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Original Research Article.

Speciation and Antimicrobial Resistance Pattern of Acinetobacter Species From Clinical Isolates in Tertiary Care Hospital

Ashok Kumar Sharma[1*], Khushboo Kumari[2], Manoj Kumar[3], Amber Prasad[4]

[1*]Associate Professor, [2]Junior Resident, [3]Professor & Head, [4]Assistant Professor,
Department of Microbiology, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India.

ABSTRACT

Introduction: Acinetobacter species are Gram negative non-fermentative bacteria. Previously these were considered just as an opportunistic pathogens, but recently have been emerged as an important nosocomial pathogen worldwide. In recent few years these have been involved in many outbreaks of hospital infections.

Aims and Objectives: The present study was conducted to type the Acinetobacter isolates and to find the sensitivity and resistance pattern of Acinetobacter in our set up.

Materials and Methods: The current study was conducted in our microbiology department, RIMS, Ranchi for a period of 1 year (January 2016 - December 2016). Different specimens were processed by standard methods and antibiotic sensitivity was performed by Kirby- Bauer disk diffusion technique as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Out of a total 1890 culture positive specimens, 110 (5.8%) Acinetobacter isolates were obtained from various specimens. Speciations were done in which predominance of A. baumannii (84.5%) was seen followed by A.lwoffii (7.26%); A. haemolyticus (4.5%) and A. junnii (3.63%). High level of resistance were seen for Ampicillin (79.1%); Cefotaxime (78.1%); Ceftazidime (73%); Ceftriaxone (70.9%) and for urine samples Nalidixic acid (70%) and Norfloxacin (70%). Significant level of resistance were observed for Ciprofloxacin (63.64%), Levofloxacin (52.73%), Doxycycline (60%), Trimethoprim-sulfamethoxazole (60%); Gentamycin (54.55%). Sensitivity to Carbapenems was 70.9% and to both Amikacin and Piperacillin- tazobactam, 63.66% were found.

Conclusion: In recent few years due to emergence of multidrug resistant strains of Acinetobacter, situation has become more problematic for the clinicians. So clinical co-relation must be undertaken to exclude commensal contaminant, before considering it to be a pathogen and prescribing appropriate antibiotic to the patient.

Keywords: Acinetobacter, Nonfermenter, Intensive Care Units, Nosocomial, Resistance.

***Correspondence to:**

Dr. Ashok Kumar Sharma
Associate Professor
Department of Microbiology
RIMS, Ranchi, Jharkhand, India.

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INTRODUCTION

Members of the genus Acinetobacter are free living, ubiquitous, aerobic, Gram negative, cocco- bacilli that preferably inhabits moist environment such as soil, water, sewage and on vegetables. It is one of the most common Gram negative bacilli carried on the skin of hospital personnel and approx upto 20-25% of healthy adults exhibit cutaneous colonisation. Sometimes identified as a contaminant in blood samples collected for culture. Fecal carriage can be detected in both healthy and hospitalised patients. Since long time it has been considered just as an opportunistic pathogens and have not been given much importance by the clinicians. But in recent few years due to emergence of its multidrug resistant variety, it has been reported as an important nosocomial pathogen worldwide and especially patients with impaired host defense have become their victim. It has been involved in the outbreaks of hospital infections especially in patients suffering from pneumonia, septicemia, urinary tract infection, wound sepsis, carditis and other chronic illness. Enhanced opportunities for transmission between patients; either via human reservoir or via inanimate material, has made possible long term survival of these bacteria in the hospital environment and ultimately has created therapeutic difficulties for these bacteria . Although frequency and significance of these multidrug resistant Acinetobacter infections are increasing, but due to their confused toxonomic status most of the clinicians and microbiologists still lack a sight on their importance. Regarding Acinetobacter infections more studies are needed worldwide. Current study has been done in an attempt to type the Acinetobacter isolates obtained from various sources using a simplified phenotypic identification scheme and also to determine their antimicrobial susceptibility.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, RIMS, Ranchi for a period of 1 year from January 2016 to December 2016. Clinical cases were selected from patients presenting in outpatient departments (OPDs) and those admitted in the wards and ICUs of RIMS, Ranchi. Clinical samples from various wards, OPDs and ICUs were received for bacterial culture and sensitivity in the microbiology department of RIMS, Ranchi taking all aseptic precautions, followed by their processing and reporting.

