Original Research Article Journal of College of Medical Sciences-Nepal, Vol-16, No 1, Jan-Mar 2020

ISSN: 2091-0657 (Print); 2091-0673 (Online) **Open Access**
DOI: 10.3126/jcmsn.v16i1.28224

Prevalence and Antibiogram of *Acinetobacter* Species Isolated from Various Clinical Samples in a Tertiary Care Hospital

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ABSTRACT

Background: Acinetobacter species has emerged as a significant hospital pathogen, and are becoming increasingly drug resistance. They cause outbreaks in intensive care units and health care units.

Methods: A cross-sectional study was conducted to determine the prevalence and antibiotic susceptibility pattern of *Acinetobacter* spp isolated from various clinical samples collected from patients admitted in various wards and intensive care units of the hospital over a period of one year (March 2018 to Feb 2019).

Results: Out of 2,623 samples, 1,201(45.78%) yielded significant growth and out of these positive cultures, 138(11.49%) *Acinetobacter* spp were isolated. Majority of isolates 24(17.39%) were isolated from General intensive care unit (GICU). Maximum sensitivity of *Acinetobacter* spp was seen towards polymyxin B 138(100%) and colistin 138(100%), followed by tigecycline 127(92.02%). Hundred and one (80.43%) isolates were found to be multidrug resistant.

Conclusions: *Acinetobacter* isolates showed multidrug resistant pattern mostly in inpatients. To avoid resistance, antibiotics should be used judiciously. There is also an urgent need for emphasizing the importance of hand washing and use of disinfectants in prevention of transmission of infection in health care setup.

Keywords: *Acinetobacter* spp; prevalence; antibiotic resistance; intensive care units; multidrug resistance.

INTRODUCTION

A Dutch microbiologist, by name Martinus Willem Beigerinck discovered in 1911, an aerobic, gram negative, non-fermentative bacterium we now know to be of the genus *Acinetobacter.*[1] The genus *Acinetobacter* are Gram-negative, strictly aerobic non-fermenting, non fastidious, non-motile, catalase -positive and oxidase negative coccobacillary bacteria. They prefer moist environment and can easily obtained from soil, water, food and sewage.[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 infections (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 group of antibiotics. More than two third of *Acinetobacter* infections are due to *Acinetobacter baumanii*. *Acinetobacter baumanii* causes health care associated infections.[5-8] *Acinetobacter baumanii* also has the ability to form biofilms, which may play a role in the process of colonization. Biofilms help the bacteria resist disinfection while also allowing the participating cells to trade resistance genes, further facilitating the persistence of the pathogen.[9] *Acinetobacter* associated infections represent a tough challenge to control in severely ill patients especially those in ICU. *Acinetobacter* species have the capacity to acquire resistance to almost all presently existing antimicrobial agents.[10] 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.[11] Because of their increasing importance of nosocomial infections and multidrug resistant pattern, further study is warranted.

In the present study attempt was made to find out the prevalence of *Acinetobacter* isolates obtained from various clinical samples collected from patients admitted in various ICUs and wards by phenotypic identification scheme and also determine their antimicrobial susceptibility at

