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Emerging importance of acinetobacter and its antibiogram in the recent era

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ABSTRACT

Background: *Acinetobacter* is widely distributed in nature as saprophytes. Recently, they have emerged as a nosocomial pathogen due to its ability for survival in the hospital environment on a wide range of dry and moist surface. They cause pneumonia, urinary tract infection (UTI), and surgical site infection (SSI) where drain tips are inserted, endocarditis, meningitis, peritonitis, and bacteremia. Antibiotic susceptibility pattern of *Acinetobacter* may vary geographically. Due to multidrug resistance patterns of *Acinetobacter*, it is imperative to know the institutional prevalent susceptibility profiles.

Aims and Objectives: This study was conducted to isolate *Acinetobacter* species from various clinical samples, to determine the antibiotic susceptibility pattern and to carry out the epidemiological investigation of the isolates.

Materials and Methods: The study was conducted in a tertiary care hospital, over a period of 2 years. After identification, the speciations of *Acinetobacter* isolates were done by biochemical tests and by VITEK 2. Antibiotic susceptibility was determined by disc diffusion method. Extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs) production were detected by the combined disc diffusion test. An epidemiological study of *Acinetobacter* was carried out.

Results: Of 5096 infected samples, 505 (9.9%) were non-fermenting Gram-negative bacilli, among which 170 (33.8%) were found to be *Acinetobacter*. The highest numbers of isolates were *Acinetobacter baumannii,* followed by *Acinetobacter lwoffii, Acinetobacter radioresistance, Acinetobacter calcoaceticus, Acinetobacter Haemolyticus,* and *Acinetobacter ursingii*. Highest incidences of susceptibility were to imipenem (60%), chloramphenicol, and gentamicin. ESBL and MBL productions were detected in 23% and 17%, respectively.

Conclusion: A high level of antibiotic resistance was observed in this study and maximum isolation rate was in SSI. Most of the patients had high-risk factors such as prolonged hospitalization, indwelling catheters, and orthopedics implants *in situ* or other catheterization and diabetes. The analysis of susceptibility pattern will be useful in understanding the epidemiology of this organism in our hospital setup.

Key words: Acb complex, *Acinetobacter* species, antibiotic sensitivity, extended-spectrum β-lactamases, metallo-β-lactamases

INTRODUCTION

Acinetobacter species are non-fermenting Gram-negative bacilli (NFGNB).[1] They are non-spore forming coccobacilli that either do not utilize carbohydrates as the source of energy or degrade them through metabolic pathway.[2] They are widely distributed in nature as saprophytes, found in soil, sewage, water, or on human skin and gut and also in hospital environment.[1,3,4] These bacteria remain stable under an extreme conditions of temperature, humidity, and pH and in the presence of commonly used detergents such as highly concentrated alcohol preparations and other antiseptics which normally inhibit the growth of other bacteria.[5] This stability offers *Acinetobacter* a growth advantage over other organisms in hospital environments. Hence, in the recent era, they have emerged as an important nosocomial pathogen due to its ability for survival in the hospital environment on a wide range of dry and moist surfaces.[4,6]

Earlier, it is believed to be non-pathogenic, but recently, they are very frequently isolated as primary pathogen. Usually, they cause hospital-acquired infection (HAI).[7] They are most commonly involved the respiratory tract, where endotracheal tubes are *in situ* or patients have undergone tracheostomy. Apart from this, the other infections caused by *Acinetobacter* are urinary tract infection (UTI), where catheter *in situ* or from wounds or surgical sites where drain tips are inserted, endocarditis, meningitis, peritonitis in patients receiving peritoneal dialysis, and bacteremia.[2,6,7]

The isolation rate of *Acinetobacter* has been increasing nowadays in tertiary care hospital. Unfortunately, a very few laboratories can

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isolate and identify them up to species level. Moreover, they pose a great threat to humankind as they are resistance to common antibiotics.[2] The antibiotic susceptibility pattern of *Acinetobacter* may vary widely geographically. However, due to unpredictable multidrug resistance (MDR) patterns of clinical strains of *Acinetobacter*, it is imperative to know the institutional prevalent susceptibility profiles. Hence, this study was conducted to isolate the *Acinetobacter* species from various clinical samples by a simplified phenotypic identification protocol and to determine the antibiotic susceptibility pattern of these isolates.[8]

MATERIALS AND METHODS

This was a prospective study. The study was conducted in the Microbiology Department of Dr. D.Y. Patil Medical College, Hospital and Research Centre, over a period of 2 years (i.e., July 2012–September 2014). A total of 15,169 clinical samples of pus, blood, body fluid (pleural fluid, peritoneal fluid, synovial fluid, etc.), urine, sputum, cerebral spinal fluid, and throat swab were carried out. The blood samples from the suspected patients of sepsis were collected in the adult and pediatric bottles of BACT/ALERT three-dimensional system. The samples were taken from the suspected patients, admitted to different wards and various intensive care units (ICU) of this hospital. A detailed history was taken. The study was approved by the Ethical Committee of our institute (Dr. D.Y. Patil Medical College and Research Centre). The statistical analysis was performed with the help of Microsoft EXCEL for WINDOWS 2007.

