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Antimicrobial susceptibility pattern of an emerging multidrug resistant *Acinetobacter baumannii* from intensive care units of a tertiary care hospital

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Abstract
Introduction: *Acinetobacter baumannii* is one of the most challenging gram negative, lactose non-lactose fermenting bacilli with ability to cause nosocomial infections and develop resistance to many classes of antibiotics. Multidrug-resistant (MDR) *A. baumannii* is a well known agent causing life-threatening infections with limited therapeutic options. In recent years, the numbers of reported carbapenem-resistant Acinetobacter spp. are increasing, which has further narrowed the treatment options and emerging as extensively drug resistant (XDR) strain. There are only a few effective drugs such as colistin, polymyxin B, and tigecycline. But given the side effects and cost effectiveness, treatment of resistant nosocomial infections by *A. baumanii* is the biggest challenge for clinicians and policy makers all over the world.
Aim:
1. To know the antibiotic susceptibility pattern of *Acinetobacter baumnii*
2. To know the prevalence of multidrug resistant *Acinetobacter baumanii* in critical areas of our tertiary care centre.

Methodology: Clinical samples from both critical and non critical areas were processed routinely. Only non lactose fermenting colonies were included for the study and *Acinetobacter baumanii* isolates were identified using standard culture method and antibiotic susceptibility testing done by disk diffusion method.
Results: Of 308 NLFs, 160 were *Acinetobacter baumanii*. 106 (66.25%) samples were from ICU and 54 (33.75%) from non critical areas. Commonest sample was endotracheal secretion 72(45%) followed by blood 38(23.75) and others. 70% of our isolates were resistant to Aminoglycosides. Among Fluoroquinolones, maximum resistance was shown for Ciprofloxacin. 59% of the isolates were resistant to Piperacillin-tazobactum and their resistance to Cephalosporins ranged from 60-80%. 42 (39.62% of our isolates were Multidrug resistant (MDR) and 19 (17.92%) were extensively drug resistant (XDR).
Conclusion: Multidrug resistant and extensively drug resistant strains of *Acinetobacter baumanii* are on the rise and slowly getting established in our healthcare set up especially the critical areas. Treatment options are very few and limited. Thus leaving us with call to take stringent measures to stop the inadvertent use of antibiotics and to review our infection control policies.

Keywords: *Acinetobacter baumannii*, ICU, MDR, XDR, antimicrobial susceptibility testing, nosocomial infection

Introduction
Acinetobacter is a Gram negative, aerobic, non lactose fermenting, hydrophilic coccobacillus which is widely distributed in nature. Acinetobacter spp. has been documented to survive in hospital environments causing major healthcare associated infections [1]. There are more than 30 species in Genus Acinetobacter among which *A. baumannii* is most commonly occurring agent. This organism can survive for long periods on both dry and moist surfaces at different temperatures and pH values and ability to acquire antibiotic resistance. As a result, it causes persistent nosocomial infections [2, 3]. It is known to cause drug-resistant nosocomial infections in the critically ill, commonly manifesting as bloodstream infections and ventillator-associated pneumonia [4, 5].

It has been isolated from blood, sputum, skin, pleural fluid, and urine, usually in device associated infections. In the recent decades, A. baumannii strains have become increasingly resistant to most of the currently available drugs because of its multidrug resistant (MDR) nature [6]. Centre for Disease Control (CDC) has defined MDROs as microorganisms, predominantly bacteria, which are resistant to one or more classes of antimicrobial agents for all epidemiological purpose [7]. Emerging Resistance to an extensive range of antibiotics, including carbapenems, is now widespread among A. baumannii isolates and posing a very big therapeutic challenge [8, 9]. Various mechanisms are in play which are responsible for development of resistance to different classes of antibiotics leading them to be either Multidrug resistant (MDR) or extensive drug resistant (XDR) organism. The bacteria produces naturally occurring Amp C β-lactamases, as well as naturally occurring oxacillinases (OXAs) with carbapenemase activity. Acquisition of inactivating enzymes or drug target mutations blocking antibiotic lethal action play a big role in development of MDR and XDR strains [10, 11]. Number of A. baumannii strains showing resistance to carbapenems has been increasing rapidly world wide [12]. These acquired alterations, which vary across isolates, act in concert with conserved mechanisms tightly linked to reduced drug penetration, including a low-permeability cell envelope and upregulation of efflux pumps [13, 14]. Insight into the intrinsic envelope-level defenses might help us to understand the reasons for resistance and also pave way for using potent bactericidal antibiotics. In our tertiary care centre, we noticed that there was an increase in frequency of isolation of Acinetobacter spp especially *A. baumanii* and they were resistant to many routinely used antibiotics. This issue was discussed with consultants from ICU and we felt that need to explore further into its antibiotic susceptibility pattern. Thus, the present study was planned and was to assess the current levels of antimicrobial susceptibility among the clinical isolates of Acinetobacter species recovered from different clinical specimens obtained from in-patients and out-patient department of a teaching hospital.

