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Speciation and antibiotic resistance pattern of *Acinetobacter* species in a tertiary care hospital in Uttarakhand

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ABSTRACT

Acinetobacter species are Gram negative nonfermentative bacteria that have now emerged as important nosocomial pathogens involved in outbreaks of hospital infections.They are considered as opportunistic pathogens that readily colonize patients with compromised host defenses especially in intensive care units (ICUs), neonatal units, and surgical wards. The current study was conducted to type the Acinetobacter isolates obtained from various sources by a simplified phenotypic identification scheme and also to determine their antimicrobial susceptibility. Specimens like blood, CSF, endotracheal aspirate, urine, sputum, pus, bronchoalveolar lavage, HVS and body fluids were processed by standard methods and the antibiotic-sensitivity was performed by Kirby-Bauer disk diffusion technique as per Clinical and Laboratory Standards Institute guidelines (CLSI). The study was conducted in a tertiary care hospital for a period of 6 months (July 2013 – Dec 2013) in which out of a total of 1272 culture positive specimens, 53 Acinetobacter isolates were obtained from various specimens. Speciation was done in which predominance of A. baumannii(90.6%) was seen while A. lwoffii and A. haemolyticus showed an isolation rate of 5.7% and 3.8% respectively. High levels of resistance were seen for Ampicillin –Sulbactam (96%), Ampicillin (94%), Aztreonam (94%), Cefuroxime (92%), ceftazidime (91%). The p-value was found to be statistically significant for all the above mentioned antibiotics except for Polymyxin B for which 100% sensitivity was recorded. Clinical co-relation must be under taken to exclude commensal contaminants, before considering it to be a pathogen and prescribing antibiotics to the patient.

Keywords: Acinetobacter, nosocomial, Resistance, Intensive care Units

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INTRODUCTION

Members of the genus *Acinetobacter* are ubiquitous, free living, small aerobic Gram negative cocco-bacilli that prefer moist environment and can be easily obtained from soil, water, food and sewage [1]. Upto 25% of healthy ambulatory adults exhibit cutaneous colonisation and are the most common Gram negative bacilli carried on the skin of hospital personnel[2]. They are usually considered to be opportunistic pathogens, and of recent have been reported to cause a number of outbreaks of nosocomial infections in hospitalized patients like septicemia, pneumonia, wound sepsis, endocarditis, meningitis and urinary tract infection (UTI) [3,4].

Such infections are often extremely difficult for the clinician to treat because of the widespread resistance of these bacteria to the major groups of antibiotics. Various mechanisms of antibiotic resistance have been recognized in these bacteria, and combination therapy is usually required for the effective treatment of *Acinetobacter* nosocomial

infections. These therapeutic difficulties are coupled with the fact that these bacteria have a significant capacity for long-term survival in the hospital environment, with corresponding enhanced opportunities for transmission between patients, either via human reservoirs or via inanimate materials[5]. Despite the increasing significance and frequency of multidrug resistant *Acinetobacter* infections, many clinicians and microbiologists still lack an appreciation of importance of these organisms because of their confused taxonomic status. In India because of their increasing importance in nosocomial infections further study is warranted. In the present study attempt was made to type the Acinetobacter isolates obtained from various sources by a simplified phenotypic identification scheme and also to determine their antimicrobial susceptibility [6].

MATERIALS AND METHODS

The present study was conducted in the department of Microbiology and Immunology at SGRRIM & HS, Patel Nagar,Dehradun over a period of 6 months from July 2013 to December 2013. Clinical cases were selected from patients presenting in out patient departments (OPDs) and those admitted in wards and ICUs of Shri Mahant Indresh Hospital (SMIH), Patel Nagar Dehradun after obtaining written informed consent. Clinical samples from OPDs and various wards, ICUs received for bacterial culture and sensitivity in the Central laboratory, Microbiology section of SMIH, Patel Nagar, Dehradun were received under all asceptic precautions, followed by their processing and reporting as per the standard methods.

Specimens like blood, CSF, endotracheal aspirate, urine, sputum, pus, bronchoalveolar lavage, HVS and body fluids like pleural and peritoneal fluids were collected from the patients, depending on the clinical condition of the patient and the suspected site of infection. All clinical specimens were transported to microbiology laboratory under all asceptic conditions. Specimens were subjected to microscopy and cultured on MacConkey agar and 5% sheep blood agar (Himedia). Urine samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar (Himedia). After overnight incubation at 37°C, isolated colonies were identified by standard biochemical tests. All non-lactose fermenters were subjected to Gram staining, Oxidase test, hanging drop and catalase test. All the *Acinetobacter* isolates in the study period, were included,while isolates other than *Acinetobacter* were excluded.The antibiotic-sensitivity was performed by Kirby-Bauer disk diffusion technique as per Clinical and Laboratory Standards Institute guidelines (CLSI). Speciation was done on the basis of hemolysis on blood Agar, growth at 37^{o}C and 44^{o}C, citrate utilization, Glucose oxidation, Arginine decarboxylation and Glucose utilization (Table 1).