Samples were processed for culture by standard conventional methods. Genus *Acinetobacter* was identified by Gram staining (Gram-negative coccobacilli), cell and colony morphology [Figure 1], positive catalase test, positive citrate test, triple sugar iron (alkaline slant/no change butt), negative oxidase test, and absence of motility. Speciation of *Acinetobacter* was performed on the basis of Hugh and Leifson oxidative-fermentative test (O-F) for glucose, sucrose, lactose, mannitol, gelatin liquefaction, beta hemolysis on blood agar media, nitrate reduction test, urease hydrolysis test (Christensen), decarboxylation of arginine, lysine, and ornithine and growth at 35°C and at 42°C for 18–24 h on two tubes of trypticase soy agar). The final identification and confirmation were done by the Vitek 2 system.[2,9]

Antibiotic susceptibility testing was determined by Kirby–Bauer disc diffusion method[2,10] Mueller-Hinton agar media was used. Commercially available HiMedia discs were used. The strength of the discs used and their zone size interpretation was carried out by National Committee for Clinical Laboratory Studies Guideline. The antibiotics, which were tested, ampicillin (10 mcg/disc), cefotaxime (30 mcg/disc), ceftazidime (CAZ) (30 mcg/disc), gentamicin (10 mcg/disc), amikacin (30 mcg/disc), norfloxacin (10 mcg/disc), cotrimoxazole (25 mcg/disc), imipenem (10 mcg/disc), chloramphenicol (30 mcg/disc), ofloxacin (5 mcg/disc), amoxicillin/clavulanic acid (20/10 mcg/disc), piperacillin/tazobactam (100/10 mcg/disc), tigecycline (15 mcg/disc), colistin (10 mcg/disc), and ertapenem (10 mcg/disc). *Acinetobacter* isolated from urine samples were also tested with nitrofurantoin (300 mcg), nalidixic acid (30 mcg), ampicillin (10 mcg/disc), norfloxacin (10 mcg), cefotaxime (30 mcg), gentamicin (10 mcg), cotrimoxazole (25 mcg), imipenem (10 mcg), amikacin (30 mcg/disc), and CAZ (30 mcg/disc).[10]

Detection of MDR Strain

The isolates which were resistance to three or more than three groups of drugs were considered as MDR strain.[11] The groups of drugs we were tested are: Penicillin (ampicillin), cephalosporin (cefotaxime, CAZ), aminoglycosides (gentamicin, amikacin), cotrimoxazole, carbapenem (imipenem, ertapenem), fluoroquinolones (norfloxacin, ofloxacin, nalidixic acid), nitrofurantoin chloramphenicol, glycylcyclines (tigecycline), and colistin.

Detection of Extended-spectrum β-lactamases (ESBLs) Production[10,12]

The combine disk diffusion test (CDDT) was used to determine the prevalence of ESBL production. Mueller-Hinton agar media was used. One CAZ (30 μg) disc was placed on a lawn culture of test isolates and at the distance of 15 mm on both sides of CAZ disc, a combination disc of CAZ/tazobactam (30/10 μg) and CAZ/clavulanic acid (30/10 μg) were placed. A ≥5 mm increased in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid or tazobactam versus the zone diameter of the agent when tested alone = ESBL producer [Figure 2].[10,12]

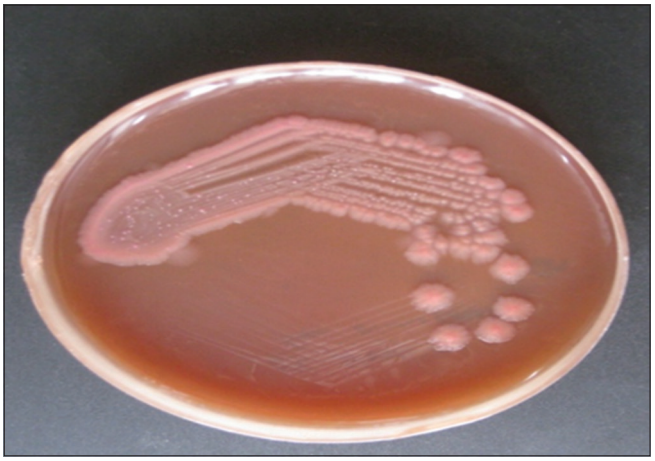

Figure 1: Colonies of *Acinetobacter radioresistance* on blood agar media

Figure 2: Detection of extended-spectrum β-lactamases by combine disc diffusion test

Detection of Metallo-β-lactamases (MBLs) Production

Mueller-Hinton agar media was used. One imipenem (10 μg) disc was placed on a lawn culture of isolates and at the distance of 15 mm a combination disc of 10 μg of imipenem and 100 μl of EDTA disc was placed. Then, it was incubated at 35°C for 18–24 h. An increase in zone size ≥7 mm around the imipenem-EDTA disc as compared to imipenem disc alone was recorded as positive [Figure 3].[10,12]

