SPECIATION OF *ACINETOBACTER* ISOLATES AND DETECTION OF RESISTANCE PATTERNS BY PHENOTYPIC AND GENOTYPIC METHOD

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THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

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APRIL 2015

CERTIFICATE

This is to certify that this Dissertation entitled **"SPECIATION OF *ACINETOBACTER* ISOLATES AND DETECTION OF RESISTANCE PATTERNS BY PHENOTYPIC AND GENOTYPIC METHOD"** is a bonafide record of work done by **DR J.THIRIVENI,** during the period of her Post graduate study from September 2013 to August 2014 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for **M.D. MICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.R. Medical University to be held in April 2015.

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DECLARATION

I declare that the dissertation entitled **"SPECIATION OF *ACINETOBACTER* ISOLATES AND DETECTION OF RESISTANCE PATTERNS BY PHENOTYPIC AND GENOTYPIC METHOD"** submitted by me for the degree of M.D. is the record work carried out by me during the period of **September 2013 to August 2014** under the guidance of Prof. **Dr.U.UMADEVI M.D.** Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D Microbiology (Branch IV) examination to be held in April 2015.

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INTRODUCTION
The Genus Acinetobacter are a group of Non-fermentative Gram negative bacteria belonging to the family Moraxellaceae. Acinetobacter are aerobic, short, stout Gram negative coccobacilli, non-capsulated, non-motile, non-sporing and oxidase negative.[1] Acinetobacter does not have fastidious growth requirements and are able to grow at various temperatures and pH. It is found extensively in natural environment. Although these organisms are not usually considered as normal human flora, the relatively high prevalence of Acinetobacter species in hospitals frequently results in colonization and infection in patients.[2] It is usually
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ABSTRACT

TITLE: SPECIATION OF *ACINETOBACTER* ISOLATES AND DETECTION OF RESISTANCE PATTERN BY PHENOTYPIC AND GENOTYPIC METHOD

Introduction:

The Genus *Acinetobacter* are a group of Non-fermentative Gram negative bacteria found extensively in natural environment, resulting in colonization and infection. *Acinetobacter species* are the second most common nonfermenter isolated from clinical specimens. The infections caused by MDR *Acinetobacter* that are capable of producing various beta lactamases are associated with significant morbidity and mortality. Hence *Acinetobacter* has been added to the list of significant microbial challenges of current era.

Aim & Objectives:

To determine the prevalent antimicrobial susceptibility pattern and various resistance patterns conferred by beta lactamases among the clinical isolates of *Acinetobacter species*, both by phenotypic and genotypic method and to correlate the clinical outcome in the patients.

Materials and Methods:

About 175 clinically significant, consecutive, non duplicate *Acinetobacter* isolates from various clinical specimens were included in this study. The isolates were identified by standard protocols. ESBL production was confirmed by CLSI phenotypic confirmatory method, AmpC production was confirmed by AmpC disc test and carbapenamase production was detected using Modified Hodge test and Imipenem-EDTA combined disc test. Carbapenem resistance gene (OXA-23, blaVIM$_1$ & blaIMP$_1$) was identified by PCR.

Results:

Acinetobacter baumannii (81.14%) was the most common species isolated followed by *A.lwoffii* (10.29%), *A.calcoaceticus* (4.57%) and *A.junii* (4%). The maximum isolates were from respiratory samples 63(36.00%) and from patients in ICU. There was a significant difference (p value <0.05) between the antimicrobial sensitivity pattern of *A.baumannii* and other species. MDR in *Acinetobacter spp*. was found to be 60%. XDR was found to be 11.43% and there were no PDR isolate in this study. 20 isolates (11.43%) were found to be resistant to meropenem. MIC values were between 32µg/ml and 256µg/ml. Modified Hodge test was positive in 9 (45%) isolates and IEDT was positive in 9(45%) isolates of the 20 meropenem resistant isolates. 61(34.86%) isolates were found to be ESBL producers and 23(13.14%) isolates were found to be AmpC producers. OXA-23 was positive in all 20 isolates (100%), blaVIM$_1$ was positive in 9isolates (45%) and blaIMP$_1$ was positive in 7isolates (35%).

Discussion:

Acinetobacter species are very notorious for their ability to acquire antibiotic resistance because of its potential to respond quickly to the changes in selective environmental pressure. *A.baumannii* was the most common species isolated and found to be more resistant when compared to other species. MDR *Acinetobacter* infections were predominant and XDR *Acinetobacter* infections have also been recorded but no PDR *Acinetobacter* were isolated in this study. Extended spectrum beta lactamases and AmpC beta lactamases were also detected in a significant number. Carbapenems remain the drug of choice for the MDR acinetobacter infections. But resistance to carbapenems due to production of various beta lactamases is of great concern as they are encoded by genes which are horizontally transmissible. There is difference between phenotypic and genotypic methods in the sensitivity of detection of

carbapenamases where genotypic methods are more sensitive and remain the gold standard.

Conclusion:

The high prevalence of *Acinetobacter* infections emphasizes the need for early detection of various beta lactamases, which would help in selection of appropriate antibiotic regimen and prevention of emergence and dissemination of MDR strains.

Key words:

MDR- Multi drug resistant, XDR- Extended drug resistant, PDR- Pan drug resistant, ESBL- Extended spectrum beta lactamase, MHT- Modified Hodge test, IEDT- Imipenem EDTA combined disc test.

INTRODUCTION

The Genus *Acinetobacter* are a group of Non-fermentative Gram negative bacteria belonging to the family **Moraxellaceae**. *Acinetobacter* are aerobic, short, stout Gram negative coccobacilli, non-capsulated, non-motile, non-sporing and oxidase negative.[1]*Acinetobacter* does not have fastidious growth requirements and are able to grow at various temperatures and pH. It is found extensively in natural environment. Although these organisms are not usually considered as normal human flora, the relatively high prevalence of *Acinetobacter* species in hospitals frequently results in colonization and infection in patients.[2] It is usually isolated from debilitated patients such as those in ICU, burns patients and those who have undergone medical instrumentation or have received multiple antimicrobial agents.

Epidemiology of Genus *Acinetobacter* is complex. Genotypic methods or a combination of genotypic and phenotypic methods are required for species identification. *Acinetobacter baumannii* is the most common species isolated from clinical samples and known to be one of the "ESKAPE" pathogen, a group of pathogens with a high rate of antibiotic resistance that are responsible for majority of nosocomial

infections. *A.lwoffii*, *A.heamolyticus, A.johnsonii* and Genomospecies 3 and 6 are also isolated from clinical specimens.[1],[3]

Acinetobacter species are opportunistic pathogens with increasing prevalance in nosocomial infections.[3] Levin et al. in 2003 and Poirel et al. in 1999 described that 10% of nosocomial infections in ICU patients were due to *Acinetobacter*. Community acquired infections are also common in *Acinetobacter.* It accounts for 10% of all community-acquired bacteremic pneumonias.[4] Similarly it is the second most common non-fermenter isolated from clinical samples.[10] It causes a wide range of clinical complications such as pneumonia, septicemia, urinary tract infections, skin and soft tissue infections, wound infections and meningitis especially in immuno compromised patients.[5]

Acinetobacter spp have been reported to cause high mortality rate of 32% to 52% in blood stream infections. Similarly mortality rates upto 70% have been reported in ICU acquired pneumonias.[4] Hence the identification of *Acinetobacter spp*. from clinical specimens is very essential.

Different *Acinetobacter* species have differences in their antimicrobial susceptibility pattern, hence it is important to identify *Acinetobacter* isolates at species level.[6]

A.baumannii is the most common species isolated from clinical specimens and is particularly formidable because of its propensity to acquire antibiotic resistance determinants. They developed 70% resistance to third generation cephalosporins, aminoglycosides and quinolones and 87% of *Acinetobacter* isolates were Multidrug resistant.[57]

The genus *Acinetobacter* have different types of resistance mechanisms, which includes antimicrobial inactivating enzymes, reduced access to bacterial targets and point mutations that change targets or cellular functions.[7] The newer beta lactamases including ESBL, AmpC, Non-metallo beta lactamases and Metallo betalactamases are the most common emerging causes for antimicrobial resistance.[56]

In India, it has been reported that 66.7% isolates were ESBL producers, 28.57% were AmpC producers, 16.67% were combined ESBL and AmpC producers and 47.6% were resistant to carbapenem drugs, in which 19% were MBL producers.[12],[56],[59],[60] For ESBL and AmpC producers, carbapenems remain the drug of choice, whereas in carbapenem resistant strains we are left with Tigecycline and polymyxins which have started developing resistance to many GNBs.[51] Hence the detection of carbapenem resistance is important in the treatment of

patients and also preventing the spread of resistant strains, as we have to go a long way for newer antibiotics.

Carbapenem resistance in *Acinetobacter* may be due to oxacillinases, metallobeta lactamases, AmpC beta lactamases or due to porin deficiency.[20] Since oxacillinases are chromosomally mediated, spread of OXA genes to other organisms is less frequent, when compared to MBL, where the spread is plasmid mediated and hence the propensity of dissemination is multifold. Also metallo beta lactamases are more potent (100-1000 fold) hydrolyzers of carbapenems when compared to OXA type carbapenamases which contribute to the carbapenem resistance to a greater extent.[59] Hence I focussed my study on identifying the most common OXA type carbapenamases OXA-23, MBL genes blaVIM_1 and blaIMP_1 in carbapenem resistant isolates.

The emergence and the rapid spread of Multidrug resistant isolates of *Acinetobacter* species causing nosocomial infections are of great concern worldwide. Because of multidrug resistance of these isolates, it poses an intriguing problem to the treating clinician and increasing the mortality of the patients. Hence invitro antimicrobial susceptibility pattern and identification of resistance pattern is important before treating *Acinetobacter* infections.

Therefore the present study was undertaken to assess the most prevalent spp. among *Acinetobacter* infections, the prevalent antibiotic sensitivity pattern, various resistance mechanisms among the isolates and the genes involved in carbapenem resistance. This may provide the necessary information to formulate a hospital antibiotic policy and also to prevent the spread of multidrug resistance strains in the community.

Aims and Objectives

AIMS AND OBJECTIVES

AIMS:

To determine the prevalent antimicrobial susceptibility pattern and various resistance patterns conferred by beta lactamases among the clinical isolates of *Acinetobacter species* and to correlate the clinical outcome in the patients.