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METHODS

The present study was conducted in the Department of Microbiology, College of Medical sciences, teaching hospital, Bharatpur, Nepal during a period of 1 year (March 2018 to February 2019). Study included all the patients who had been admitted in various wards, ICUs and whose various clinical samples were sent to the microbiology laboratory for routine culture and antibiotic susceptibility tests. The samples were inoculated onto Blood Agar and MacConkey Agar plates. Urine Samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar. All isolates obtained were further processed and identified by standard routine microbiological processes. Genus *Acinetobacter* was identified by Gram staining as Gram negative coccobacilli, colony morphology, non-motile, oxidase negative, catalase positive, TSI reaction (K/K) and citrate utilization test positive. Speciation of *Acinetobacter* was done on the basis of glucose oxidation (OF test), hemolysis on blood agar, growth at 37°C and 44°C, citrate utilization, Arginine decarboxylation, Glucose utilization.[12,13] as shown in (Table 1). Antibiotic susceptibility testing was performed by standard Kirby Bauer disc diffusion method for the following antimicrobial agents- Ceftazidime (30µg) Cefoperazone/ Sulbactam (50/50µg), Levofloxacin (5µg), Ciprofloxacin(5µg), Cotrimoxazole (25µg), Amikacin (30µg), Gentamycin (10µg), Ciprofloxacin (5µg), Polymyxin B (300 units), Tigecycline (µg), Imipenem (10 µg) Meropenem (10µg), Colistin (10µg), Ceftriaxone (30µg), Piperacillin-Tazobactam (100/10µg), Nitrofurantoin (300 µg) and Norfloxacin (10µg) for urine samples. The zones of inhibition were measured and interpreted as per Clinical and Laboratory Standards Institute guidelines (CLSI).[14] All dehydrated media and antibiotic discs were procured from HiMedia labs, Mumbai, India.

Table 1. Speciation scheme of *Acinetobacter* species.

Species	Hemolysis on Blood agar	Growth at 37°C	Growth at 44°C	Citrate utilization	Glucose oxidation fermentation	Arginine decarboxylation	Glucose utilization
Acinetobacter baumannii		+	+	+	+	+	+
Acinetobacter lwoffi		+					
Acinetobacter calcoaceticus	+	+			+	+/-	+
Acinetobacter junii		+				+	+
Acinetobacter radioresistens		+				+	+

RESULTS

Out of the total 2,623 samples, 1,201(45.78%) were culture positive, and out of the total 1,201 culture positive samples, 138 (11.49%) were *Acinetobacter* (Table 2).

Table 2. Distribution of culture of total sample.

Cultured Sample	Number of isolates	Percentage
Culture positive sample	1,201	45.78%
***Acinetobacter* organism**	138	11.49%
Other organism	1063	40.52%
Culture negative	1422	54.15%

Acinetobacter isolates were obtained from various specimens. The isolation rate of *Acinetobacter* spp. was maximum in General ICU 24(17.39%) followed by General medical ward 19(13.76%), Neurosurgical ward and Coronary care unit 15 (10.86% (Table 3). There was higher incidence of *Acinetobacter* infection in males (59.0%) then females (41.0%). *Acinetobacter* spp. was more common in patient with age group of >55yrs. with an incidence of (72.0 %). The isolation rate of *Acinetobacter* spp was maximum from sputum sample 44(31.88%), followed by endotracheal tip/

Table 3. Distribution of isolates in various wards/ ICUs. (n=138)

Unit	Number of isolates	Percentage
G. ICU	24	17.39
G. Medical ward	19	13.77
Neurosurgical ward	15	10.87
Coronary care unit	15	10.87
Surgical ward	12	8.69
Neurosurgical ICU	11	7.97
Surgical ICU	11	7.97
Orthopedic Ward	10	7.25
Post-op Ward	8	5.80
Gynecology	6	4.35
Neonatal ICU	4	2.90
Pediatric Ward	3	2.17

aspirates 23(16.66%), followed by pus/swab 21 (15.21%), other tips 17(12.31%) which include drain tip, catheter tip, suction tip, CVP tip (Table 4). The most predominant species of *Acinetobacter*

Table 4. Specimen distribution of *Acinetobacter* isolates. (n=138)

Sample	Number	Percentage
Sputum	44	31.89
Endo tracheal tip/ aspirate	23	16.67
Pus/swab	21	15.22
Tips	17	12.32
Urine	14	10.14
Body fluids	12	8.69
Blood	5	3.62
High vaginal swab	2	1.45

isolated was *A. baumanii* 108 (78.26%) followed by *A. lwoffi* 19 (13.77%). *A. baumanii* was also the predominant species isolated from general medicine ward 38(35.18%) followed by neurosurgical wards 35(32.40%). Only one *A. radioresistens* was isolated from neonatal ICU 1 (0.72 %) (Table 5).