An epidemiological study of *Acinetobacter* was carried out by means of in-use test [Figure 4]. With a sterile pipette, transferred 1 ml of the used disinfectant into 9 ml of nutrient broth in a sterile universal container and placed 0.02 ml drops of this mixture onto 10 different areas of two well-dried nutrient agar plates. Incubate one plate at 37°C for 3 days and another one at room temperature for 7 days. Read the test as showing failure of disinfection if there was growth in more than five drops in either place.[1,2] To tract the source, 25 samples were isolated from inanimate objects and from disinfectants of different wards and ICUs.[1]

RESULTS

In this study, out of 15,169 clinical samples, a total number of culture-positive isolates were 5096 (33.59 %) among which 1921 (37.69%) were Gram-positive cocci and 3175 (62.3%) were GNB. Of 3175 GNB, 505 (15.9%) were NFGNB. Of the total 505 isolates, 170 (33.66%) were different species of *Acinetobacter*. *Acinetobacter* species were predominantly isolated from different types of body fluids and from various catheter tips 61 (35.88%) followed by pus 57 (33.53%), blood samples 29 (17.05%), sputum 13 (7.64%), and urine samples 10 (5.88%). Maximum *Acinetobacter* species isolated were from surgical ward (23%) followed by medicine ICU (10.8%) next to it was medicine ward (9.6%). There was a higher incidence of infection among males (69.8%). *Acinetobacter* infection was more common in patients in the age group of 51–60 years, comprises 16.40% followed by 41–50 years (16%) [Figure 5].

The highest number of isolates were *Acinetobacter baumannii,* comprises 56.47% followed by *Acinetobacter lwoffii* 20.58%, then *A. baumannii* complex (ABC) 10.58% next to it was *Acinetobacter radioresistance* comprises 4.7%, *Acinetobacter calcoaceticus* 4.11%, *Acinetobacter haemolyticus* 2.94%, and *Acinetobacter ursingii* 0.58% [Table 1].

Highest number of *Acinetobacter* species were isolated from surgical site infection (SSI), comprises 35.88%, followed by 21.17% isolates were yield from the patients who were suffering from respiratory tract infection and 20.58% isolates were obtained from the patients who have developed septicemia [Table 2]. In this study, we have analyzed the risk factors for colonization and infection with *A. baumannii*. Major surgeries, trauma, SSI, prolonged hospitalization, mechanical ventilation, indwelling foreign devices (especially orthopedic implants), diabetes mellitus (DM), and debilitating disease such as tuberculosis and previous antimicrobial therapy all have identified as risk factors which are predisposing to acquisition this infection. In this study, 24.56%

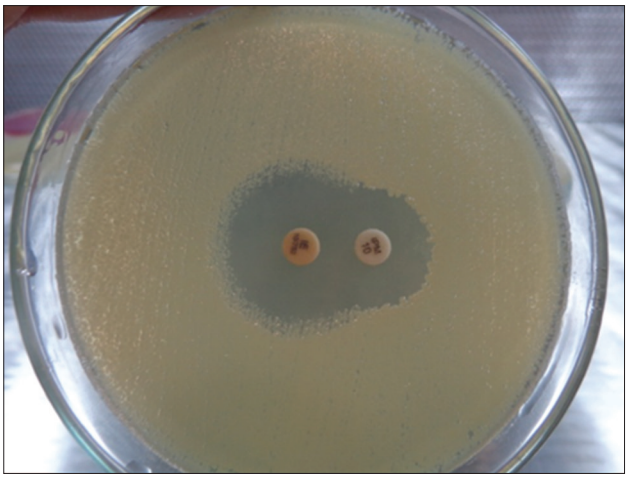

Figure 3: Detection of metallo-β-lactamases by combine disc diffusion test

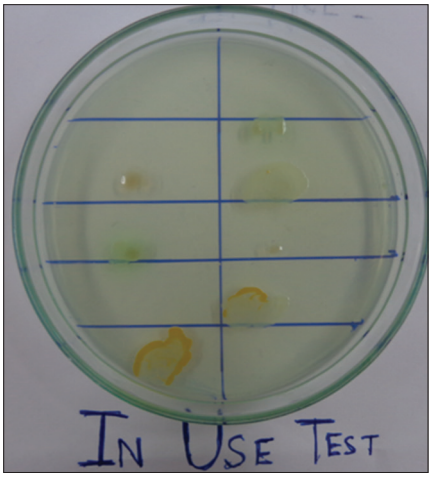

Figure 4: In-Use test

Table 1: Distribution of *Acinetobacter* in different clinical samples (*n*=170)

