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Research Article

CHARACTERIZATION, ANTIBIOTIC RESISTANCE PATTERN AND EXTENDED SPECTRUM BETA- LACTAMASE (ESBL) PRODUCTION OF *ACINETOBACTER* SPECIES IN A TERTIARY CARE HOSPITAL OF NORTH KERALA

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Abstract- Background: *Acinetobacter* spp. is non-fermenting Gram negative coccobacilli, associated with nosocomial infections and show widespread resistance to various antibiotics. **Aim:** To characterize *Acinetobacter* Species from clinical samples and to study their antibiotic resistance pattern and Extended Spectrum Beta-Lactamase (ESBL) Production in a tertiary care hospital of north Kerala. **Materials and Methods:** A retrospective study carried out on clinical samples -blood, urine, respiratory secretion and exudates- from January 2016 to December 2016 according to standard protocol. Data Analysis was done by using *WHO NET ANTIBIOTIC RESISTANCE SURVEILLANCE SOFTWARE;* data was analyzed using EPI INFO 2013 software. **Result:** Out of 10803 samples taken up for study, culture is positive in 3218 and *Acinetobacter* species is isolated in 192, giving an overall isolation rate of 6.0 %. The most vulnerable age group is between 61 to 70 years and those above the age of 50 constituted 67.7 %. Out of 192 *Acinetobacter* isolates, *A. baumanii* is predominant species having recovered from 175(91.1%). The most active antibacterial agents are carbapenems. **Conclusion:** As *Acinetobacter* spp. is associated with nosocomial infections with widespread resistance to antibiotics, characterization and determination of antibiotic resistance pattern is mandatory for proper management of infections caused by them

Keywords- *Acinetobacter,* Characterization, Extended Spectrum Beta- Lactamase (ESBL), nosocomial infections.

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Introduction

Acinetobacter, an aerobic, catalase- positive, oxidase- negative, Gram –negative coccobacillus is ubiquitous in nature and is associated with health care infections. [1] They are saprophytes in nature or commensals in human beings and are commonly seen in hospital environment [2] and have been associated with a wide variety of illnesses in hospitalized patients, especially in intensive care units. [3] The pathogenic potential has been proved beyond doubt by their frequent isolation from clinical samples and their association with disease. [4] Members of the genus *Acinetobacter* survive a long time in the hospital environment and exhibit widespread resistance to various antibiotics including beta lactam and carbapenems and infections caused by them are often difficult to treat. [5] A. baumannii is the species most often responsible for nosocomial infections [6] exhibiting increased antimicrobial resistance and Extended spectrum beta lactamase (ESBL) associated resistance among Acinetobacter species is now known. [3] This study was undertaken to characterize the *Acinetobacter* spp isolated from various clinical samples, analyze their anti-microbial resistance pattern and identify the production ESBLs as early diagnosis and appropriate antibiotic treatment is mandatory for management of infections due to A*cinetobacter* Spp.

Materials and Methods

After getting approval from Institutional ethics committee, a retrospective study was carried out, based on review of records of 10803 patients from whom samples of blood, urine, respiratory secretion and pus were collected and processed in the diagnostic section of Department of Microbiology, from January 2016 to December 2016, meeting inclusion and exclusion criteria. The basic socio-demographic information and data regarding age, gender and *Acinetobacter* isolates from samples of blood, urine, respiratory secretion, and pus and their antibacterial resistance pattern were collected using predesigned Performa, according to standard protocol.

All specimens of blood, urine, respiratory secretion and pus were subjected to culture by inoculating onto 5% sheep Blood and McConkey agar plates and incubated at 37°C for 48 hours and Gramstain.[7] The bacterial isolates were identified based on the study on colony morphology, gram stain and biochemical reactions[8].The following biochemical tests- fermentation of 10% lactose, decarboxylation of arginine, ornithine and lysine, oxidative fermentation of (OF) of Hugh-Leifson- glucose, lactose, sucrose, mannitol, maltose and xylose and arabinose fermentation tests were done**.** Antibiotic susceptibility testing for the isolates was performed on Muller Hinton agar by the 'Kirby Bauer disc diffusion' method as recommended by the Clinical and Laboratory Standards Institute. [9]

The antimicrobials for the *Acinetobacter* isolates, gentamycin (10µg), amikacin (30µg), ceftazidime (30µg), ceftazidime+ clavulanic acid (30µg+10µg), cefipime (30µg), ciprofloxacin (5 µg), piperacillin+ tazobactam (100µg+10µg), imipenem (10 µg), meropenem (10 µg) were used. The resistance and susceptibility were interpreted according to CLSI guidelines. ESBL production among *Acinetobacter* Spp. was detected by the combined Disc Diffusion Test, using ceftazidime (30 mcg) and ceftazidime + clavulanic acid (30+10 mcg) discs according to the CLSI guidelines. Organism was considered as ESBL producer if there was >/= 5 mm increase in zone of inhibition of ceftazidime + clavulanic acid disc, as compared to disc with ceftazidime alone.

Pseudomonas aeruginosa (ATCC 27853) was used as reference strains. Data Analysis was done by using WHO NET ANTIBIOTIC RESISTANCE SURVEILLANCE SOFTWARE; data was entered in Excel and analyzed using EPI INFO 2013 software.