Table 5. Species distribution of *Acinetobacter* isolates. (n=138)

Acinetobacter species	Number	Percentage
Acinetobacter baumannii	108	78.26
Acinetobacter lwoffi	19	13.77
Acinetobacter calcoaceticus	7	5.07
Acinetobacter junii	3	2.17
Acinetobacter radioresistens	1	0.72

Out of the total 138 *Acinetobacter* isolates, 78 (56.52%) were monomicrobial and 60(43.47%) were polymicrobial samples (Table 6).

Table 6. Comparison between monomicrobial and polymicrobial infections. (n=138).

Types of infection	Number of isolates	Percentage
Monomicrobial	78	56.52
Polymicrobial	60	43.48

Pseudomonas aeruginosa (31.3%) was the most commonly associated organism with *Acinetobacter* followed by *Escherichia coli* (24.3%), *Enterobacter* species (22.7%) and *Klebsiella pneumoniae* (20.0%). *Staphylococcus aureus* (1.7%) was found to be rarely associated organism with *Acinetobacter*. Among 138 isolates of *Acinetobacter*, all showed 100% sensitivity to colistin and polymyxin B. High levels of resistance were seen for ceftazidime (90.58%) and ceftriaxone (87.69%). The p-value was found to be statistically significant for these resistant antibiotics (Table 7).

Norfloxacin was tested only in urine isolates and (63.04 %) of isolates were resistant to this antibiotic. The percentage of drug resistant *Acinetobacter* isolates which were multi drug resistant (MDR) was 111 (80.43%) (Figure 1).

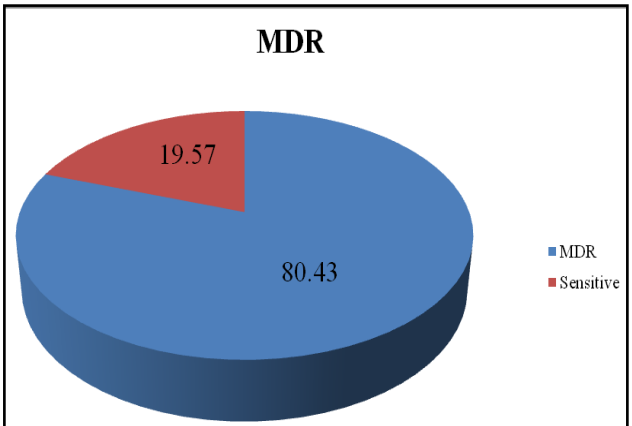

Figure 1. Distribution of multidrug resistant *Acinetobacter* isolates.

All MDR isolates were resistant to at least one agent in three or more antimicrobial categories; penicillins, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems.[15,16] Out of 80.43% extensively drug resistant isolates, 33.5% were isolated from the sputum sample followed by ET tips (24.8%) and pus/swab (18.6%). A higher

Table 7. Antibiotic resistance pattern of *Acinetobacter* isolates in various wards and ICUs.

Antibiotic	Sensitive No.	Sensitive %	Resistance No.	Resistance %	p-value
Ceftriaxone	17	12.31	121	87.69	<0.001
Gentamicin	35	25.36	103	74.64	<0.001
Meropenem	76	55.07	62	44.93	0.268
Amikacin	53	38.40	85	61.59	<0.001
Cotrimoxazole	40	28.98	98	71.01	<0.001
Ciprofloxacin	34	24.64	104	75.36	<0.001
Levofloxacin	79	57.25	59	42.75	0.106
Ceftazidime	13	9.42	125	90.58	<0.001
Cefoperazone-sulbactum	101	73.19	37	26.81	<0.001
Colistin	138	100	-	-	
Polymixin B	138	100	-	-	
Piperacillin-Tazobactum	66	47.83	72	52.17	0.670
Tigecycline	127	92.02	11	7.97	<0.001
Ampicillin	11	7.97	127	92.02	<0.001
Norfloxacin	51	36.95	87	63.04	<0.001
Cefepime	57	41.30	81	58.69	<0.001
A/S	22	15.94	116	84.05	<0.001

Note: Method of p-value calculation: By using SPSS16 version, one sample test.

prevalence rate of MDR pattern was seen towards the General ICU (34.8%), followed by neurosurgical ward (14.0%) and General medical ward (12.7%) respectively.