Materials and Methods

The present cross sectional prospective study was conducted at SS Institute of Medical Sciences and Research Centre, Davangere which is a tertiary care hospital. Study was conducted from 2019 June-2021 December. A total of 308 NLFs were isolated from various samples, of which 160 turned out to be *Acinetobacter baumanii* and were included in the study. Standard microbiological techniques (by manual method) were used for the isolation and identification of bacteria. All the samples except Urine were inoculated onto Blood agar/ Chocolate agar and MacConkey agar plates using a sterile wire loop. Urine samples were inoculated onto CLED agar and inoculated agar plates were incubated aerobically at 37°C for 24 hours. After 24 hours of incubation culture plates were examined for growth of bacteria. Each isolate was identified using standard colony morphology, microscopy and biochemical reactions. All the isolates that turned out to be *Acinetobacter baumanii* were included in this study.

Antimicrobial susceptibility testing

A modified Kirby-Bauer disk diffusion method was used to detect the susceptibility pattern of bacterial isolates according to the Clinical Laboratory Standards Institute (CLSI) 2021 guidelines [15]. Bacterial isolate suspension was made by emulsifying bacterial colonies with 3-4 mL of normal saline, to achieve 0.5% McFarland standard. Lawn culture was done on Muller-Hinton agar plates. Once it dried, a set of standard antimicrobial disks were placed aseptically and plates were incubated aerobically at 37°C for 24 hours. Zones of inhibition was measured and interpreted according to recent CLSI guidelines. Zones were reported as sensitive, intermediate and resistant.

Inclusion criteria: Only those isolates that turned out to be *Acinetobacter baumanii* have been included in this study.

Exclusion criteria: Isolates other than *Acinetobacter baumanii* have been excluded from the study.

Results

Of all the samples subjected for culture in the laboratory during a period of June – 2019 to Nov 2021 (2 years, 5 months), a total of 308 NLFs were isolated from various samples from both ICU and non-ICU (Wards, OPDDs). Of 308 NLFs, 210 were Acinetobacter species of which 160 turned out be *Acinetobacter baumanii*.