A total of 4260 specimens like urine, blood, CSF , sputum, pus, endotracheal aspirates, body fluids like pleural and peritoneal fluids were collected from the patients of different age groups according to their clinical condition and suspected site of infection. All clinical specimens were transported to microbiology lab on proper time. Further specimens were subjected to microscopy and cultured on 5% sheep blood agar and Mac Conkey agar. Urine samples were inoculated on Cystein Lactose Electrolyte Deficient (CLED) agar. After overnight incubation at 37 degree celsius, all isolations obtained were identified by their morphology on culture plate and standard microbiological and biochemical tests. For blood samples, brain heart infusion broth was used as a primary culture medium. All non-lactose fermenters (NLFs) were subjected to Gram staining, oxidase test, hanging drop and catalase test. Acinetobacter species were identified as Gram negative coccobacilli, non-motile, oxidase negative and catalase positive.

Speciation was done on the basis of growth at 37 degree Celsius and 44 degree celsius, hemolysis on blood agar, citrate utilization, Glucose oxidation, Arginine decarboxylation and Glucose utilization (Table1).

Antibiotic- sensitivity testing was done by Kirby -Bauer disk diffusion technique as per Clinical and Laboratory Standards Institute (CLSI) guidelines for Ampicillin, Ampicillin -sulbactam, Piperacillin-tazobactam, cefepime, cefotaxime, ceftazidime, ceftriaxone, Imipenem ,Meropenem ,Gentamycin, Amikacin, Doxycycline, Ciprofloxacin, Levofloxacin, Trimethoprim – sulfamethoxazole and for urine samples Nalidixic acid, Norfloxacin, Nitrofurantoin. Sterile commercially available antibiotic discs were used.

Table 1: Identification scheme of Acinetobacter species.

Species	Hemolysis on blood agar	Growth at 37 degree celsius	Growth at 44 degree celsius	Citrare utilization	Glucose oxidation fermentation	Arginine decarboxylation	Glucose utilization
A.baumannii	-	+	+	+	+	+	+
A.lwoffii	-	+	-	-	-	-	-
A.haemolyticus	+	+	-	-	+	+/-	+
A.junii	-	+	-	-	-	+	+

RESULTS

During the study period a total of 4260 specimens were processed. Out of which 1890 (44.3%) were culture positive and 2370 (55.7%) showed no growth. Among 1890 (44.3%) culture positive specimens, 110 (5.8%) Acinetobacter isolates were obtained from various specimens. Maximum isolations of Acinetobacter species were from the age group 40-60 (45%); followed by the patients in the age group of above 60 (30%). 10% isolations were from age group 20-40 and 15% were from below 20 years. In our study male to female ratio was 1.75:1. Our study shows 108(98.19%) isolates were from hospitalised cases and 2(1.81%) were from OPD cases. In present study 66 (60%) isolates were from ICU settings followed by surgical ward 35(30.18%). While few isolations were from other wards.

Table 3 shows that maximum isolations of Acinetobacter species were from pus sample 44 (40%), followed by urine 20 (18.18%); sputum 17 (15.45%); blood from I.V Catheter 6 (5.4%); endotrachial tube 2 (1.8%). A single isolation was from C.S.F.

Considering pre-disposing factores, highest percentage of Acinetobacter infections were seen in patient on antibiotic intake > 3days – 77 (70%), followed by the patient with ICU stay 66 (60%), mechanical ventilation > 3days – 45 (40%), endotracheal intubation 44 (40%), post-operative cases 20(1.82%), chronic illness 39 (35.44%).