DISCUSSION

In early days in most of the clinical microbiological laboratories, non fermentative gram negative bacilli (NFGNB) other than pseudomonas aeruginosa are not taken seriously as a pathogen.[17] We took up this study when we regularly encountered isolates of NFGNB from various clinical samples, especially from the various ICUs patients. These isolates were identified as *Acinetobacter* spp as per standard criteria.[18]

Acinetobacter spp has emerged as a cause of ICUs infection. Multiresistant *Acinetobacter* spp including the leading species, *A. baumanii*, are developing as real infectious threat mainly in the intensive care units (ICUs).[19] Their ubiquitous nature in the ICU environment and inadequate infection control practice has continuously raised the incidence of *Acinetobacter* infection over the past two decades.[20] All the isolates of *Acinetobacter* spps were isolated from different ICUs and wards, which indicate all isolates were nosocomial. The same observations has been reported by Dimple *et al* and Lahiri *et al* wherein nosocomial isolates of *Acinetobacter* spps from hospital patients were 98.1% and 82.9%.[11,21] A number of risk factors enhances the spread and persistence of *Acinetobacter* spp like mechanical ventilation, admission to ICUs, underlying chronic debilitating conditions and prolonged hospital stay have been found to be significant risk factors for the spread of this organism in the hospital environment.

Majority of the isolates were recovered from the General ICU patients (17.39%) followed by patients admitted in general medicine wards (13.76%) while lower percentage of isolation were observed from other wards in the current study. Various other studies have reported the rate of isolation varying from 4.25% to (20.1 %).[22,23] This variation can be attributed to the varying prevalence rates of different *Acinetobacter* spp in the hospital environment and the community in different geographical areas.

Maximum number of *Acinetobacter* isolates were from sputum (31.88%) followed by endotracheal tip/aspirate (16.66%), pus (15.21%), tips (12.31%) and urine (10.14%) in the present study. This is in variance with other studies as by Lahiri et al. and Raina et al. in which the isolates were maximum from tips (43.4%), Oberoi et al found maximum isolates from pus samples (86.2%).[24] Apoorva et al. found maximum number of *Acinetobacter* isolates from respiratory samples (35.78%) followed by pus (32.84%).[25] Pooja et al. also isolated 25.6% of the *Acinetobacter* isolates from respiratory tract. This indicates that *Acinetobacter* infections were most frequently involved in the respiratory tract of intubated patients.[26]

In this study, the most common *Acinetobacter* species isolated from the clinical samples of our institute was *Acinetobacter baumanii* (78.26%), followed by *Acinetobacter lwofii* (13.76%). Almost similar results were observed in studies conducted by Apoorva et al, who found 74.50% and 24.50% of *Acinetobacter baumanii* and *Acinetobacter lwofii* respectively. Predominance of *A. baumanii* isolated from various samples were observed by Raina et al. and also by Lone R et al.[11,27] There are three major factors possibly contributing to the persistence of *A. baumanii* 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. baumanii* strains with a selective advantage in an environment, such as the modern ICU, when microorganisms are confronted with extensive exposure to antimicrobials.[28] Therefore in ICUs, where the pathogen is endemic, empirical antibiotic therapy should include drugs that are effective according to microbiological ecology.[29]

High levels of resistance were seen for ampicillin (92.02%), ceftazidime (90.58%), ceftriaxone (87.64%), ampicillin/sulbactam (84.05%. Significant levels of resistance were also recorded for ciprofloxacin (75.36%), gentamicin (74.64%), cotrimoxazole (71.01%). The p-value was found to be statistically significant for all the above mentioned antibiotics except for polymyxin B and colistin for which 100% sensitivity was recorded. Norfloxacin was tested only in urinary isolates and (63.04%) isolates were resistant to this antibiotic.