TABLE 1:Identification scheme of *Acinetobacter* species

Species	Hemolysison blood agar	Growth at 37^{0}C	Growth at 44^{0}C	Citrate utilization	Glucose oxidation fermentation	Arginine decarboxylation	Glucose utilization
A. baumannii	_	+	+	+	+	+	+
A. lwoffii	_	+	_	_	_	_	_
A. haemolyticus	+	+	_	_	+	+/-	+
A. radioresistens	_	+	_	_	_	+	+
A. junii	_	+	_	_	_	+	+

Sterile commercially available antibiotic discs (BD, BBL DIFCO) were used. The isolates were tested for Ampicillin, Ampicillin-sulbactam, Piperacillin-tazobactam, Cefuroxime, Cefixime, Ceftazidime, Cefipime, Aztreonam, Meropenem, Amikacin, Trimethoprim-sulfamethoxazole, Tigecycline, Polymyxin B, Levofloxacin and Nitrofurantoin in case of urine samples.

RESULTS

Out of total of 1272 culture positive specimens, 53 *Acinetobacter* isolates were obtained from various specimens.The isolation rate of *Acinetobacter spp.* was maximum in the age group of <10 years (22.6%) followed by patients in the age group of 41-50yrs (20.8%). It was found that the male to female ratio was 1.5:1. Of the total 53 *Acinetobacter* isolates percentage of isolation from hospitalized cases was 98.1% and that of OPD cases was

1.9%. Majority of the isolates were recovered from ICU patients 31(58.5%), followed by patients admitted in surgical ward 11 (20.8%), while lower percentage of isolation was observed from other wards (Table 2).

Table 2: Distribution of isolates in various wards (n=53)

WARD/ICU	NO. OF ISOLATES	PERCENTAGE
ICU	31	58.5%
Surgical Ward	11	20.8%
Neurology Ward	3	5.7%
Medical Ward	2	3.8%
Private Ward	2	3.8%
Obstetric Ward	1	1.9%
Paediatric Ward	1	1.9%
Orthopaedic Ward	1	1.9%
OPD	1	1.9%
Total	53	**100%**

The isolation rate of *Acinetobacter spp.*was maximum in patients with wound infections and with post- operative infections (52.8%) followed by patients diagnosed with respiratory problems (26.4%) and patients with various CNS conditions showed an isolation rate of 20.8% for *Acinetobacter* infections. Highest percentage of *Acinetobacter* infections was seen in patients on antibiotic intake for >72hrs (86.8%) followed by the patients with ICU stay (58.5%) and patients who were mechanically ventilated (52.8%) as shown in Table 3.

Table 3:Isolation rate of *Acinetobacter* on the basis of pre-disposing factors (n=53)

PRE-DISPOSING FACTORS	NO. OF PATIENTS	PERCENTAGE ISOLATION
Antibiotic Intake >72hrs	46	86.8%
ICU Stay	31	58.5%
Mechanical Ventilation >48hrs	28	52.8%
Endotracheal Intubation	23	43.4%
IV or/and Catheterization >48hrs	17	32.0%
Urinary Catheterization >48hrs	15	28.3%
Post-Operative Cases	12	22.6%
Burn	2	3.8%

Table 4 depicts that the isolation of *Acinetobacter* was maximum from tips (43.4%), followed by pus (26.4%) and blood (17%). Two (3.8%) *Acinetobacter* isolates were isolated from CSF.

Table 4: Specimen distribution of *Acinetobacter* isolates (n=53)

SPECIMEN	NO. OF ISOLATES	PERCENTAGE
Tip	23	43.4%
Pus	14	26.4%
Blood	9	17%
CSF	2	3.8%
Urine	2	3.8%
HVS	1	1.9%
Miscellaneous	2	3.8%
TOTAL	**53**	**100%**

Species distribution of *Acinetobacter* isolates showed predominance of *A. baumannii* (90.6%) isolates while *A. lwoffii* and *A. haemolyticus* showed an isolation rate of 5.7% and 3.8% respectively(Table 5).

