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CLINICAL AND ANTIMICROBIAL PROFILE OF ACINETOBACTER SPECIES AT TERTIARY CARE HOSPITAL IN CENTRAL INDIA

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ABSTRACT: BACKGROUND: Acinetobacter are the "superbugs" of the modern hospital environment causing significant proportion of infections and in particular nosocomial infections with high mortality rates. The aim of this study was to isolate Acinetobacter species from clinical specimens and to study the antimicrobial susceptibility pattern of Acinetobacter isolates. **MATERIAL AND METHODS:** Two hundred and four clinical isolates of Acinetobacter species were processed for species identification by standard microbiological procedures. Antimicrobial susceptibility of these isolates was performed by Kirby-Bauer disc diffusion method. **RESULTS:** Out of 204 Acinetobacter isolates, 125(61.27%) isolates were from ICU and 79(38.72%) were from general wards. A baumannii was the most common species isolated (74.50%), followed by A.lwoffii (24.50%) and A.haemolyticus (0.98%). A.baumannii showed maximum sensitivity to IPM (52.63%) followed by MRP(36.18%), AK(28.28%), PIT(26.31%), TCC(21.71%), CIP(21.05%) G(17.76%) and COT(05.26%). Maximum resistance was observed to CTX(1.31%) followed by CAZ(1.97%), CTR(1.97%) and CPM(1.97%) respectively. A.lwoffii showed maximum sensitivity to IPM(94%) followed by AK(90%), and MRP(84%). Statistically significant difference (p value <0.001) was noticed between antibiotic resistance of A.baumannii and A.lwoffii. **CONCLUSION**: Continued surveillance of drug resistant strains in ICUs, combined with preventive measures remains absolutely essential to prevent or limit the spread of Acinetobacter species in hospital.

KEYWORDS: Acinetobacter.

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

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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.

MATERIAL AND METHODS: The study was carried out in the Department of Microbiology from January 2012 to December 2014. Relevant clinical specimens were collected from ICU and Wards by standard collection procedures. No specific exclusion criteria envisaged. Specimens were processed by standard microbiological techniques.[3] Non-fermenters were initially separated and further identified as Acinetobacter spp. 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 Mac Conkey 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 Mac Conkey 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 isolates confirmed by the above standard microbiological tests were further speciated as per the following scheme of identification.[3, 5] [Table 1].

Antimicrobial susceptibility testing [3] was performed by modified Kirby Bauer method [8] as per the Clinical and Laboratory Standards Institute guidelines.[9] Antibiotics tested were Amikacin (AK), Gentamicin (GM), Ciprofloxacin (CIP), Trimethoprim- Sulfamethoxazole (COT), Ceftazidime (CAZ), Ceftriaxone (CTR), Cefotaxime (CTX), Cefepime (CPM), Piperacillin-tazobactam (PIT), Ticarcillin-clavulanic acid (TCC), Imipenem (IPM), Meropenem (MRP), Colistin (CL), Polymyxin (PB).

Statistical analysis: P value was reported and a value of $P < 0.05$ was considered as a significant. The statistical analysis was performed using the Chi-square test.

RESULTS: A total of 204 non duplicate, non consecutive Acinetobacter isolates were processed for species identification, and antimicrobial susceptibility. Out of 204 Acinetobacter isolates, 125(61.27%) isolates were from ICU and 79(38.72%) were from general wards. Significantly higher percentage of Acinetobacter strains were found in ICU compared with general wards.

In the present study maximum number of Acinetobacter isolates were from respiratory (35.78%), followed by pus (32.84%), blood (23.52%), body fluids (03.92%) and urine (03.92%). Most common Acinetobacter species isolated was Acinetobacter baumannii (74.50%), followed by Acinetobacter lwoffii (24.50%) and Acinetobacter haemolyticus (0.98%) [Table 2]

A. baumannii was the most common species responsible for pneumonia (34.21%), wound infection (27.63%), septicaemia (30.26%), peritonitis (03.94%) and urinary tract infection (03.94%), [Table 2].

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The antimicrobial susceptibity testing pattern of A.baumannii and A.lwoffii is shown in Table 3. On comparing the antibiotic resistance between A.baumannii complex and A.lwoffii significant difference in terms of p value (<0.001) was observed for most of the antibiotics.