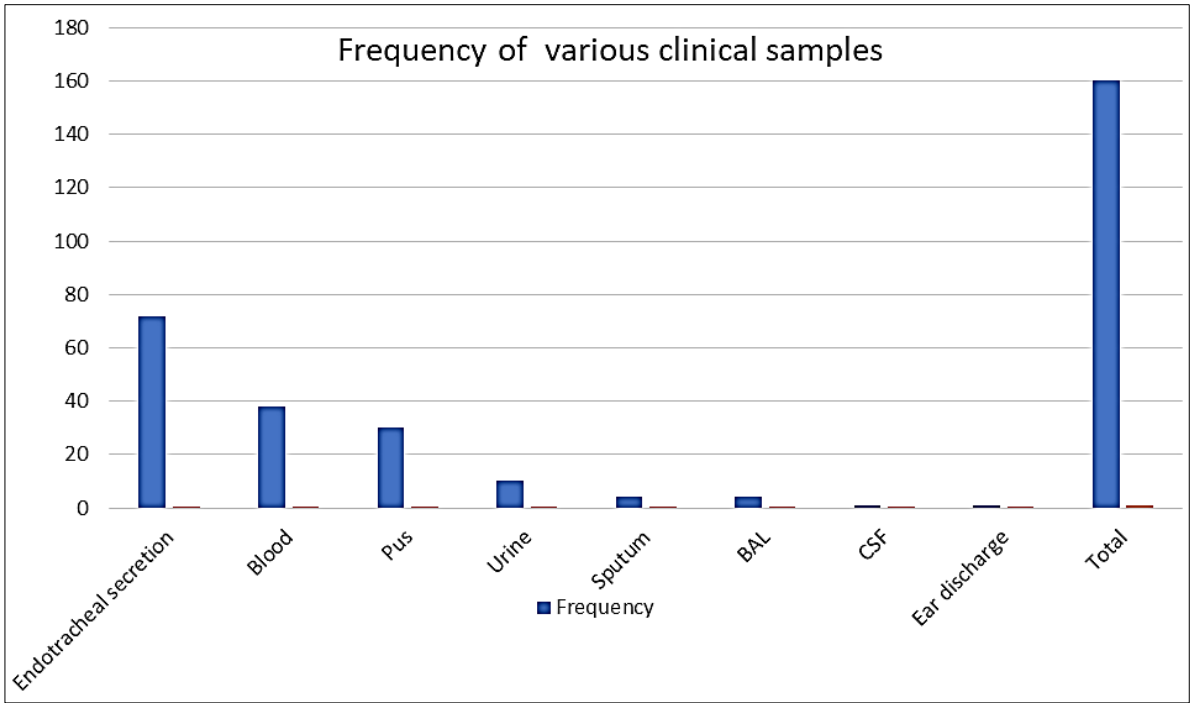
Of the 160, 48 were from females and 122 were from males. Distribution of samples from different areas (ICU and Non ICU) are shown in table 1. Samples from ICU were more from Non ICU areas. Of all the samples sent during the study period, endotracheal secretion was the commonest followed by blood and others as shown in graph-1.

Table 2 gives a cumulative data of AST pattern. After applying CLSI guidelines and CDC guidelines, it was seen that a total of 42 *Acinetobacter baumanii* were Multidrug resistant and 19 were Extensive drug resistant as shown in table – 3.

Data was entered in the excel spread sheet. Descriptive statistics of the explanatory and outcome variables were calculated by mean, standard deviation for quantitative variables, frequency and proportions for qualitative variables. SPSS (Statistical Package for Social Sciences) version 20. was used to perform the statistical analysis.

Table 1: Table showing no of samples from ICU & Non- ICU

AREA	Frequency	Percent
NON ICU	54	33.75
ICU	106	66.25
Total	160	100



Graph 1: Graph showing the frequency of various clinical samples

Raw data for the above graph is given below.

Sample	Frequency	Percent
Endotracheal Secretion (ET)	72	45%
Blood	38	23.75%
Pus	30	18.75%
Urine	10	6.25%
Sputum	4	2.5%
BAL	4	2.5%
CSF	1	0.62%
Ear Discharge	1	0.62%
Total	160	100

Table 2: Table showing cumulative data of Antibiotic susceptibility pattern of Acinetobacter baumanii from both ICU and Non ICU areas

Antibiotic sensitivity pattern		I		R		S		Total	
AK	NON ICU	1	1.9%	29	53.7%	24	44.4%	54	100.0%
	ICU	1	.9%	74	69.8%	31	29.2%	106	100.0%
	Total	2	1.3%	103	64.4%	55	34.4%	160	100.0%
GEN	NON ICU	2	3.7%	31	57.4%	21	38.9%	54	100.0%
	ICU	2	1.9%	73	68.9%	31	29.2%	106	100.0%
	Total	4	2.5%	104	65.0%	52	32.5%	160	100.0%
CPM	NON ICU	5	9.3%	30	55.6%	19	35.2%	54	100.0%
	ICU	12	11.3%	68	64.2%	26	24.5%	106	100.0%
	Total	17	10.6%	98	61.3%	45	28.1%	160	100.0%
CAZ	NON ICU	1	1.9%	40	74.1%	13	24.1%	54	100.0%
	ICU	2	1.9%	89	84.0%	15	14.2%	106	100.0%
	Total	3	1.9%	129	80.6%	28	17.5%	160	100.0%
CIS	NON ICU	4	7.4%	20	37.0%	30	55.6%	54	100.0%
	ICU	19	17.9%	38	35.8%	49	46.2%	106	100.0%
	Total	23	14.4%	58	36.3%	79	49.4%	160	100.0%
CIP	NON ICU	1	1.9%	47	87.0%	6	11.1%	54	100.0%
	ICU	0	0.0%	93	87.7%	13	12.3%	106	100.0%
	Total	1	.6%	140	87.5%	19	11.9%	160	100.0%
CTX	NON ICU	2	3.7%	44	81.5%	8	14.8%	54	100.0%
	ICU	1	.9%	89	84.0%	16	15.1%	106	100.0%
	Total	3	1.9%	133	83.1%	24	15.0%	160	100.0%
CTR	NON ICU	0	0.0%	45	83.3%	9	16.7%	54	100.0%
	ICU	1	.9%	92	86.8%	13	12.3%	106	100.0%
	Total	1	.6%	137	85.6%	22	13.8%	160	100.0%
LE	NON ICU	5	9.3%	27	50.0%	22	40.7%	54	100.0%
	ICU	11	10.4%	52	49.1%	43	40.6%	106	100.0%