Table 5:: Species distribution of *Acinetobacter* isolates (n=53)

SPECIES	NO. OF ISOLATES	PERCENTAGE
A. baumannii	48	90.6%
A. lwoffii	3	5.7%
A. hemolyticus	2	3.8%
Total	**53**	**100%**

A. baumannii was also the predominant species in ICUs (54.2%) followed by wards and OPD while *A. lwoffii* and *A. haemolyticus* were isolated only from ICUs (100%). Growth was monomicrobial in 81.1% samples while it was polymicrobial in 18.9% samples. *Klebsiella pneumoniae* (40%) was the most common associated organism followed by *Proteus vulgaris* (20%) and *Pseudomonas aeruginosa* (20%) while *Staphylococcus aureus* (10%) and *Candida albicans* (10%*)* were found to be rarely associated. High levels of resistance were seen for Ampicillin –Sulbactam (96%), Ampicillin (94%), Aztreonam (94%), Cefuroxime (92%), ceftazidime (91%). The p-value was found to be statistically significant for all the above mentioned antibiotics except for Polymyxin B for which 100% sensitivity was recorded (Table 6).

Table 6: *In vitro* activity of various antimicrobial agents against *Acinetobacter* isolates (n=53)

Antibiotic	Sensitive	Resistant	p- value
Ampicillin	3 (6%)	50(94%)	< 0.0001
Ampicillin-sulbactam	2(4%)	51(96%)	< 0.0001
Piperacillin-tazobactam	9(17%)	44(83%)	< 0.0001
Cefuroxime	4(8%)	49(92%)	< 0.0001
Ceftazidime	5(9%)	48(91%)	< 0.0001
Cefipime	9(17%)	44(83%)	< 0.0001
Aztreonam	3(6%)	50(94%)	< 0.0001
Amikacin	9(17%)	44(83%)	< 0.0001
Levofloxacin	10(19%)	43(81%)	<0.001
Meropenem	14(26%)	39(74%)	<0.05
Polymyxin B	53(100%)	0	…….
Trimethoprim-sulfamethoxazole	9(17%)	44(83%)	< 0.0001

A shift of the resistance pattern was seen more towards the ICU isolates. The p-value was found to be statistically significant for Antibiotics like Ampicillin, Cefipime, Aztreonam, Amikacin, Trimethoprim- sulfamethoxazole and Levofloxacin. For Piperacillin-tazobactam and meropenem , no statistical association was found (Table 7).

Table7:Antiibiotic resistance pattern of *Acinetobacter* in ICUs and wards

Antibiotic resistance pattern					
Antibiotic	**ICUs**		**WARDS**		
	Sensitive	**Resistant**	**Sensitive**	**Resistant**	**p-value**
Ampicillin	1(3%)	30 (97%)	2(9%)	20 (91%)	<0.05
Ampicillin-sulbactam	1(3%)	30 (97%)	1(5%)	21(95%)	……
Piperacillin-tazobactam	4(13%)	27 (87%)	5(23%)	17 (77%)	>0.05
Cefuroxime	2(6%)	29 (94%)	2(9%)	20(91%)	……
Ceftazidime	2(6%)	29(94%)	3 (14%)	19 (86%)	=0.05
Cefipime	5(16%)	26 (84%)	4(18%)	18 (82%)	<0.05
Aztreonam	1 (3%)	30(97%)	2(9%)	20(91%)	<0.05
Amikacin	5 (16%)	26 (84%)	4(18%)	18 (82%)	<0.05
Levofloxacin	6(19%)	25 (81%)	4(18%)	18 (82%)	<0.05
Meropenem	8(26%)	23(74%)	6 (27%)	16(73%)	>0.05
Polymyxin B	31 (100%)	0	22 (100%)	0	…….
Trimethoprim-sulfamethoxazole	5(16%)	26 (84%)	4(18%)	18(82%)	<0.05

The percentage of drug resistant *Acinetobacter* isolates which were XDR was 37(69.8%) while only one isolate (1.9%) was MDR and all XDR isolates belonged to *A. baumannii* species whereas one MDR isolate was *A. lwoffii.* Out of 69.8% extensively drug resistant isolates 56.8% were isolated from the tip specimens followed by pus specimens wherein 32.4% of these isolates were found. CSF, blood and urine also reported the presence of these isolates. The only MDR isolate was reported from the blood specimen.

