The Study on the prevelance, speciation and antibiogram of Acinetobacter isolated from heterogeneous samples in a tertiary care hospital Chhattisgarh: A lead towards antimicrobial stewardship

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***Abstract*: Acinetobacter spp. is an important nosocomial pathogen especially in intensive care settings and is resistant to commonly available antimicrobial agents. Active surveillance is therefore necessary in order to determine appropriate antibiotic for the treatment. The purpose of this study was to determine the prevalence and antibiogram of Acenatobactor spp. isolated in patients attending the tertiary care hospital in Chhattisgarh.**

It is a prospective study conducted in the Department of Microbiology, Shri shankaracharya Hospital Bhilai Chhattisgarh. Acinetobacter from various clinical samples were included in this study during eight months period from October 2021 to May 2022.The isolates were identified using conventional and automated methods (Vitek2 COMPACT, bioMérieux) and the susceptibility was done using the Kirby-Bauer disk diffusion method. During the study period, a total of 127 Acinetobacter spp. was isolated from various clinical specimens, out of which 73(57.48%) isolation was from ICUs. Highest isolation was observed from pus samples 35 (27.55%) and endotracheal tube aspirates 35(27.55%) followed by blood 25 (19.68%) and urine and sputum samples 11 (8.66%). 73.47% isolates were MDR (MultidrugResistant), however they remained susceptible to colistin127(100%) and tetracycline88 (70.07%). It is necessary to regularly monitor the resistance phenotypes of Acinetobacter. Enhanced surveillance of MDR Acinetobacter is critical for guiding the appropriate use of antibiotics and reducing the incidence of hospital acquired infection.

***Keywords*: Antibiotic Susceptibility; Multi-Drug Resistance (MDR); Acinetobacter spp.; BAL:Bronchioalveolar Lavage, CVP: Central Venous Pressure**

Introduction

Acinetobacter spp are usually considered to be opportunistic pathogens,. They are recognized as an important nosocomial pathogen and recently reported to cause a number of outbreaks of nosocomial infections in hospitalized and immune compromised patients like septicemia, pneumonia, wound sepsis, endocarditis, meningitis and urinary tract infections (UTI).[1,2]These remain as one of the most challenging pathogens owing to their uniqueness and multiplicity of their resistance mechanisms [3]. Some risk factors for acquisition of infection by Acinetobacter spp. include prolonged hospitalization, immune compromised status of patients, mechanical ventilation, cardiovascular or respiratory failure, previous infection and antimicrobial therapy, and presence of indwelling catheters such as central venous or urinary catheters [4]. More than two third of Acinetobacter infections are due to Acinetobacter baumanii.

Acenatobactor 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.[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.[6]But 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 Shri shankaracharya institute of medical sciences Bhilai Chhattisgarh .Acinetobacter spp. as pathogens are developing resistance at a very rapid pace to almost all antimicrobial agents that are available which includes, aminoglycosides, quinolones and broad-spectrum β lactams [7]. Almost, 60 - 70% of these bacteria have developed resistance to many antibiotics, including carbapenems. And they are associated with higher patient morbidity and mortality, and few or no antimicrobials remain effective for their treatment [8].

Materials and Methods

It is a prospective study carried out in the Shrishankaracharya hospital and Department of Microbiology. It was conducted for a period of 8 months from 1st oct 2021 - 31st May 2022 .The 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. In case of urine samples, the isolates were subjected to biochemical tests and antimicrobial susceptibility only if the colony count was significant (> 100000 CFU/ ml). Acinetobacter spp. were identified by colony characteristics (Non-Lactose-fermenting , glistening , small mucoid colonies etc),

All isolates obtained were further processed and identified by standard routine microbiological processes. .[9,10] and VItek 2. After identification by phenotypic methods and Vitek, antibiotic susceptibility was performed for each isolate by the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar using 0.5 MacFarland Turbidity standard. The following antibiotic discs were used: Ampicillin (100 mcg), Piperacillin-tazobactam (100/10 mcg), ceftazidime (30 mcg), cefepime (30 mcg), ceftriaxone (30 mcg), cefotaxime (30 mcg), imipenem (10 mcg), meropenem (10 mcg), gentamicin (10 mcg), tobramycin (10 mcg), amikacin (30 mcg), ciprofloxacin (5 mcg), levofloxacin (5 mcg), tetracycline (30 mcg), trimethoprim sulfamethoxole (25 mcg), colistin (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).[11] All dehydrated media and antibiotic discs were procured from Hi Media labs, Mumbai, India.

OBSERVATION

Out of the total 1,201(56.65%) culture positive samples, A total of 127 (10.57%) non-duplicated Acinetobacter spp were isolated from patients admitted and attending the OPD at Shrishankaracharya Hospital, during the study period (1st Oct2021 to 31st May 2022). Total 127 isolates of Acinetobacter spp. were recovered from various clinical specimens and there unit wise distribution are shown in table 1 & table2 respectively.