Name of the organism	Pus (%)	Body fluid (%)	Blood (%)	Sputum (%)	Urine (%)	Total *n*=170 (%)
A. baumannii	36	29	18	7	6	96 (56.47)
A. lwoffii	13	13	4	4	1	35 (20.58)
ABC	4	8	4	1	1	18 (10.58)
A. radioresistance	0	6	1	0	1	8 (4.7)
A. calcoaceticus	3	1	1	1	1	7 (4.11
A. haemolyticus	1	3	1	0	0	5 (2.9)
A. ursingii	0	1	0	0	0	1 (0.58)
Total (%)	57 (33.53)	61 (35.88)	29 (17.05)	13 (7.64)	10 (5.88)	170

A. baumannii: Acinetobacter baumannii, A. lwoffii: Acinetobacter lwoffii, ABC*: Acinetobacter baumannii* complex*, A. radioresistance: Acinetobacter radioresistance, A. calcoaceticus: Acinetobacter calcoaceticus, A. haemolyticus: Acinetobacter haemolyticus, A. ursingii: Acinetobacter ursingii*

isolates were obtained from the patients who have admitted in this hospital for a long tenure. Around 21.05% isolates were obtained from the patients, who were on mechanical ventilators, whereas 17.54% isolates were yield from the patients who were suffering from DM. We had been isolated around 11.40% *Acinetobacter* species from the immunocompromised patients who were on chemotherapy. 18.42% isolates were yield from the patients who had indwelling catheters or orthopedics implants *in situ* [Table 3].

The isolates of *A. baumannii* obtained from pus, blood, body fluid, and sputum revealed good susceptibility to imipenem (60%), chloramphenicol (52%) next to it was gentamicin (48.9%) and amikacin (47.8%) [Figure 6]. The other species of *Acinetobacter* other than *A. baumannii* revealed good susceptibility to imipenem (70%), chloramphenicol (62.85%), amikacin (58.57%), and gentamicin 54.28% [Figure 7]. The *Acinetobacter* species isolated from urine samples revealed 90% sensitivity to imipenem followed by 80% sensitivity to norfloxacin and nitrofurantoin [Figure 8]. Around 94 (55.29%) isolates of *Acinetobacter* species were MDR strains, among which 35 isolates (37.23%) were ESBL producer and 29 isolates (30.85%) were MBL producer [Table 4].

25 samples from inanimate objects and from disinfectant were collected from different wards and ICUs. Of these 25 samples, 9 (36%) were culture positive among which 44.44% were *A. baumannii* [Table 5].

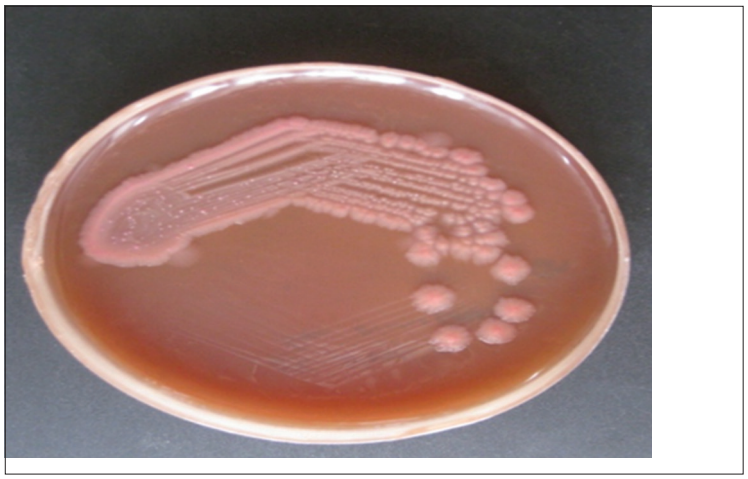

Figure 5: Age distribution of the patients

Table 2: Diagnosis wise distribution of the *Acinetobacter* species (*n*=170)

Name of the organism	SSI	RIT	Sepsis	GIT	CA	UTI	Surface non-healing ulcers	Burn	Total
A. baumannii	33	17	22	10	7	3	3	1	96
ABC	5	9	2	0	0	1	1	0	18
A. lwoffii	14	7	7	3	3	0	1	0	35
A. radioresistance	3	0	2	2	1	0	0	0	8
A. calcoaceticus	3	2	0	0	0	2	0	0	7
A. haemolyticus	2	1	2	0	0	0	0	0	5
A. ursingii	1	0	0	0	0	0	0	0	1
Total (%)	61 (35.88)	36 (21.17)	35 (20.58)	15 (8.82)	11 (6.47)	6 (3.52)	5 (2.94)	1 (0.58)	170

SSI: Surgical site infection, RIT: Repeat infection timeframe, UTI: Urinary tract infection, *A. baumannii: Acinetobacter baumannii,* ABC*: Acinetobacter baumannii* complex, *A. lwoffii: Acinetobacter lwoffii, A. radioresistance: Acinetobacter radioresistance, A. calcoaceticus: Acinetobacter calcoaceticus, A. haemolyticus: Acinetobacter haemolyticus, A. ursingii: Acinetobacter ursingii*