	Total	16	10.0%	79	49.4%	65	40.6%	160	100.0%
OF	NON ICU	4	7.4%	36	66.7%	14	25.9%	54	100.0%
	ICU	12	11.3%	68	64.2%	26	24.5%	106	100.0%
	Total	16	10.0%	104	65.0%	40	25.0%	160	100.0%
TE	NON ICU	1	1.9%	40	74.1%	13	24.1%	54	100.0%
	ICU	5	4.7%	80	75.5%	21	19.8%	106	100.0%
	Total	6	3.8%	120	75.0%	34	21.3%	160	100.0%
PTZ	NON ICU	6	11.1%	26	48.1%	22	40.7%	54	100.0%
	ICU	12	11.3%	63	59.4%	31	29.2%	106	100.0%
	Total	18	11.3%	89	55.6%	53	33.1%	160	100.0%
IPM	NON ICU	7	13.0%	23	42.6%	24	44.4%	54	100.0%
	ICU	18	17.0%	52	49.1%	36	34.0%	106	100.0%
	Total	25	15.6%	75	46.9%	60	37.5%	160	100.0%
MRP	NON ICU	8	14.8%	26	48.1%	20	37.0%	54	100.0%
	ICU	18	17.0%	51	48.1%	37	34.9%	106	100.0%
	Total	26	16.3%	77	48.1%	57	35.6%	160	100.0%
COT	NON ICU	0	0.0%	46	85.2%	8	14.8%	54	100.0%
	ICU	1	.9%	85	80.2%	20	18.9%	106	100.0%
	Total	1	.6%	131	81.9%	28	17.5%	160	100.0%

Table 3: Table showing the frequency and percentage of Multidrug resistant (MDR) and extensively drug resistant (XDR) Acinetobacter baumanii isolates

Sl. No	MDR from ICU samples only Resistant to All cephalosporins & inhibitor combinations + Fluoroquinolones + Aminoglycosides	XDR from ICU samples only MDR + Resistant to Carbapenems
	42 (39.62%)	19 (17.92%)
Total No of Acinetobacter spp isolated from ICU	106 from ICU	

Discussion

Through this present study we wanted to know the extent of Acinetobacter species prevalent in our hospital especially from different Intensive care units (ICUs). We also checked the samples from noncritical areas too. We also wanted to look into the prevalence of Multidrug Resistant (MDR) and extensively drug resistant (XDR) Acinetobacter. Prior use of broad spectrum antibiotics, cross infection by hand of hospital staff, ventilator machine are all potential risk factors for development of multidrug-resistant Acinetobacter infection in hospital [16]. Resistance to an extensive range of antibiotics, including formerly last-resort agents such as carbapenems, is now widespread among A. baumannii isolates, with the emergence of strains resistant to all available antibiotics now documented [8, 9].

We found that frequency of Acinetobacter isolated from males was more than the females. 66% of the total samples were from ICU and remaining 34% were from other areas. 75% of the samples were endotracheal secretions from critical areas like ICU or post op wards followed by blood (24%) and pus (19%).