Table 2: Distribution of isolates in different wards/ICU (n= 110)

WARD/ ICU	NO OF ISOLATES	PERCENTAGE
ICU	65	60%
Surgical ward	35	30.18%
Neurological ward	1	.9%
Medical ward	2	1.81%
Obstetric ward	1	.9%
Paediatric ward	1	.9%
Orthopaedic ward	2	1.81%
OPDs	2	1.81%
Total	110	100%

Table 3: Acinetobacter isolates from various samples.

SPECIMEN	NO. OF ISOLATES	PERCENTAGE
Pus	44	40%
Urine	20	18.18%
Blood from central venous catheter	6	5.4%
Peripheral blood	9	8.2%
Tracheal aspirates	11	10%
Endotracheal tube	2	1.8%
C.S .F.	1	.9%
TOTAL	110	100

Table 4: Isolation rate of Acinetobacter on the basis of pre-disposing factors (n=110)

Pre-disposing factors	No. Of patients	Percentage isolations
Antibiotic intake>3 days	77	70%
ICU stay	66	60%
Mechanical ventilation >3 days	45	40.1%
Intravenous catheterisation	42	38.1%
Urinary catheterisation	33	30%
Endotracheal intubation	44	40%
Post- operative cases	20	1.82%
Chronic - illness	39	35.44%
TOTAL	110	100%

Table 5: Species distribution of Acinetobacter isolates (n=110)

SPECIES	NO. OF ISOLATES	PERCENTAGE
A.baumannii	93	84.5%
A.lowffii	8	7.27%
A.junni	4	3.63%
A.haemolyticus	5	4.5%
TOTAL	110	100%

Table 6: Antimicrobial susceptibility profile of Acinetobacter species (n=110)

Antibiotics	Sensitive	Resistance
Ampicillin	23(20.9%)	87(79.1%)
Ampicillin-sulbactam	50(49.5%)	60(50.5%)
Piperacillin- tazobactam	70(63.66%)	40(36.34%)
Cefepime	39(35.45%)	71(64.55%)
Cefotaxime	24(21.9%)	86(78.1%)
Ceftazidime	29(26.1%)	31(73.9%)
Imipenem	78(70.9%)	32(29.1%)
Meropenem	78(70.9%)	32(29.1%)
Gentamycin	50(45.45%)	60(54.55%)
Amikacin	70(63.66%)	40(36.34%)
Doxycyclin	44(40%)	66(60%)
Ciprofloxacin	40(36.36%)	70(63.64%)
Levofloxacin	52(47.27%)	58(52.73%)
Trimithoprim- sulfamethoxazole	44(40%)	66(60%)
Urine samples- Norfloxacin	33(30%)	77(70%)
Nalidixic acid	33(30%)	77(70%)
Nitrofurantoin	99(90%)	11(10%)

In current study there is predominance of A.baumannii (84.5%) isolates, followed by A.lwoffii (7.26%), A.haemolyticus (4.5%), A.junnii (3.63%) isolates. Growth were monomicrobial in 89(80.1%) of samples, whereas 21 (19.9%) samples were polymicrobial. Pseudomonas aeruginosa (25%) was the most associated organism followed by E.coli (20%); Klebsiella pneumonia (20%); Staphylococcus aureus (18%) and Candida (15%).

In current study high level of resistance were seen for Ampicillin (79.1%); Cefotaxime (78.1); Ceftazidime (73.9%); Ceftriaxone (70.9%) and for urine samples- Nalidixic acid (70%); Norfloxacin (70%). Significant level of resistance were found for Ciprofloxacin (63.64%); Levofloxacin (52.73%); Doxycycline (60%); Trimethoprim-sulfamethoxazole (60%); Gentamycin (54.55%). Sensitivity level of Carbapenems (for both Imipenem and Meropenem) were reported 78 (70.9%) followed by Piperacillin- tazobactam 70 (63.66%) and Amikacin 70 (63.66%).

