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A Study On Phenotypic Characterisation And Antimicrobial Susceptibility Pattern Of Acinetobacter Isolates From Various Clinical Samples In A Tertiary Care Hospital

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Abstract : Acinetobacter spp. have emerged as an important nosocomial pathogen,especially in intensive care settings1,2. Despite their low pathogenic potential they are being reported increasingly as the causal organism of numerous hospital outbreaks in several countries3,4 .In a recent international multicentre study,Acinetobacter spp were ranked amongst the 10 organisms most commonly causing septicaemia in 18 of 44 large European hospitals5.Different levels and patterns of antimicrobial susceptibilities have been found among different Acinetobacter species6.Several studies reported a higher occurrence of multidrug resistance in A.baumannii compared with non-A.baumannii species7,8.Intraspecies diversity of antimicrobial susceptibilities has also been reported.Hence this study was done to speciate and to determine the Antimicrobial susceptibility of Acinetobacter isolates in our Tertiary care hospital. In this study,a total of 6553 samples like blood,CSF,and other bodyfluids were analyzed.The period of study was for 3 months from August 2011-October 2011. Totally 543 non-fermenters were isolated ,out of which 100 were Acinetobacter.Speciation was done using simplified phenotypic tests,which showed A.calcoaceticus-baumanii complex (87),A.lwoffi (7),A.junii(4),A.hemolyticus(2). Resistance pattern to various drugs were cefoperazonesulbactum (11), Amikacin (43), Ofloxacin(49), Ceftazidime(55), Gentamicin(57), Chloramphenicol (93).

Keyword : Acinetobacter,Non-fermenter,Nosocomial <!-- /* Font Definitions */ @font-face {font-family:Calibri; panose-1:2 15 5 2 2 2 4 3 2 4; mso-font-charset:0; mso-generic-font-family:swiss; mso-font-pitch:variable; mso-font-signature:-520092929 1073786111 9 0 415 0;} /* Style Definitions */ p.MsoNormal, li.Mso Normal, div. Mso Normal {mso-style-unhide: no; mso-style—qformat: yes; mso-style-parent:""; margin-top:0cm; margin-right:0cm; margin-bottom:10.0pt; margin-left:0cm; line-height:115%; mso-pagination: widow-orphan; font- size : 11.0pt; font-family:" Calibri","sans-serif"; mso-ascii-font-family: Calibri; mso-ascii-theme-font:minor-latin; mso-fareast-font-family: "Times New Roman"; mso-fareast-theme-font: minor -fareast; mso-hansi-font-family:Calibri; mso-hansi-theme-font: minor-latin; mso-bidi-font-family:"Times New Roman"; mso- bidi-theme-font:minor-bidi; mso-ansi-language:EN-US; mso-fareast-language:EN-US;} .MsoChpDefault {mso-style-type: export-only; mso-default-props:yes; font-family:"Calibri","sans-serif";mso-ascii - font—family: Calibri; mso - ascii - theme-font:minor- latin; mso-fareast-font-family:"Times New Roman"; mso- fareast-theme - font:minor-fareast; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi; mso-ansi-language: EN-US; mso-fareast-language:EN-US;} .MsoPapDefault {mso-style-type:export-only; margin-bottom:10.0pt;line-height:115%;} @page WordSection1 {size:612.0pt 792.0pt; margin:72.0pt 72. 0pt 72.0pt 72.0pt; mso-header-margin:36.0pt; mso-footer-margin:36.0pt; mso-paper-source:0;} div.WordSection1 {page:WordSection1;} -->

Introduction Acinetobacter species are ubiquitous and commonly present in soil and water as free living saprophytes.Bacteria belonging to the Genus Acinetobacter are non-motile, non-fermentative, Gram negative coccobacilli that can be easily isolated with appropriate enrichment techniques from soil,water,sewage,human skin and a variety of large foodstuffs.They are isolated as commensals from skin and throat . Acinetobacter has emerged as an important nosocomial pathogen involved in outbreaks of hospital infections.This organism has been recovered from hospital environment,from colonized or infected patients or from hospital staff (Hand carriage)[9].In India,very few studies of Acinetobacter spp have been reported.In view of their increasing importance in nosocomial infections,further study is warranted in this part of the world[10,11].

