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ISOLATION AND IDENTIFICATION OF ACINETOBACTER SPECIES FROM VARIOUS CLINICAL SAMPLES AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN

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ABSTRACT

The present study, totally 2710 samples taken, number of Acinetobacter positive cases were 58 (2.1%). Out of total 775, 1699 and 236 samples of wound/pus, urine and sputum samples number of Acinetobacter isolated were 36 (27.1 %), 16(13%) and 6(12.5 %) respectively. Out of total 58 Acinetobacter positive cases, maximum was from wound/pus (36) and least was from sputum (6) samples. A. baumannii species was the maximum (34) out all the samples, compared to other species. Out of these 34 cases of A. baumannii, maximum positive cases were isolated from wound (22) and least from sputum (2) samples. Of the total 58 Acinetobacter isolated, maximum was from ICU (20cases) and least from Gynaecology and Urology departments (2cases each). Out of total 58 Acinetobacter positive cases, more number of cases was between the age group 31-45 years and least between 0-15 years. 30 cases (52%) were males and 28 (48%) were females. In the whole year maximum isolation of Acinetobacter was between the months of July to September. Out of 16 antimicrobials tested, for 34 A. bowmannii isolates (by Kirby Buer Disk Diffusion method and vitek for colistin), maximum resistance was for Ciprofloxacin (97%) and 100% susceptibility was seen for Colistin, Tobramycin, Minocycline and Tigecycline. Acinetobacterbowmannii showed more resistant to antibiotics compared to other species (A. lwoffii, Acalcoaceticus, A.hemolyticus). 21 isolates (61.8%) of Acinetobacterbowmannii was multi drug resistant and mainly from ICU patients i.e., 15 isolates (71.4%). No extensively drug resistant and pan drug resistant strains were found in this study. Out of 16 antimicrobials tested for all 58 Acinetobacter isolates (by Kirby Buer Disk Diffusion method and vitek for colistin), maximum resistance was for Ciprofloxacin (82.8%) and 100% susceptibility was seen for Colistin, Tobramycin, Minocycline and Tigecycline.

Keywords: Acinetobacter, wound/pus, urine, sputum and ciprofloxacin

Introduction

Acinetobacter is a non-fermenting gram negative bacilli or cocco bacilli belonging to family Moraxellacae. They are oxidase negativeand catalase positive. They are commensals isolated from digit webs, groin, and axilla of 25 percent of the population **(1).** In hospital personnel and in hospitalized patients, Acinetobacter normally colonize the skin and the gut.

Acinetobacter are saprophytic and ubiquitous. Presence of Acinetobacter in inanimate objects, ventilators, catheters and diverse medical or surgical equipment are noted. ICU patients may get infected from various sources and nosocomial infections occur either occasionally in the form of sporadic cases or as outbreaks in hospitals. Wide spectrum of bacterial infections e.g., pneumonia, urinary tract infections, bacteremia, super added infections in burn patients and secondary meningitis have been caused by Acinetobacter during the last few decades. Acinetobacter species accounts to 9-14% of the total nosocomial infections worldwide. Acinetobacter is also responsible for community Acquired bacterial infections (2). The severity of infection and development of multi drug resistance by these organisms to major antibiotic classes, confronts a great problem to the treating clinicians.

In last two decades, Acinetobacter spp. has progressively and significantly gained importance, due to the development of resistance mechanisms against major antibiotic classes like carbapenems, carboxypenicillins, third generation cephalosporin and broad spectrum β –

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lactams. Most strains are resistant to fluoroquinolones and produce a wide range of aminoglycoside-inactivating enzymes (3).

Knowledge of virulence factors of Acinetobacter is still in the elementary stage. Production of extracellular enzymes and toxins, their ability to adhere to epithelial cells, polysaccharide capsule and surface components, protecting bacteria from opsonisation and phagocytosis are some of the virulence factors of Acinetobacter[(2)].

There are 32 species in the genus Acinetobacter. Growth at different temperatures, production of acid from glucose, gelatin hydrolysis and assimilation of 14 different carbons sources are the commonly used phenotypic identification methods for species identification (4). Based on bio-chemical reactions, in many laboratories the organism is reported as AcinetobactercalcoaceticusA.baumannii complex or the sacchrolyticAcinetobacter species which includes A. baumannii, A.calcoaceticus, Acinetobactergenomospecies 3, and Acinetobactergenomospecies 13TU and non sacchrolyticAcinetobacter spp. A. lwoffii. DNA-DNA hybridization is the gold standard for definitive identification of the Acinetobacter species which is available only in reference laboratories. Plasmid profiling, ribotyping, PFGE, RAPID analysis, REP PCR, AFLP, infrequent-restriction-site PCR and recently MLST and PCR ESI-MS aresome of the molecular typing methods to investigate the molecular epidemiology of A. baumannii[(2)]. A. baumannii has emerged as a leading opportunistic Multi drug resistant pathogen, showing resistance to major antimicrobial agents used for treatment of nosocomial infections [(5)]. Chromosomes, plasmids and transposon are responsible for transfer of MDR antibiotic resistance genes [(6)]. Antibiotic susceptibility pattern of Acinetobacter may vary widely geographically and between various units of the same hospital at various point of time. The variations in Acinetobacter resistogram, necessitates periodic surveillance of these pathogens to achieve appropriate selection of therapy [(7)]. Due to unpredictable multidrug resistance patterns exhibited by various clinical strains of Acinetobacter, it is imperative to know the institutional prevalence and susceptibility profile. Hence this study was conducted to isolate Acinetobacter species from various clinical samples by phenotypic identification protocol and determine their antibiotic susceptibility pattern.

MATERIALS AND METHODS

Inclusion criteria

1. Acinetobacter isolates from all the routine clinical samples (wound/pus, urine and sputum) received for culture and sensitivity testing were included in this study.
2. Patients belonging to all age groups and both the sexes were included in the study.

Exclusion criteria

Bacteria other than Acinetobacter isolates were excluded from this study.

METHODOLOGY

Permission to conduct this study was obtained from the institutional ethical committee. This prospective study was conducted in SreeBalaji medical college and hospital Chennai. The period of study was for 12months from (January 2016 to December 2016). Samples such as wound swab, pus, sputum and urine from various departments like ICU, orthopedics, medicine, surgery gynecology, urology, and pediatrics were included. The various clinical materials sent to

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department of microbiology-central diagnostic lab, were subjected to microscopy, bacteriological culture, biochemical identification and antibiotic susceptibility testing.

Collection of specimen:

Under aseptic precautions, wounds were wiped clean with sterile saline and two swabs were taken from the depth of the wound and if discharge was less, then edges were squeezed to expel the contents. The samples were collected aseptically and sent to lab in sterile, screw capped tubes or screw capped containers. In case of abscess, sterile syringes were used to collect pus. Sterile containers were used to collect coughed out sputum and clean catch midstream urine.

Sample Processing:

Day 1:

Except blood cultures, all clinical samples received in our lab, according to our standard protocol, were subjected to Gram staining and if Gram negative bacilli seen they were inoculated and streaked on Nutrient agar, 5% sheep blood agar and MacConkey agar and incubated at 37 c overnight.

Day 2:

Colony morphology on culture plates:

On Nutrient agar plate: 1-2mm, mucoid, low convex, opaque colonies (Fig 1).

On Blood agar plate: smooth, opaque, raised, creamy and small colonies, hemolytic or non-hemolytic colonies (Fig2). On MacConkey agar plate: NLF, but colonies exhibit a lavender or purplish hue (Fig 3).

These colonies were selected for Gram staining and motility.

Microscopic appearance:

Gram staining shows 1-1.5 by 1.5-2.5 µ, short, plump, Gram-negative to Gram variable cocco bacilli arranged singly, in pairs or chains (Fig 4).

Hanging drop procedure for motility:

A small amount of paraffin-petrolatum is placed around the lip of the well on the under surface of the hanging drop glass slide. Colony to be examined is placed in the center of the coverslip, into a small drop of saline or water. The slide is carefully brought to an upright position for direct examination under low power microscope. Acinetobacter species are seen as non-motile organisms.

COLONIES WHICH SHOWED GRAM NEGATIVE, NON MOTILE, COCCOBACILLARY FORMS WERE SUBJECTED TO FOLLOWING BIOCHEMICAL TESTS ON DAY 2 AND REACTIONS NOTED ON DAY 3 -

Catalase test, Oxidase test, Indole test, Citrate test, Urease test, Triple sugar iron agar, oxidative-fermentative test & rapid utilization of 10% Lactose, Growth at 44 ˚C and 42˚ C, Nitrate reduction, Decarboxylation of Arginine for identification, confirmation and speciation of Acinetobacter species were done.

BIOCHEMICAL TESTS:

TABLE 10: preliminary biochemical tests for Acinetobacter identification:

Name of the test	Results for Acinetobacterspp
Oxidase test	Negative
Catalase test	Positive

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Indole test	Negative
TSI test	Alkaline slant/alkaline butt (K/K) no H2S and no gas
Urease test	Negative
Nitrate reduction	Negative

TUBE CATALASE TEST

Procedure: To 0.5 ml of 3% hydrogen peroxide in a test tube introduce the 18-24hr culture of the organism with a sterile glass rod and appearance of rapid and sustained bubbles or effervescence within 20-30 seconds constitutes a positive test (Fig 5).