DISCUSSION

In routine clinical microbiology lab findings, non-lactose fermentative Gram negative bacilli (NLFGNB) other than Pseudomonas aeruginosa are not taken seriously as a pathogen and neglected just as a contaminants. In our set up NLFGNB isolates from various clinical samples were regularly encountered, which drag our attention. Acinetobacter species were identified as per standard criteria. In recent few years Acinetobacter species has emerged as an important pathogen with increasing trend towards drug resistance. In present study 110 (5.85%) Acinetobacter species has been isolated from 1890 culture positive specimens. Our findings are slightly higher than that of Raina Dimple et al(2016); Vijaya S Rajmane et al (2015); Manjunath P. Salmani et al(2015), where Acinetobacter species isolation rate was 4.1%,3.1% and 4.3% respectively but lower than that of Oberoi et al (2009); who reported in his studies 8.4% isolations of Acinetobacter species. In our study male to female ratio was 1.75:1, which is in line with the studies done worldwide. Cause behind it may be that more male patients come to the hospital for the treatment than female patients. Acinetobacter infections were more common in patients of the age group 40-60(45%), followed by the patients in the age group of >60 years (30%); which are in line with the findings of Cucunawangsih et al, who reported maximum isolations from patients of 14-65 years old (73.8 %); followed by elderly patients(19%). Whereas Raina Dimple et al reported maximum isolations in the age group of <10 years (22.6%) followed by patients in the age group of 41-50 years (20.8%). In current study isolations of Acinetobacter species from hospitalised cases and 0PD cases were 98.19% and 1.81% respectively which is consistent with the report of Raina Dimple et al who reported 98.1% isolates were nosocomial and 1.9% were community acquired. Lahiri KK et al reported 82.9% and 17.1% from hospitalised cases and from OPDs respectively. In current study we observed that 66(60%) isolates were from ICU settings followed by surgical ward 35(30.18%). Lower isolations were reported from other wards. Raina Dimple et al (2016) reported (58.5%) isolations were from ICUs which are in line with our study. Vijaya S Rajmane et al (2015) and Anupurva S et al (2005) reported 82.55% and 20.8% isolations from ICUs respectively. Worldwide studies indicate increased trend of isolations towards ICUs in recent years. In ICUs significant risk factors such as intravascular catheterisation, mechanical ventilation, endotracheal intubations, urinary catheterisations are present; which contributes towards persistence and spread of Acinetobacter species in hospital environment. In our study some independent risk factors such as ICU stay (60%); Antibiotic intake>3 days (70%); Mechanical ventilations >3days (40.1%); endotracheal intubation (40%); chronic illness (35.44%); post-operative cases (1.82%) has been reported to be associated with the isolations of Acinetobacter species, which is consistent with the findings of Vijaya S Rajmane et al(2015) and Rubina et al. Study carried out by Prasanth et al showed no significant associations between Acinetobacter species isolations and ICU stay as well with mechanical ventilation.

Our study shows predominance of Acinetobacter baumannii (84%) followed by A.lwoffii (7.26%) , A.hemolyticus (3.8%), A.junni (3.63%) isolates; whereas Raina Dimple et al reported A. baumannii (90.6%); A. lwoffi (5.7%) A.hemolyticus (3.8%) in their studies. Our findings are in line with the report of Manjunath P. Salmani et al. and Prasanth et al (2004). Singla P et al reported A.baumannii (74.6%) and A.lwoffii (24.3%). Our study shows 80.1% samples were polymicrobial, whereas 19.9% samples were monomicrobial which is consistent with the finding of Manjunath P. Salmani et al and Raina Dimple et al. In our study Pseudomonas aeruginosa (25%) was the most associated organism whereas Raina Dimple et al and Manjunath P.Salmani et al reported it as Klebsiella pneumoniae and E.coli respectively. Present study shows isolations of Acinetobacter was maximum from pus(40%) followed by urine(18.18); sputum(15.45%); blood from I.V. Line (5.4%) and peripheral blood (8.2%).Our findings are in consistent with Oberoi et al and Manjunath P. Salmani et al who reported 86.2% and 29% isolations from pus samples respectively. This is in contrast with the study of Lahiri et al who reported maximum isolations were from urine samples (51.3%).