Table 3: Risk factors wise distribution of the organisms (*n*=114)

Name of the organism	Prolonged hospitalization	Ventilation	DM	Chemotherapy due to malignancy	Orthopedics implants	Indwelling intravascular catheters	TB	Total
A. baumannii	18	12	15	10	7	5	8	75
ABC	1	5	0	1	2	2	0	11
A. lwoffii	6	4	4	1	2	1	0	18
A. radioresistance	1	1	0	1	0	0	0	3
A. calcoaceticus	0	0	1	0	1	1	0	3
A. haemolyticus	1	2	0	0	0	0	0	3
A. ursingii	1	0	0	0	0	0	0	1
Total (%)	28 (24.56)	24 (21.05)	20 (17.54)	13 (11.40)	12 (10.52)	09 (7.89)	8 (7.02)	114

A. baumannii: Acinetobacter baumannii, ABC: *Acinetobacter baumannii* complex, *A. lwoffii: Acinetobacter lwoffii, A. radioresistance: Acinetobacter radioresistance, A. calcoaceticus: Acinetobacter calcoaceticus, A. haemolyticus: Acinetobacter haemolyticus, A. ursingii: Acinetobacter ursingii,* DM: Diabetes mellitus, TB: Tuberculosis

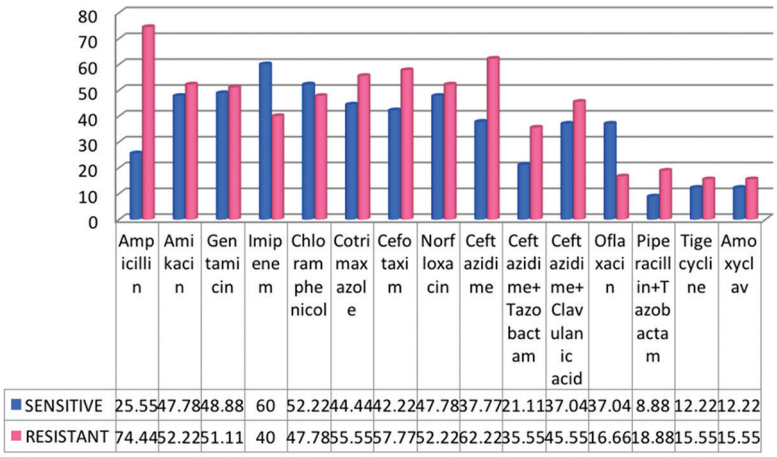

Figure 6: Antibiotic susceptibility pattern of *Acinetobacter baumannii* isolates was from pus, body fluid, blood, and sputum

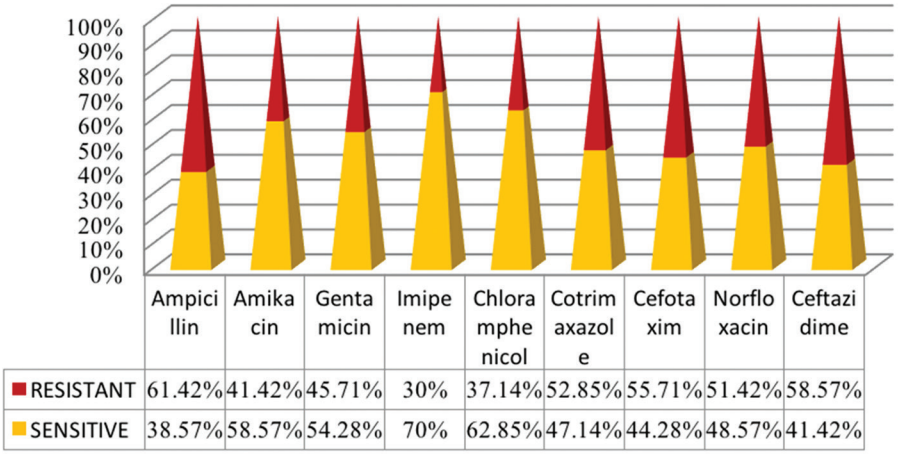

Figure 7: Antibiotic susceptibility pattern of *Acinetobacter* species, other than *Acinetobacter baumannii* isolates were from pus, body fluid, blood, and sputum