Positive control:*Staphylococcus aureus*ATCC *25923* and

Negative control:*Strptococcuspyogenes ATCC 19615*

OXIDASE TEST (KOVAC'S METHOD):

Procedure: Commercial oxidase disc impregnated with the substrate 1% tetramethyl p-phenylenediamine hydrochloride was kept on the glass slide and colony to be tested is removed with glass rod and kept on the disc and development of dark purple within 10 seconds is considered positive. Acinetobacter is oxidase negative (Fig 6).

Positive control: Pseudomonas aeruginosa and Negative control: Escherichia coli.

BIOCHEMICAL TESTS DONE FOR CONFIRMATION OF THE ISOLATE.

INDOLE PRODUCTION TEST:

Inoculate the peptone water with the test colony and incubate at 37.C for 18-24hrs and add 0.5ml of Kovac reagent along the inner wall of tube. Positive reaction- development of bright fuchsia red color within seconds is indicative of splitting of tryptophan to indole. No red ring means negative reaction. Acinetobacter does not produce indole (Fig 7).

Positive control: Escherichia coli and Negative control: Klebsiellapneumoniae.

UREASE TESTS (CHRISTENSEN'S METHOD):

Test colony is streaked over the surface of a urea agar slant at 37.C for 18- 24 hrs. Positive reaction- color change to magenta indicative of production of urease enzyme. If no color change (butt remain light orange) is negative.

Acinetobacter is urease negative (Fig 8).

Positive control: Proteus vulgaris and Negative control: Escherichia coli.

NITRATE REDUCTION TEST:

The colony to be tested is inoculated in the nitrate medium (KNO_3) and incubated at 37.C for 7 days. At the end of the incubation, 5 drops of sulfanilic acid and 5drops of alpha-naphtlhylamine added and look for the development of red color due to the formation of a red diazonium dye, p-sulfobenzene-azo-αnaphthylamine within 3 minutes indicating the presence of nitrites. Absence of the color indicates negative reaction. Acinetobacter species shows negative reaction (fig 9).

Positive control -E. coli Negative control- Acinetobacterbaumannii (VITEK confirmed).

TRIPLE SUGAR IRON AGAR (TSI) TEST:

Three sugars glucose, lactose, sucrose in a ratio of 1:10:10 is distributed in tubes with butt and slant in triple sugar iron agar medium. Colony to be tested stabbed to butt and streaked on the

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slant and incubated at 37.C for 1824hrs. Alkaline slant/alkaline butt (K/K) with no hydrogen sulphide and gas indicates glucose, lactose, sucrose nonutilizer, with no production of carbon dioxide and hydrogen gas and hydrogen sulphide production. Acinetobacter species showed no fermentation in the TSI Agar as they are nonfermenters (Fig 10).
Alkaline slant/alkaline butt (K/K) no H2S and no gas: Pseudomonas aeruginosa
Acid slant /acid butt (A/A) with gas: E. coli, were used as controls.

CITRATE UTILIZATION TEST:
Inoculate Simmons citrate agar with a test colony of 18-24hrs old culture, by touching lightly the tip of the needle and incubate at 37.C for 24- 48 hrs. Look for development of deep blue color which is indicative of citrate utilization as sole source of carbon. Negative test is absence of growth with no color change (remains green). Acinetobacterlwoffii is citrate negative. Acinetobacterbowmannii is citrate positive (Fig 11 and Fig 12).
Positive control: Klebsiellapneumoniae and Negative control: Escherichia coli.

OXIDATION-FERMENTATION TEST (HUGH AND LEIFSON):
Inoculate the colony to be tested in two tubes containing OF medium, each containing single carbohydrate (10% Glucose, 1% Lactose, 1% Sucrose were tested for oxidative utilization) by stabbing a straight inoculating needle 4 to 5 times to a depth of 1cm. one tube is overlaid with sterile mineral oil and other tube remained open. Incubate both tubes at 37.C for seven days. Acinetobacter species utilized only 10% glucose and 1% lactose (Fig 13 and Fig 14).

Interpretation:

TUBE WITH NO MINERAL OIL	**TUBE OVERLAID WITH MINERAL OIL**	**METABOLISM**	**ISOLATE**
Acid (yellow)	Alkaline (green)	Oxidative	A. bowmannii, A. calcoaceticus
Acid (yellow)	Acid (yellow)	Fermentative	E.coli
Alkaline (green)	Alkaline (green)	Asaccharolytic	A.lwoffii, A. johnsonii

Control:
Note: Appropriate organisms depend on which carbohydrate has been added to the basal medium. Glucose is used as an example. Fermenter: Escherichia coli. Oxidizer:
Pseudomonas aeruginosa**.**

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GROWTH AT 42.C AND 44.C:

Inoculate test colony on three tubes of nutrient agar/trypticase soya agar by lightly touching a needle and streaking the slant. Incubate one at 37.C, 42.C and 44.C respectively. A. bowmannii grows at37.C, 42.C and 44.C.

A. calcoaceticus, A. lwoffii and A. hemolyticusgrows at 37C [24] (Fig 15 and Fig 16).

DECARBOXYLATION OF ARGININE (MOELLER`S METHOD):

Colony to be tested must be inoculated in two tubes of Moeller decarboxylase medium one containing, amino acid arginine and other devoid of amino acid arginine which is used as a control tube. Sterile mineral oil is overlaid over both tubes for about 1cm on the surface and incubate at 37.C for 18-24 hrs. Initial stages both tubes turn yellow. Control tube turns yellow indicating organism is viable and the pH of the medium is sufficiently lowered to activate decarboxylase enzymes. Reversion of the tube containing arginine to purple color indicates positive test due to the formation of amines. A. calcoaceticus, A.bowmannii, and A. hemolyticus are arginine decarboxylase positive (Fig 17).

ANTIBIOTIC SENSITIVITY TESTING:

All the isolated Acinetobacter strains were subjected to antibiotic sensitivity testing by the "Disc diffusion" method of Kirby Bauer method. Hi media discs were used. A Mueller-Hinton agar plate of 100mm diameter (maximum of 5 disks) was used. For confirmation all the isolates were run through Vitek 2 COMPACT successively. Acinetobacter species was confirmed by Vitek Gram–negative identification and AST cards and MIC values of Colistin (according to CLSI 2016) were taken for the study. For Tigecycline the guidelines laid down by F.D.A. were used [44], [45].

DISC DIFFUSION SUSCEPTIBILITY TESTING:

Four to five colonies of same morphology were inoculated into 4-5 ml of tryptic soy broth at 37.c for 2-6 hrs. The turbidity of the organism suspension was compared to commercially available 0.5 McFarland Standard against white background with a contrasting black line which corresponds to 1.5×10^8 colony forming units per millimetre. Within 15 minutes of adjusting the turbidity, a sterile nontoxic cotton swab was dipped in to the inoculum suspension and swab was rotated several times with firm pressure on inside wall to remove the excess fluid. Dried surface of the Mueller-Hinton agar plate which was brought to room temperature was streaked with swab for 3 times over the entire surface rotating the plate approximately 60 degrees each time for even distribution of inoculum. After rim of the agar was swabbed, lid was replaced in Petridish and after 3 to 5 minutes, appropriate antimicrobial – impregnated disks were placed using forceps. Each disk was gently tamped down onto the agar for uniform contact and was placed uniformly, no closer than 24mm from center to center. Plates were inverted and incubated at 37.c for 18-24hrs (46).

Day 3:

Using ruler, the zones of complete growth inhibition around each disks were measured to within the nearest millimetre, diameter of the disk were included in the measurement. Results were interpreted as sensitive, intermediate and resistant by comparing the inhibition zone diameters with the ranges recommended by CLSI guidelines 2016. With each batch of tests, a control for each antibiotic was also set up. The control strains were obtained from American Type Culture Collection (ATCC). The control strains were included as per the CLSI guidelines (Fig 18 and Fig 19).

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Table 11: Disk diffusion of control strains against **Antimicrobial agents used and their zone of inhibition diameters** [38]

Sl no	Antimicrobial agents	Disk Content	Pseudomonas aeruginosa ATCC® 27853	Escherichia coli ATCC® 35218 [b,c]	Escherichia coli ATCC® 25922
1	AMPICILLIN-SULBACTAM	10/10 µg	-	17-22 mm	-
2	PIPERACILLIN-TAZOBACTAM	100/10 µg	-		-
3	CEFTAZIDIME	30 µg	22-29 mm	-	-
4	CEFEPIME	30 µg	24-30 mm	-	-
5	CEFOTAXIME	30 µg	18-22 mm	-	-
6	CEFTRIAXONE	30 µg	-	-	26-34 mm
7	MEROPENEM	10 µg	-	-	19-25 mm
8	COLISTIN	10 µg	11-17 mm	-	-
9	GENTAMICIN	10 µg	17-23 mm	-	-
10	TOBRAMYCIN	10 µg	20-26 mm	-	-
11	AMIKACIN	30 µg	18-26 mm	-	-
12	TETRACYCLINES	30 µg	-	-	18-25 mm
13	MINOCYCLINES	30 µg	-	-	19-25 mm
14	CIPROFLOXACIN	5 µg	25-33 mm	-	-
15	TRIMETHOPRIM-SULFAMETHOXAZOLE	1.25/23.75 µg	-	-	23-29 mm
16	TIGYCYCLINE	15µg	9-13 mm	-	-

Footnotes

a. ATCC® is a registered trademark of the American Type Culture Collection

b. QC strain recommended when testing β-lactam/βlactamase inhibitors.

c. It is essential that E. coli ATCC® 35218 maintains its ability to produce β-lactamase in order to adequately perform QC for β-lactam/β-lactamase inhibitor agents.

Antimicrobial pattern of Acinetobacter according to CLSI guidelines 2016 for test/report groups (38).

