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A Prevalence Study of Acinetobacter Species and Their Sensitivity Pattern in a Tertiary Care Hospital Rajkot City of Gujarat (India): A Hospital Based Study.

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Abstract
Background: *Acinetobacter species has emerged as an important pathogen globally in various infections especially in hospital acquired infections.*
Objectives: *This study was conducted to determine the prevalence, and antibiotic resistance pattern of Acinetobacter species from various clinical samples.*
Materials and Methods: *The study included a total of 1000 clinical samples, collected from patients treated in P.D.U. Hospital Rajkot-a tertiary care hospital in Gujarat, India were included in study period from November 2012 to August 2014. Isolation , Identification and sensitivity of Acinetobacter species were performed by manual method.*
Results: *48 (4.8%) patients clinical samples showed growth of Acinetobacter species. Acinetobacter species isolation rate from blood were 24(50%), pus 15(31.25%), urine 4(8.33%), CSF 2(4.18%), sputum 1(2.08%), plural fluid 1(2.08%) and tracheal aspirate 1(2.08%). Resistance observed to Meropenem was 41.67%, Piperacillin -Tazobactum 58.34%, Amikacin 52.09%, Ceftazidime 79.71%, Gentamicin 62.5% and Levoflaxacin 68.75%. This data suggest that Acinetobacter isolated from hospital exhibits resistanas to multiple antimicrobial drugs.*
Conclusion: *The study will help to implement better infection control strategies and improve the knowledge of antibiotic resistance patterns of Acinetobacter species in our region.*
Keywords: *Acinetobacter species, antibiotics, multidrug resistance, nosocomial*

I. Introduction

Members of the genus Acinetobacter are ubiquitous, free living organisms that prefer moist environment and can be easily obtained from soil, water, food and sewage [1]. 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 septicaemia, pneumonia, wound sepsis, endocarditis, meningitis and urinary tract infection (UTI) [2,3]. Although acknowledged to be an opportunist in hospitalised patients, community acquired infections are reported and they can cause infections in virtually every organ system [4]. Interpreting the significance of isolates from clinical specimens is often difficult, because of the wide distribution of Acinetobacter in nature and its ability to colonise healthy or damaged tissue [5]. This study was undertaken to determine the prevalence, and antibiotic resistance pattern of Acinetobacter species from various clinical samples.

II. Material And Method

The study was undertaken in Department of Microbiology, P.D.U. Medical College, Rajkot (Gujarat, India), from period November 2012 to August 2014. Total 1000 clinical samples received in our laboratory from patients treated at P.D.U. Hospital Rajkot were included in this study. All the clinical samples were inoculated on MacConkey agar and blood agar. Inoculated plates were incubated at 37°C for 24 - 48 hours. Colonies of *Acinetobacter species* were white/cream coloured, smooth, circular with entire edges on blood agar and nonfermenter with a pinkish tint on MacConkey agar. Microscopy showed gram negative coccobacilli on gram stain. Oxidase test was negative [6,7].

Identification scheme of Acinetobacter species.

Test	MOTILITY	Oxidase test	Indole Test	Metyl Red Test	Citrate Test	TSI Test	Urease Test	/OF Test	Hemolysis on Blood agar
Acinetobacter species	Non motile	Negative	Negative	Negative	Variable	Alkaline slant / No change in butt	Negative	Oxidative Asaccharolytic	Mostly non hemolytic

Antibiotic sensitivity testing of Acinetobacter species were performed by Kirby Bauer disc diffusion test. Antimicrobials tested were Amikacin, Gentamicin, Cefepime, Ceftazidime, Levofloxacin, Ampicillin-Sulbactam, Piperacillin-Tazobactam, Cotrimoxazole, Cefoperazon-Sulbactam, Tetracycline, Meropenem as per CLSI [8].

'Multidrug resistant (MDR) Acinetobacter spp.' is defined as isolate that is resistant to at least three classes of antimicrobial agents - all Penicillins and Cephalosporins (including inhibitor combinations), Fluoroquinolones and Aminoglycosides. 'Extensive drug resistant (XDR) Acinetobacter sp.' shall be the MDR isolate that is also resistant to Carbapenems. [9]

III. Result

Total 1000 patients clinical samples were included in present study, out of which 48(4.8%) showed growth of Acinetobacter species.

Acinetobacter species were isolated highest from blood 24 (50%), followed by pus 15 (31.25%), urine 4 (8.33%), CSF 2 (4.18%), sputum 1 (2.08%), plural fluid 1 (2.08%) and tracheal aspirate 1 (2.08%) **(Table 1).**

Table 1: Prevalence of Acinetobacter in various clinical samples.

Clinical Sample	**Isolation rate (n=48)**
Blood	24 (50 %)
Pus	15 (31.25%)
Urine	04 (8.33%)
CSF	02 (4.18%)
Sputum	01 (2.08%)
Plural fluid	01 (2.08%)
Tracheal aspirate	01 (2.08%)

Number of Acinetobacter species were more from paediatric ward followed by surgical ward. Most of the isolates from paediatric ward was from preterm babies **(Table 2).**

Table 2: ward wise distribution of Acinetobacter Species .

Ward	**Isolation rate (n=48)**
Paediatric	23 (47.94 %)
Surgical	13 (27.08%)
Medical	05 (10.41%)
Obst and Gynec	05 (10.41%)
TB and Chest	02 (4.16%)

Acinetobacter infection was found nearly equal in male 23(47.9%) and female 25(52.1%).

In Antibiotic Sensitivity Testing, highest resistance was observed to Cefepime (87.5%) and lowest to Meropenem (41.67%) **(Table 3).**

Table 3. Resistance pattern of Acinetobacter Species to different antibiotics.