In the current study high level of resistance were seen for Ampicillin (79%); Cefotaxime (78.1%), Ceftazidime (73.9%); Ceftriaxone (70.9%); and for urine samples Nalidixic acid and Norfloxacin (both showed 70% resistance. Our findings are in variance with that of Raina Dimple et al who reported in his studies that resistance of Acinetobacter to Ampicillin (94%); Ampicillin-sulbactam (96%); Cefuroxime (92%); Ceftazidime (91%). Similarly Shareek et al also reported 85-90% resistance to beta- lactams, which is in line with the findings of Raina Dimple et al. Vijaya S.Rajmane et al reported resistance of Ampicillin (77.95%); Cefotaxime (86.04%). Findings of Rubina et al was in consistent with Vijaya S Rajmane et al. S.V Wankhade et al reported resistance to piperacillin-55%; Ceftriaxone-77% , which is in line with our findings. Prashanth et al reported almost all strains were resistant to Cephazolin and 50% and 58% were resistant to Cefotaxime and Ceftazidime respectively. Alireza et al reported 100% resistance to Cefotaxime and Ceftazidime. Significant level of resistance were observed to Ciprofloxacin (63.64%); Levofloxacin (52.73%); Doxycycline (60%); Trimethoprim- sulfamethoxazole (60%); Gentamycin (54.55%). Which is in variance with the findings of Raina Dimple et al who reported 81% and 83% resistance to Levofloxacin and Amikacin respectively. Shareek et al reported 80-72% resistance to Amikacin; Ciprofloxacin and Cotrimoxazole which is in line with the findings of Raina Dimple et al. S.V.Wankhade et al

reported resistance of Amikacin 44%; Gentamycin 55%; Co-trimoxazole 66%; Ofloxacin44%. Vijaya S Rajmane et al reported resistance of Amikacin 55.81%; Gentamicin 74.41%; Ofloxacin 55.97%. Ayisha Javed et al in Pakistan reported resistance to Amikacin 47%, Ciprofloxacin 43.5%; Gentamycin 50%; Co-trimoxazole 56.5%. Carbapenems were the most sensitive drugs. Both Imipenems and Meropenems showed 70.9% sensitivity, which are higher than observations of Raina Dimple et al and Shareek et al. They reported sensitivity to Carbapenems 26% and 25% respectively. Jaggi et al also reported much less sensitivity to Carbapenems (11%). Observations of S.V Wankhade et al and Silpa K Gokale et al is consistent with our findings who reported 56% and 85% sensitivity to Imipenem respectively. Findings of Arora et al also is in line with our data. Cucunwangsih et al in Indonesia and Ayisha Javed et al in Pakistan reported 70% and 82.6% sensitivity to Carbapenems respectively. The Emergence of multidrug resistant strains of Acinetobacter in particular with carbapenems resistance had made the situations more problematic for the clinicians leading to a decrease in therapeutic options.

CONCLUSION

These days various studies throughout the world has indicated that Acinetobacter species is an emergent and global nosocomial pathogen. 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 . Resistance pattern of Acinetobacter spp. is quite alarming in our healthcare setting also, especially in ICUs. So infection control practices and judicious use of antibiotics is mandatory. More studies is needed regarding this Acinetobacter species worldwide and its prevention and control should be in priority of every health care centers.