Acinetobacter species are showing increasing resistance to most of the commonly used antibiotics like aminoglycosides, fluoroquinolones, and broad-spectrum β-lactams. Pathogenesis in A. baumannii infections is an outcome of multiple virulence factors, including porins, capsules, and cell wall lipopolysaccharide, enzymes, biofilm production, motility, and iron-acquisition systems, among others [17]. Isolates from the ICU showed considerable level of resistance to Aminoglocosides (Amikacin – 68.9% & Genatamicin- 69.8%). Resistance to gentamicin was similar to a study by Rit K, [18] - Amikacin (14.29%) and Gentamicin (70.13%).The resistance to aminoglycoside from different studies was found to vary among the different agents under this antimicrobial class. It was seen that more than half of isolates showed resistance to Quinolones like Ciprofloxacin (87.7%), Levofloxacin (49.1%) and Ofloxacin (64.2%). The majority of strains are resistant to cephalosporin class of antimicrobials (84%, 64.2%) and resistance to carbapenems is also being reported increasingly. In our study, resistance to Carbapenems like Imipenem was about 49.1% and to meropenem it was, 48.1%. Around 80-85% isolates were resistant to commonly used Cephalosporins which is similar to findings by Ayenew Z *et al.* [19]. Carbapenems which were once the mainstay therapy are no longer effective against Acinetobacter baumanii as it has developed resistance. Thus, infection with carbapenem resistant A. baumannii needs to be treated with the so called "last-line" antibiotics such as colistin, polymyxin B, or tigecycline [20]. Sulbactam, a β-lactamase inhibitor, has good *in vitro* activity against Acinetobacter species is being used with some promising results for treating carbapenem-resistant strains [21, 22]. Globally, treating resistant infections is posing a big challenge for especially ICU consultants as this organism is developing resistance many antibiotics at a very fast rate. Availability of higher antibiotics over the counter and inadvertent use of the same has brought us to the brink dealing with Multidrug resistant (MDR) strains. In our study we found that 42 (39.62%) isolates from our hospital are Multidrug resistant showing resistance to three or more than three classes of drugs. This rate is much less than the that found in Saudi Arabia (74%) **23** and Bosnia and Herzegovina (78.4%) [24]. However, if the situation is not handled properly and the problem at hand is not addressed, we will not be far from reaching the findings from study conducted in Jimma which isolated 57.2% MDR Acinetobacter species [25] and also in Iran (56.7%) [11]. In the recent years, *Acinetobacter baumanii* is also showing considerable level of resistance to Carbapenems too. This extensive resistance to antimicrobial agents may be due to enzymatic modification of antibiotics, target gene mutation, altered outer membrane permeability, and upregulated multidrug efflux pumps.

Though this gives a sense of less burdensome MDR data, we do see an alarming situation where 19 (17.92%) MDR isolates from ICU samples showed resistance to Carbapenems as well thus being extensively drug resistant (XDR). Such a considerable percentage of isolates being XDR could be due to inadvertent use of carbapenems before admission to our hospital, overtreatment of infections or there may be actually XDR stains are getting established in our hospital. Probable causes for their wide spread could be due to transfer of patients between hospitals and regions over the course of time, and also selective pressure from antibiotics might have also led to their increasing numbers although in many cases there is no evidence for this. Use of contact isolation precautions, enhanced environmental cleaning, removal of sources of infection from the hospital environment, and prudent use of reserved drugs like cefoperazone/sulbactam, tigecycline, polymyxin, Colistin could control their spread. [26]A thorough and detailed multicentric studies need to be done in this regard to go to the root of the problem with multidrug resistant and extensively drug resistant *Acinetobacter baumanii* especially in the critical areas. This strongly calls for the immediate attention of clinicians and policy makers to address this alarming situation.

Limitations

1. We could not segregate the patients or collect data of patients already on antibiotics before getting admitted to our hospital. This would have given us a clearer reason for isolation of MDR or XDR strains.
2. Colistin was not tested as we did not have the facility to do microbroth dilution method as per CLSI guidelines.
3. Only phenotypic confirmation of MDR and XDR strains was done. Geneotypic confirmation could not be done due to lack of facility to conduct the test.

Conclusion

Acinetobacter baumanii has evolved as a dynamic organism with as extensive and elaborate capacities to adapt to highly unfavorable environment. This adaptability gives it a greater advantage to survive even in highly sterile areas like ICUs. Treating infections due to such resistant strains, with a very few reserved antibiotics is the biggest challenge that we are facing. Clinicians and policy makers need to take stringent measures to mitigate further development and spread of resistant strains of *Acinetobacter baumanii*.

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