OBJECTIVES:

1. To identify and speciate the clinically significant *Acinetobacter* isolates from various clinical specimens.
2. To identify the prevalent antimicrobial susceptibility pattern of these isolates.
3. To determine the Meropenem MIC for the meropenem resistant isolates by Macrobroth dilution method as per CLSI guidelines.
4. To identify the Carbapenem resistance conferred by beta lactamases (Non-Metallobeta lactamases, Metallo betalactamases and AmpC beta lactamases) in meropenem resistant isolates.
5. To detect Extended spectrum beta lactamases (ESBL) and AmpC beta lactamases in *Acinetobacter* isolates by phenotypic methods.

6. To identify the carbapenem resistance by genotypic method- by identifying Oxacillinase gene OXA-23 and Metallo betalactamases genes bla-IMP_1 and bla- VIM_1 in meropenem resistant isolates by PCR.
7. To analyze the clinical outcome of the patients in the study group.

Review of Literature

REVIEW OF LITERATURE

The Genus *Acinetobacter* are a group of Taxonomically diverse, non fermentative Gram negative rods.[4] They all share the common phenotypic features like failing to acidify the butt of Klingler iron medium or Triple sugar iron medium or of oxidative fermentative media and grow only under aerobic conditions using oxygen as the final electron acceptor in the respiratory pathway.[10],[46]

3.1 TAXONOMY:

3.1.1 HISTORY:

The Genus *Acinetobacter* has colourful taxonomic history. They were identified in the first decade of 20th century.[4] *Acinetobacter* was frequently misidentified due to lack of differentiating features.

Genus *Acinetobacter* are a group of Gram negative bacteria belonging to **Gammaproteobacteria**.[11] It was first described in 1908 as ***Diplococcus mucosus***. The lack of distinctive characteristics was a driving force in the evolving nomenclature: *Micrococcus* (small), *Mima* (mimics), *Achromobacter* (colourless), *Acinetobacter* (motionless), and *anitratus* (nitrate not reducing).

The first strain of *Acinetobacter spp* were isolated by M.W. Beijerinck, a Dutch Microbiologist in 1911 from soil and were named as ***Micrococcus calcoaceticus***.[8] In the year 1930s and 1940s, De Bord proposed a new tribe, ***Mimaeae*****,** to encompass these organisms. Later Brisou and Prevot in 1954 proposed the genus *Acinetobacter* to include colourless, nonmotile, saprophytic gram-negative bacilli regardless of the oxidase activity.

In the year 1971, the ***Subcommittee on the Taxonomy of Moraxella and Allied Bacteria*** proposed that the genus *Acinetobacter* should include only the oxidase negative strains.[8] In the year 1984, Bergey's Manual of Systematic Bacteriology classified *Acinetobacter* in the family ***Neisseriaceae,*** but more recently the molecular taxonomic studies have resulted in the reclassification of this organism in the new family ***Moraxellaceae*** in 1991.[11] This family also includes *Moraxella, Psychrobacter* and related organisms. The genus *Acinetobacter* belongs to,

Phylum	-	Proteobacteria
Class	-	Gammaproteobacteria
Order	-	Pseudomonadales
Family	-	*Moraxellaceae*
Genus	-	*Acinetobacter*

3.1.2 CLASSIFICATION:

The genus characteristics of *Acinetobacter* was made clear by 1971. They are gram negative rods or coccobacilli, catalase positive, oxidase negative, non- motile, non-sporing and may be capsulated. Phenotypic identification is possible using a scheme proposed by Bouvet and Grimont.[27] The presumptive identification is possible with the above mentioned characteristics.

Differentiation of *Acinetobacter spp.* is difficult with the means typically available in most clinical microbiology laboratories. The first species identified was *A.calcoaceticus.* Initially the scientists had distinguished the species based on the ability to produce acid from glucose or not. By this *A.calcoaceticus* was distinguished into two variants, *A.calcoaceticus var.anitratus* which produce acid from glucose and *A.calcoaceticaus var.lwoffii* which do not produce acid.[4]

Other methods of species identification includes bacteriocin typing, phage typing, characterization of outer membrane proteins, serotyping, phenotyping, ribotyping, transfer ribonucleic acid (tRNA), genomic fingerprinting and DNA homology studies.[8]

The Epidemiological identification of a strain was done using pulsed field gel electrophoresis, amplified fragment length polymorphism(AFLP), randomly amplified polymorphic DNA-polymerase chain reaction(RAPD-PCR), MLST, electrospray ionization mass spectrometry (PCR/ESI-MS) or ribotyping.[9] Fluorescent Lactose Denitrification (FLN) was used to identify the different species of bacteria in this genus by the amount of acid produced due to metabolism of glucose.[46]

In 1986, based on the DNA-DNA hybridization studies Bouvet & Grimont identified 12 genomic species. In 1989 it is increased to 17, now 33 different genomic species have been identified, of which 17 have been named and others will carry the genomic species number.[13]

Acinetobacter Nomenclature:

Acinetobacter calcoaceticus (genomic species 1), *A.baumannii* (genomic species 2), *A.haemolyticus* (genomic species 4), *A.junii* (genomic species 5), *A.johnsonii* (genomic species 7), *A.lwoffii* (genomic species 8/9), *A.radioresistens* (genomic species 12), *A.baylyi*, *A.bouvetii*, *A.gerneri*, *A.grimontii*, *A.parvus*, *A.schindleri*, *A.tandoii*, *A.tjernbergiae*, *A.towneri*, *A.ursingii*, *A.venetianus* and *Acinetobacter* species unnamed.

Genomospecies 1,2,3 and 13 of Tjernberg and Ursing may be difficult to distinguish in the clinical laboratory and have been referred to as *Acinetobacter calcoaceticus-baumannii complex*.[8] *A.johnsonii, A.lwoffii* and *A.radioresistens* are the natural inhabitants of human skin, commensal in oropharynx and vagina. *A.schindleri* are commonly isolated from vagina, cervix, throat, nose, ear, conjunctiva and urine, it is mostly regarded as clinically insignificant.[23]

A.baylyi, A.bouvetii, A.grimontii, A.tandoii, A.tjernbergiae and A.towneri were not commonly isolated from human specimens.

3.1.3 MORPHOLOGY:

Members of the genus *Acinetobacter* are gram negative rods or coccobacilli. During the exponential phase they appear bacillary to coccobacillary forms but become more coccoid or diplococcal in the stationary phase and in non selective agars.[8] Individual cells are 1 to 1.5 by 1.5 to 2.5µm in size sometimes difficult to decolourise with a tendency to retain the crystal violet.[10] Hence clinical Microbiologist must be aware that *Acinetobacter* species may appear as gram positive in initial cultures.

3.1.4 CULTURAL CHARACTERISTICS:

Members of the genus *Acinetobacter* are strictly aerobic, catalase positive, oxidase negative gram negative rods growing at a wide range of temperatures and pH, optimally at 37˚C. They may be capsulated in older cultures, non-motile occasionally an odd twitching motility can be demonstrated and non- sporing.[8] They do not reduce nitrates to nitrites, this distinguishes these organisms from *Enterobacteriaceae*.[4] They are not fastidious and most strains grow in defined media containing single carbon and energy source which accounts for its prevalence in nature.[9]

The colonies are 1-2mm in diameter which are smaller than *Enterobacteriaceae*, dome shaped, smooth, slightly mucoid and opaque with grayish white or yellowish pigmentation. *Acinetobacter* are nonlactose fermenters but it may produce a pinkish hue on MacConkey agar.[8] Hemolytic property on Blood agar is variable.

Some special media like Leeds *Acinetobacter* Medium and Liquid Enrichment medium have been used for the isolation of *Acinetobacter* species from various clinical specimens and from environmental samples.[13]

3.1.5 BIOCHEMICAL CHARACTERISTICS:

Organism	Genomo species	Growth at 37˚C	Growth at 44˚C	Haemo lysis	OF glucose	Arginine hydrolysis	Malonate utilization
A.calcoaceticus	1	+	-	-	+	+	+
A.baumannii	2	+	+	-	+	+	+
A.haemolyticus	4	+	-	+	V	+	-
A.lwoffii	8/9	+	-	-	-	-	-
A.junii	5	+	-	-	-	+	-
A.johnsonii	7	+	-	-	-	V	V

The Genus *Acinetobacter* does not form Indole, does not acidify the butt of TSI, citrate is not utilized, urease is not produced and nitrate is not reduced to nitrites.[1] Main differentiation between the species is based on the saccharolytic property. It acidifies most OF carbohydrates, in particular definitive identification is made by demonstrating the rapid production of acid from 1% or 10% lactose.

3.1.6 AUTOMATED METHODS FOR IDENTIFICATION:

Vitek 2 and Phoenix are the two methods which are available for detection of *Acinetobacter* from specimens, but their detection rate to identify the organism at species level is poor. Hence it is not used routinely.

3.2 EPIDEMIOLOGY:

Acinetobacter species are ubiquitous in the environment.[8] *Acinetobacter* species may be isolated from a common source such as computer key boards, Bp cuffs, parenteral nutrition or dust in the interior of mechanical ventilator or from the dialysis machine.[9]

They are normally isolated from moist areas like axilla, groin and toe webs. They are commensals in the respiratory tract (7%) and exhibit 25% of cutaneous colonization in healthy adults.[4] *A.lwoffii* (58- 61%) is the most common skin colonizer followed by *A.johnsonii* (20%), genomospecies 15 (12%), *A.junii* (10%), *A.radioresistens* (8%), genomospecies 3 (5%) and *A.baumannii* (0.5-3%). Generally it colonizes the human skin 44% in non-hospitalized and 75% in hospitalized patients.[9] *A.baumannii* colonization is very low in normal individuals but it has higher end during hospitalization. In 1986 Larson et al. in his study found that *acinetobacters* were the most common gram negative organism found on the hospital personnel.[8]

3.2.1 BURDEN OF DISEASE:

WORLDWIDE:

The multidrug resistant *Acinetobacter spp* especially *Acinetobacter calcoaceticus baumannii* complex isolation were showing a rising trend all over the world. The first carbapenamase enzyme in a resistance strain

was found in Scotland in 1985.[16] Till 2002 carbapenem resistance was not a major health problem in Europe. From 2003 the isolation of resistant strains has been increased.