VITEK 2 COMPACT PRINCIPLE:

The vitek, automated microbiology system utilizes growth based technology. It makes use of colorimetric reagent cards that are incubated and interpreted automatically. It has compliance for electronic records and signatures and a colorimetric reagent card to identify Gram negative, Gram positive and yeast. The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalization, enzyme hydrolysis and growth in the presence of inhibitory substances. An optimally clear film

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present on both sides of the card allows for the appropriate level, of oxygen transmission while maintain a sealed vessel that prevents contact with the organism substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date and a unique identifier that can be linked to the sample either before or after loading the card onto the system [47].

Uses:

1. Identification and speciation of isolates.
2. AST by MIC method.

Colony morphology and Gram staining

Figure 1: Acinetobacter colonies plate

Figure 2: Acinetobacter colonies on nutrient agar on Blood agar plate.

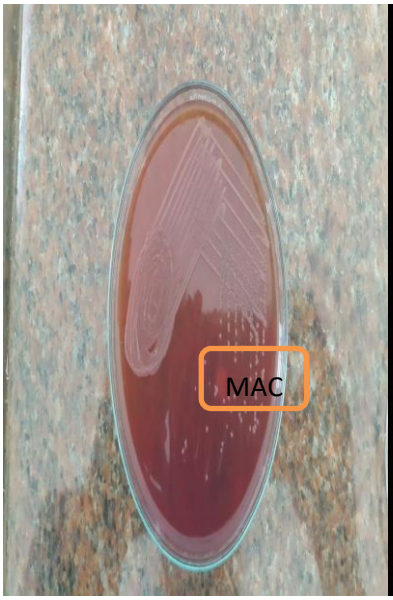

Figure 2: Acinetobacter colonies

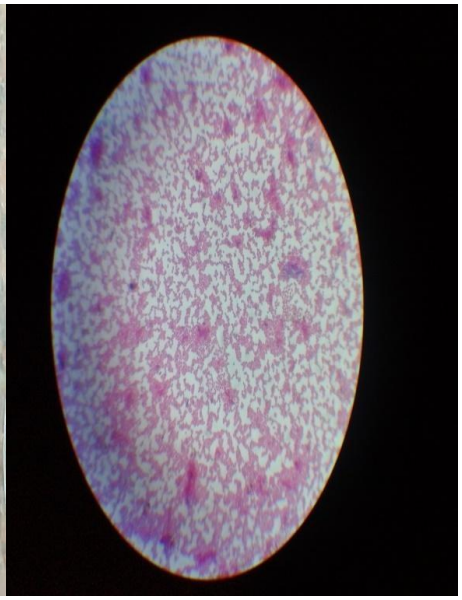

Figure 1: Gram stain: Gram on MacConkey agar plate. Negative coccobacilli

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BIOCHEMICAL TESTS FOR ACINETOBACTER IDENTIFICATION

Figure 5: Tube catalase test positive by Acinetobacter.
A. Positive control- Staphylococcus aureus ATCC 25923, **B**. Test –positive (Acinetobacterspp), **C**. Negative control- Streptococcuspyogenes ATC C 19615

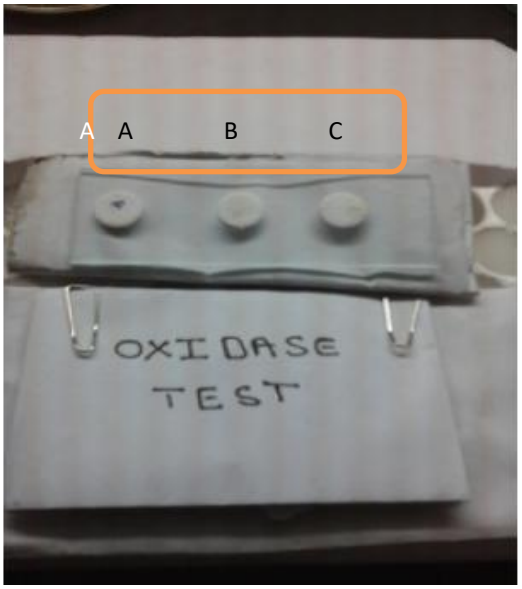

Figure 6: oxidase test negative by Acinetobacter

A. Positive control- Pseudomonas aeruginosa ATCC 27853, **B**. Test –Negative (Acinetobacterspp), **C.** Negative control- E. coli ATCC 25922

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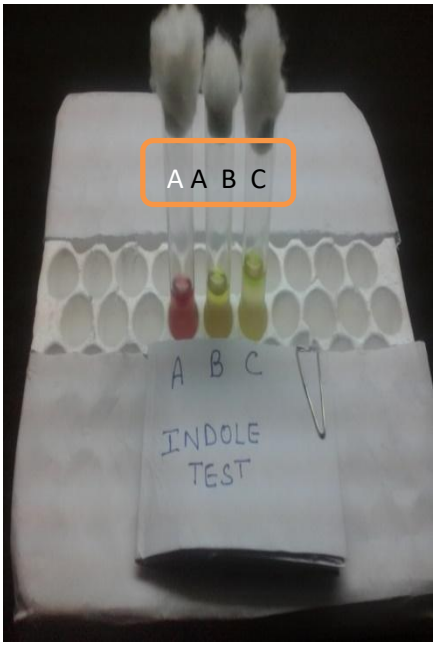

Figure 7: Indole test: indole not produced by Acinetobacter,
A. Positive control - E. coli ATCC 25922, **B**. Test – indole not produced (Acinetobacterspp) **C.** Negative control - Klebsiellapneumoniae

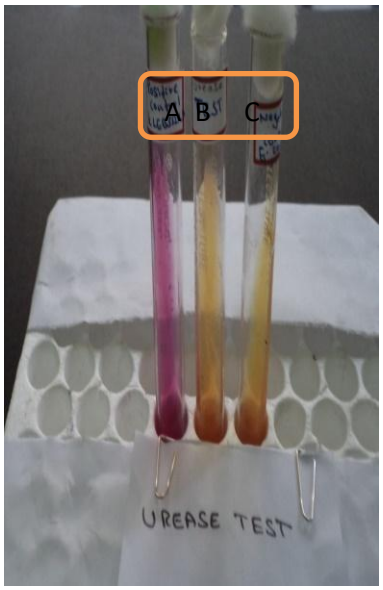

Figure 8: Urease test: urea not hydrolysed by Acinetobacter.
A. Positive control – Proteus vulgaris, **B.** Test – negative (Acinetobacterspp), **C.** Negative control - E. coli ATCC 25922

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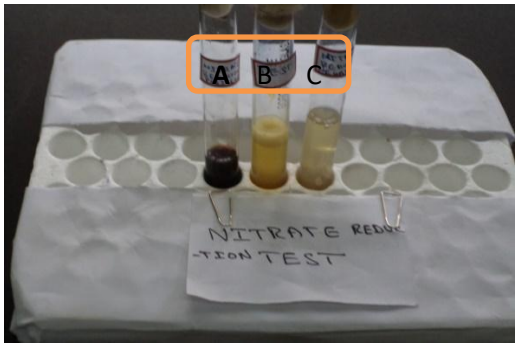

Figure 9: Nitrate Reduction Test

A. Positive control - E. coli ATCC 25922, **B.** Test - Negative (Acinetobacterspp), **C.** Negative control - Acinetobacterbaumannii (VITEK conformed).

Fgure 10: TSI Test

A.Uninnoculated tube **B**. Test – alkaline/alkaline, no gas and no H2S (Acinetobacterspp) Controls: **C**. A/A with gas – E.coli ATCC 25922 **D.** K/KPseudomonasaeruginosa ATCC 27853

BIOCHEMICAL TESTS FOR ACINETOBACTER SPECIATION

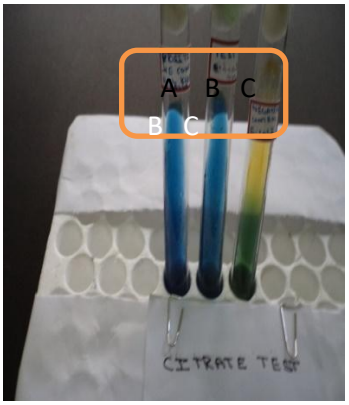

Figure 11: Citrate test: citrate utilized by A.buamannii

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A. Positive control - Klebsiellapneumoniae, **B.** Test – positive (Acinetobacterbowmannii), **C.** Negative control - E. coli ATCC 25922

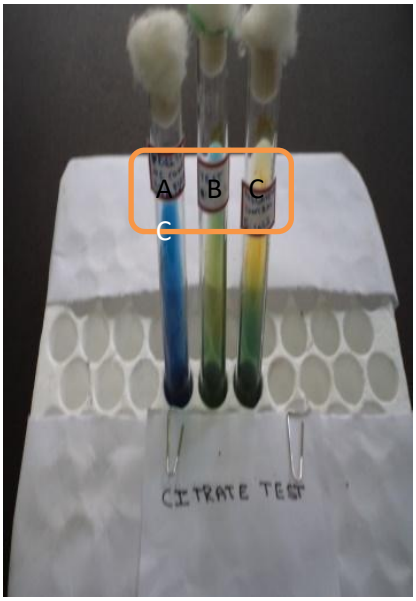

Figure 12: Citrate test: citrate not utilized by A. lwoffii,
A. Positive control - Klebsiella pneumonia, **B.** Test – Negative (A. lwoffii), **C.** Negative control - E. coli ATCC 25922