A.baumannii showed maximum sensitivity to IPM (52.63%) followed by MRP (36.18%), AK (28.28%), PIT (26.31%), TCC (21.71%), CIP (21.05%) G(17.76%) and COT (05.26%). Maximum resistance was observed to CTX (1.31%) followed by CAZ (1.97%), CTR (1.97%) and CPM (1.97%) respectively. A.lwoffii showed maximum sensitivity to IPM (94%) followed by AK (90%), and MRP (84%).

DISCUSSION: Acinetobacter is an important nosocomial pathogen with high mortality rates..Acinetobacter spp. is the second most commonly isolated non-fermenter in human specimens (afterPseudomonas aeruginosa). They rank fourth (after P. aeruginosa, Stapylococcus aureus and Klebsiella pneumoniae) among the most frequent hospital acquired infectious agents.[10]

Acinetobacter spp. has emerged as a cause of ICUs infection. Multiresistant Acinetobacter spp. have become established as "alert" pathogens, particularly in ICUs and are associated with outbreaks of infection.[11] Their ubiquitous nature in the ICU environment and inadequate infection control practice have continuously raised the incidence of Acinetobacter infections over the past two decades. [12]

In our study, out of 204, 125(61.27%) isolates were from ICU and 79(38.72%) were from general wards. Acinetobacter infections were more common in ICU as compared with general wards. Various other studies have reported the rate of isolation varying from 4.25% to 20.1%.[13, 14, 15.] This variation can be attributed to the varying prevalence rates of different Acinetobacter species in the hospital environment and the community in different geographical areas. Like many other previous studies the species most commonly isolated from the clinical samples in our institution was A. baumannii 152(74.50%), followed by A. lwoffii 50 (24.50%) and A. haemolyticus 02 (0.98%).[13, 16,17]

The most common infection caused by Acinetobacter species in our study was the pneumonia followed closely by the wound infection. Occurrence of Acinetobacter is contributed by several factors including immunosuppressed hosts, patients with severe underlying disease, previous use of antibiotics, duration of hospital stay and more frequent use of antibiotics in ICU. 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.[18] The development of ICU-acquired infections is strongly related to prolonged ICU stay and is associated with worse outcomes including increased morbidity and mortality.[19]

In the present study maximum number of Acinetobacter isolates were from respiratory (35.78%), followed by pus (32.84%), blood (23.52%), body fluids (03.92%) and urine (03.92%). Most common Acinetobacter species isolated was Acinetobacter baumannii (74.50%), followed by Acinetobacter lwoffii (24.50%) and Acinetobacter haemolyticus (0.98%) [Table 2].

Pooja single et al isolated 25.6% of Acinetobacter isolates from the respiratory tract.This concludes that Acinetobacter infections most frequently involve the respiratory tract of intubated patients.[20] Study also shows 32.84% isolation of Acinetobacter species from pus followed by blood (23.52 %). [Table 2] Almost similar results were observed in study conducted by Isahi S et al, who isolated 36.95% and 23.91% Acinetobacter species from pus and blood respectively.[21] Study also

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revealed 03.92% isolation body fluids and urine each, while study conducted by Purti et al isolated 0.94% and 12.14% from body fluids and urine respectively.[22]

In our study, out of the 204 Acinetobacter isolates, A. baumannii (74.50%) was the most common species to cause Acinetobacter infection [Table 2]. From 140 Acinetobacter isolates, Joshi et al.[23] 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 [24] isolated 71.42% A. baumannii, 10.02% A. lwoffii, 4.08% A. haemolyticus and 2.04% strains of A. junii.

Acinetobacter spp. is notorious for their ability to acquire antibiotic resistance. [25]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 classes 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.

A.baumannii showed maximum sensitivity to IPM (52.63%) followed by MRP (36.18%), AK (28.28%), PIT (26.31%), TCC (21.71%), CIP (21.05%) G (17.76%) and COT(05.26%). Maximum resistance was observed to CTX (1.31%) followed by CAZ (1.97%), CTR (1.97%) and CPM (1.97%) respectively. A.lwoffii showed maximum sensitivity to IPM (94%) followed by AK (90%) and MRP (84%).None of the isolate was resistant to colistin and polymyxins. 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.