The National Infection Surveillance System showed Imipenem resistance has been increased from 0% to 20%. In USA Imipenem resistance was reported as 39.8%.[101] Similarly army personnel returning from Afghanistan Iraq conflict showed Imipenm resistance of 37%.[100] Imipenem resistance in Australia is started from 1999.[16]

Carbapenem resistance seems to be highest in the countries of Turkey, Greece, Italy, and England and the rates appear to be the least in countries of Germany and The Netherlands. Pneumonia due to *Acinetobacter* in critically ill patients is more in Asia (4-44%) and European (0-35%) hospitals than in US hospitals (6-11%). The *Acinetobacter* isolates from Asian and European countries were resistant to aminoglycosides and Piperazillin Tazobactum in higher proportion when compared to United States. This data suggests the growing threat of *Acinetobacter* infection in critically ill patients especially in Asia and Europe.[21]

3.2.2 INDIA:

In India, it has been reported that 66.7% isolates were ESBL producers, 28.57% were AmpC producers, 16.67% were combined ESBL and AmpC producers and 47.6% were resistant to carbapenem drugs, in which 19% were MBL producers.[12],[56],[59],[60]

Carbepenem resistance is reported from various parts of India. A study conducted by Sinha et al. in 2011 in North India showed 87% of isolates were MDR and 20% were resistant to Meropenem.[5] Similarly a study conducted in the same year showed 14.8% of *A.baumannii* isolates were Meropenem resistant and 86% were MDR.[18]

An incidence of 14.2% *Acinetobacter* strains resistant to carbapenem was documented in a study from Christian Medical College, Vellore.[17] Similarly a study from All India Institute of Medical Sciences, New Delhi in 2005 has given a prevalence of 34.7% resistance to meropenem , from St. John's Medical College, Bangalore resistance rate of 14% and from Chandigarh in 2003 resistance rate of 20% have been documented.[19],[20]

Gaynes et al. have documented 17% of XDR and Jyoti Sharma et al. have documented 22.38% of PDR *Acinetobacter spp.* in India.[95],[96]

3.3PATHOGENESIS:

3.3.1 VIRULENCE FACTORS:

- **Lipopolysaccharide** – Because of lipopolysaccharide, *Acinetobacter* O antigen display a marked hydrophobocity with the ability to grow on hydrophobic substrates.

- **Capsule** – The presence of polysaccharide capsule protects against phagocytosis.

- **Fimbriae** – Fimbriae facilitate the adhesion to human epithelial cells.

- **Protein S layers** and **Slime** also potentially enhance the virulence of the organism.

- Certain strains of *Acinetobacter* have been shown to produce **siderophores** and iron-repressible outer membrane receptor proteins.

- **Enzymes** – Enzymes such as butyrate esterase, caprylate esterase and leucine arylamidase potentially involved in damaging tissue lipids.

- **Bacteriocin** production may enhance the survival of *Acinetobacter*.

- **Biofilm** – Biofilm formation is a well known pathogenic mechanism in device associated infections. The excess polysaccharide formation in *A.baumannii* leads to difficulty in antibiotic penetration and also the differences in cell physiology in biofilm increases the drug resistance.[22]

3.3.2 CLINICAL MANIFESTATIONS:

The major drawback in the identification of *Acinetobacter* infection is the interpretation in the significance of isolates from clinical samples.[4]

RESPIRATORY TRACT:

This is the most common site of infection due to pharyngeal colonization. Community acquired bronchiolitis and tracheobronchitis have been reported in healthy children.[4] Similarly 10% of community acquired pneumonia in adults is due to *Acinetobacter* and it accounts for 20% of gram negative pneumonia.[15] The major impact is the ventilator associated pneumonia in ICU patients due to nosocomial spread. It is also associated with high mortality rate of 40% to 60%.[9] Corbella et al. indicate that colonization of digestive tract is an epidemiological reservoir in the development of ventilator associated pneumonia.[14]

BACTEREMIA:

The bacteremia due to *Acinetobacter* will occur late during hospitalization. It is mainly followed by respiratory tract infections and through the indwelling catheters. *A.baumannii* was the tenth most common cause for monomicrobial blood stream infection and the mortality rate was 17% to 46%, followed by *A.lwoffii, A. junii and A.parvus*.[9]

URINARY TRACT:

Acinetobacter though colonizes the lower urinary tract, it is rarely invasive. Indwelling bladder catheter or nephrolithiasis may cause cystitis and pyelonephritis due to *Acinetobacter*.[4]

SOFT TISSUE INFECTION:

The major pathogen in traumatic wounds, postoperative incisions and burns is *Acinetobacter* because of its ability to thrive in the devitalized tissues.

MISCELLANEOUS INFECTIONS:

Acinetobacter spp may also be reported from various clinical syndromes like intra cranial infections, soft tissue infections,

conjunctivitis, endophthalmitis, endocarditis, arthritis, osteomyelitis, pancreatitis and liver abscess.[100],[102]

3.3.3 RISK FACTORS:

Acinetobacters are generally non pathogenic but it may cause infections in debilitated individuals. It is the second most nonfermenter isolated from the human specimens next to *P.aeruginosa.*[10]

Risk factors for community acquired infection include alcoholism, cigarette smoking, chronic lung disease and diabetes mellitus.[15] For nosocomial infections length of hospital stay is the important cause followed by surgery, wounds, previous infection, fecal colonization, indwelling catheters, admission to ICU or burns unit, parenteral nutrition, mechanical ventilation and breaches in infection control protocols.

3.4 ANTIMICROBIAL SUSCEPTIBILITY PATTERN:

Different species of *Acinetobacter* exhibit differences in antimicrobial susceptibility pattern.[6] Hence species identification and its specific susceptibility pattern is very essential. Initially *Acinetobacter* infections were treated with beta lactam antibiotics like third generation cephalosporins, extended spectrum penicillins, penicillins-beta lactam inhibitor combinations and fluoroquinolones.[49] Nowadays due to the

development of various resistance mechanisms, the antibiotic treatment regimen for *Acinetobacter* infection is very much narrowed.[103]

3.5 MECHANISM OF RESISTANCE:

During 1970s, the *Acinetobacter* isolates were susceptible to the major group of antibiotics. From 1975, most of the isolates were resistant to first and second generation cephalosporins retaining susceptibility to third and fourth generation caphalosporins, aminoglycosides, fluoroquinolones and carbapenems. At that time all the isolates were 100% susceptible to Imipenem.[18]

The Worldwide emergence and spread of Imipinem resistant *Acinetobacter* strains started appearing in the late 1980s and 1990s.[26] Till that time Carbapenem remained the only drug to treat severe *Acinetobacter* infections. Due to the emergence of Carbapenem resistant strains, the Polymyxins and Tigecycline came to use, which also developed resistance in recent years.[18],[37],[39],[40] The extent of antimicrobial resistance in *Acinetobacter spp.* can be explained with varied definitions.

- Multi Drug Resistant (MDR) – The isolate resistant to at least three classes of antimicrobial agents including all penicillins, cephalosporins, fluoroquinolones and aminoglycosides.

- Extensive Drug Resistant (XDR) - The isolate will be resistant to carbapenems in addition to the above mentioned drugs.

- Pan Drug Resistant (PDR) – The isolate will be resistant to all the available drugs, including polymyxins and tigecycline.

The mechanism of resistance in *Acinetobacter* involves the following three broad categories [7]:

(1) Antimicrobial inactivating enzymes.

(2) Reduced access to bacterial targets.

(3) Point mutations that change targets or cellular functions.

These mechanisms may combine to act in the same microorganisms as in other gram negative bacteria.

3.5.1 Resistance to Betalactam Antibiotics:

A wide array of beta-lactamases are present in *Acinetobacter*.[42] The beta-lactamases causes hydrolysis of beta lactam ring and confer resistance to penicillins, caphalosporins and carbapenems.[27] The resistance to betalactam antibiotics is not only mediated by beta-lactamases enzymes but also by the efflux pumps.

The beta-lactamases are classified by two systems:

Ambler's classification – It is a molecular classification based on amino acid sequences.

Bush-Jacoby Medeiros classification – It is a functional classification.

- **Ambler's Classification:**

  Class A - Penicillinase (eg.TEM, SHV)

  Class B - Metallo betalactamase (eg.IMP, VIM)

  Class C - Cephalosporinase –AmpC (eg.CMY,NMC)

  Class D - Oxacillinase (eg.OXA 23, OXA 58)

  Class A, C and D require serine moieties for their function, similarly Class B require zinc for its action.

- **Bush-Jacoby Medeiros classification**[65],[66]

Group	Enzyme	Molecular class	Inhibited by Clavulanic acid
1	Cephalosporinase	C	No
2a	Penicillinase	A	Yes
2b	Broad spectrum	A	Yes
2be	Extended spectrum	A	Yes
2br	Inhibitor resistant	A	Diminished
2c	Carbenicillinase	A	Yes
2d	Cloxacillinase	D or A	Yes
2e	Cephalosporinase	A	Yes
2f	Carbapenamase	A	Yes
3	Carbapenamase	B	No
4	Penicillinase		No

The beta-lactamases may be chromosomal or plasmid mediated.

Carbapenamases:

The first serine carbapenamase in *A.baumannii* was isolated from blood culture in 1985, in Scotland.[16] This was followed by its occurrence in Spain, France, Japan, Singapore, Cuba, Brazil, China and Kuwait. Class D (OXA) carbapenamases are the main cause for carbapenem resistance. *A.baumannii* inherently exhibit OXA-51 hence it is used for the confirmation of *A.baumannii* isolates. Expression of these enzymes also require insertion sequence element ISAba 1.

Generally carbapenamases are classified based on sequence homology into: OXA-23 (includes OXA-27 and OXA-49), OXA-24 (includes OXA 25, 26 and 40) and OXA 58. OXA-23 was the first iaolated carbapenamase enzyme and most common carbapenamase gene in *Acinetobacter*. It is both chromosomal and plasmid mediated. OXA-24 is both chromosomal and plasmid mediated, less frequent than OXA-23, restricted to United States and Europe. OXA-58 is recently isolated from France.[30],[31]

Acinetobacter also express Ambler class B Metallo-beta-lactamases (MBLs) like IMP, VIM and SIM-1.[43] MBL genes are mobile

genetic elements that can be transferred easily which pose a great threat of spread.[25] These genes have been isolated from various parts of the world. The first MBL gene was isolated in Pseudomonas aeruginosa in 1991. In India, MBL production among *A. baumannii* isolates has been reported as 42%. Anuradha et al. from Bombay reported 33.33% of MBL in 2008.[50]The most prevalent MBL gene was *bla*IMP-1.[23] Another study from south India states that blaVIM was the most common gene in Metallobeta-lactamase producing *A.baumannii* isolates.[24],[41]MBL is also expressed by *A.junii*.