Figure 13: Oxidation fermentation Test, A. Oxidizer-Pseudomonas aeruginosa ATCC 27853, **B. Test** – Oxidizer (A. bowmannii, A. calcoaceticus) **, C.** Fermenter-E. Coli ATCC 25922

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Figure 14: Oxidation fermentation Test
A. Oxidizer—Pseudomonas aeruginosa ATCC 27853, **B.** Test - Asaccharolytic (Acinetobacterlwofii), **C.** Fermenter-E.coli ATCC 25922

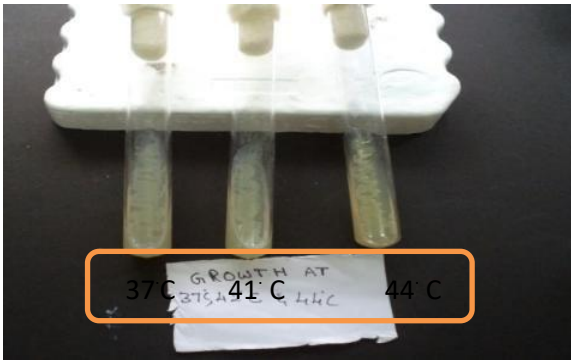

Figure 15: A. bowmannii grown at 37° C, 41°Cand 44° C

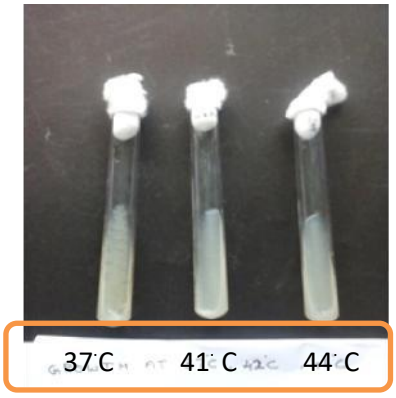

Figure 16: A. lwofii, A.calcoaceticus, A. haemolyticus, grown at 37° C and not grown at 41°C and 44° C.

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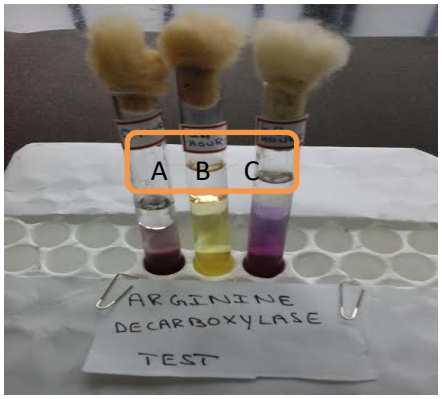

Fig 17: Decarboxylation of arginine (Moeller`s method): A. Test Positive - A. calcoaceticus, A. bowmannii, and A. hemolyticus, **B.** Control tube- only base without amino acid, **C.** Uninoculated tube

ANTIMICROBIAL SUSCEPTIBILITY TESTING: DISK DIFFUSION BY KIRBY BAUER METHOD

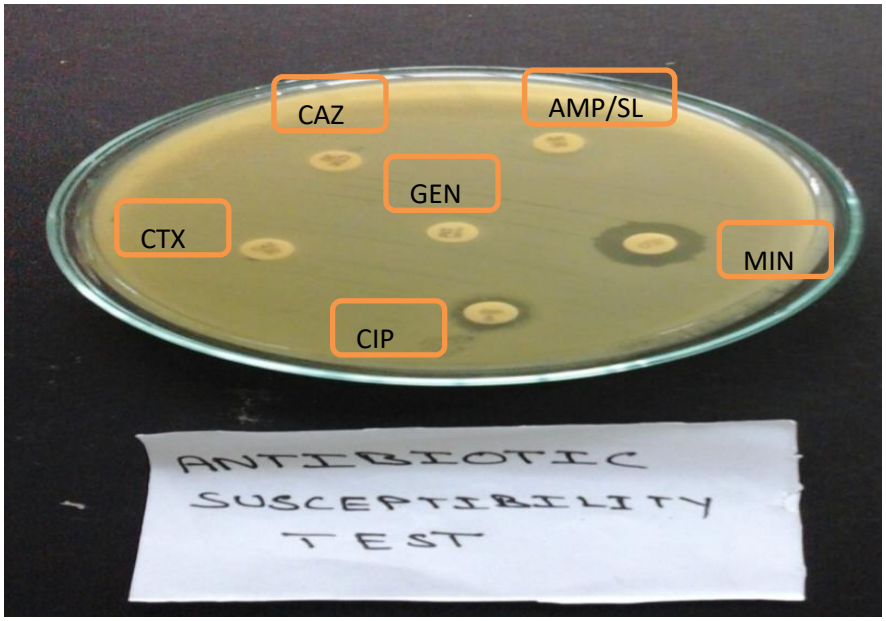

Figure 18: Antimicrobial susceptibility testing: AMP/SLB- Ampicillin-sulbactam, CAZ-Ceftazidime, CTX- Cefotaxime, MIN-Minocycline, CIP- Ciprofloxacin, and GM-Gentamicin

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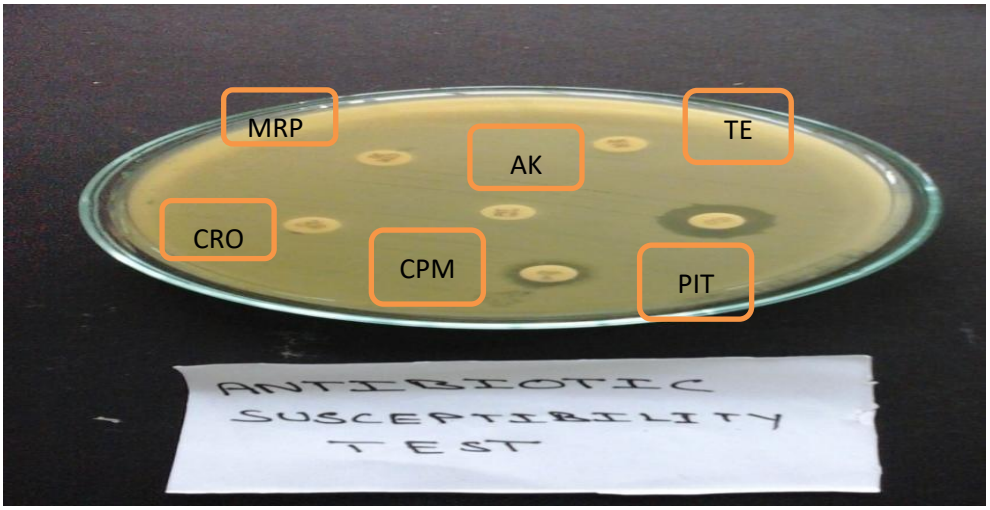

Figure 19: Antimicrobial susceptibility testing: MRPMeropenem, CPM- Cefepime, CRO-Ceftriaxone, PITPiperacillintazobactum, AK-Amikacin, TE-tetracycline

RESULTS

TABLE 12: DISTRIBUTION OF DIFFERENT BACTERIAL GROUPS ISOLATED FROM THE STUDY SAMPLES.

TOTAL NO. OF SAMPLES	NO GROWTH	NO. OF GPC ISOLATED	NO. OF GNB ISOLATED
2710	800	593	1317
PERCENTAGE (%)	29.5	21.9	48.6

Out of 2710 samples processed, 1317 samples were GNB.

From a total of 2710 samples processed, 800 (29.5%) showed no growth, 593(21.9%) samples were Gram positive cocci and 1317 (48.6%) Gram negative bacilli.

TABLE 13: DISTRIBUTION OF ACINETOBACTER IN VARIOUS SAMPLES

S. NO	TYPE OF SAMPLE	NO.OF SAMPLES	NO. OF ENERTOBACTERIACAE GROUP ISOLATED	NO.OF NON-FERMENTERS ISOLATED	NO. OF ACINETOBAC-TER (PERCENTAGE)
1	WOUND	775	296	133	36 (27.1 %)
2	URINE	1699	590	123	16(13%)
3	SPUTUM	236	127	48	6(12.5 %)
4	TOTAL	2710	1013	304	58 (2.1%)

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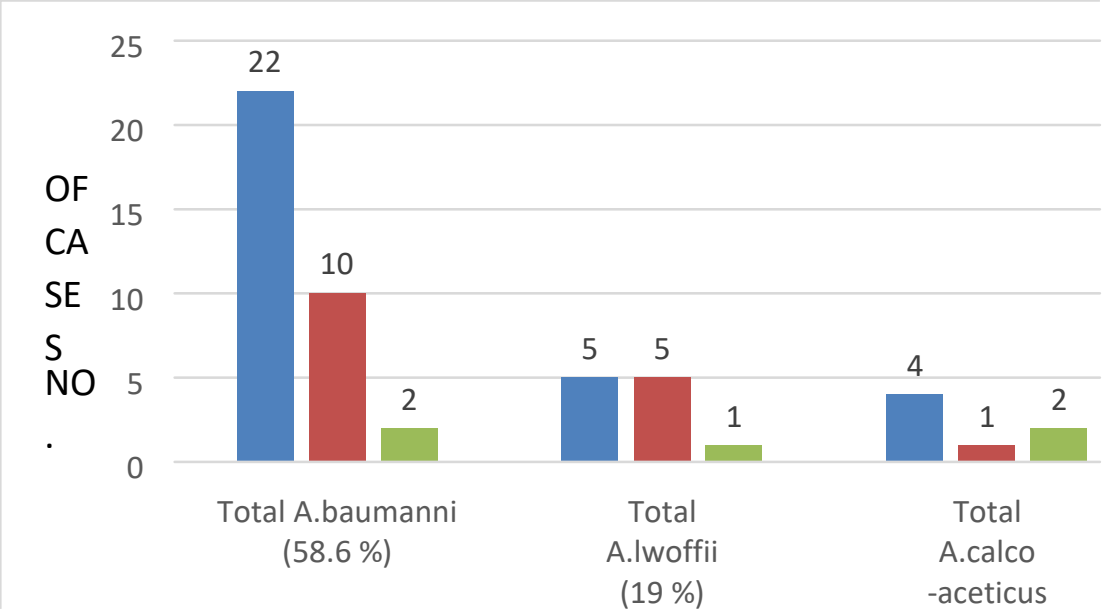
Out of 775, 1699 and 236 samples of wound, urine and sputum samples no. of Acinetobacter isolated were 36 (27.1 %), 16(13%) and 6(12.5 %) respectively. From total 2710 samples received, no.ofAcinetobacter positive cases were 58 (2.1%).