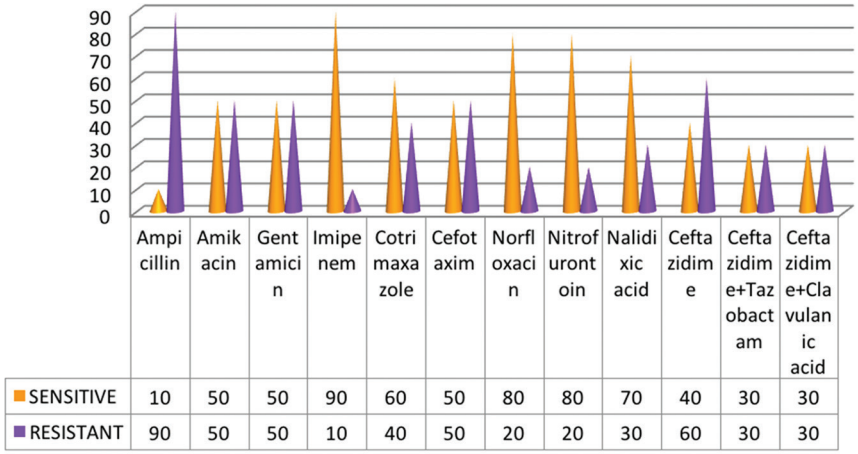

Figure 8: Antibiotic susceptibility pattern of *Acinetobacter* species of urinary isolates

A. baumannii isolated from pus, body fluid, blood, and sputum revealed good susceptibility to imipenem (60%), followed by chloramphenicol (52.22%) and gentamicin (48.88%).

The isolates of *Acinetobacter* species, other than *A. baumannii* revealed a good sensitivity to imipenem (70%) followed by chloramphenicol (62.85%) and amikacin (58.57%).

Table 4: Distribution of MDR strains of *Acinetobacter* species

Name of the organisms	Total number of isolates	Total number of MDR	ESBL	MBL
A. baumannii	96	55	23	17
ABC	18	8	1	2
A. lwoffii	35	20	7	7
A. radioresistance	8	3	2	1
A. calcoaceticus	7	6	1	2
A. haemolyticus	5	2	1	0
A. ursingii	1	0	0	0
Total (%)	170	94 (55.29)	35 (37.23)	29 (30.85)

MDR: Multidrug resistance, ESBLs: Extended-spectrum β-lactamases, MBLs: M etallo-β-lactamases, *A. baumannii: Acinetobacter baumannii,* ABC: A*cinetobacter baumannii* complex, *A. lwoffii: Acinetobacter lwoffii, A. radioresistance: Acinetobacter radioresistance, A. calcoaceticus: Acinetobacter calcoaceticus, A. haemolyticus: Acinetobacter haemolyticus, A. ursingii: Acinetobacter ursingii*

Table 5: Epidemiological study

Inanimate objects or disinfectants	Ward	Growth
Washbasin	MSW	*P. aeruginosa*
Washbasin	FSW	MRSA
Washbasin	MMW	*A. baumannii*
Washbasin	FMW	MSSA
Air condition machine	NICU	No Growth
Warmer	NICU	No growth
Humidifier	PICU	No growth
Humidifier	SICU	No growth
Disinfectant	MSW	*A. baumannii*
Disinfectant	FSW	MRSA
Disinfectant	MOW	*A. baumannii*
Disinfectant	FOW	MSSA
Disinfectant	OBGY	*A. baumannii*
Disinfectant	M. OPTHAL WARD	No growth
Disinfectant	F. OPTHAL WARD	No growth
Disinfectant	M. ENT	No growth
Disinfectant	F. ENT	No growth
Disinfectant	PICU	No growth
Disinfectant	NICU	No growth
Disinfectant	SICU	No growth
Disinfectant	MICU	No growth
Disinfectant	OT (NEURO OT)	No growth
Disinfectant	OT (OPTHAL OT)	No growth
Disinfectant	OT (ORTHO OT)	No growth
Disinfectant	OT (SURGERY OT)	No growth
TOTAL	25	Growth in 9 samples

P. aeruginosa: Pseudomonas aeruginosa, A. baumannii: Acinetobacter baumannii, MSSA: Methicillin-susceptible *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*

Here, imipenem revealed a very good sensitivity rate, 90%, followed by norfloxacin and nitrofurantoin, individually comprises 80%, and next to it was nalidixic acid 70%.

DISCUSSION

Acinetobacter is ubiquitous in nature. However, recently, they have emerged as primary opportunistic pathogens in hospitalized patients as well as immunocompromised patients and responsible for causing variant infections.[12] They are very hard to desiccate, difficult to eradicate and have numerous intrinsic and acquired mechanisms of drug resistance, thus they possess a great threat to the clinician as well as to microbiologists. They can stay alive within disinfectants and can create problem in health-care facilities spreading by cross-contamination.[13] They are posing a great threat to human race as they are resistant to routinely used antibiotics. The abuse and the unjudicial practice of antibiotics are responsible for the burgeoning resistance of commonly used antibiotics toward *Acinetobacter*. Moreover, the MDR among these organisms makes the treatment of this infection difficult and expensive.[12]

A total of 15,169 clinical samples of pus, wound swab, different body fluid, blood, sputum, and urine were carried out. Of these total sample processed, 5096 (33.59%) were culture positive. A total of 505 (15.9%) NFGNB were obtained from the culture-positive samples. Among these 505 NFGNB, the leading number of isolates was different species of *Pseudomonas* (189 isolates), followed by 170 isolates of different species of *Acinetobacter*. All these isolates of *Acinetobacter* are potential to cause nosocomial infection, ventilator-associated pneumonia (VAP), SSI, bacteremia, respiratory tract infection, and UTI in catheterized patients.