The carbapenem resistance is also mediated by AmpC betalactamases when present along with decreased membrane permeability or due to alterations in penicillin binding proteins. [32],[47]

AmpC Betalactamases:

AmpC type cephalosporinase are inherently expressed by *A.baumannii* known as Acinetobacter derived cephalosporinases (ADC). These will hydrolyse aminopenicillins and extended spectrum cephalosporins. Inducible type of AmpC is not expressed by *Acinetobacter*. Its expression is mediated by the presence of upstream insertion sequence known as ISAba 1 and ISA 1135.[28],[29]

Extended-spectrum Betalactamases (ESBLs):

Ambler class A beta-lactamases are reported in *Acinetobacter species*. The first ESBL reported in *A.baumannii* was PER-1which was confined to Turkey initially, but now it has spread throughout the World. It is either chromosomal or plasmid mediated requires insertion sequence ISPa 12 for its expression. The other ESBLs reported in *A.baumannii* are PER-2, VEB-1, TEM-1, TEM-2 and carbenicillinase CARB-5. CTX-M-2 and CTX-M-43 also been reported. [33],[34]

Co-production of ESBL and AmpC Betalactamases:

The genus *Acinetobacter* has high level expression of the natural production of AmpC type beta lactamases. The AmpC producing organism can act as a hidden reservoir for the ESBLs. The high level expression of AmpC β-lactamases may mask the recognition of the ESBLs and it may result in fatal and inappropriate antimicrobial therapy.[56] Hence the detection of ESBL among the AmpC producers helps in the appropriate treatment of the patients. The coproduction of ESBL and AmpC in *A.baumannii* was reported as 16.67%.[56]

3.5.2 Resitance to Quinolones:

Flouroquinolone resistance is mediated by DNA topoisomerase, acquisition of mobile genetic elements or through efflux pumps. The mutation in topoisomerase enzyme like gyrA and parC leads to the

modifications of lipopolysacharides which also confers resistance in *A.baumannii*.[36],[49]

3.5.3 Resistance to Aminoglycosides:

Plasmid or transposons coded Aminoglycoside-Modofying Enzymes (AMEs) or efflux pumps are involved in aminoglycoside resistance.[35]

3.5.4 Resitance to Tigecycline:

The overexpression of AdeABC multidrug efflux pump confers resistance to Tigecycline and also to many other antibiotics like tetracycline, aminoglycosides and quinolones.[25],[38],[51]

3.5.5 Resitance to Colistin:

The modification in the lipopolysaccharides of the bacterial cell membrane due to point mutation interfere with the binding of the antimicrobial agents like Colistin.[37],[51]

3.6 LABORATORY METHODS TO DETECT RESISTANCE MECHANISMS:

3.6.1 Phenotypic Methods:

Screening Methods:

Carbapenamase Detection:

Carbapenamases are capable of hydrolyzing carbapenems, other betalactams and betalactamase inhibitors with the exception of Aztreonam. With reference to CLSI 2014 document, disc diffusion testing using discs with 10µg potency of Imipenem and Meropenem is used as a screening test for carbapenamase production. The isolates having zone diameter of ≤22mm and ≤14mm with Imipenem & Meropenem respectively are categorized as resistant.[48]

AmpC Betalactamase Detection:

AmpC betalactamases are resistant to beta lactamase inhibitors, all betalactams including Cephamycins except Carbapenems. Isolates showing resistance to Cefoxitin (zone size < 18mm) should be considered as probable AmpC producers.[60],[63],[64]

Extended spectrum Betalactamase Detection:

ESBLs are capable of hydrolyzing penicillins – oxyiminocephalosporins and Monobactams (Aztreonam) and are inhibited by betalactamase inhibitors but have no detectable activity against Cephamycins or Carbapenems. Isolates exhibiting resistance to one or more third generation cephalosporins like cefotaxime (30µg/ml), ceftrioxone (30µg/ml) and ceftazidime (30µg/ml) with reference to CLSI 2014 AST interpretive criteria are considered to be ESBL producers.[48]

Confirmatory Methods:

Carbapenamase Detection

- Imipenem-EDTA combined disc test[53]
- Imipenem EDTA double disc synergy test[56]
- EDTA disc potentiation test[54]
- Modified Hodge test[54]
- MBL E test[53]

AmpC Betalactamases

- Modified three dimensional test[56]
- AmpC disc test[54]
- Detection by cefoxitin agar media[60]
- Detection by inhibitor based method[60]
- AmpC betalactamase E test[60]

Extended spectrum β lactamase

- CLSI phenotypic confirmatory method[48]
- Double disc diffusion synergy test[56]
- Three dimensional test
- Modified Three dimensional test
- Inhibitor potentiated disc diffusion test[56]
- ESBL E strip method
- Automated methods.

3.6.2 Molecular Methods:

Polymerase Chain Reaction (PCR) is a technique which amplifies a specific DNA target region, so as to obtain a million or more copies which can then be easily visualized by using DNA staining techniques for the identification of resistance conferring genes. PCR is the gold standard procedure to determine the resistant genes, since the phenotypic methods have not yet been standardized for NFGNBs but cost prohibiting.

3.7 THERAPEUTIC OPTIONS:

The therapeutic options for the management of MDR, XDR and PDR of *Acinetobacter spp.* infections have declined due to emergence and dissemination of antimicrobial resistance even to many last line drugs.

3.7.1 Treatment of MDR *Acinetobacter* species:

Carbapenem remains the drug of choice for the treatment of MDR *Acinetobacter spp.* MYSTIC surveillance program documented that Imipenem is more potent than Meropenem for MDR, because efflux pumps affect Meropenem to a greater degree when compared to Imipenem. Hence both Imipenem and Meropenem susceptibility should be done.[25]

3.7.2 Treatment of XDR and PDR *Acinetobacter* species:

The treatment of XDR *Acinetobacter spp.*infections include Polymyxins and Tigecycline as the last resort. [38]

Tigecycline:

This drug is a glycycline agent which received approval from the Food and Drug Administration in June 2005.[61],[62] It is a broad-spectrum, parenteral and bacteriostatic agent. Its acts by blocking the protein synthesis. There is no interpretive criteria for tigecycline as per CLSI guidelines.[48] The guidelines laid down by FDA was used in many studies.[61] The major side effects are nausea, vomiting and diarrhea. Tigecycline can be used as combination therapy.[103] High degree of resistance to tigecycline has also been reported in *Acinetobacters*.[51]

Polymyxins:

Because of limited therapeutic options, polymyxins like polymyxin B and polymyxin E (colistin) came into use.[39],[40] Colistimethate is produced by Bacillus colistinus. It is hydrolyzed to colistin. It has bactericidal activity and its effect is concentration dependant. Colistin is a cationic detergent which acts by increasing the cell permeability by altering the lipopolysaccharide component in the bacterial cell membrane leading to cell death. Its use is limited because of its major adverse effects like nephrotoxicity, neurotoxicity and pulmonary toxicity. Many studies

have proved that colistin is effective in the treatment of MDR *Acinetobacter spp.* and other MDR organisms.[44]

Betalactamase Inhibitors:

Acinetobacter strains are intrinsically susceptible to Sulbactam, a betalactamase inhibitor. The combination therapy involving beta lactam antibiotic with its inhibitor combination does not have any significant role when comparing to betalactamase alone.[45]

Combination Therapy:

The combination therapy including sulbactam, rifampicin, aminoglycosides, carbapenems and colistin can be tried for treating XDR and PDR *Acinetobacter spp.*[40],[44],[46]

3.8 FACTORS AFFECTING OUTCOME OF THE PATIENT:

The presence of chronic and acute co-morbid conditions influences the patient outcome. The chronic conditions include diabetes, hypertension and immunosuppressive states and the acute conditions include dyselectrolytemia, cardiovascular compromise etc.

3.9 CONTROL MEASURES:

The persistence of MDR *Acinetobacter spp.* in health care settings could be due to several factors including the presence of susceptible

patients, the patients with colonization or infection, selective pressure from antimicrobial use and poor infection control procedures. Hence stringent measures should be taken to control and prevent the spread of MDR *Acinetobacter* infections.[25]

Infection control practices:[25]

1. Standard precautions, environmental cleaning and disinfection.
2. Point source control effective during the outbreak.
3. Contact barrier precaution to health care personnel.
4. Cohorting of patients.
5. Cohorting of healthcare personnel.
6. Clinical unit closure during outbreak to interrupt transmission and for thorough environmental disinfection.
7. Judicious use of antimicrobials to prevent drug resistance by antimicrobial stewardship.
8. Passive and active surveillance to identify colonized or infected patients, so that interventions can be implemented.

Materials and Methods

MATERIALS AND METHODS

Place of study:

This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai.

Study period:

The study period was for one year from September 2013 to August 2014.

Ethical consideration:

Approval was obtained from the Institutional ethics committee before the commencement of the study. Informed consent was obtained from all the patients participated in this study. All patients satisfying the inclusion criteria were included. Patients were interviewed by structured questionnaire.

Statistical analysis:

Statistical analyses were carried out using Statistical Package for Social Sciences (SPSS). The proportional data of this cross sectional study were tested using Pearson's Chi Square analysis test &Fisher Exact test.

Study Population:

A total of 175 clinically significant, consecutive, non duplicate isolates of *Acinetobacter spp.* were enrolled in this study. The isolates were from various clinical specimens sent to the Microbiology department for bacteriological culture, biochemical identification and antibiotic susceptibility testing. Isolates included in this study were obtained from blood, sputum, endotracheal aspirate, bronchial wash, pleural fluid, ascitic fluid, peritoneal dialysis fluid, cerebrospinal fluid, urine and wound swabs.

Inclusion criteria:

1. Clinically significant, consecutive, non duplicate isolates were included in the study. The significance of the isolate was based on two or more of the following criteria - clinical history, presence of organism in Gram stain, presence of intracellular forms of the organism and pure growth in culture with a significant colony count wherever applicable.
2. Patients aged more than 18 years.