Out of total 775 wound samples processed, 296 were Gram negative bacteria (Enterobacteriaecae group), 133 were non-fermenters and 36 were Acinetobacter species. Out of total of 1699 urine samples processed, 590 were Gram negative bacteria (Enterobacteriaceae group), 123 were non-fermenters and 16 were Acinetobacter species. Out of total of 236 sputum samples processed, 127 were Gram negative bacteria (Enterobactiraceae group), 48 were non-fermenters and 6 were Acinetobacter species. Acinetobacter species were predominantly isolated from wound samples 36 (27.1%), followed by urine 16 (13%) and sputum 6 (12.5%). Out of the total number of samples (2710) taken for the study, Acinetobacter isolated was 58 (2.1%).

GRAPH 1: DISTRIBUTION OF ACINETOBACTER SPECIES AMONG THE TOTAL ACINETOBACTER POSITIVE SAMPLES IN VARIOUS CLINICAL SAMPLES



Maximum isolated species were Acinetobacterbaumannii 34 (58.6%), followed by Acinetobacterlwoffii 11 (19%), Acinetobactercalcoaceticus 7 (12.01%) and Acinetobacterhemolyticus 6 (10.3%). Acinetobacterbaumannii was maximum isolated from wound samples 22 followed by urine samples10 and minimum from sputum samples 2. Acinetobacterlwoffii was maximum isolated from wound samples 5 and urine samples 5 and minimum from sputum 1. Acinetobactercalcoaceticus was maximum isolated from wound samples 4, followed by sputum samples 2 and minimum from urine samples 1. Acinetobacterhemolyticus was maximum isolated from wound samples 5, followed by sputum samples 1and was not isolated from urine samples at all.

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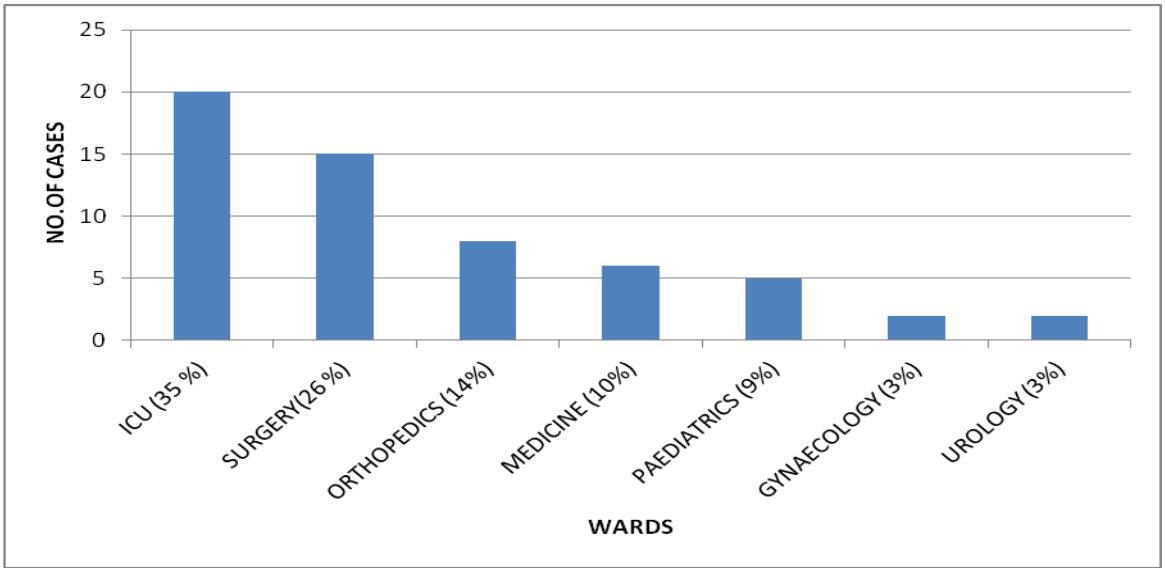
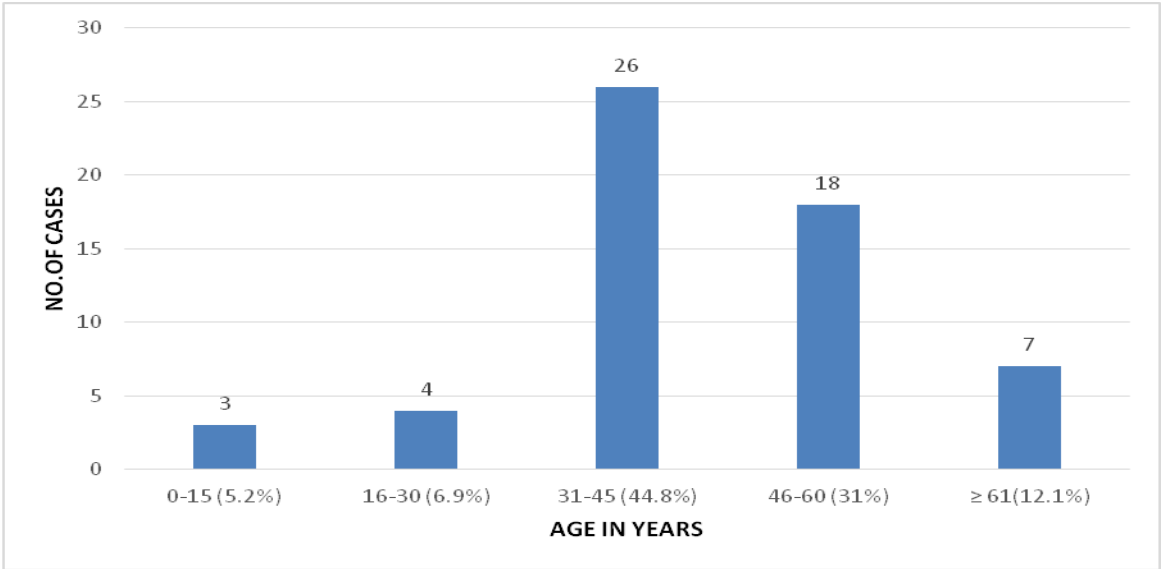
GRAPH 2: PERCENTAGE OF ACINETOBACTER ISOLATED FROM VARIOUS INPATIENT DEPARTMENTS IN S.B.M.C.H



Out of total 58 Acinetobacter isolated, maximum was from ICU 20cases (35%).

Maximum Acinetobacter species was isolated from Intensive care unit 20 (35%), followed by surgery ward 15 (26%), orthopedic ward 8 (14%), medicine ward 6(10%), pediatric ward 5 (9%) gynecology ward 2(3%) and urology ward 2 (3%).

GRAPH 3: ISOLATION OF ACINETOBACTER WITH RELATION TO AGE.



Out of total 58 Acinetobacter positive cases, maximum number of patients was between the age group 31-45 years and least between 0-15 years.

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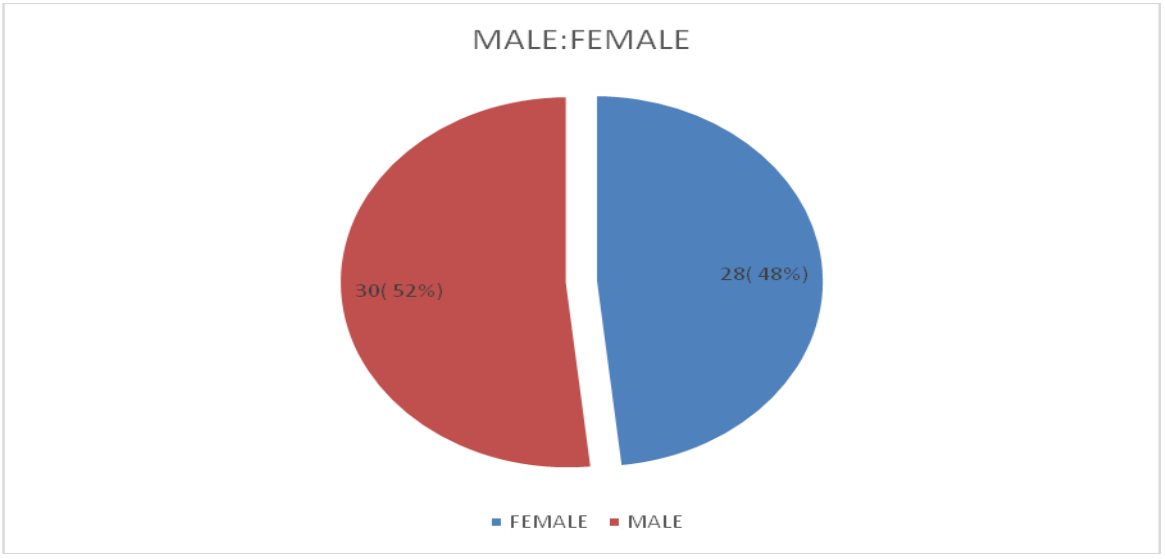
Acinetobacter infection was more common in patients age group of 31 to 45 years (44.8%), followed by 46 to 60 years (31%), ≥ 61 years (12.1%), 16 to 30 year(6.9%) and 0 to 15 years (5.2%)

