXA-23 producing highly CR-*Ab* isolates [24-26]. These finding demonstrate that *in vitro* activity of antibiotic combinations in CR-*Ab* may be strain dependent [24-26]. Hence, with the demonstrated high prevalence of $bla_{\text{OXA-23}}$ and bla_{VIM} genes in our isolates may be responsible behind better outcome with colistin-non carbapenem combination compared to colistin-carbapenem combination therapy against our isolates.

Although it may seem paradoxical at first sight, carbapenems have been commonly prescribed against CR-*Ab*, particularly as adjuvant drug in combination with another active agent [3, 27]. Preclinical studies have suggested synergistic effect with enhanced antibacterial activity against CR-*Ab* for carbapenem-sulbactam and carbapenem-aminoglycoside combination [3, 27-29]. Non colistin containing carbapenem combination strategy may be potentially useful against CR-*Ab*, but more clinical data are needed to routinely recommend such practice.

Despite the reported findings, our study has several limitations. First, the study is uncontrolled in nature so there is no comparison of outcomes, including mortality and cure of infection with the control group. Second, with varied combination of antibiotics used to treat the infection caused by CR-*Ab*, individual data for each type of infection and the treatment regimen followed for the same becomes too limited to comment on its statistical significance. Third, clinical isolates were not tested for the susceptibility to rifampicin and plain sulbactam.

CONCLUSION

CR-*Ab* was prevalent in our setup. Coexistence of multiple carbapenemase genes including bla_{NDM} in clinical isolates of CR-*Ab* is a worrying trend and it underlines the need for a stringent infection control practices along with antimicrobial stewardship to curb these *Acinetobacter*. The optimal treatment of infection caused by CR-*Ab* is still a debatable topic. Present study demonstrated better outcome with colistin-non carbapenem combination compared to colistin-carbapenem combination against *Acineobacter baumannii* with high level carbapenem resistance due to OXA-23 carbapenemases and MBLs. It is clear that resistance mechanism plays a crucial role in deciding the effectiveness of various antibacterial combinations [27]. Thus, the knowledge of prevalent resistant mechanism among CR-*Ab* in an institutional setup may guide clinicians in choosing a preemptive therapy for CR-*Ab* infections and warrants further investigation.

CONFLICT OF INTERESTS

Declared none

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