Exclusion criteria:

1. Isolates of repeated samples from the same patient were not included in the study.

2. Patients with colonization of *Acinetobacter* with no apparent clinical illness.
3. Patients aged less than 18 years were not included.

❖ Preliminary identification of isolates belonging to Genus *Acinetobacter* was done based on the following characteristics,

4.1 COLONY MORPHOLOGY:

- **On Nutrient agar** - Translucent colonies 1-2 mm in diameter with smooth surface, slightly mucoid and opaque with grayish white pigmentation and some strains with yellow pigmentation.

- **On Blood agar -** circular colonies with or without haemolysis.

- **On MacConkey agar -** Lactose non fermenting colonies but sometimes with pinkish hue.

- **On CLED** - circular, 1-1.5mm in diameter lactose non fermenting colonies.

➢ The isolates obtained were subjected to preliminary tests like Gram staining, Catalase test, Oxidase test and Motility by Hanging drop method.(APPENDIX - II)

- The isolates which were Gram negative bacilli or Gram negative coccobacilli, catalase positive, oxidase negative and non-motile by hanging drop were subjected to biochemical reactions for further confirmation.

- The following preliminary biochemical reactions were done with appropriate controls - Mannitol motility medium for mannitol fermentation, Triple sugar iron agar medium for sugar fermentation and hydrogen sulphide production, Indole production using Kovac's reagent and Citrate utilization on Simmons Citrate Medium.

- Isolates giving the following reactions were further processed in the study:-

  - **Mannitol motility medium-** mannitol not fermented, non motile.

  - **Triple sugar iron agar**- Alkaline slant/alkaline butt, no gas or hydrogen sulphide production.

  - **Indole** was not formed on adding Kovac's reagent to 24hr broth culture.

  - Absence of growth and absence of change in colour from apple green to blue denotes non utilization of citrate.

4.2 SPECIATION OF ACINETOBACTER ISOLATES:

Phenotypic characterization:

The isolates which were identified as belonging to the Genus *Acinetobacter* were subjected to the following biochemical reactions for speciation.

- **Hugh Leifsons OF medium**: A set of semisolid medium containing 1% glucose was inoculated with a young agar slope culture. One of the tube was immediately overlaid with sterile paraffin oil to produce anaerobic condition. The species which utilizes carbohydrates produces an acid reaction in the open tube only.

- **Arginine dehydrolase test**: Isolated colonies were stab inoculated into Moller decarboxylase medium with Arginine and overlaid with a 5mm layer of sterile paraffin oil, incubated at 37ºC for 24 hrs. Violet discolouration of the medium denotes positive reaction, and a yellow discolouration as negative reaction.

- **Malonate utilization test**: Malonate broth was inoculated with a young agar slope culture and incubated at 37˚C for 48hours. The

change in colour from green to blue indicates positive reaction, no colour change indicates negative reaction.

- **Nitrate reduction test:** Nitrate reduction broth was inoculated with a young agar slope culture and incubated at 37˚C for 96hours. After incubation 0.1ml of a mixture containing alpha napthylamine and sulfanilic acid in equal proportion was added. The development of red color within a few minutes indicates the presence of nitrite and the ability of the organism to reduce nitrate. The absence of red color indicates the inability of the organism to reduce nitrate.

- **Heamolytic property:** The culture from a young agar slope was inocualted onto 5% sheep blood agar for the identification of heamolysis, as *A.haemolyticus* produces haemolytic colonies and other species are non-haemolytic.

- **Growth at variable temperature:** The culture from a young agar slope was inocualted onto two Nutrient agar plates and was incubated at 37˚C and at 42˚C respectively. The presence/absence of growth at two different temperatures was used in the species identification.

BIOCHEMICAL CHARACTERISTICS:

Organism	Growth at 37˚C	Growth at 44˚C	Haemolysis	OF glucose	Arginine hydrolysis	Malonate utilization
A.calcoaceticus	+	-	-	+	+	+
A.baumannii	+	+	-	+	+	+
A.haemolyticus	+	-	+	V	+	-
A.lwoffii	+	-	-	-	-	-
A.junii	+	-	-	-	+	-
A.johnsonii	+	-	-	-	V	V

4.3 ANTIMICROBIAL SENSITIVITY TESTING:

4.3.1 Disc diffusion method:

Antibiotic sensitivity was performed for all the isolates by Kirby - Bauer disc diffusion method using cation adjusted Mueller-Hinton agar plate. Three to four colonies were suspended in nutrient broth and were incubated for two hours at 37ºC, so as to get the organism in the logarithmic phase. The density of the suspension was standardized with nutrient broth, visually equivalent to 0.5 McFarland units. Within fifteen minutes of preparation of the suspension, a sterile cotton-wool swab was dipped into the suspension and the surplus was removed by rotating the swab against the side of the test tube. With this swab, the agar plate was inoculated by even streaking of the swab over the entire surface of the plate in three directions so as to obtain a lawn culture. After brief drying,

the antibiotic disc was placed, 5 on each plate. All the batches of antibiotics were quality checked as per standard guidelines. The control strains were included as per the CLSI guidelines.

The panel of drugs used for antimicrobial sensitivity testing was as follows;

ANIBIOTICS	RESISTANT (mm)	INTERMEDIATE (mm)	SENSITIVE (mm)
Cefotaxime (30µg)	≤ 14	15-22	≥ 23
Ceftazidime (30µg)	≤ 14	15-17	≥ 18
Cefepime (30µg)	≤ 14	15-17	≥ 18
Amikacin (30µg)	≤ 14	15-16	≥ 17
Gentamycin (10µg)	≤ 12	13-14	≥ 15
Ciprofloxacin (5µg)	≤ 15	16-20	≥ 21
Piperazillin / Tazobactum 100/10µg)	≤ 17	18-20	≥ 21
Trimethoprim /Sulfamethoxazole (1.25/23.75µg)	≤ 10	11-15	≥ 16
Imipenem (10µg)	≤ 18	19-21	≥ 22
Meropenem (10µg)	≤ 14	15-17	≥ 18
Tigecycline (15µg)	≤12	13-15	≥16
Polymyxin B (300U)	≤10	-	≥14
Colistin (10µg)	≤11	-	≥14

- ❖ Interpretations were made using the Clinical and Laboratory Standards Institute, USA guidelines (January 2014, M100-S24-Volume 34 No.1, Table 2B-2, Page 62/63).

- ❖ Journal reference was used for Polymyxin B and Colistin Disc diffusion standards as no CLSI guidelines exist for the same.[57],[58]

- ❖ For Tigecycline the guidelines laid down by F.D.A. were used.[61],[62]

4.3.2 Minimum inhibitory concentration (MIC) for detecting Meropenem Resistance Using Macrobroth Dilution Method:

MIC was determined for the isolates which were showing resistance to Meropenem (< 18mm) by disc diffusion method.

1. Culture media: cation adjusted Mueller Hinton broth (pH 7.2-7.4).

2. Preparation of antibiotic stock solution:

Meropenem used for preparing stock solution was obtained from Macleods.

- ❖ Antibiotic stock solution was prepared using the formula,

$$W = \frac{1000}{P} \times V \times C$$

Where P= potency of the antibiotic in relation to the base. (For Meropenem, P= 675/1000 mg)

V = volume of the stock solution to be prepared (10ml)

C = final concentration of the antibiotic solution (1024µg/ml)

W = weight of the antibiotic to be dissolved in the volume V

15.17 mg of drug was mixed with 10ml of distilled water which contains 1024µg/ml concentration of drug.

3. Preparation of antibiotic dilutions:

- Two rows each of 14 sterile test tubes were arranged in the rack (1 row for the test & 2nd row for ATCC control).
- Using micropipette 1ml of MH broth was transferred to all the tubes in the rack.
- From the stock solution 1 ml was transferred to the first tube in each row and mixed well.
- From the first tube 1 ml of the antibiotic solution was transferred to second tube.
- This procedure was repeated till the 14th tube.
- One tube containing only antibiotic solution was kept as control.

4. Inoculum preparation for the test and ATCC control:

- 9.9 ml of MH broth was taken in a sterile test tube.
- 0.1ml of 0.5 McFarland turbidity matched test organism was added to broth and mixed well.
- From the above inoculum 1 ml was transferred to each tube containing antibiotic dilutions.
- One tube containing only test inoculum was kept as control
- Same procedure was repeated for ATCC control strain.

5. Incubation:

The test tubes were incubated at 37ºC overnight.

Interpretation:

- MIC of ATCC control strain and the test organism was observed.
- The lowest concentration of the antibiotic which shows clearing was considered as the MIC for the ATCC strain & for the test isolate.

MIC of Meropenem[48]:

≤ 2µg/ml - Susceptible

4µg/ml - Intermediate

≥8µg/ml - Resistant

4.4 DETECTION OF ANTIMICROBIAL RESISTANCE MECHANISMS:

Phenotypic Method:

All the isolates which were included in this study were subjected to Carbapenemase screening test using Meropenem disc, AmpC screening test by Disc antagonism test using Cefoxitin disc and ESBL screening test using Cefotaxime and Ceftazidime discs. The screen test positive isolates were subjected to respective confirmatory tests using appropriate antibiotic discs that were quality checked.

4.4.1 Carbapenamase detection:

The isolates which were resistant to Meropenem by disc diffusion method as per CLSI guidelines was used as the indicator for carbapenamase production and tested for Oxacillinase, Metallo betalactamase and AmpC betalactamase production.[69],[70]

4.4.1A Oxacillinase detection by Modified Hodge Test:

Lee et al. has described the MHT for detection of Carbapenamase.[52] A 0.5 McFarland standard suspension of E.coli ATCC 25922 from an overnight culture was prepared and was diluted 1:10 in saline or broth. The MHA plate was inoculated with the suspension as for disc diffusion procedure and allowed to dry for 5 to 10

minutes. The 10µg of Ertapenem disc (Himedia) was placed in the centre. The test isolate was then streaked from the edge of the disc to the periphery of the plate along with positive and negative controls and incubated at 37˚C for 24 hours. The length of the streak should be 20 to 25mm.[53],[54]

Interpretation:

Enhanced growth of the test strain towards the zone of inhibition - positive for carbapenamase production.

No enhanced growth of the test strain towards the zone of inhibition - negative for carbapenamase production.