Table 1 showing distribution of isolates among various clinical samples

Sample	**No. of isolates(n)**	**Percentage** (%)
Pus	35	27.55%
Endo tracheal Aspirate	**35**	**27.55%**
Blood	25	19.68%
Urine	11	08.66%
Sputum	11	08.66%
BAL	04	03.14%
Swab(Gluteal abscess)	01	00.78%
Throat swab	01	00.78%
CVP Tip	01	00.78%

Table 2 Unit wise distribution of isolates among various clinical samples

Unit	**Number of isolates**	**Percentage**
G .ICU	24	18.89%
G.Medical ward	19	14.96%
Neurosurgical ICU	15	11.46%
Coronary care unit	15	11.46%
Surgical ward	12	09.44%
Neurosurgical ICU	11	08.66%
Surgical ICU	11	08.66%
Orthopedic ward	10	07.87%
Post –op ward	08	02.96%
Gynecology	06	04.72%
Neonatal ICU	04	03.14%
Pediatric ICU	03	02.36%

Table 3 Species identification of Acinetobacter isolates by Vitek 2

Acinetobacter species	**Number**	**Percentage(%)**
Acinetobacter baumannii	107	84.25%
Acinetobacter Iwoffi	07	5.51%
Acinetobacter calcoaceticus	07	5.51%
Acinetobacter haemolyticus	07	5.51%
Acinetobacter radioresistans	03	2.23%
Acinetobacter junii	03	2.23%
Acinetobacter ursingii	03	2.23%

There was higher incidence of Acinetobacter infection in males (53.54%) compare to females(46.45%). Most of the patients from whom Acinetobacter spp. were isolated were in the age group of 16-50years (40.15%), followed by age group >50 years (37.79%); 0-15 years (22.04%) respectively (Table 4).

Table 4: Age wise distribution of Acinetobacter

Age	No of isolates(n)	Percentage (%)
0-15yrs	28	22.04%
16-50yrs	51	40.15%
>50yrs	48	37.79%

Among 127 isolates of Acinetobacter, all showed 100% sensitivity to colistin and polymyxin B. High levels of resistance were seen for cefotaxim (81.88%) ampicillin (79.52%) ,ceftriaxone (79.52%) ceftazidime (73.22%) .(Table 5)

Table 5: Antimicrobial susceptibility of Acinetobacter isolates.

Antibiotics	**Sensitive no.**	**Sensitive %**	**Resistance no.**	**Resistance %**
Cefotaxime	24	18.89%	104	81.88%
Ceftriaxone	26	20.47%	101	79.52%
Gentamycin	43	33.85%	84	66.14%
Meropenem	41	32.28%	86	67.71%
Amikacin	42	33.07%	85	66.92%
Cotrimoxazole	41	32.28%	86	67.71%
Ciprofloxacin	39	30.70%	88	69.29%
Levofloxacin	44	34.64%	83	65.35%
Ceftazidime	34	26.77%	93	73.22%
Colistin	127	100%	00	00%
Polymixin B	127	100%	00	00%
Piperacillin - Tezobactum	41	32.28%	86	67.71%
Tigecycline	107	84.25%	20	15.74%
Ampicillin	26	20.47%	101	79.52%
Norfloxacin	57	44.88%	70	55.11%
Cefepime	31	24.40%	96	75.59%
A/S	37	29.13%	90	70.86%
Tobramycin	41	32.28%	86	67.71%
Piperacillin	42	33.07%	85	66.92%
Doxycyclin	61	48.03%	66	51.96%

DISCUSSION

In past most of the clinical microbiological laboratories, non fermentative gram negative bacilli (NFGNB) other than pseudomonas aeruginosa were not taken seriously as a pathogen.[12] We took up this study when we frequently 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.[13] Acinetobacter spp. specially, A. baumanii, are developing as real infectious threat mainly in the intensive care units (ICUs).[14] it has emerged as a cause of ICUs infection because their ubiquitous nature in the ICU environment and inadequate infection control practice. Multi resistant antimicrobial pattern has continuously raised the incidence of Acinetobacter infection over the past two decades.[15] Various 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 Infections caused by multidrug resistant .Acinetobacter are difficult to treat and are a major cause of increased morbidity and mortality in hospitalized patients [16] The frequency of antibiotic resistance in Acinetobacter is worrisome since there are hardly any antibiotics in development process which have suitable activity against these multi-resistant strains of organism [17]. Until recently, carbapenem class of antibiotics were the drug of choice against this pathogen. However, with the development of resistance against carbapenems by Acinetobacter spp. the entire scenario has changed, making the pathogen difficult to treat [18]

