Antimicrobial Profile of *Acinetobacter* spp. an Emerging Nosocomial Superbug

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A B S T R A C T

Recently, *Acinetobacter* has emerged as significant hospital pathogen, notoriously known to acquire antibiotic resistance to most of the commonly prescribed antimicrobials. Many risk factors are associated with *Acinetobacter* infections, especially in patients in intensive care unit (ICU). This study aims to isolate *Acinetobacter* from various clinical specimens and to determine its antimicrobial sensitivity pattern. Identification, speciation and antimicrobial sensitivity testing were performed using the standard microbiological techniques. From the processed clinical specimens, 88 *Acinetobacter* strains were isolated. Significantly higher percentage of *Acinetobacter* strains was found in ICU compared with general wards. Most common *Acinetobacter* samples were from blood. Infections were more common in males and were associated with major risk factors such as post-surgical, diabetes mellitus, catheterization, extended hospital stay and prolonged antibiotic usage. *Acinetobacter baumannii* was the most common species isolated from blood, (septisemias) pus (wound infection), etc. Imipenem was most sensitive drug (73 (82.95%) followed by colistin 62(70.45%), Tygycyclin 59(67.05%), cproflaxacin 55(62.50%), cefoperazone + sulbactum 55(62.50%) oflaxacin 54(61.36%), amikacin 53(60.23%), gentamycin 52 (59.09%). Highest resistance is seen in ampicillin.70 (79.35%) followed by cefixime 66(75.00%), ceftriaxone 62(70.45%), amoxicillin + clavulanic acid (61(69.23%), cephotoxime 57(64.77%), ticarcillin+clavulanic acid 54(61.35%) co-trimoxazole 49(55.68%), piperacillin + tazobactim 40(45.45%). *Acinetobacter* nosocomial infections resistant to most antimicrobials have emerged, especially in ICU. Early identification and continued surveillance of prevalent organism will help prevent the spread of *Acinetobacter* in hospital environment.

Keywords: *Acinetobacter*, Antimicrobial resistance, Nosocomial pathogen.

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Introduction

*Acinetobacter* are Gram-negative *Coccobacilli*, strictly aerobic, non-motile, catalase positive, oxidase negative and lack pigmentation. (1) They are ubiquitous (2) free living saprophytes in soil and water. (3)

Up to 25% of healthy ambulatory adults exhibit cutaneous colonization by *Acinetobacter* and are the most common Gram-negative bacteria carried on the skin of hospital personnel. (4) They are usually opportunistic pathogens reported to cause a number of outbreaks of nosocomial infections such as septicemia, pneumonia, wound sepsis, endocarditis, meningitis, urinary tract infections and peritonitis, (5) but their predominant role is in ventilator associated pneumonia (VAP), in intensive care units.
(ICUs). (1) Predisposing factors for Acinetobacter infections include the presence of prosthesis, endotracheal intubation, intravenous (I.V.) catheters and prior antibiotic therapy in a seriously ill patient in hospital. (3) Such infections are often extremely difficult to treat because of widespread resistance to the major groups of antibiotics and long-term survival of bacteria in the hospital environment. (1)

Resistance to all known antibiotics has now emerged in Acinetobacter spp. with the majority of strains still being susceptible to carbapenems. (6) Multidrug-resistant (MDR) Acinetobacter infections are associated with increased time on mechanical ventilation, in the ICU and in the hospital.

Treatment options are severely limited; carbapenems and colistin are the agents of choice. More research and greater emphasis on the prevention of health-care associated transmission of MDR Acinetobacter infection are essential. (7)

The aim of this study was to isolate Acinetobacter species from clinical specimens and to study the antimicrobial susceptibility pattern of Acinetobacter isolates.

Materials and Methods

The study was carried out in the central laboratory of Microbiology Narayana Medical College Nellore South India from August 2015 to September 2016. Relevant clinical specimens sputum, blood, pus, urine were collected from patients by standard collection procedures. No specific exclusion criteria envisaged. Specimens were processed by standard microbiological techniques. (3) In Gram stain of direct smears Acinetobacter appeared as tiny, Gram-negative coccobacillary cells often appearing as diplococci. (5) All specimens were inoculated on 10% sheep blood agar and MacConkey agar and incubated at 37°C for 18-24 h. (3) Colonies on blood agar were 0.5-2 mm diameter, translucent to opaque (never pigmented), convex and entire. On MacConkey agar a faint pink tint was produced. (5) Gram stain, catalase, oxidase and motility tests were performed. Acinetobacter are Gram-negative Coccobacilli, non-motile, strictly aerobic, catalase positive and oxidase negative. Rapid utilization of 10% glucose was seen with O-F medium. Acinetobacter species identification done. Acinetobacter baumannii 66 (75.00%) Acinetobacter lwoffii 22 (2.005%).