DISCUSSION

During routine clinical microbiology work being done in most of the laboratories, non-lactose fermentative Gram negative bacilli (NFGNB) other than *Pseudomonas aeruginosa* are not taken seriously as a pathogen [7]. Most of the times they are not pursued for identification and are dismissed as contaminants. We took up this study when we regularly encountered isolates of NFGNB from various clinical samples, especially those from the various ICU patients. These isolates were identified as *Acinetobacter spp* as per standard criteria[8]. Infections caused by them are difficult to control due to multidrug resistance, which limits therapeutic options in critically ill and debilitated

patients, especially from ICUs, where prevalence of the organism is the most noted [9]. In table 2 out of the 53 isolates of Acinetobacter species, 52 (98.1%) isolates were nosocomial, isolated from various wards including ICUs whereas only 1 (1.9%) was community acquired from an OPD case indicating predilection of *Acinetobacter* isolates to cause nosocomial infections as compared to community acquired infections. The same observation has been reported by Lahiri KK et al wherein nosocomial isolates from the hospital patients were 82.9% as compared to the 17.1% community acquired isolates from the OPD [10]. In the hospital settings a number of risk factors are operational which can permit the spread and persistence of *Acinetobacter spp*. Significant risk factors such as mechanical ventilation, admission to ICUs, underlying chronic debilitating conditions and a prolonged hospital stay have been found to be major contributing conditions that facilitate the persistence and spread of *Acinetobacter spp.* in hospital environment.

Majority of the isolates were recovered from ICU patients (58.5%), followed by patients admitted in surgical wards (20.8%), while lower percentage of isolation was observed from other wards in the current study. Most of them had undergone invasive procedures like intravascular catheterization, mechanical ventilation and prior surgery. In a study conducted by Anupurba S et al in 2005, 20.8% of *Acinetobacter isolates* were isolated from ICU, whereas in present study it is 58.5%. This shows increasing trend of *Acinetobacter* to cause nosocomial infections [11]. Mechanical ventilation and admission to ICU were found to be independent risk factors for *Acinetobacter* infections in a study conducted by Lone R et al and in other studies[9,12,13]. Hence the amalgamation of these high risk factors in high risk units as ICUs provides an optimum infrastructure for the *Acinetobacter* isolates to emerge as the most important nosocomial pathogens as has been reported in a number of research journals

The highest percentage of *Acinetobacter* infections was observed in patients on antibiotic intake >72hrs (86.8%) followed by the patients admitted in various ICUs (58.5%), patients who were mechanically ventilated (52.8%) and intubated for >48hrs (43.4%) in this study. In other studies also, as reported by Vincent et al, and Lee SO et al, previous exposure to antimicrobial agents and ICU stay were found to be significant risk factors for the *Acinetobacter* infections as majority of the isolates in their studies respectively were recovered from ICU patients and the past medical records of majority of the indoor patients of their study showed that they were treated either by extended spectrum cephalosporins or fluoroquinolones before getting admitted to the health care facility [14,15]. This is concurrent with the observations in our study where exposure to antimicrobial agents and ICU stay were identified as potential risk factors for MDR and XDR strains. Thus exposure of patients to nosocomial pathogens particularly in ICU settings where many patients are on mechanical ventillatory support, catheterized, on a diet of broad spectrum antibiotics and above all with weak defence systems, the ouster of commensals paves way for the development and persistence of the pathogenic and resistant strains which are offered a selective advantage in these settings [9].

In the current study isolation of *Acinetobacter* was maximum from tips (43.4%), followed by pus (26.4%) and blood (17%). Two (3.8%) *Acinetobacter* isolates were isolated from CSF. This is in variance with other studies as by Lahiri et al in which majority of isolates were found in urine samples (51.3%) and Oberoi A et al where maximum isolation rate was reported from pus samples (86.2%) [10,16]. The rate of isolation was maximum from the ICUs in the present study as the majority of samples received from ICUs comprised of tips as there is more frequent use of invasive devices in the ICUs including endotracheal tube, central venous catheter, and tracheostomy tubes [17,18]. Morever the patients who stay longer in the ICU may be sicker, and require more invasive monitoring and therapeutic procedures to survive; therefore, they are predisposed to the development of lower respiratory tract infections like pneumonia particularly VAP in patients on mechanical ventilation and *Acinetobacter* has been found to be one of the most common pathogens involved in these infections as reported by Garnacho-Montero J et al and Diaz O et al [19,20].

Predominance of *A. baumannii* (90.6%) isolated from various samples and various wards was observed in the current study. *A. lwoffii*and *A. haemolyticus* showed an isolation rate of 5.7% and 3.8% respectively. This has also been reported in a study by Singla P wherein 74.6% of the isolates were identified as *A. baumannii* followed by *A. lwoffii* (24.3%)[21].