CONCLUSION: 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, Acinetobacter spp. are prone to persist indefinitely in the hospital environment and 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.

It is therefore necessary to improve microbiological techniques for early and more accurate identification and laboratory vigilance to prevent inappropriate empirical treatment. Nevertheless, continued surveillance of prevalent organisms in ICUs, combined with preventive measures 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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Species	Haemolysis on sheep blood agar	Hugh-Leifson (Oxidative/Fermentative) test	Citrate Test	Arginine dihydrolase test	Gelatin Liquifaction
A.baumannii	-	+	+	+	-
A.lwoffii	-	-	-	-	-
A.haemolyticus	+	+	-	-	+
Table 1: Differentiation of Acinetobacter species					

Acinetobacter Infections	Clinical Sample (n=204)	A.baumannii (n=152)	A.lwoffii (n=50)	A.haemolyticus (n=02)	Total (n=204)
Pneumonia	Respiratory	52 (34.21)	21 (42)	00	73 (35.78)
Wound Infection	Pus	42 (27.63)	23 (46)	02 (100)	67 (32.84)
Septicaemia	Blood	46 (30.26)	02 (04)	00	48 (23.52)
Peritonitis	Ascitic Fluid	06 (03.94)	02 (04)	00	08 (03.92)
Urinary Tract Infection	Urine	06 (03.94)	02 (04)	00	08 (03.92)
Total	204	152 (74.50)	50 (24.50)	02 (0.98)	204
Table 2: Distribution of Acinetobacter species					

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Antimicrobial agents	A.baumannii (n=152)	A.lwoffii (n=50)	P value
Amikacin	43 (28.28)	45 (90)	< 0.001
Gentamicin	27 (17.76)	37 (74)	< 0.001
Ciprofloxacin	32 (21.05)	34 (68)	< 0.001
Cotrimoxazole	08 (05.26)	29 (58)	< 0.001
Ceftazidime	03 (1.97)	03 (06)	< 0.005
Ceftriaxone	03 (1.97)	16 (32)	< 0.001
Cefotaxime	02 (1.31)	21(42)	< 0.001
Cefepime	03 (1.97)	15 (03)	< 0.001
Piperacillin-Tazobactum	40 (26.31)	34 (68)	< 0.001
Ticarcillin-clavulanic acid	33 (21.71)	41(82)	< 0.001
Imipenem	80 (52.63)	47 (94)	< 0.001
Meropenem	55 (36.18)	42 (84)	< 0.001
Colistin	152 (100)	152 (100)	-
Polymyxin	152 (100)	152 (100)	-
Table 3: Comparison of antimicrobial susceptibility of A.baumannii and A.lwoffii			

Antimicrobial agents		Amikacin	Gentamicin	Ciprofloxacin	Cotrimoxazole	Ceftazidime	Ceftriaxone	Cefepime	Piperacillin-tazobactum	Ticarcillin-clavulanic acid	Imipenem	Meropenem
A.baumannii (n=152)	ICU=98	16 (16.32)	-	-	-	02 (2.04)	01 (1.02)	02 (2.04)	14 (14.28)	13 (13.26)	47 (47.95)	22 (22.44)
	Ward=54	27 (50)	27 (50)	32 (59.25)	08 (14.81)	01 (1.85)	02 (3.70)	01 (1.85)	26 (26.53)	20 (37.03)	33 (61.11)	33 (61.11)
A.lwoffii (n=50)	ICU=25	21 (84)	17 (68)	14 (56)	14 (56)	01 (4)	-	03 (12)	15 (60)	19 (76)	24 (96)	19 (76)
	Ward=25	24 (96)	20 (80)	20 (80)	15 (60)	02 (8)	16 (64)	12 (48)	19 (76)	22 (88)	23 (92)	23 (92)
A.haemolyticus (n=02)	ICU=02	01 (50)	-	01 (50)	02 (100)	-	-	-	02 (100)	-	02 (100)	-
	Ward=00	-	-	-	-	-	-	-	-	-	-	-
Table 4: Antimicrobial susceptibility pattern of Acinetobacter species recovered from ICU and Ward patients												

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