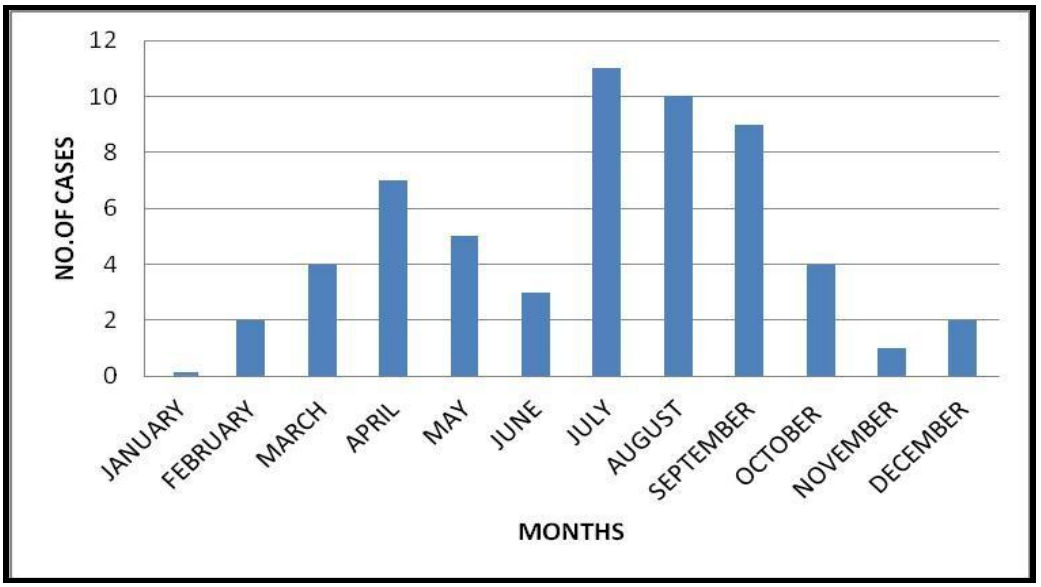
PIE CHART 1: MALE: FEMALE RATIO IN ACINETOBACTER POSITIVE CASES



Out of total 58 Acinetobacter cases isolated, 30 cases (52%) were males and 28 (48%) were females.

There was higher incidence of Acinetobacter infection among male patients i.e. 30 (52%). Male: Female ratio is 30:28 (52%:48%).

GRAPH 4: ISOLATION OF ACINETOBACTER WITH RELATION TO DIFERENT



MONTHS.

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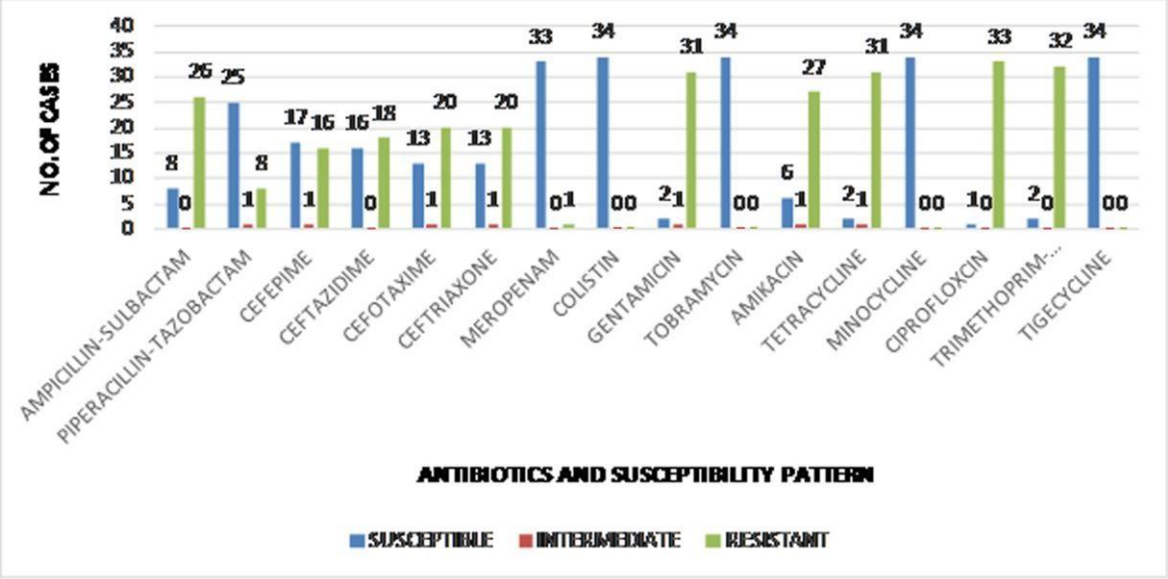
Maximum isolation of Acinetobacter was between the months of July to September

Isolation rate of Acinetobacter species for the month of January was nil, February- 2 cases, March -4 cases, April-7 cases, May-5 cases, June-3 cases, July-11 cases, August-10 cases, September-9 cases, October-4 cases, November-1cases, December-2 cases.

GRAPH 5: ANTIMICROBIAL SUSCEPTIBILITY OF A. BOWMANNII (N=34)



Susceptible, intermediate and resistance pattern of Acinetobacterbaumannii for various antimicrobial agents.

The antimicrobial susceptibility pattern (Kirby disc diffusion method) of Acinetobacterbaumannii showed maximum resistance to ciprofloxacin 97%, cotrimoxazole 94.1 %, gentamicin 91.2%, tetracycline 91.2%, ampicillinsulbactum 76.4%, cefotaxime 58.8%, ceftriaxone 58.8%, Ceftazidime (52.9%), Cefepime (47%), piperacillintazobactum 23.5% and meropenam 2.9%. Maximum susceptibility was recorded for Colistin {by Vitek MIC} 58 (100%), Tigecycline 58 (100%), Minocycline 58 (100%) and Tobramycin 58 (100%).

Out of 16 antimicrobials tested for 34 A. bowmannii isolated (by Kirby Buer Disk Diffusion method and MIC for Colistin by Vitek), maximum resistance was for Ciprofloxacin (97%) and 100% susceptibility was for Colistin, Tobramycin, Minocycline and Tigecycline.

TABLE 14: Antimicrobial susceptibility among various Acinetobacter species isolated other than A. bowmannii.

SL	ANTIMICROBIAL AGENT	ABBREVATION	A. lwoffii	A. calcoaceticus	A. hemolyticus

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			S	I	R	S	I	R	S	I	R
1	AMPICILLIN-SULBACTAM	AMP/SLB	8	2	1	5	2	O	3	3	0
2	PIPERACILLINTAZOBACTAM	PIT	8	2	1	4	1	2	3	2	1
3	CEFEPIME	CPM	3	8	0	3	4	0	3	3	0
4	CEFTAZIDIME	CAZ, CZ	2	2	7	2	3	2	4	2	0
5	CEFOTAXIME	CTX CX	2	2	7	2	2	3	3	1	2
6	CEFTRIAXONE	CRO CTR	5	1	5	3	2	2	3	3	0
7	MEROPENEM	MRP MR	11	0	0	7	0	0	5	1	0
8	COLISTIN	CL	11	0	0	7	0	0	6	0	0
9	GENTAMICIN	GM GEN	5	2	4	5	2	0	4	2	0
10	TOB RAMYCIN	TOB	11	0	0	7	0	0	6	0	0
11	AMIKACIN	AK	4	1	6	5	1	1	4	2	0
12	TETRACYCLINE	TE	2	1	8	2	1	4	3	1	2
13	MINO CYCLINE	MIN	11	0	0	7	0	0	6	0	0
14	CIPROFLOXACIN	CI	3	1	7	2	0	5	1	2	3
15	TRIMETHOPRIM-SULFAMETHOXAZOLE	COT	4	0	7	2	1	4	2	2	2
16	TIGECYCLINE	TGC	11	0	0	7	0	0	6	0	0

Antibiotic resistance of A.lwoffi was more compared to A.calcoaceticus and A.hemolyticus.

A. baumannii was found to be more resistant to all antimicrobial agents compared to other 3 Acinetobacter species.

DISCUSSION

Total numbers of samples taken for a period of 1 year (Jan-Dec 2016) for this study was 2710. Samples consisted of wound swab, pus, urine and sputum from inpatients admitted in various specialty wards including ICU of SreeBalaji Medical College and hospital, a tertiary care hospital in Chennai, South India. In this study ,under proper aseptic condition specimens were collected and processed in the Microbiology lab and organisms identified based on cultural characteristics, motility test, Gram staining and biochemical reactions, zone of inhibition for recommended antibiotics by AST and was reported after comparison with the standard chart of CLSI guidelines 2016. In this study, from a total of 2710 samples processed, 800 (29.5%) showed no growth, 593(21.9%) samples were Gram positive cocci and 1317 (48.6%) Gram negative bacilli. From the total 1317 GNB isolates, 304 were nonfermentors, out of which 58 isolates were Acinetobacter. 58 (2.1%) Acinetobacter were isolated from various clinical samples in my study.

