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 baumanii* was the most common species isolated from blood, (septisemeas) 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%), cefriaxone 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

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. *Acenetobactder baumanni* 66(75.00%) *Acenetobacter lowfii* 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, cephotoxime, cefizime, co-trimoxazole, ciprofloxacin, oflaxacin, gentamicin, amikasin, tygecyclin, amoxillin with clavulanic acid, ceforazolewith sulbactim, ticarcillin with clavulanic acid, piperacillin with tazobzctim, imipenem, cifriaxone 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 (59.095) 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 baumanni 66 (75.00%), followed by *Acenitobacter lowfii* 22 (25.00%). Imipenem was most sensitive drug 73 (82.95%) followed by colistin 62 (70.45%), Tigecyclin 59 (67.05%), ciproflaxacin 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%), cefriaxone 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 *Coccobacilli* 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%
	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%
		64	72.73%		24	27.27%	88	100%

Table.4 Antimicrobial pattern

Antimicrobial AgenT	NO OF Resistance/%	NO OF Sensitivity/%	TOTAL
Ampicillin	70	18	88
	79.55%	20.45%	100.00%
Cephotaxime	57	31	88
	64.77%	35.23%	100.00%
Cefizime	66	22	88
	75.00%	25.00%	100.00%
Co-trimoxazole	49	39	88
	55.68%	44.32%	100.00%
Ciproflaxacin	33	55	88
	37.50%	62.50%	100.00%
Oflaxacin	34	54	88
	38.64%	61.36%	100.00%
Gentamycin	36	52	88
	40.91%	59.09%	100.00%
Amikacin	35	53	88
	39.77%	60.23%	100.00%
Tigecyclin	29	59	88
	32.95%	67.05%	100.00%
Amoxicillin +clavulanic	61	27	88
	69.32%	30.68%	100.00%
Ceperazone+sulbactim	33	55	88
	37.50%	62.50%	100.00%
Tecarcillin +clavulanicacid	54	34	88
	61.36%	38.64%	100.00%
Piparacillin+tzobactam	40	48	88
	45.45%	54.55%	100.00%
Imepenium	15	73	88
	17.05%	82.95%	100.00%
Cefriaxone	62	26	88
	70.45%	29.55%	100.00%
Colistin	26	62	88
	29.55%	70.45%	100.00%

Currently at least 31 *Acinetobacter* genomospecies have been described. *Acinetobacter johnsonii*, *Acinetobacter lwoffii* and *Acinetobacter radioresistant* seem to be natural inhabitants of human skin and commensals in human oropharynx and vagina.(5) The digestive tract of patients within ICUs often serve as reservoirs for multiresistant *A. baumannii* strains involved in hospital outbreaks.(2) The most common site for *A. baumannii* infection is the respiratory tract and the most common manifestation is VAP and bloodstream infections. *A. lwoffii* has been more commonly associated with meningitis, *A. junii* rarely causes ocular infection and bacteremia.(5) In our study, out of 88 *Acinetobacter* isolates, *A. baumannii* 66(75.00%) was the most common species to cause *Acinetobacter* infection followed by acenitobacter lowfii 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. lwoffii*, 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.(22) 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.(7) 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.(5) *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%), cproflaxacin 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%), cefriaxone 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).

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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