Despite the increasing significance and frequency of multidrug resistant Acinetobacter infections ,many clinicians still lack an appreciation of importance of these organisms because of their confused taxonomic status [12].The aim of this study is to speciate the Acinetobacter isolates obtained from various clinical samples by a simplified phenotypic identification method and also to determine their antimicrobial susceptibility pattern.

Materials and Methods : The study was conducted in a tertiary care Hospital,Chennai from August 2011 to October 2011.A total of 6553 samples like Blood,Sputum,Pus,CSF and other body fluids were subjected to phenotypic identification and Antimicrobial Susceptibility testing was done. Presumptive identification of Acinetobacter was made by inoculation on Mac conkey agar medium,incubated at 37 C for 24 hrs.All non lactose fermenting colonies were subjected to Gram staining,catalase,Oxidase ,Triple sugar iron medium inoculation and motility testing by hanging drop method. Acinetobacter species are Gram negative coccobacilli, catalase positive,

oxidase negative and non-motile.Bacteria showing the above features were taken and further speciation was done using special tests.(Table-4) .Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method (Table-7)

Results: During the study period ,a total of 6553 samples were examined from patients of different age groups admitted in various wards in Govt General Hospital.Nonfermenter isolates accounted for 8.2% of the total organisms and Acinetobacter isolates accounted for 18.4% of total number of non-fermenters isolated. Male to female ratio was 1.2:1. Acinetobacter infection was more common in patients of age more than 40 years.

Table 1:
AGE DISTRIBUTION OF PATIENTS

Age	No of patients
0-20	8
21-40	29
41-60	37
>60	26

Table2:
GENDER DISTRIBUTION OF PATIENTS

Sex	Number	Percentage
Male	56	56%
Female	44	44%

Table 3: NUMBER OF NONFERMENTERS AND ACINETOBACTER SPECIES ISOLATED FROM VARIOUS CLINICAL SAMPLES

Specimen	Total number of samples (n=6553)	Nonfermenters isolated	Acinetobacter isolated
Pus/swab	2539	229	34
Sputum	651	161	39
Bronchial wash/pleural fluid	548	22	2/1
Blood	1811	51	9
Asciticfluid, peritoneal dialysis fluid, cerebrospinal fluid	468	26	1/2/1
Others (tracheal culture, endotracheal tip)	536	54	11
Total	6553	543	100

Table 4:
IDENTIFICATION METHOD OF ACINETOBACTER

Species (Total number)	Hemolysis on BAP	Growth 37c/42c		OF test	Arginine	Malonate utilization	Gelatin liquefaction	Chloramphenicol sensitivity
A.baumanii (73)	-	+	+	Saccharolytic(S)	+	+	-	R
A.calcoaceticus (14)	-	+	-	S	+	+	-	R
A.lwoffi (7)	-	+	-	NS	-	+ (2%)	-	S
A.juni (4)	-	+	-	NS	+	-	-	R
A.hemolyticus (2)	+	+	-	S(75%)	+	-	+	R

(OF Hugh and leifson's oxidative-fermentative test, NS-Non saccharolytic, S-sensitive, R-resistant.)