The present study was conducted to know the prevalence of Acinetobacter spp. infection in our hospital and to know their antibiotic susceptibility profiles and resistance patterns. Amongst a total of 1201 bacterial isolates cultured from various (2105) clinical specimens over a period of 8 months 127 (6.03%) isolates were identified as Acinetobacter spp. similar prevalence of 3% and 3.36% of Acinetobacter isolates was reported by Dash., et al. in Odisha and Gupta., et al. [19,20]. Higher prevalence rate of 14% and 9.6% was reported by Mostofi., et al. in Tehran, Iran and Joshi., et al. [21,22]. in United States, where the rise in isolation of Acinetobacter was seen among these age groups [23]. Maximum number of Acinetobacter strains in this study were isolated from pus (35/27.55%)and Endotracheal aspirate (35/27.55%) followed by blood (25/19.68%); urine (11/08.66%) and sputum (11/08.66%). A similar observation has been reported in the study done by Shivaranjani V., et al. in South India (2013) which showed 38.5% isolates from pus, followed by 20.4% isolates from endotracheal aspirate [24]. Maximum number of Acinetobacter isolates were from pus(27.55%) and endotracheal tip/aspirate (27.55%) followed by,blood (21.25%), sputum(08.661%), tips (12.31%) urine (08.66%) and tips(07.081%) 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%).[25] Apoorva et al. found maximum number of Acinetobacter isolates from respiratory samples (35.78%) followed by pus(32.84%).[26] 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.[27]

Commonest species isolated in human clinical specimens is Acinetobacter baumannii [28]. In our study 107 (84.25%) isolates were A. baumannii complex, followed by Acinetobacter lwoffii and Acinetobacter haemolyticus 07 (5.51%) each and Acinetobacter radioresistens, Acinetobacter ursingii, Acinetobacter junii 03 (2.5%) each. This again is in concordance with the study done by Gupta N., et al. in 2015 [29] where 72% were A. baumannii complex, followed by A. lwoffii, A. haemolyticus and 1% A. radioresistens, A. junii. in the present study, Acinetobacter spp. were found to be resistant to most commonly used antimicrobial agents as a routine and prevalence of 77.5% MDR was observed. Similar reports of MDR Acinetobacter isolates have been reported with 88.02% resistance to commonly applied antibiotics [30]. Other study done by Rajkumari et al. (2020) shows similar76.81% MDR in Acinetobacter spp.

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.[6] In our study, 100% sensitivity was recorded for colistin and polymixin B. Raina et al.[6] 100% sensitivity. Acinetobacter is ubiquitous in the hospital setting. It has the ability to survive for longer periods and also demonstrates a number of antimicrobial resistance genes which has made Acinetobacter a successful hospital pathogen [32] Acinetobacter were highly resistant to cephalosporins (81.88%) correlating with studies done by Guckan R., et al. in 2015 and Shivaranjani V., et al. in 2013 [22,33]. Acinetobacter shows resistance towards piperacillin (66.92%) which correlates with the study done by Shivaranjani V., et al. in South India (2013) [33].Resistance towards imipenem and meropenem was seen to be 55% and 67.71% respectively. Data of the antibiotic susceptibility patterns of Acinetobacter from different geographical areas revealed that the resistance of Acinetobacter spp. to imipenem rose from 0% resistance to 40% (2000 - 2004) [34]. The prevalence of imipenem resistance in A. baumannii isolated from a burns unit of the USA was 8% earlier (2007) [35].

Resistances to major antimicrobial drugs as well as disinfectants are the major factors that make it a successful and persistent hospital pathogen [36]. No resistance to colistin was seen in this study which is similar to the studies published by Dash., et al, Shareek., et al. and Nazir A [19,37,38] Initial concern about multidrug resistant (MDR) and carbapenem resistant Acinetobacter baumannii (CRAB) associated infections began when the first hospital wide outbreak occurred in New York City in 1991 [39]. Since then, reports of CRAB from other parts of the world including India [38,40] are coming in.

Out of the total isolates 84 (66.14%) were multidrug resistant (MDR) in this study. Other studies conducted by Dash., et al. (2013) and Rekha., et al. (2011) reported MDR isolates to be 55% and 74% respectively [19,41]. Other Studies done by B Apoorva .,et al.(2020} that most of Acinetobacter spp. were isolated from patients admitted in the high-risk settings like Intensive Coronary Care Unit (ICCU) in our study (57.48%). our results are similer with the results seen in the study conducted by Mera RM., et al. (2010) and Gupta., et al. (2015), where an increased number of Acinetobacter isolates were recovered from Intensive Care settings [15,42]. The emergence of antibiotic resistant strains in ICU is because of higher use of antimicrobial agents per patient and per surface area [42].

Conclusion

The rate of Acinetobacter resistance to routinely used antibiotics is increasing rapidly. MDR Acinetobacter isolates remained susceptible to colistin and tetracycline, which can be used as the treatment option for management of most of the cases of infections caused by this organism, however with caution as colistin is and should be a last resort . Patients infected with MDR Acinetobacter is widely spread in our hospital specially in patients admitted to intensive care settings and the reason/s behind this alarming situation need to be ascertained and taken care of at regular intervals. It is necessary to regularly monitor the resistance phenotypes of these isolates. Enhanced surveillance of MDR Acinetobacter is critical for guiding the rational use of antibiotics and reducing the incidence of Hospital Infection Control (HIC).

In our study, Acinetobacter were resistant to most commonly used antibiotics. Emergence of carbapenem resistance is worrisome. 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 hospitals.

Conflict of Interest

There are no conflicts of interest.

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