Result

Out of the total of 10803 samples of blood, urine, respiratory secretion and pus taken up for the study, culture was positive for growth of microorganisms in 3218 and *Acinetobacter* species was isolated in 192, giving an overall isolation rate of 6.0 %. Specimen wise distribution of *Acinetobacter* Spp. is given in [Table-1].The source of *Acinetobacter* Spp. was most often respiratory samples (13. 1%)

Of these, the incidence was 59.3% and 40.7% in male and female patients respectively. In relation to age factor, the most vulnerable age group was between 61 to 70 years and the incidence was 30.7% and those above the age of 50 constituted 67.7 %. Age and gender distribution among 192 culture positive samples with growth of *Acinetbacter* Spp is shown in [Table-2]. Out of a total of 192 *Acinetobacter* isolates, *A. baumannii* was recovered from 175(91.1%), followed by *A. lowffi* in 10 (5.2%) and *A. hemolyticus* in 7(3.7%) of patients. [Fig-1]

The *Acinetobacter* isolates show least resistance to carbapenems, 22.4% and 26.6% to meropenem and imipenem respectively. Antibiotic resistance pattern of *Acinetobacter spp*is depicted in [Table-3]: Of all the isolates 68.5% were ESBL producers detected by the combined Disc Diffusion Test, using ceftazidime (30 mcg) and ceftazidime + clavulanic acid (30+10 mcg) discs.

Table-1 *Specimen- wise distribution of Acinetobacter Spp.*

Specimen	Total number of specimens	Total number of culture positive specimens	Number of *acinetobacter* Spp. isolated from culture positive specimens	Percentage of *acinetobacter* Spp out of culture positive samples
Blood	3269	327	23	7
Respiratory secretion	1699	686	90	13.1
Pus	2106	973	25	2.6
urine	3729	1232	54	4.4
Total	10803	3218	192	6

Table-2 *Age and gender distribution among 192 culture positive samples with growth of Acinetbacter Spp.*

Age in years	Gender		Number of patients
	M	F	
NB	2	2	4(2.1)
<10 yrs	3	2	5 (2.6)
11-20 yrs	4	6	10 (5.2)
21-30 yrs	10	5	15 (7.8)
31-40 yrs	5	5	10 (5.2)
41-50 yrs	12	6	18 (9.4)
51-60 yrs	18	10	28 (14.6)
61-70 yrs	34	25	59 (30.7)
>70 yrs	26	17	43 (22.4)
Total	114(59. 3%)	78(40.7)	192(100.0%

Figures in parenthesis indicate percentage

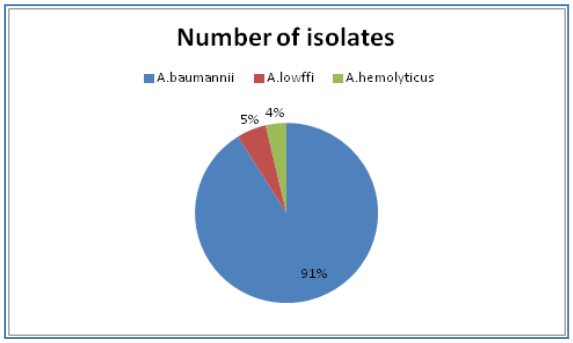

Fig-1 Species-wise distribution of *Acinetobacter* organisms

Table-3 *Antibiotic resistance pattern of Acinetobacter Spp(192)*

Antibiotic	Number of organisms showing resistance	% of resistance
Meropenem	43	22.4
Imipenem	51	26.6
Piperacillin/ tazobactem	67	35
Cefipime	67	35
Amikacin	74	38.5
Gentamicin	79	41.1
Ciprofloxacin	98	51
Ceftazidime	125	65

Total number of isolates given in parenthesis.

Discussion

The percentage of isolation of *Acinetobacter* Spp. from clinical samples varied from 3.3% -12.9%as reported by different authors.[2,10,11and 12]. In our study, out of a total of 3218 culture positive samples, 192 showed growth of *Acinetobacter* Spp giving an isolation rate of 6 %. Among the *Acinetobacter* Spp., *A. baumannii* is the predominant species having isolated from 175 samples (91.1%) followed by *A.lowffi* in 10 (5.2%) and *A. hemolyticus* in 7(3.7%). Similar studies too show preponderance of *Acinetobacter baumannii* over other species like *A. lowffi, A. hemolyticus, A. johnsonii, A. junii* and the isolation rate differs from 54 % to 78 % [2, 4, 12, and 13]

In a study by Gales the carbapenems were the most active antimicrobials against *Acinetobacter* species showing 11% resistance.[14] In our study also the most active antibacterial agents against *Acinetobacter* species are carbapenems, as the isolates show least resistance to carbapenems, 22.4% and 26.6% to meropenem and imipenem respectively, as compared to other agents. However a study by Abouseda reports 78 % of A. Baumannii as carabapenamase producers detected by the use of matrix assisted laser desorption ionisation time of flight mass spectrometry (MALDI TOF MS). [15] The percentage of ESBL production by *Acinetobacter* varied from 28 to 78.5% isolates as reported by various authors. [3, 12, 13 and16] In our study the ESBL production was shown by 68.5% of all the isolates. They possess different types of Beta-lactamases like SHV, TEM and others which lead to treatment failure in case of infections due to this pathogen. [13]. Multidrug resistant Acinetobacter Spp can cause outbreak of infection in the neonatal unit as reported by Mittal N. [17]

Conclusion

Acinetobacter spp. is non-fermenting Gram negative coccobacilli ubiquitous in nature and is associated with various nosocomial infections. Members of the genus *Acinetobacter* show widespread resistance to various antibiotics. They possess different types of Beta-lactamases that can lead to treatment failure. Therefore characterization and determination of antibiotic resistance pattern of *Acinetobacter* Spp is mandatory for proper management of infections caused by them.

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Author Contributions All authors equally contributed

Conflict of Interest Nil

Ethical Committee Approval Number: No. IEC/MES/23/2017

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