Table 5:

Name of ward	Number of organisms isolated
Thoracic Medicine	31 (31%)
Surgical ward	27 (27%)
Intensive care unit	25 (25%)
Medicine	17 (17%)
Total	100

DISTRIBUTION OF ACINETOBACTER IN WARDS

Table 6:
DISTRIBUTION OF ACINETOBACTER SPECIES IN VARIOUS CLINICAL SAMPLES

Species N=100	Sputum	Pus	Tracheal c/s	Blood	Bronchial wash	Peritoneal dialysis fluid	Ascitic fluid	Pleural fluid	CSF
A.baumanii (73)	26	24	11	6	2	1	1	1	1
A.calcoaceticus (14)	8	3	-	2	-	1	-	-	-
A.lwoffi (7)	2	5	-	-	-	-	-	-	-
A.junii (4)	1	2	-	1	-	-	-	-	-
A.hemolyticu (2)	2	-	-	-	-	-	-	-	-

Table 7: SENSITIVITY PATTERN OF ACINETOBACTER ISOLATED TO DIFFERENT ANTIBIOTICS
N=100

Antibiotics	Sensitivity (s)	Resistant
Cefoperazonesulbactum (CS) 75/30µg/disc	89%	11%
Imipenam (I) 10µg/disc	100%	-
Amikacin (AK) 30µg/disc	57%	43%
Ofloxacin(OF) 5µg/disc	51%	49%
Ceftazidime(CZ) 30µg/ disc	45%	55%
Gentamicin(G)	43%	57%
10µg/disc		
Chloramphenicol(C)	7%	93%
30µg/disc		

Discussion : A total of 6553 samples were processed. 543 were non-fermenters which accounted for 8.2% of total number of organisms isolated.Pseudomonas was the most common non-fermenter isolated (81.6% of total nonfermenters). Acinetobacter accounted for18.4% of total non-fermenters. In this study 87% of strains belongs to Acinetobacter calcoaceticus baumannii complex.other species include A.lwoffi 7 isolates (7%),A.junii 4 isolates (4%),A.hemolyticus 2 isolates (2%). Isolation rate was higher from sputum followed by pus.In all the samples A.baumannii is the most common isolate,but predominant isolation is from sputum.A.calcoaceticus is predominantly isolated from pus.A.lwoffi and A.junii were commonly isolated from pus. The two strains of A.hemolyticus were isolated from sputum. They suffered from recurrent bronchiectasis. Among the 8 strains isolated from blood ,four strains were isolated three to four times repeatedly.

Most of these patients had respiratory problems like chronic obstructive pulmonary disease (COPD),bronchial asthma and respiratory failure.Also majority of them had wound infections from postoperative site and cellulitis of wound. One isolate was from CSF. The patient was a 9 year old boy,who suffered from cerebellar astrocytoma. He was given ventriculoperitoneal shunt ,which was infected and removed . In this study Acinetobacter were isolated more commonly from thoracic medicine wards(31%) followed by surgical wards(27%) followed by ICU(25%). Studies from various countries have shown predominant isolation from tracheobronchial secretions (24.8-48.8%)[13].In the present study Acinetobacter was isolated from sputum (39%),and other respiratory tract secretions (14%). The male to female ratio is 1.2:1 in this study .This is similar to the study done in Hong Kong by TK et al in 1993-1994[14]. In a study conducted by Anupurba S et al[15] in 2005,20.8% of Acinetobacter were isolated from ICU ,whereas in present study it is 25%.This shows increasing trend of Acinetobacter to cause nosocomial infections .Genus Acinetobacter has the ability to develop antibiotic resistance extremely rapid in response to challenge with new antibiotics.In the present study , 93% of strains were resistant to chloramphenicol,52% to gentamycin,55% to ceftazidime,49% to ofloxacin,43% to amikacin ,11% to cefaprazone sulbactum.This is similar to study conducted by Capoor et al[16]. Acinetobacter baumannii calcoaceticus complex showed more resistance to Aminoglycosides and cephalosporins than other species.

Conclusion: There is an increased rate of isolation of non-fermentative gram negative bacilli now-a-days. Pseudomonas aeruginosa is the most common non-fermenter followed by Acinetobacter.Acinetobacter is emerging as an important nosocomial pathogen and also causes community acquired infections.Hence identification and speciation of Acine tobacter should be done as a routine .Traditional typing methods like phenotyping and antibiogram have advantage over genotyping as they are readily available and cost effective.

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