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In previous study by Neetugupta et al., 3.36% Acinetobacter were isolated from various clinical samples [48]. Study shows maximum number of Acinetobacter in pus/wound (27.1%), followed by urine (13 %) and sputum (12.5%). Study by Joshi et al.,where the percentage of wound infection was 27.5% and also maximum isolation was from pus followed by urine and sputum [49]. Study by N. Sinha et al., where the percentage of urine infection was 13.57% and pragyaRani et al., percentage of Acinetobacter in sputum was 12.70% which coincides with this study [50] [51].

Higher prevalence of Acinetobacter was found in the age group of 31 to 45 years (44.8%), followed by 46 to 60 years (31%), ≥ 61 years (12.1%), 16 to 30 year (6.9%) and 0 to 15 years (5.2%) which is similar to study by Cucuhawangish et al., in which age ranged between 14-65 year old had higher incidence (73.8%), 0-14 year had less incidence (7.2%) and ≥ 65 year 19%(52). A slightly higher incidence in males to females (52%:48%) is comparable to other reports from Indian studies Bhattacharya et al., where the gender ratio is 1.46:1 (male: female) [53]. Frequency is more in males and age group 31 to 45 years probably due to exposure to outside working environment.

Between July, August and September month's number of Acinetobacter species isolated from clinical samples were more. Which is consistent with previous reports by NeetuGuptaet al., and Gootz et al., (48), (54). This is correlated with atmospheric temperature changes (when the temperature is hot and humid) high isolation rate is noted. In this study, maximum Acinetobacter species were isolated from ICU (35%) followed by surgery (26%) similar to the study by HossienFazeli et al., where 50% of isolates were from ICU [54]. Higher rate of Acinetobacter infections were found in ICU patients with decreased immune status as co-morbid and severely ill patients are easily infected /colonized with this organism. In this study ICU patients showed maximum isolates compared to ward patients, which was the same with Pragya Rani et al study, which showed 53.69% isolates from ICU and 38.42% in wards [51]. Significant risk factors in our study were previous ICU stay, prior exposure to 3rd generation cephalosporin's, hospitalization and invasive procedures such as indwelling catheter, intubation and catheter lines.

The predominant species was A. baumannii 58.6% followed by A. lwofii 19%, A. calcoaeticus 12.1% and A. hemolyticus 1 0.3% which was same as the study done by Sangramsingpatel et al., where A. baumannii was 60% and Neetu Gupta et al., where the A. lwofii and A. hemolyticus was 14% and 12% respectively (48) (55).

In this study, Acinetobacter species had maximum resistance to ciprofloxacin 82.8%, trimethoprimsulphamethoxazole 77.6%, tetracycline 77.6%, gentamicin 60.3%, amikacin 58.6%, cefotaxime 55.2%, ampicillinsulbactum 46.5%, ceftriaxone 46.5%, cefepime 27.6% ,ceftazidime 46.5%. More susceptible to minocycline 100%, tobramycin 100% tigycycline 100%, colistin 100% meropenam 96.5% and piperacillin-tazobactum 20.7% which is consistent with observation by Neetu Gupta et al., from western part of India where cefepime 44%, ceftazidime 46% , cefotaxime 43% , ceftriaxone 46%, amikacin 42% and ciprofloxacin was 23% resistant (48) . A study by N Sinha et al., from North India showed high resistance to Amikacin 84.8%, gentamicin 85.7%, ceftriaxone 89.35%, cefotaxime 89.3%, ciprofloxacin 85.7%, tetracycline 81.3% cotrimoxazole 85.7 % and meropenam was 95.5% susceptible [50]. Study by Reena set from western part of India showed 47.36% resistance to ampicillin-sulbactum which was similar to this study [56].

This study shows, Acinetobacterbaumannii alone had maximum resistance to ciprofloxacin 97%, cotrimoxazole 94.1 %, gentamicin 91.2%, tetracycline 91.2%, ampicillinsulbactum 76.4%, cefotaxime 58.8%, ceftriaxone 58.8%, piperacillin-tazobactum 23.5%, meropenam 2.9%, and colistin was 100% susceptible. A study in North India by Sangramsingh Patel et al., showed resistance to ciprofloxacin 65%, cotrimoxazole 57 %,

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gentamicin 56%, tetracycline 55%, ampicillin-sulbactum 77%, cefotaxime 56%, ceftriaxone 60%, piperacillin-tazobactum 23%, meropenam 13%, and colistin 11% which was similar to this study(55) . 21 isolates (61.8%) of Acinetobacterbowmannii was MDR and mainly from ICU patients i.e., 15 isolates (71.4%). No XDR and PDR strains found in this study.

CONCLUSION

Acinetobacter species have become important in recent times because of increasing antibiotic resistance shown by these bacteria, parallel to development of newer antibacterial agents. One of the major bacterial species causing hospital acquired infections is Acinetobacter. More serious problem will be caused by high prevalence of Acinetobacter species in immunosuppressive patients in ICU and other wards. Major source of infection is contaminated clothing, bedding, medical instruments and other fomites in hospital environment. Incidence of Acinetobacterspp though low in our hospital setup (compared to other studies) was predominantly drug resistant especially, in ICU patient, where the selective pressure of antibiotics is already high. In order to control nosocomial spread, administer appropriate antibiotic therapy to patients, periodic surveillance studies to know the prevalence rate are needed by the hospital infection control committee at regular intervals.

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Ethical approval: The study was approved by the Institutional Ethics Committee

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

[1] S.PeterBorriello, Patrick R,Murray and Guide Funke. Acinetobacter and Moraxella.Topley and Wilson`s microbiology and microbial infections bacteriology.10 editions; volume 2; 2005. 1301-1311.

[2] Eugenie B ergogne-Berezin. Herman Friedman. Mauro Bendinelli.Infectious agents and pathogenesis Acinetobacter Biology and Pathogenesis. Springer publishers; 2010. 1-18, 19-45, 61-83, 85-104,145-154, 175-196, 211-216.

[3] Heritier C., Poirel L., and Nordmann P. 2006. Cephalosporinase over expression as a result of insertion of ISA bal in Acinetobacterbaumanni .ClinMicrobiol Infect 12:123-130.

[4] Bouvet P.J.M and Grimont P.A.D. 1986. Taxonomy of the genus Acinetobacter with the recognition of Acinetobacterbaumanii sp. Nov., Acinetobacterhaemolyticussp.Nov.,Acinetobacterjohnsonnii sp. Nov., and Acinetobacterjunnii sp. Nov., and embedded descriptions of Acinetobactercalcoaceticus and Acinetobacterlwoffii . Int J S yst Bacterial 36:228-240.

[5] Villegas MV, Hartstein AI. Acinetobacter outbreaks,1997-2000. Infect control HospEpidermiol 2003; 24:284-95.

[6] Peleg AY, Seifert H, Paterson DL. Acinetobacterbaumanii: Emergence of a successful pathogen. ClinMicrobiol Rev2008; 21:538-82.

[7] Lone R, shah A ,kadri SM , Lone S, Shah F. Nosocomial multi-drug resistant Acinetobacter infections-clinical findings, risk factors and demographic characteristics . Bangladesh J Med microbial 2009; 03:34-38.

[8] Mandell, Douglas and Bennett's. Principles and practice of infectious disease. Acinetobacter species. 7th edition; volume 2; pp2881-2885.

[9] Jonathan cohen, WilliamG. Powderlysteven M. opal.Pseudomonas spp. Acinetobacter spp. And miscellaneous Gram-negative bacilli. Infectious diseases .3rd edition. Volume 2 published 2010. 17041722.

[10] Patrick R. Murray/Ellen JoBaronJameshh. Jorgensen /Marie Louise Landry Michael. A. Pfaller. Acinetobacter,Achromobacter, Chryseobacterium, Moraxella, and Other Nonfermentative Gram-Negative Rods. Manual of clinical microbiology. Volume 1 .9 thed 2007. 770-794

[11] Bergogne-Berezin. E. and K.J.T owner.1996. Acinetobacter spp.as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin .Microbiol. Rev.9.148-165.

[12] Glew RH, Moellering RC Jr,KunzLJ.Infections with Acinetobactercalcoaceticus (Herelleavaginicola): Clinical and laboratory studies, Medicine (Baltimore).1977;56:79-97.

[13] Lortholary .O, Fagon J-Y, Hoi AB, etal.Nosocomial acquisition of multi-resistant Acinetobacterbaumannii: Risk factors and prognosis. ClinInfect Dis.1995; 20:790-796

[14] O`Connell CJ, Hamilton R. Gram-negative rod infections: II. Acinetobacter infections in general hospital. N Y State J Med.1981; 81:750-753.

[15] Leung WS, Chu CM, Tsang KY, et al. Fulminant community – acquired Acinetobacterbaumanii pneumonia as a distinct clinical syndrome. Chest. 2006; 120; 102-109.

[16] National Nosocomial Infections Surveillance System. National Nosocomial Infections Surveillance (NNIS) System Reports, data summary from January 1992 through june2004, issued oct 2004.Am J Infect control.2004;32:470-485.

[17] Fagon J-Y,Chastre J, Domart Y, et al. Mortality due to ventilator associated pneumonia or colonization with Pseudomonas or Acinetobacter species: Assessment by quantitative culture of samples obtained by protected specimen brush. Clin infect Dis. 1996; 23:538-542.

[18] .Seifert H, StrateA, Pulverer G. Nosocomial bacteremia due to Acinetobacterbaumannii .Clinical features, epidemiology and predictors of mortality. Medicine (Baltimore).1995; 74:340-349.