Taneja et al.[30] in their study reported that the resistance of *Acinetobacter* to gentamicin and ciprofloxacin was 79.5% and 72.8% respectively and is in accordance with our study. Resistance towards meropenem was recorded to be 44.93%. In a study by Amandeep et al,[31] resistance towards imipenem and meropenem were recorded to be 42.6% and 55.4% respectively and is also in accordance with our study. However, Shareek et al.[32] and Dimple et al.[11] reported that 75% and 74.1% of the strains were resistant to carbapenems, which is higher than our findings.

A high level resistance was also recorded for ampicillin/sulbactam (84.05%). This correlates with the studies by Amandeep et al.[31] and Raina et al.[11] In our study, 100% sensitivity was recorded for colistin and polymixin B. Raina et al.[11] also recorded 100% sensitivity for colistin. In another

study published by Dash et al,[33] all isolates were sensitive to colistin which is also in accordance with our findings. Amandeep et al,[31] recorded a resistance rate of 2.2% and 4.2% respectively towards polymixin B and colistin. Nahar et al,[34] reported 10.5% resistance of *Acinetobacter* towards colistin.

Among a total of 138 *Acinetobacter* isolates, 106 (76.81%) isolates were MDR. All MDR isolates belonged to *A. baumanii* except for two isolates which belonged to *A. lwofii*. Similar studies by Uwingabiye et al. [35] and Kaur et al.[36] reported MDR *Acinetobacter* of 77.4% and 76.6% respectively and the figure is near about similar with our studies. Amatya et al.[37] in a study in Nepal reported a rate of 71.3% MDR *Acinetobacter* isolates. In the studies by Dash et al.[33] and Bhatacharya et al,[38] reported a lower rate of MDR *Acinetobacter*, 54.7% and 29.0% respectively.

This wide variation can be due to various factors like patients co-morbid conditions, compliance of infection control programs, type of strains, their survival in the environment and further colonization of the patients. According to our study, highest MDR *Acinetobacter* were isolated from sputum (31.88%), followed by endotracheal tip 23 (16.66%) and pus 17(12.31%). These findings were comparable with the studies done by Sivaranjan et al.[39] Whereas, in the study done by Uwingabiye et al,[35] highest percentage of MDR *Acinetobacter* was demonstrated in vascular catheter.

Susceptibilities of *Acinetobacter* against antimicrobials are considerably different among countries, centers and even among wards of same hospital. Therefore, such type of local surveillance studies and around are important in deciding the most adequate therapy for *Acinetobacter* infections.

CONCLUSIONS

These days the rate of isolation of *Acinetobacter* spp indicated by various studies indicates its role as nosocomial pathogen, especially in critically ill patients admitted in ICUs. It is a great challenge for the physicians to treat MDR *Acinetobacter* spp. In our study, *Acinetobacter* were resistant to most commonly used antibiotics. Emergence of carbapenem resistance is worrisome. In our study, colistin and polymyxin B was the most sensitive antibiotics against *Acinetobacter* with a resistant rate of zero percent. However, colistin resistant is emerging slowly. Rational use of antibiotic is necessary to prevent microbial resistance. Though the organism has developed multidrug resistance, it has largely remained susceptible to disinfectants and antiseptics. A strict control of the hospital environment, hand hygiene and optimizing/ judicious use of antibiotics is recommended in order to reduce the resistance rates and also to reduce the MDR frequency in the hospital.

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Citation: Rajkumari S, Pradhan S, Sharma D, Jha B. Prevalence and Antibiogram of *Acinetobacter* Species Isolated from Various Clinical samples in a Tertiary Care Hospital, Bharatpur, Chitwan, Nepal. JCMS Nepal. 2020; 16(1):26-32.