Antimicrobial susceptibility testing (3) was performed by modified Kirby Bauer method (10) as per the Clinical and Laboratory Standards Institute guidelines. (11) Antibiotics tested were ampicillin, cephamoxime, cefazime, co-trimoxazole, ceftazidime, gentamicin, amikacin, tygecyclin, amoxicillin with clavulanic acid, cefuroxime with sulbactam, ticarcillin with clavulanic acid, piperacillin with tazobactam, imipenem, ceftriaxone and colistin.

Results and Discussion

In total, 88 Acinetobacter strains were isolated. Out of these 88 Acinetobacter isolates, 36 isolates were from general wards and 52 were from ICU. Significantly higher percentage of Acinetobacter strains were found in ICU 56 (59.09%) compared with general ward 36 (40.90%). Acinetobacter infections were more common in males 64 (72.73%) as compared with females 24 (27.27%) (Table 1). Most infections are between 40 to 69 years as per table 3. The most common Acinetobacter isolates are from blood 45 (51.14%) followed by pus 18 (20.15%), urine 15 (17.05%) and sputum (11.36%) (Table 2). Most common Acinetobacter species isolated was
Acinetobacter baumannii 66 (75.00%), followed by Acinetobacter lwofii 22 (25.00%). Imipenem was most sensitive drug 73 (82.95%) followed by colistin 62 (70.45%), Tigecyclin 59 (67.05%), ciprofloxacin 55 (62.50%), oflaxacin 54 (61.36%), amikacin 53 (60.23%) gentamycin 52 (59.09%). Highest resistance is seen in ampicillin 70 (79.35%) followed by cefixime 66 (75.00%), ceftriaxone 62 (70.45%) amoxicillin + clavulanic acid 61(69.23%), cephotoxime 57 (64.77%), ticarcillin + clavulanic acid 54 (61.35%) co-trimoxazole 49 (55.68%), piperacillin + tazobactim 40 (45.45%) (Table 4).

Acinetobacter spp. is Gram-negative Cocobacilli that contribute profoundly to the burden of modern medicine. Acinetobacter spp. is the second most commonly isolated non-fermenter in human specimens (after Pseudomonas aeruginosa). They rank fourth (after P. aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae) among the most frequent hospital acquired infectious agents. (12) Acinetobacter spp. have emerged as a cause of ICUs infection. Multiresistant Acinetobacter spp. has become established as “alert” pathogens, particularly in ICUs and is associated with outbreaks of infection. (13) Their ubiquitous nature in the ICU environment and inadequate infection control practice has continuously raised the incidence of Acinetobacter infections over the past two decades. The understanding and recognition of Acinetobacter infections in the ICU is critically needed. (14)

In our study, a total number 88 Acinetobacter strains were isolated from processed clinical specimens. Houang et al., (15) reported a total of 1.32%. Patients in ICU are sicker and require more invasive monitoring and therapeutic procedures to survive. ICU environmental contamination appears to be another important source of Acinetobacter infection. (14) The development of ICU-acquired infections is strongly related to prolonged ICU stay and is associated with worse outcomes including increased morbidity and mortality.(18) In the present study, most common infections septicemias (blood 45-51.14%) followed by wound infections (pus 18-20.25%) pneumonia (sputum 20-11.36%) urinary tract infections (urine 15-17.05%). Joshi et al., (19) reported that 27.5 wound infections were caused by Acinetobacter. Acinetobacter ICU-acquired infections during the last decade represent a growing concern among clinicians and researchers. These infections most frequently involve the respiratory tract of intubated patients.(18)

In the present study, Acinetobacter infections were more common in males 64 (72.73%) as compared with females 24 (27.27%). This may be due to the fact that the males report more frequently to the hospitals compared with females. Prashanth and Badrinath(16) reported the infections to be more common in males (58.00%) compared with females (42.00%). Joshi et al., (19) reported 50.20% infection in males.