[19] Lindberg RB Wetzler TF, Newton A, et al. The bacterial flora of te bloodstream in the Korean battle casualty.Ann surg.1955:141: 366-368.

[20] Kozub WR, Bucolo S, Sami AW, et al. Gonorrhea-like urethritis due to Mimapolymorphavar.oxidans: Patient summary and bacteriological study. Arch Intern Med.1968; 122:514-516.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 2254 - 2279
Received 15 December 2020; Accepted 05 January 2021.

[21] Gradon JD, Chapnick EK, Lutwick LI, Infective endocarditis of a native valve due to, Acinetobacter : case report and review. Clin Infect Dis.1992; 14: 11451148.

[22] Preyman GA, Vastine DW, Diamond JG. Vitrectomy and intra-ocular gentamicin management of Herelleaendophthalmitis after incomplete phacoemulsification. Am.J Ophthalmol.1975; 80:764-765.

[23] Wand M,OliveGM,MangiaracineAB. Corneal perforation and iris prolapse due to Mimapolymorpha.Arch Ophthalmol.1975; 93; 239-241.

[24] Patricia M.Tille. Bailey and scotts Diagnostic Microbioloy. 13 thed ELSEVIER: Mosby-year Book; 2014.pp 209,329-334.

[25] Towner K. The Genus Acinetobacter. Prokaryotes. 2006(6):746-58.

[26] Prasanth kenchappa,badrinath sreenivasmurthy. Simplified panel of assimilation tests for identification of Acinetobacterspecies.IndianJPatholMicrobiol 2003:46 (4): 700-706.

[27] Sofia constantiniu, Angela Romaniiuc, Romaniuc, LuminitaSmarandalancu, RalucaFilimon, IulianaTarasi. Cultural and biochemical characteristics of Acinetobacter spp. stains isolated from hospital units. The journal of preetive medicine 2004; 12 (3-4): 35-42.

[28] Data from Bouvet and Grimont, Int.J. Syst. Bacteriol. 36: 228-240, 1986.

[29] JeolFishbain, Anton Y. Peleg. Treatment of Acinetobacter Infections. Oxford Journals. Medcine and Health Clinical Infectious Diseases. Volume 51, Issue 1.79-84.

[30] Clinical and laboratory standards institute, M100S, Performance Standards for Antimicrobial Susceptibility Testing. Acinetobacter species. 2B-2. 27th edition, 46-69.

[31] Getchell-White, S.I.Donowitz, L.G and Groschel, D.H 1989. The inanimate environment of an intensive care unit as a potential source of nosocomial bacteria: evidence for long survival of Acinetobactercalcoaceticus. Infect. Control Hosp. Epidemiol.10:402407.

[32] Musa, E. K., Desai, N.andCasewell.M.W. 1990. The survival of Acinetobactercalcoaceticus inoculated on fingertips and on formica. J.Hosp. Infect.15:219-227.

[33] Somerville and Noble , W.C.1970. A note on the gram-negative bacilli ofhumanskin.Eur. J. Clin.Biol Res. 40:669—670.

[34] Rosenthal and Tager. I.B. 1975. Prevalence of gramnegative rods in the normal pharyngeal flora. Ann.Intern.Med. 83:355-357.

[35] Goldstein, F.W., Labigne-Roussel, A.Gerbaud, G..Carlier.C.,Collatz. E and Courvalin P. 1983. Transferable plasmid-mediated antibiotic resistaance in Acinetobacter. Plasmid Ribera. A.Ruiz, J.Jimenez de Anta. M. T. and Vila, J. 2002. Effect of an efflux pumps inhibitor on the MIC of nalidixic acid for Acinetobacterbaumannii and Stenotrophomonasmaltophilia.J.Antimicrob. C hemother.49:697-702.

[36] Da Silva, G. J.Correia. M., Vital, C.Ribeiro, G., Sousa, LJ. C., Leitao, R., Peixe, L. and Duarte, A.2002. Molecular characterization of bla(IMP-5), a new integrin-borne metallo-

beta-lactamase gene from an Acinetobacterbaumannii nosocomial isolate in Portugal. FEMS Microbiol. Lett. 215:33-39.

[37] Clinical and laboratory standards institute, M100S, Performance Standards for Antimicrobial Susceptibility Testing. 26th edition, 66-67, 156-158, 214-219.

[38] Gootz, Thomas D; Marra, Andrea. Acinetobacterbaumannii: an emerging multidrug-resistant threat. Expert review of anti-infective therapy 6.3 (junnnn 2008):309-25.

[39] A.P. Magiorakos, A. Srinivasan, R.B. Carey, Y. Carmell, M.E. Falgas, C.G. Giske,S. Harbarth, J.F.Hindler, G.Kahlmeter, B. Olsson-Liljequist,D.L. Monnet. Multidrug-resistant,extensively drug-resistant and pandrug-resistant bacteria:an international expert proposal for interim standard definitions for acquired resistance.CMI Clinical microbiology and infection. 27 july 2011.

[40] Kyungwon Lee, Dongeun Yong, SeokHoonJeong, and Yunsop Chong. Review Article Multidrug-Resistant Acinetobacter spp.: Increasingly Problematic Nosocomial Pathogens. Yonsei Med J 52(6):879-891, 2011.

[41] HossienFazeli ; AzadehTaraghian; RazieKamali;FarkhondehPoursina; Bahram Nasr Esfahani; and ShararehMoghim. Molecular Identification and antimicrobial resistance profile of Acinetobacterbaumannii isolated from Nosocomial infection of a teaching hospital in Isfahan, Iran. Avicenna Journal of Clinical Microbiology and infection. 2014 October; 1(3):e21489.

[42] Bhattacharyya S, Bhattacharyya I, Rit K,

[43] Mukhopadhyay PK, Dey JB, Ganguly U, Ray R. Antibiogram of Acinetobacter spp. isolated from various from clinical specimens in a tertiary care hospital in West Bengal, India. Biomedical Research 2013; 24 (1): 43-46.

[44] Daniel Curcio,FranciscoFernandez.Tigecycine Disk Diffusion breakpoints of Acinetobacter spp. a clinical point of view.Journal of Clinical Microbiology.2007 jun;45(6)2095-2096.

[45] Tygacil(tigecycline)-FDA (https//www. accessdata. fda.gov/drugsatfda docs/label/2013/021821s026s031ibl. pdf).

[46] Washington Winn, Jr. Stephen Allen, William Janda, Elmer Koneman, Gary Procop, Paul Schreckenberger, Gail Woods. Koneman`s Color Atlas and Textbook of Diagnostic Microbiology.6th edition2006.Lippincott Williams and Wilkins. 1-66, 1480.

[47] David H Pincus. Microbial Identification using BiomeuriuxVitek 2 system. 2-3.

[48] Neetu Gupta, NageswariGandham, SavithaJadhav and RavindraNath Mishra. Isolation and identification of Acinetobacter species with special reference to antibiotic resistance.J Nat SciBiol Med.2015 Jan-Jun; 6(1):159-162.

[49] Joshi, Suresh; Litake, Geetanjali; Satpute, Meenakshi; Telag, Nilima: Ghole, Vikram ; et al. Clinical and demographic features of infection caused by Acinetobacter species. Indian journal of medical sciences 60.9 (sep 2006): 351-60.

[50] N Sinha, J Agarwal, S Srivastava, M Singh. Analysis of carbapenem-resistant Acinetobacter from a tertiary care setting in North India. Indian Journal of Microbiology, (2013)3(1):60-63.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 2254 - 2279
Received 15 December 2020; Accepted 05 January 2021.

[51] Dr. Pragya Rani, Dr.MadhaviLatha B, Dr. SukruthaGopal Reddy and Dr Anil Kumar Bilolikar. A study of Acinetobacter from various clinical specimens and its antibiotic sensitivity pattern in a tertiary care hospital. J Med Sci Res.2015;3(4):162-165.

[52] Cucunawangsih , Veronica Wiwing, NataPratamaHardjoLugito. Antimicrobial Susceptibility of Multidrug-Resistant Acinetobacterbaumanii in a teaching hospital: A two-year Observation.Open Journal of Medical Microbiology, 2015, 5, 85-89.

[53] Bhattacharyya S, Bhattacharyya I, Rit K, Mukhopadhyay PK, Dey JB, Ganguly U, Ray R. Antibiogram of Acinetobacter spp. isolated from various from clinical specimens in a tertiary care hospital in West Bengal, India. Biomedical Research 2013; 24 (1): 43-46.

[54] HossienFazeli ; AzadehTaraghian; RazieKamali;FarkhondehPoursina; Bahram Nasr Esfahani; and ShararehMoghim. Molecular Identification and antimicrobial resistance profile of Acinetobacterbaumannii isolated from Nosocomial infection of a teaching hospital in Isfahan, Iran. Avicenna Journal of Clinical Microbiology and infection. 2014 October; 1(3):e21489.

[55] Sangram Singh Patel, Bhawna, Dilip Kr. Singh Kushawaha, Ravi Kr. Sharma, VishakhaSrivastava, Jitendra Prasad. Antibiogram of Acinetobacter species isolated from various clinical specimens in a Tertiary care Hospital from North India. Annals of International

[56] Medical and Dental Research, vol (2), Issue (1): 284287.

[57] Reena set, OmprakashBobade, JayanthiShastri. Bacteriology profile among patients with ventilatorassociated pneumonia from a medical intensive care unit at a tertiary care center in Mumbai. Indian journal of pathology and microbiology -54 (2), April-June 2011.