Quality control Organisms:

Positive control - K.pneumoniae ATCC BAA-1705

Negative control - K.pneumoniae ATCC BAA-1706

4.4.1B Metallo Beta lactamase Detection by Imipenem(IMP)-EDTA Combined Disc Test:

The test isolate was inoculated onto MHA plates as for disc diffusion procedure. Two Imipenem discs (10µg) (Himedia) were placed on the MHA plate. 10µl of EDTA solution (750µg) was added to one of the Imipenem disc. The plate was incubated at 37˚C for 24hrs. The

increase in zone size of ≥7mm around Imipenem EDTA as compared to Imipenem was interpreted as a positive result.[52],[53],[93]

4.4.1C AmpC Betalactamase Detection by AmpC Disc Test:

A lawn culture of ATCC E.coli 25922 was prepared on a MHA plate. A disc containing 10µg of Cefoxitin (Himedia) was placed on the surface of agar. Sterile disc was moistened with 20µl sterile saline and inoculated with several colonies of the test organism. The inoculated disc was then placed beside the Cefoxitin disc almost touching on the inoculated plate. The plates were incubated overnight at 37˚C. A positive test appears as flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc.[54],[63],[64]

4.4.2 AmpC Betalactamase Detection by AmpC Disc Test:

The isolates with zone size of less than 18mm for cefoxitin disc was considered as screening test positive for AmpC. They were further confirmed with AmpC disc test as described above.

4.4.3 ESBL Detection by CLSI Phenotypic Confirmatory Method:

In this method a lawn culture of test isolate was made as for disc diffusion procedure.[48] Ceftazidime clavulanic acid disc(30µg/10µg) (Himedia) and ceftazidime disc 30µg (Himedia) were placed on the surface of the plate. The test isolate was considered to produce ESBL, if

the zone size around the β lactamase inhibitor combination disc was increased by ≥5mm. The test was performed with appropriate controls.

4.5 MOLECULAR METHOD:

Polymerase chain reaction:

The isolates which were resistant to Meropenem by Kirby Bauer Disc diffusion method irrespective of phenotypic methods were subjected to conventional PCR for the detection of Oxacillinase gene OXA-23 and Metallo Beta Lactamases genes bla-IMP_1 and bla-VIM_1.[72],[73]

DNA extraction:

5-10 Acinetobacter colonies were inoculated into nutrient broth and incubated overnight at 37ºC. 1.5ml of overnight broth culture was transferred into 2.5ml of centrifuge tube and centrifuged at 10,000 rpm for 3 minutes. Supernatant was discarded, excess medium was removed by gently tapping the tube on a paper towel.

Procedure:

1. The pellet obtained was suspended in 200µl of PBS.
2. 180µl of Lysozyme digestion buffer and 20µl of Lysozyme were added.
3. Above mixture was mixed well and incubated at 37 ºC for 15min.

4. After incubation 200µl of Lysis buffer and 20µl of Proteinase K [10mg/ml] were added and incubated at 56ºC for 10min in waterbath.
5. Then 300µl of Isopropanol was added and mixed well.
6. The whole lysate was transferred into PureFast spin column and centrifuged at 10000rpm for 1min.
7. Flow through was discarded and 500µl of Wash buffer-1 was added to spin column and centrifuged at 10000rpm for 1min.
8. Flow through was discarded and 500µl of Wash buffer-2 was added to spin column and centrifuged at 10000rpm for 1min. washing was repeated one more time.
9. Flow through was discarded and the spin column was centrifuged for additional 2 minutes to remove any residual ethanol.
10. The DNA was eluted by adding 100µl of Elution buffer and centrifuged for 1min. The eluted DNA was used as the template for PCR.

Primers: [Designed by HELINI Biomolecules, Chennai]

GENE	PRIMER SEQUENCES	AMPLICON SIZE
OXA-23 (F)	5'-CTTGCTCGTGCTTCGACCGAGT-3'	**160bp**
(R)	5'-CGCCTAGGGTCATGTCCTTTTC-3'	
VIM_1 (F)	5'-GTGCTTTGACAACGTTCGCT-3'	**422bp**
(R)	5'-TCCACGCACTTTCATGACGA-3	
IMP_1 (F)	5'-TTTTGCAGCATTGCTACCGC-3'	**220bp**
(R)	5'-CACGCTCCACAAACCAAGTG-3	

PCR Procedure:

1. Reactions were set up as follows;

Components	Quantity
HELINI 2X PCR Master Mix	10µl
Primer Mix 10pmoles/Reactions	5µl
Genomic DNA	5µl
Total volume	20µl

2. All the components were mixed gently and placed into Corbett Reseach thermocycler and programmed it as follows,

Cycle Number	Denaturation	Annealing	Extension
1	94ºC for 5 min	-	-
35	94ºC for 30sec	58ºC for 30sec	72ºC for 30sec
1	-	-	72º C for 5 min

Agarose gel electrophoresis:

1. 2% agarose gel was prepared [2gm of agarose in 100ml of 1x TAE buffer] with eight wells.
2. 8µl 6X Gel loading dye was mixed to each PCR vial.
3. 15µl from each PCR vial was loaded.
4. Then 100bp DNA ladder and appropriate controls were loaded.
5. Electrophoresis was run at 50V for 45 min and the bands were observed using UV Transilluminator.

Interpretation:

The amplified PCR products and 100bp DNA molecular markers were seen as bright fluorescent bands with satisfactory controls. A 160bp corresponds to OXA-23, 422bp corresponds to blaVIM$_1$ and 220bp corresponds to blaIMP$_1$ gene.

Results

RESULTS

This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College in association with various other Departments at the Rajiv Gandhi Government General Hospital, Chennai during the period of Sep 2013 to Aug 2014. A total of 175 clinically significant, consecutive, non duplicate isolates of *Acinetobacter species* from various clinical specimens were included in the study. All the isolates were identified by standard procedures.

TABLE 1: GENDER DISTRIBUTION OF PATIENTS (n= 175)

SEX	NO. OF PATIENTS	PERCENTAGE%
MALE	**120**	**68.57%**
FEMALE	**55**	**31.43%**

There was a male predominance (68.57%) among the isolates obtained from the patients.

FIGURE-1: GENDER DISTRIBUTION OF PATIENTS (n =175)

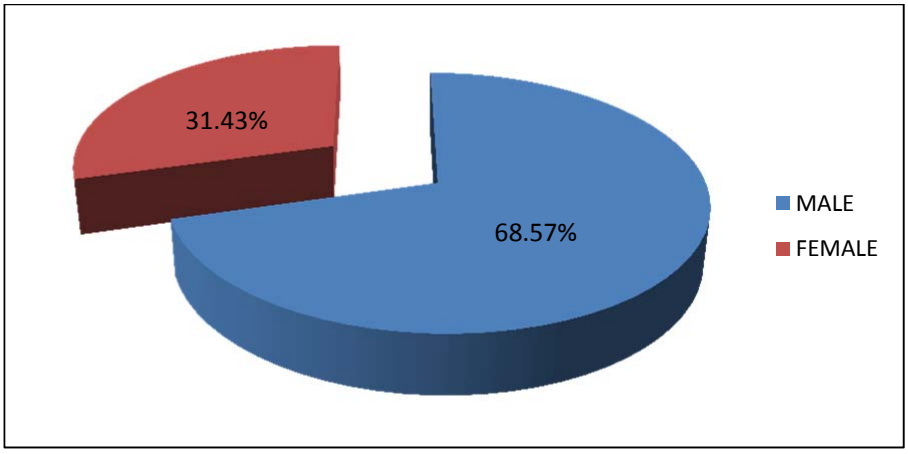


TABLE 2: AGE DISTRIBUTION OF PATIENTS (n=175)

AGE IN YEARS	NO. OF PATIENTS			PERCENTAGE %
	MALE	FEMALE	TOTAL	
18-20	7	3	10	5.71%
21-40	28	20	48	27.43%
41-60	59	25	84	48.00%
>60	26	7	33	18.86%

The maximum number of isolates were from the patients in the age group of 41 – 60 years (48.0%) followed by 21 – 40 years (27.43%).

FIGURE-2: AGE AND GENDER DISTRIBUTION OF PATIENTS (n =175)

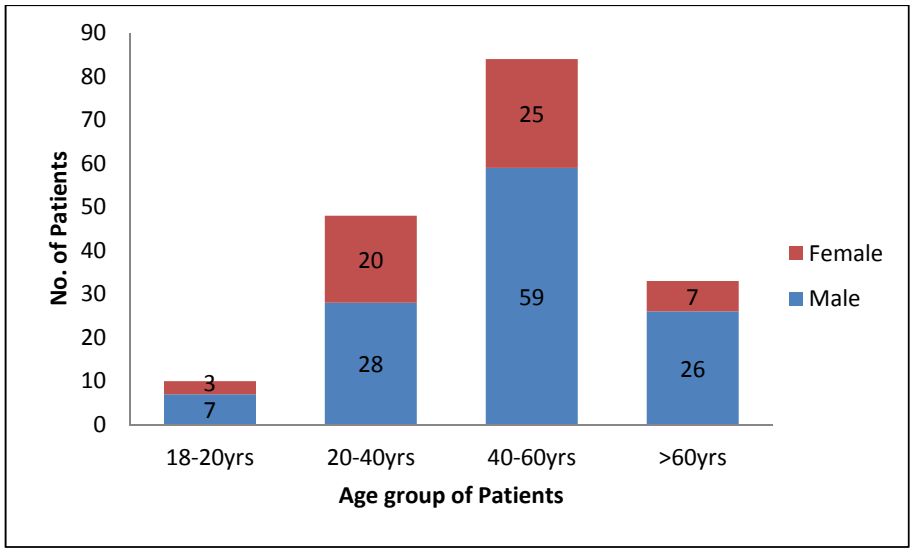


TABLE 3: DISTRIBUTION OF *ACINETOBACTER* ISOLATES FROM VARIOUS CLINICAL SPECIMENS (n =175)

SAMPLES	TOTAL NUMBER	PERCENTAGE %
Urine	45	25.71%
Endotracheal aspirate	41	23.43%
Wound swab	41	23.43%
Blood	17	9.71%
Sputum	12	6.86%
Bronchial wash	7	4.0%
PD fluid	5	2.86%
CSF	3	1.71%
Pleural fluid	2	1.14%
Ascitic fluid	2	1.14%

FIGURE-3: DISTRIBUTION OF *ACINETOBACTER* ISOLATES FROM VARIOUS CLINICAL SPECIMENS (n =175)