REFERENCES

1. Mandell G, Bennett JE, DolinR. Principles and practice of infectious diseases. Churchill Livingstone 5th ed 2000;2:239-41.
2. Gerner-Smidth P. Taxonomy and epidemiology of Acinetobacter infections. Rev Med Microbiol 1995; 6:186-97.
3. Towner KJ. Clinical importance and antibiotic resistance of Acinetobacter spp. J Med Microbiol 1997; 46:721-46.
4. Levi I, Rubinstein E. Acinetobacter infections- overview of clinical features. In: Bergogne- Berezin I, Joly- Guilloo MI, Towner KJ, editors. Acinetobacter: microbiology, epidemiology, infections, management .Boca Raton, CRC Press. 1996;101-15.
5. Koneman EW, Allen SD, Jande WM, Schreckenberger PC, WinnJr WC. Colour atlas and text book diagonistic Microbiology. Lippncot 5thed1997;286-7.
6. Lahiri K, Mani NS, Purai SS. Acinetobacter species as nosocomial pathogen: Clinical significance and antimicrobial sensitivity. Med J Armed Forces India.2004; 60:7-10
7. Oberoi A, Aggarwal A, Lal M.A decade of an underestimated nosocomial pathogen- Acinetobacter in a terciary care hospital in Punjab. www.jkscience.org.2009;11(1):24-26
8. Singhla P, Sikka R, Deep A, Seema, Chaudhary U. Pattern of antimicrobial resistance in clinical isolates of Acinetobacter species at a tertiary level health care facility in northern India. Journal of Evolution of Medical and Dental Sciences.2013;2(2):159-165.
9. Taneja N, Singh G, Singh M. Emergence of tigecycline and colistin resistant Acinetobacter baumannii in patients with complicated urinary tract infections in north India. Indian J Med Res.2011; 133:681-4.
10. Shareek PS, Sureshkumar D, Ramgopalakrishnan et al. Antibiotic Sensitivity Pattern of Blood Isolates of Acinetobacter species in a Tertiary Care Hospital: A Retrospective Analysis.Am Infect. Dis.2012;8(1) 65-69.
11. Raina Dimple, Sharma Nupur, Mahawal B. S., Khanduri Ankit and Pandita Ajay. Speciation and antibiotic resistance pattern of Acinetobacter species in a terciary care hospital in Uttarakhand. Int J Med Res Health Sci.2016,5(4):89-96.
12. Jaggi N, Sissodia P and Sharma L. Acinetobacter baumannii isolates: Epidemiology, antibiogram and nosocomial status studies over a 25 month period in a tertiary care hospital in India. Proceeding of the International Conference on Prevention and Infection Control, Jun .29-Jul.2,Geneva, Switzerland.2011.
13. Manjunath P. Salmani and Preeti B. Mindolli.Speciation and Antimicrobial Susceptibility Pattern of Acinetobacter from Clinical Isolates in a Tertiary Care Centre. Int. J.Current. Microbial. App. Sciences (2015) 4(10):973-976
14. Cucunawangsih, Veronica Wiwing,Nata Pratama Hardijo Lugito. Antimicrobial Susceptibility of Multidrug- Resistant Acinetobacter baumannii in a Teaching Hospital: A Two-Year Observation.Open Journal of Medical Microbiology.2015;5,85-89.
15. Anupurba S, Sen MR. Antimicrobial resistance profile of bacterial isolates from ICU: Changing trends. J Common Dis 2005;37(1):58-65.
16. Prasanth K, Badrinath S. Simplified phenotypic tests for identification of Acinetobacter spp. And their antimicrobial susceptibility status. J Med Microbial 2000;49:773-778.
17. S.V. Wankhede, D.P. Ghadage, T.R.Patil and A.V.Bhore. Isolation and study of multidrug resistant Acinetobacter from varios clinical samples ina tertiary care hospital. International Journal of Medical Microbiology and Tropical Diseases, April-June,2016;2(2):56-59.
18. Alireza Japoni- Nejad, Masoomeh Sofian, Alex van Belkum, Ehsanollah Ghaznavi-Rad. Nosocomial outbreak of extensively and pan drug-resistant Acinetobacter bauminnii in tertiary hospital in central part of Iran. Jundishapur J Microbiol 2013; 6(8):e9892
19. Rubina Lone, Azra Shah, Kadri SM, Shabana Lone, Shah Faisal. Acinetobacter- An Emerging Nosocomial Pathogen.Middle East Journal of Family Medicine 2009;7(2):13-16
20. Ayisha Javed, Aizza Zafar et al. Frequency and antimicrobial susceptibility of Acinetobacter species isolated from blood samples of paediatric patients. Pak J Med Sci 2012 28(3).
21. Vijaya S Rajmane, Shivkumar T Rajmane and T Mohite.Study of Incident , Risk Factors and Antibiotic Sensitivity Pattern of Acinetobacter baumannii in a tertiary Care Hospital.Journal of Krishna Institute of Medical Sciences University,Vol.4, No.1,Jan- Mar 2015.

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