We also observed that the infection was common in the patients of the age group of 51–60 years followed by 41–50 years' age group. In a study by Mindolli *et al*. isolates *Acinetobacter* were in the age group of >45 years possibly due to weakened immune system and associated chronic diseases in these age groups.[4,8]

There is burgeoning incidence of *Acinetobacter* species causing serious nosocomial infection worldwide, among which the most prevalent is *A. baumannii*. In 2011, Sinha *et al*. studied 9756 samples from which they received 140 different species of *Acinetobacter* among which *A. baumannii* was the predominant species (92.14%) one.[14] In this present study also, we have isolated different species of *Acinetobacter* amidst which quite a considerable number of isolates are *A. baumannii*. The majority of *A. baumannii* were isolated from pus or wound discharge swab accounting for 36 (37.5%), followed by 29 (30.21%) isolates from different type of body fluids. *A. baumannii* is the most prevalent human pathogen among all *Acinetobacter* species and creates a challenge to health-care personnel in terms of treatment and infection control.[15]

Nosocomial infections caused by other named *Acinetobacter* species are burgeoning recently. Similarly, in this study, apart from *A. baumannii*, other isolates of this genre were *A. lwoffii* 35 (20.58%), ABC 18 (10.58%), *A. radioresistance* 8 (4.71%), *A. calcoaceticus* 7 (4.11%), *A. haemolyticus* 5 (2.94%), and only one isolate of *A. ursingii* (0.58%) was yield from the drain tip of a patient admitted in male surgery word.

The highest number of isolates of *A. lwoffii* was yield from pus and as well as from body fluids, each accounting for 13 isolates. Whereas, only eight isolates of ABC were isolated from body fluid. Six isolates of *A. radioresistance* were obtained from body fluid, three isolates of *A. calcoaceticus* yield from pus, three isolates of *A. haemolyticus* revealed from body fluid, and only one isolate of *A. ursingii* obtained from the drain fluid of cholecystectomy patients.

In this study, majority of *Acinetobacter* species, including *A. baumannii*, yield from SSI or from the specimen of operated site, accounting for 61 (35.88%) isolates, followed by 36 (21.17%) isolates from the respiratory samples. VAP is the another frequent clinical manifestation of hospital-acquired *Acinetobacter* infection. However, sometimes, it is difficult to distinguish upper respiratory tract colonization from true infection.[16] We have also observed that the sepsis due to *Acinetobacter* species is another common finding. Almost 35 (20.58%) isolates were yield from blood samples. To rule out the colonization, two sets of blood samples were collected from each patient.

Many studies have analyzed the risk factors for colonization and infection with *Acinetobacter.* Major surgeries, trauma, SSI, prolonged and previous hospitalization, prolonged hospital stay (more than 72 h), mechanical ventilation, indwelling foreign devices, invasive procedures, and previous antimicrobial therapy all have identified as risk factors predisposing to acquisition of infection with *Acinetobacter*.[16] In this study, 24.56% isolates were obtained from the patients who had a prolonged hospital stay and in 18% with DM as predisposing factor. Around 21.05% isolates were obtained from the patients, who were on mechanical ventilators, whereas 8% isolates were yield from the patients who had indwelling catheters or orthopedic implants *in situ*.

ABC and Heterogeneous Group of Bacteria

The genus *Acinetobacter* comprises a complex and heterogeneous group of bacteria. However, *A. baumannii* as well as its close relatives belonging to genomic species 3 (*Acinetobacter pittii*) and 13TU (*Acinetobacter nosocomialis*), are important nosocomial pathogens, often associated with epidemic outbreaks of infection, that are only rarely found outside of a clinical setting. In 2011, the genus *Acinetobacter* includes 23 species for which a formal name has been assigned and 11 other recognized additional genomic species without a name, although names have recently been proposed but are not yet formally assigned for genomic species 3 and 13TU. *A. baumannii, A. calcoaceticus*, genomic species 3 (*Acinetobacter pittii*), and genomic species 13TU (*Acinetobacter nosocomialis*) are closely related according to DNA-DNA hybridization studies, and can hardly be distinguished according to phenotypic or chemotaxonomic criteria. For convenience, many laboratories often group these genomic species together in the so-called, "*A. calcoaceticus-A. baumannii* (Acb) complex."[16]

In our laboratory, we were able to yield four ''*A. calcoaceticus-A. baumannii* (Acb) complex,'' comprising *A. baumannii, A. calcoaceticus*, genomic species 3 (*A. pittii*), and genomic species 13TU (*A. nosocomialis*) with the help of VITEK-2 system. One was isolated from the drain tip of a cholecystectomy patient, who was admitted in ICU for a long tenure, the case was an intraoperative sample. The another was again a drain tip and one was isolated from the blood of a patient admitted in ICU with diagnosis of septicemia for a long time. The remarkable thing about all these four isolates is that these isolates obtained from the typical cases of HAI.