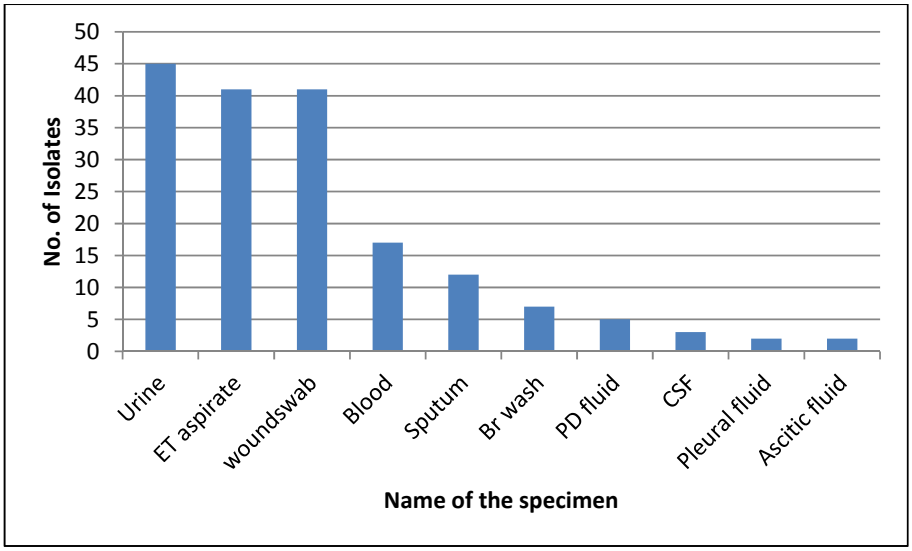


TABLE4: DISTRIBUTION OF *ACINETOBACTER* ISOLATES IN VARIOUS CLINICAL SPECIMENS FROM DIFFERENT WARDS (n = 175)

SPECIALITY	SPECIMEN	NUMBER	TOTAL	PERCENTAGE %
ICU	ET aspirate	26	36	20.57%
	Blood	4		
	Urine	4		
	Sputum	1		
	Wound swab	1		
MEDICAL WARD	Urine	19	31	17.71%
	Blood	6		
	Sputum	2		
	Ascitic fluid	2		
	Pleural fluid	2		
NEURO SURGERY	ET aspirate	15	26	14.86%
	CSF	3		
	Urine	3		
	Wound swab	5		
SURGICAL WARD	Wound swab	20	24	13.71%
	Urine	4		
NEPHROLOGY	Urine	9	21	12%
	Blood	5		
	PD fluid	5		
	Sputum	2		

SPECIALITY	SPECIMEN	NUMBER	TOTAL	PERCENTAGE %
ORTHO PAEDICS WARD	Wound swab	13	15	8.57%
	Urine	2		
THORACIC MEDICINE	Sputum	7	14	8%
	Bronchial wash	7		
OTHERS		8	8	4.57%

Most of the isolates were from the Intensive care unit (20.57%), followed by Medicine unit (17.71%), Neuro surgery (14.86%) and Nephrology (12%).

FIGURE 4: DISTRIBUTION OF *ACINETOBACTER* ISOLATES IN VARIOUS CLINICAL SPECIMENS FROM DIFFERENT WARDS (n =175)

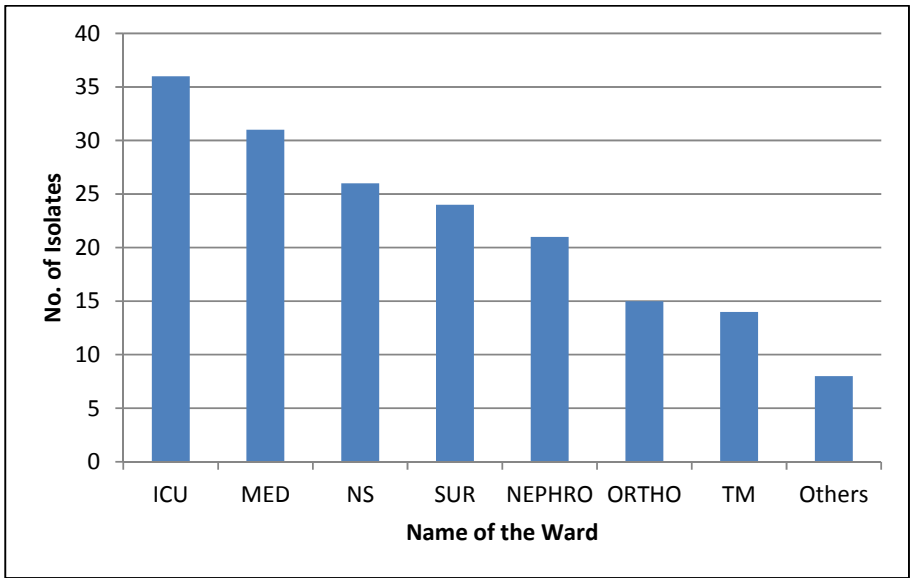


TABLE 5: SPECIES OF *ACINETOBACTER* ISOLATED (n =175)

S.NO.	SPECIES ISOLATED	NO. OF ISOLATES	PERCENTAGE %
1	***A.baumannii***	**142**	**81.14%**
2	***A.lwoffii***	**18**	**10.29%**
3	***A.calcoaceticus***	**8**	**4.57%**
4	***A.junii***	**7**	**4%**

Acinetobacter baumannii (81.14%) was the most common species isolated followed by *A.lwoffii* (10.29%), *A.calcoaceticus* (4.57%) and *A.junii* (4%).

FIGURE-5: DISTRIBUTION OF *ACINETOBACTER* SPECIES

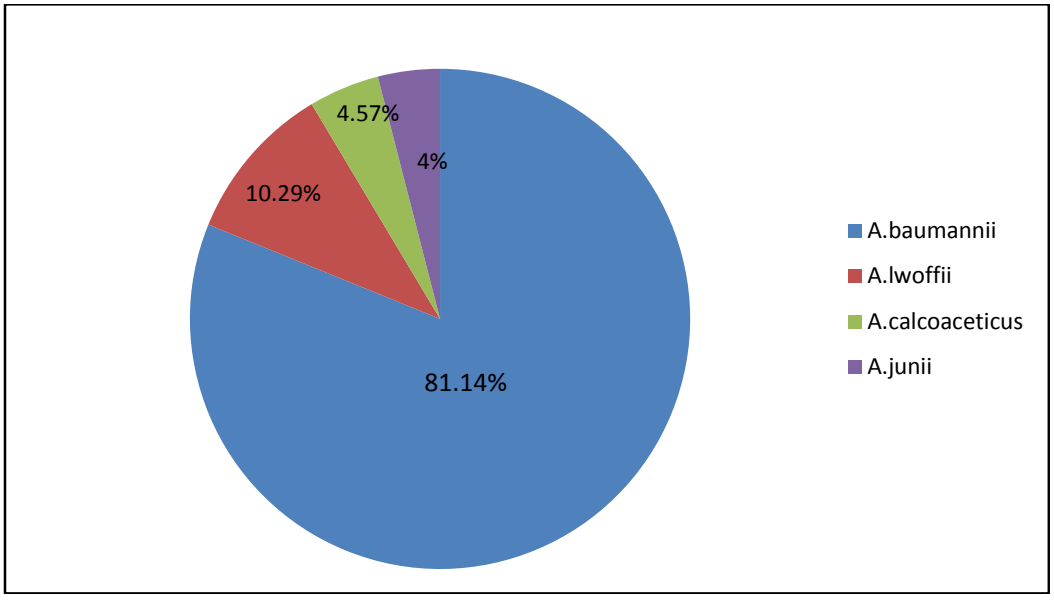


TABLE 6: DISTRIBUTION OF *ACINETOBACTER* SPECIES IN VARIOUS CLINICAL INFECTIONS (n =175)

INFECTIONS	A.baumannii	A.lwoffii	A.calcoaceticus	A.junii
Respiratory tract infections	61	-	1	-
Wound infections	27	7	3	4
Urinary tract infections	34	6	2	1
Septicemia	9	3	-	2
Cirrhosis	2	1	1	-
CKD	6	1	1	-
Hydrocephalus	3	-	-	-

A.baumannii was isolated from respiratory tract infections followed by wound infections, urinary tract infections and septicemia. *A.lwoffii* was isolated from wound infections and UTI. *A.calcoaceticus* was isolated from wound infections. *A.junii* was isolated from wound infections and septicemia.

TABLE 7: DISTRIBUTION OF *ACINETOBACTER* SPECIES IN VARIOUS CLINICAL SPECIMENS (n =175)

SPECIES	SPECIMEN	NUMBER	TOTAL	PERCENTAGE%
A.baumannii	**ET aspirate**	**41**	**142**	**81.14%**
	Urine	**34**		
	Wound swab	**27**		
	Sputum	**11**		
	Blood	**10**		
	Bronchial wash	**7**		
	PD fluid	**5**		
	CSF	**3**		
	Pleural fluid	**2**		
	Ascitic fluid	**2**		
A.lwoffii	**Wound swab**	**7**	**18**	**10.29%**
	Urine	**6**		
	Blood	**5**		
A.calcoaceticus	**Urine**	**4**	**8**	**4.57%**
	Wound swab	**3**		
	Sputum	**1**		
A.junii	**Wound swab**	**4**	**7**	**4%**
	Blood	**2**		
	Urine	**1**		

A.baumannii was isolated from ET aspirate, urine, wound swab, blood, sputum, bronchial wash and body fluids. *A.lwoffii, A.calcoaceticus* and *A.junii* were isolated from wound swab, blood and urine.