Acinetobacter baumanii as a predominant pathogen and responsible for 72% of infections has also been reported in a study by Lone R et al [9]. There are three major factors possibly contributing to the persistence of *A. baumannii* in the hospital environment, i.e. resistance to major antimicrobial drugs, resistance to dessication, and resistance to disinfectants. Resistance to antibiotics may provide certain *A. baumannii* strains with a selective advantage in an

environment, such as the modern ICU, where microorganisms are confronted with extensive exposure to antimicrobials [22,23]. Therefore in ICUs where this pathogen is endemic, empirical antibiotic therapy should include drugs that are effective according to the microbiological ecology [24].

High levels of resistance were seen for Ampicillin–sulbactam (96%), Ampicillin (94%), Aztreonam (94%), Cefuroxime (92%), ceftazidime (91%). Significant levels of resistance were also recorded for Piperacillin-tazobactam 85 (83%), Cefipime (83%), Amikacin (83%), Trimethoprim- sulfamethoxazole (83%) and Levofloxacin (81%). The p-value was found to be statistically significant for all the above mentioned antibiotics except for Polymyxin B for which 100% sensitivity was recorded.Taneja et al in their study have reported that the resistance of *Acinetobacter* to gentamicin, amikacin and ciprofloxacin was 79.5%, 73.2% and 72.8% respectively [25]. Shareek et al reported that 75% of the strains were resistant to carbapenems and only 25% were sensitive to carbapenems, 10-15% of the strains were sensitive to β-lactams and 20-28% of the strains were sensitive to amikacin, ciprofloxacin and cotrimoxazole[26]. Even in our study a high level of carbapenem resistance (74%) was seen whereas only 26% of the isolates were sensitive for carbapenems.

An analysis of the resistance pattern for various antibiotics used against *Acinetobacter* infections in ICUs and wards showed a shift of the resistance pattern more towards the ICU isolates. The p-value was found to be statistically significant (<0.05) for Antibiotics like Ampicillin, Cefipime, Aztreonam, Amikacin, Trimethoprim-sulfamethoxazole & Levofloxacin. For Carbapenems like meropenem no statistical association was found as the percentage resistance for ICU isolates was 74% and for ward isolates it was almost near at 73% thus implying that carbapenem resistance is emerging as a huge threat not only in ICUs but even in the wards. In Delhi, India the prevalence of carbapenem resistance in *Acinetobacter spp.* isolated from different clinical samples was found to be almost 35% by Sinha et al but the latest studies by Jaggi et al show resistance to carbapenem is seen in up to 89% of isolates[27,28].

The percentage of *Acinetobacter* isolates showing extensively drug resistance pattern i.e XDR was 69.8% while only one (1.9%) isolate was MDR. All XDR isolates belonged to *A. baumannii* species whereas one MDR isolate was *A. lwoffii*. On comparing the percentage of occurrence of XDR isolates to the MDR isolates in our hospital set up a significant difference in terms of p-value (<0.0001) was observed implying the emergence and dominance of Carbapenem resistance spp. in our hospital. A study by Singla P et al has also reported 51% of the *Acinetobacter* isolates as XDR and 11% as MDR [21].. The emergence of carbapenem resistance particularly in *A. baumannii* species largely through clonal spread has also been found in a study by Fernández-Cuenca F et al, leading to a decrease in therapeutic options [29]. Such levels of antimicrobial resistance in *A. baumannii*, as seen in our study also, have been attributed to antimicrobial inactivating enzymes, reduced access to bacterial targets and mutations changing bacterial targets as has been reported in research articles by Fernández-Cuenca F et al and Rice LB et al [29,30]. The global spread of XDR *Acinetobacter spp.* is a major challenge for the healthcare industry and other drugs such as Colistin and Polymyxin B, and newer drugs such as Tigecycline and Doripenem, are being tried for treating such infections [31].

CONCLUSION

These days the rate of isolation of *Acinetobacter spp* indicated by various studies indicates its role as nosocomial pathogen and also as an etiological agent in community acquired infection. Overall various infections caused by *Acinetobacter spp.* provide an impressive demonstration of the increasing importance of this genus as an important human pathogen. Thus the high potential of this genus to develop antibiotic resistance, leading to a considerable selective advantage in environments with widespread and heavy use of antibiotics, especially with relation to hospital environment and nosocomial infections makes it an important emerging nosocomial pathogen.

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