The isolates of *A. baumannii* obtained from pus or wound swab, body fluid, blood, and sputum revealed 60% were sensitive to imipenem followed by 52.2% susceptibility to chloramphenicol and 48.9% to gentamicin, next to it was amikacin and norfloxacin each comprises 47.8%, in contrast to this study, another study by Rit *et al*. revealed low susceptibility to chloramphenicol (28%) and gentamicin (24%).[17] In our study, CAZ shows a bit low sensitivity pattern, accounting for 37.8 %. Similarly, a study by Rit *et al*. reported 28% sensitivity to CAZ.[17]

Antibiotic susceptibility pattern of *Acinetobacter* species, other than *A. baumannii*, yield from pus, body fluid, blood, and sputum reveal a good sensitivity to imipenem (70%), chloramphenicol (62.85%), followed by amikacin (58.57%), next to it was 54.28% sensitivity to gentamicin. This study revealed a moderate susceptibility to cotrimoxazole (47.14%), and cefotaxime shows 44.28% sensitivity.

Antibiotic susceptibility pattern of *Acinetobacter* species of urinary isolates reveals 90% sensitivity rate to imipenem followed by norfloxacin and nitrofurantoin, individually comprises 80%, and next to it was nalidixic acid 70% and cotrimoxazole 60%. Amikacin, cefotaxime, and gentamicin each of them show 50%. CAZ and ampicillin show 40% and 10%, respectively.

Acinetobacter poses a threat to health-care community as they represent the problem of MDR to the commonly used antibiotics. There are limited data on β-lactamase producing *Acinetobacter* species from India. In our study, 57.38% of *Acinetobacter* species were ESBL producer and 22.5% were MBL producers by the CDDT. Kansal *et al*. and Kumar *et al*. found the 75% of ESBL producing and 21% of MBL producing isolates in their study, respectively.[7,18,19] Due to different antimicrobial susceptibility pattern in different hospitals, these surveillance studies are valuable in deciding the most adequate therapy for *Acinetobacter* infections. In our study, 61 (35.6%) isolates were MDR strains. *Acinetobacter* appears to have a propensity to develop antibiotic resistance extremely rapidly, perhaps as a consequence of its long-term evolutionary exposure to antibiotic-producing organisms in soil environment.[7,20] The emergence of antibiotic-resistant strains in ICUs is because of higher use of antimicrobial agents per patient and per surface area.[7,21]

As rates of infection have increased, so the incidence of infection with MDR isolates of *Acinetobacter* species is also increasing. Providing effective treatment for infections caused by MDR *Acinetobacter* is a challenge. MDR strains typically require therapy with colistin, an older and relatively toxic polymyxin antimicrobial, and aminoglycosides or with the newer antimicrobial agent like tigecycline.[21,22] In our study, the *Acinetobacter* isolated which is pan resistant, i.e., resistant to all the antibiotics we have used; colistin was the last resort for drug of choice for these isolates.

CONCLUSION

A large number of *Acinetobacter* are isolated as primary pathogen from different clinical specimens of the patients, admitted in different wards and ICUs in this institution. The remarkable thing about all these isolates is that these isolates obtained from

the typical cases of HAI. Most of the patients had high-risk factors such as prolonged hospitalization, immunocompromised due to chemotherapy, indwelling catheters and orthopedics implants *in situ* or other catheterization (urinary or intravenous), diabetes mellitus, and burns. These organisms have possibly come from inanimate objects such as ventilator, humidifier, washbasin, and from diluted disinfections.

Most effective antibiotics are imipenem, amikacin, gentamicin, ciprofloxacin, cefotaxime, and cotrimoxazole. Most of the strains of *Acinetobacter* were resistant to penicillin, whereas carbapenem groups of drugs showed good sensitive.

A quite high number of isolated *Acinetobacter* species are MDR strains and most of them are resistant to commonly used antibiotics. This is an alarming indication that *Acinetobacter* species should need to be taken more seriously as primary pathogen and should not be discarded as mere contaminant or non-pathogen. Hence, proper isolation and identification of these organisms can enlighten their prevalence rate and the role of pathogenicity among hospitalized patients.

There is a widespread variability of antibiotic profile in common hospital for these pathogens. The antibiotic susceptibility can change from hospital to hospital set up, and there may be a gross geographical variation. Hence, it is imperative that every hospital should monitor a proper antibiogram profile for these isolated from time to time to serve as a basic empirical therapy to prevent the development of MDR cases.

Treating these pathogens should be based on the laboratory data after identifying the proper causative agents and antibiotic susceptibility result. Minimized the use and abuse of antimicrobial agents, proper surveillance of antibiotic panel, strict infection control measures, and even simple yet proper handwashing method and using disinfection of inanimate objects, can prevent the emergence *Acinetobacter* and can reduce the rate of MDR strains.

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