| Table.1 Age wise distribution of Acenitobacter spp |
|---------------------------------------------------|
| TOTAL MALE | 64 | 72.73 |
| TOTAL FEMALE | 24 | 27.27% |
| TOTAL | 88 | 100% |
### Table 2 Various clinical samples of *Acinetobacter* spp

| Sample   | Positive | %     |
|----------|----------|-------|
| BLOOD    | 45       | 51.14%|
| PUS      | 18       | 20.45%|
| URINE    | 15       | 17.05%|
| SPUTAM   | 10       | 11.36%|
| **Total**| 88       | 100.00%|

### Table 3 Age wise distribution

| <10 | NO OF MALE | 3 | 3.41% | NO OF FEMALE | 0 | 0.00% | 3 | 3.41% |
|-----|------------|---|-------|--------------|---|-------|---|-------|
| 10 TO 19 | NO OF MALE | 4 | 4.55% | NO OF FEMALE | 2 | 2.27% | 6 | 6.82% |
| 20 TO 29 | NO OF MALE | 8 | 9.09% | NO OF FEMALE | 4 | 4.55% | 12 | 13.64% |
| 30 TO 39 | NO OF MALE | 9 | 10.23% | NO OF FEMALE | 3 | 3.41% | 12 | 13.64% |
| 40 TO 49 | NO OF MALE | 11 | 12.50% | NO OF FEMALE | 7 | 7.95% | 18 | 20.45% |
| 50 TO 59 | NO OF MALE | 12 | 13.64% | NO OF FEMALE | 3 | 3.41% | 15 | 17.05% |
| 60 TO 69 | NO OF MALE | 14 | 15.91% | NO OF FEMALE | 3 | 3.41% | 17 | 19.32% |
| >70 | NO OF MALE | 3 | 3.41% | NO OF FEMALE | 2 | 2.27% | 5 | 5.68% |
| **Total** | **64** | **72.73%** | **24** | **27.27%** | **88** | **100.00%** |

### Table 4 Antimicrobial pattern

| Antimicrobial Agent | NO OF Resistance/% | NO OF Sensitivity/% | TOTAL |
|---------------------|---------------------|---------------------|-------|
| Ampicillin          | 70                  | 18                  | 88    |
| Cephotaxime         | 79.55%              | 20.45%              | 100.00%|
| Cefizime            | 57                  | 31                  | 88    |
| Co-trimoxazole      | 66                  | 22                  | 88    |
| Ciproflaxacin       | 49                  | 39                  | 88    |
| Cefixime            | 75.00%              | 25.00%              | 100.00%|
| Oflaxacin           | 34                  | 54                  | 88    |
| Gentamycin          | 36                  | 52                  | 88    |
| Amikacin            | 35                  | 53                  | 88    |
| Tigecyclin          | 39.77%              | 60.23%              | 100.00%|
| Amoxicillin +clavulanic | 61                | 27                  | 88    |
| Cepazone+subactin   | 69.32%              | 30.68%              | 100.00%|
| Cefotazone-clavulanicacid | 64              | 34                  | 88    |
| Piparacillin-tizobactam | 61            | 48                  | 88    |
| Imipenem            | 15                  | 73                  | 88    |
| Cefriaxone          | 17.05%              | 82.95%              | 100.00%|
| Colistin            | 70.45%              | 29.55%              | 100.00%|
| **Total**           | **88**              | **88**              | **100.00%** |
Currently at least 31 *Acinetobacter* genomspecies have been described. *Acinetobacter johnsonii*, *Acinetobacter lwofii* and *Acinetobacter radioresistant* seem to be natural inhabitants of human skin and commensals in human oropharynx and vagina. The digestive tract of patients within ICUs often serve as reservoirs for multiresistant *A. baumannii* strains involved in hospital outbreaks. The most common site for *A. baumannii* infection is the respiratory tract and the most common manifestation is VAP and bloodstream infections. *A. lwofii* has been more commonly associated with meningitis, *A. junii* rarely causes ocular infection and bacteremia. In our study, out of 88 *Acinetobacter* isolates, *A. baumannii* 66 (75.00%) was the most common species to cause *Acinetobacter* infection followed by acetonobacter lwofii 22 (25.00%) from 140 *Acinetobacter* isolates. Joshi et al., (19) isolated 70.00% *A. baumannii*, 1.40% *Acinetobacter calcoaceticus*, 6.40% *Acinetobacter haemolyticus*, 8.60% *A. junii* and 1.40% *A. johnsonii*. Prashanth and Badrinath (16) isolated 71.42% *A. baumannii*, 10.02% *A. lwofii*, 4.08% *A. haemolyticus* and 2.04% strains of *A. junii*.