Drug	Resistance pattern
Ampicillin Sulbactam(A/S)10/10ug/disc	64.58%
Ceftazidime(CAZ) 30ug/disc	79.17%
Levofloxacin(LE)5ug/disc	68.75%
Meropenem(MRP)10ug/disc	41.67%
Gentamicin(GEN)10ug/disc	62.5%
Amikacin(AK)30ug/disc	52.09%
Piperacillin Tazobactam(PIT)100/10 ug/disc	58.34%
Piperacillin(PI)100ug/disc	64.59%
Cefepime(CEP)30ug/disc	87.5%
Cefotaxime(CTX)30ug/disc	75%
Tetracycline(TE)30ug/disc	66.67%
Cotrimoxazol(COT)1.25/23.75ug/disc	62.5%

IV. Discussion

Acinetobacter spp. are the second most common Non-fermenting bacteria after *Pseudomonas* species that are isolated from human specimens, especially among nosocomial infections.[10] In recent years, this species has emerged as the causative agent of important nosocomial infections in the ICUs, which is probably related to the increasingly invasive procedures used, the greater quantity of broad-spectrum antimicrobials used, and prolonged duration of stay in the hospital. Development of resistance to antimicrobials is a major problem in the treatment of *Acinetobacter* infections.[11]

Isolation rate of Acinetobacter species in present study was 4.8%, which is quite comparable with Lone et al (4.8%) [12] and Mindolli PB et al(4.25%) [13]. Higher prevalence rates of 14% and 9.6% among hospital isolates were observed by Mostofi *et al*. (Iran) and Joshi *et al*. (India), respectively[14,15]. *Acinetobacter spp.* can colonize skin, wounds, respiratory and gastrointestinal tracts.[16] It is a pathogen of tropical and humid environment, but some species can survive environmental desiccation for weeks, a characteristic that promotes transmission through fomite contamination in hospitals.[17]

In present study Acinetobacter species were isolated highest from blood 24 (50%), followed by pus 15 (31.25%), urine 4 (8.33%), CSF 2 (4.18%), sputum 1 (2.08%), plural fluid 1 (2.08%) and tracheal aspirate 1 (2.08%). Highest isolation was from blood, most of them were from preterm babies, where due to lower immunity, more chances of bacterial infection. In a study conducted by A. Asensio et al in 2008 Acinetobacter was isolated from respiratory tract (42.2%), surgical wound (15.1%), urinary tract (12.9%), skin (11.7%)[18].

In our study, 20.83% isolates were MDR & 20.83% isolates were XDR. The other studies conducted by Bhattacharyya *et al*. in West Bengal [19] and Mostofi *et al*. in Tehran[14] reported the MDR isolates to be 29% and 54%, respectively. *Acinetobacter* is ubiquitous in the hospital setting. Its ability to survive for long periods coupled with its ability to demonstrate a number of antimicrobial resistance genes has made *Acinetobacter* a successful hospital pathogen.[20, 21]

Most of the patients who were admitted in our hospital had previously attended primary and secondary care hospitals and usually received combination of β-lactam antibiotics like third and fourth generation Cephalosporins along with Aminoglycosides or Fluoroquinolones. Majority of the isolates in our study were resistant to commonly used antibiotics such as Ceftazidime(79.17%), Cefepime(87.5%), Gentamicin(62.5%), Amikacin(52.09%), Levofloxacin(68.75%), and Ampicillin/sulbactam(64.58%). This suggest that MDR isolates are increasing, probably due to indiscriminate use of these antibiotics in healthcare settings. It is re-emphasized that broad spectrum antibiotics should be used with caution. We found that, Meropenem(41.67%) and Piperacillin/Tazobactam(58.34%) were also showing resistance against this pathogen suggesting increased

XDR isolates. Mostofi *et al*. in their study had reported resistant drug Meropenem (31%) and Piperacillin/Tazobactam (40%)[14]. Differences observed between the different studies, could be due to the methods, the resistance patterns and and the antimicrobial patterns used [22]. Although antibiotic resistance is a worldwide concern, it is first and foremost a local problem – selection for and amplification of resistant members of a species that are occurring in individual hospitals and communities, which can then spread worldwide[23] There are many measures that may impact on antimicrobial resistance; reducing and restricting the use of antimicrobials to only those situations where they are warranted, at proper dose and for the proper duration is the most appropriate solution.[24]

Carbapenems have been the drug of choice for treating *Acinetobacter* infections, but unfortunately, Carbapenem resistant *Acinetobacter* is becoming common worldwide[25, 26]

V. Conclusion

In the present study Acinetobacter spp. accounted for 4.8% of total culture. Resistance observed to Meropenem was 41.67%, Piperacillin -Tazobactam 58.34%, Amikacin 52.09%, Ceftazidime 79.17%, Gentamicin 62.5%, Levoflaxacin 68.75% which suggested that Acinetobacter isolated from hospital exhibit resistance to multiple antimicrobial drugs.

Traditional typing methods like phenotyping and antibiogram typing have an advantage over genotyping as they are readily available in all clinical microbiology laboratories. Simple identification schemes and antimicrobial susceptibility testing provide a cost effective approach for typing Acinetobacter spp. Although above systems have certain limitations when compared to molecular methodologies, the distinction between resistant and susceptible Acinetobacters atleast, is useful for effective clinical management of the infection caused by this group of organisms.

Overall infections caused by Acinetobacter spp. Provide an impressive demonstration of the increasing importance of this genus as human pathogen because of the high potential of this genus to develop antibiotic resistance leading to a considerable selective advantage in environment with widespread and heavy use of antibiotic, especially with relation to hospital environment and nosocomial infections. To avoid resistance, antibiotics should be used judiciously and empirical antibiotic therapy should be determined based on local antibiotic sensitivity pattern of the prevalent organisms of the hospital.

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