As noted by the Infectious Disease Society of America, *Acinetobacter* is “a prime example of mismatch between unmet medical need and the current antimicrobial research and development pipeline.” *Acinetobacter* spp. are notorious for their ability to acquire antibiotic resistance. Antimicrobial resistance among *Acinetobacter* spp. has increased substantially in the past decade and has created a major public health dilemma. The most potent antibiotic drug class currently available are the carbapenems, but resistant strains have emerged. We have studied the antimicrobial resistance pattern among *Acinetobacter* isolates by Kirby-Bauer disc diffusion method. In our study, *Acinetobacter* isolates showed resistance to most of the antibiotics available. *Acinetobacter* spp. is universally resistant to penicillin, ampicillin and cephalothin. Various susceptibility to second and third generation cephalosporins have been reported. *Acinetobacter* species possess a wide array of β-lactamases that hydrolyze and confer resistance to penicillins, cephalosporins and carbapenems. AmpC cephalosporinases are chromosomally encoded and confer resistance to broad-spectrum cephalosporins. Class D oxacillin-hydrolyzing-type enzymes, Class B metallo β-lactamases (MBLs), hydrolyze a broad array of antimicrobial agents, including carbapenems. Increasing antimicrobial resistance leaves few therapeutic options for MDR *Acinetobacter* infection. In our study, Imipenem was most sensitive drug 73 (82.95%) followed by colistin 62 (70.45%), Tygycyclin 59 (67.05%), cprofloxacin 55 (62.50%), oflaxacin 54 (61.36%), amikacin 53 (60.23%) gentamycin 52 (59.09%). Highest resistance is seen in ampicillin. 70 (79.35%) followed by cefixime 66 (75.00%), ceftriaxone 62 (70.45%) amoxicillin + clavulanic acid 61 (69.23%), cephotoxime 57 (64.77%), ticarcillin + clavulanic acid 54 (61.35%) cotrimoxazole 49 (55.68%), piperacillin + tazobactim 40 (45.45%) (Table 4).

Sinha et al., (23) reported 35.00% Imipenem resistant *Acinetobacter*. Lee et al., (24) reported 21.18% Corbella et al.,(25) reported 36.00% carbapenem resistant *A. baumannii* from the patients admitted to ICU.

*Acinetobacter* are the “superbugs” of the modern hospital environment causing significant proportion of infections in specific patient populations, especially in critically-ill patients in the ICU. As ubiquitous organisms (fortunately of low virulence), with few requirements for growth and survival, *Acinetobacter* spp. are prone to persist indefinitely in the hospital environment and to
cause infections periodically when iatrogenic factors are present, i.e., overuse of broad spectrum antibiotics and high-risk patients. This situation, together with the fact that Acinetobacter isolates have inherent and/or easily acquired mechanisms of resistance against many of the available antimicrobial agents, makes this pathogen one of the most significant microbial challenges of the current era. Antibiotic resistance is attributed to production of extended spectrum beta-lactamase, MBL, loss of outer membrane proteins, efflux pumps and biofilm formation. Are there ways to control or limit the spread of these multiresistant strains? Is it still possible to treat Acinetobacter infections? First, it is necessary to improve microbiological techniques for early and more accurate identification and laboratory vigilance to prevent inappropriate empirical treatment.

Second, newer strategies for antibiotic use should be employed to reduce selection pressure, including more frequent rotation of antibiotic groups or sequential use of antibiotic classes. The development of totally new antibiotics with novel bacterial molecular target sites may constitute therapeutic alternatives within the next few years. Nevertheless, continued surveillance of prevalent organisms in ICUs, combined with preventive measures (e.g., isolation precautions, hand disinfection, efficient sterilization of instruments) remains absolutely essential in efforts to prevent or limit the spread of Acinetobacter infection. Continued awareness to maintain good housekeeping, control of the environment including equipment decontamination, strict attention to hand washing, isolation procedures and control of antibiotic usage, especially in high-risk areas, appear most likely measures to control the spread of Acinetobacter spp. in hospitals.

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