TABLE 8: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *A.baumannii*

Drugs	Total Number (n = 142)		Percentage %	
	Sensitive	Resistant	Sensitive	Resistant
Cefotaxime	19	123	13.38	86.62
Ceftazidime	29	113	20.42	79.58
Cefepime	47	95	33.09	66.91
Amikacin	61	81	42.96	57.04
Gentamycin	38	104	26.76	73.24
Ciprofloxacin	27	115	19.01	80.09
Pip - Taz	88	54	61.97	38.02
Cotrimoxazole	27	115	19.01	80.09
Imipenem	125	17	88.03	11.97
Meropenem	122	20	85.92	14.08
Tigecycline	118	24	83.10	16.90
Polymyxin- B	137	5	96.48	3.52
Colistin	142	-	100	-

FIGURE 6: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *A.baumannii*

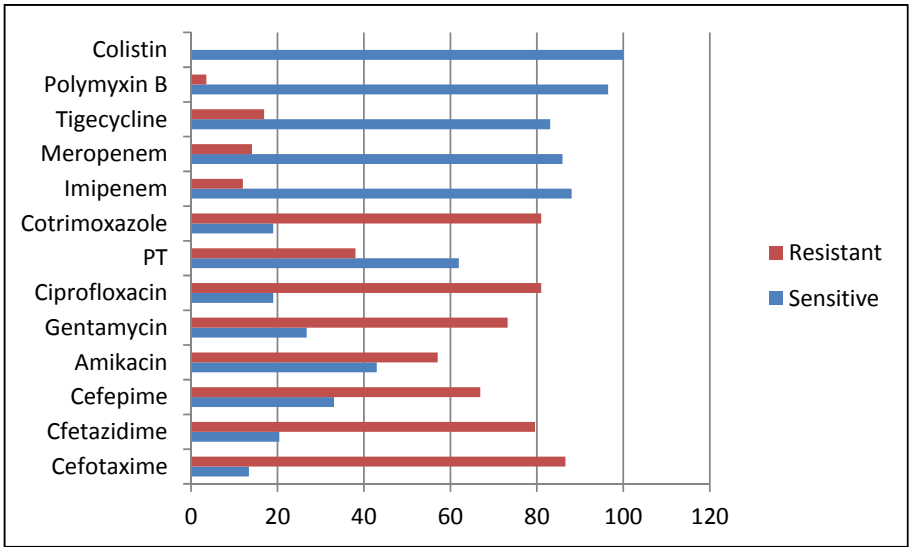


TABLE 9: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *A.lwoffii*

Drugs	Total Number (n = 18)		Percentage %	
	Sensitive	Resistant	Sensitive	Resistant
Cefotaxime	6	12	33.33	66.67
Ceftazidime	10	8	55.56	44.44
Cefepime	13	5	72.22	27.78
Amikacin	11	7	61.11	38.89
Gentamycin	8	10	44.44	55.56
Ciprofloxacin	5	13	27.78	72.22
Pip - Taz	15	3	83.33	16.67
Cotrimoxazole	3	15	16.67	83.33
Imipenem	18	-	100	-
Meropenem	18	-	100	-
Tigecycline	17	1	94.44	5.55
Polymyxin- B	18	-	100	-
Colistin	18	-	100	-

FIGURE 7: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *A.lwoffii*

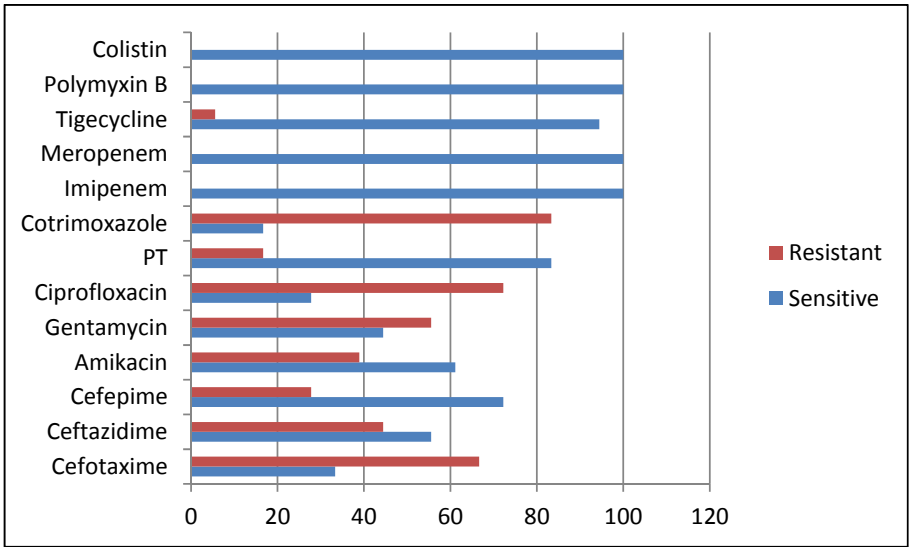


TABLE 10: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *A.calcoaceticus*

Drugs	Total Number (n = 8)		Percentage %	
	Sensitive	Resistant	Sensitive	Resistant
Cefotaxime	6	2	75	25
Ceftazidime	8	-	100	-
Cefepime	8	-	100	-
Amikacin	7	1	87.50	12.50
Gentamycin	6	2	75	25
Ciprofloxacin	3	5	37.50	62.50
Pip - Taz	8	-	100	-
Cotrimoxazole	3	5	37.50	62.50
Imipenem	8	-	100	-
Meropenem	8	-	100	-
Tigecycline	7	1	87.50	12.50
Polymyxin- B	8	-	100	-
Colistin	8	-	100	-

FIGURE 8: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *A.calcoaceticus*

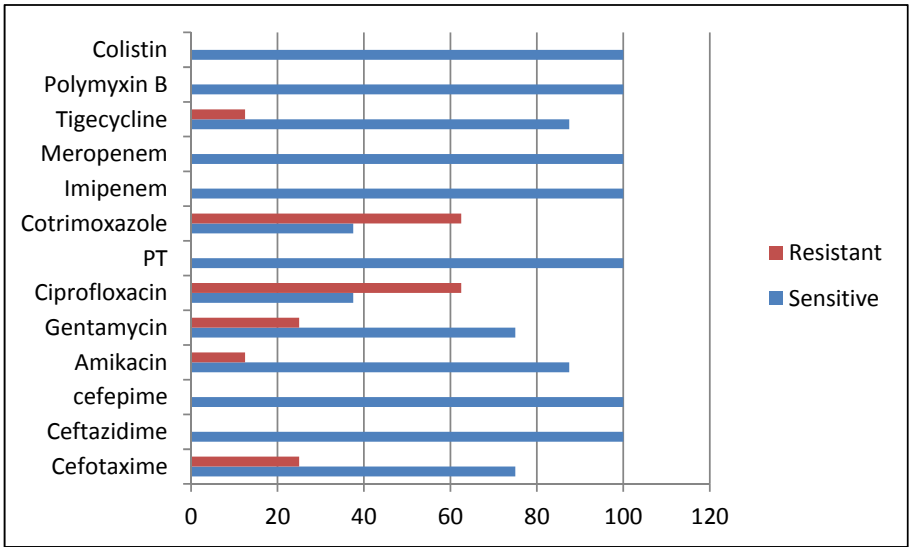


TABLE 11: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *A.junii*

Drugs	Total Number (n = 7)		Percentage %	
	Sensitive	Resistant	Sensitive	Resistant
Cefotaxime	4	3	57.14	42.86
Ceftazidime	5	2	71.43	28.57
Cefepime	5	2	71.43	28.57
Amikacin	3	4	42.86	57.14
Gentamycin	1	6	14.29	85.71
Ciprofloxacin	1	6	14.29	85.71
Pip - Taz	6	1	85.71	14.29
Cotrimoxazole	1	6	14.29	85.71
Imipenem	7	-	100	-
Meropenem	7	-	100	-
Tigecycline	6	1	85.71	14.29
Polymyxin- B	7	-	100	-
Colistin	7	-	100	-

FIGURE 9: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *A.junii*

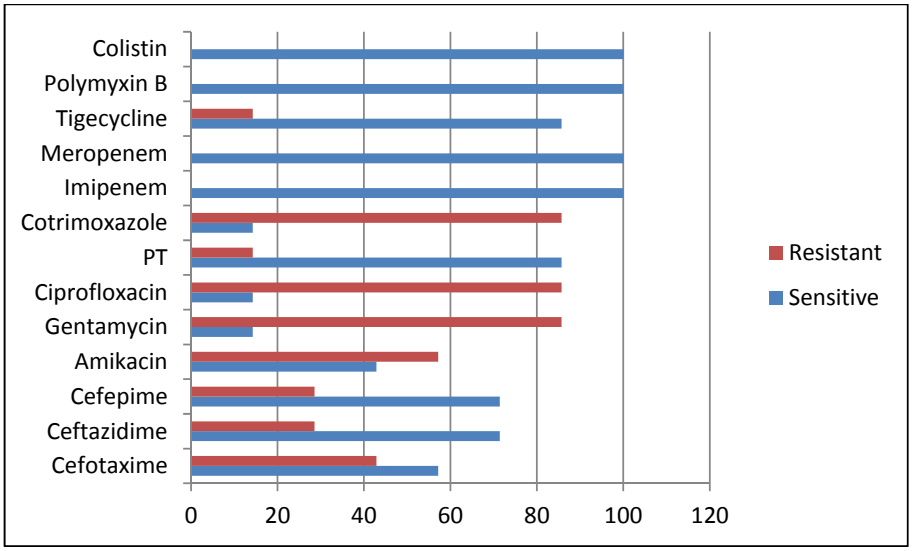


TABLE 12: COMPARISON OF ANTIMICROBIAL SENSITIVITY PATTERN AMONG ACINETOBACTER SPECIES (n =175)

DRUGS	A.BAUMANNII (n =142)		OTHER SPP. (n=33)		TEST	P VALUE	SIGNIFI CANCE
	SEN	RES	SEN	RES			
Cefotaxime	19	123	16	17	Chi-square	0.001	Significant
Ceftazidime	29	113	23	10	Chi-square	0.001	Significant
Cefepime	47	95	26	7	Chi-square	0.001	Significant
Amikacin	61	81	21	12	Chi-square	0.032	Significant
Gentamycin	38	104	15	18	Chi-square	0.035	Significant
Ciprofloxacin	27	115	9	24	Chi-square	0.290	Significant
PT	88	54	29	4	Chi-square	0.004	Significant
Cotrimoxazole	27	115	7	26	Chi-square	0.774	Non-Significant
Imipenem	125	17	33	0	Fisher's Exact	0.045	Significant
Meropenem	122	20	33	0	Fisher's Exact	0.016	Significant
Polymyxin-B	137	5	33	0	Fisher's Exact	0.585	Non-Significant
Tigecycline	118	24	30	3	Chi-square	0.004	Significant

There was a significant difference between the antimicrobial sensitivity pattern of *A.baumannii* and other species since p value is <0.05 for cephalosporins, aminoglycosides, quinolones and carbapenems. But there was no significant difference for cotrimoxazole and polymyxin-B.

TABLE 13: DETECTION OF MEROPENEM RESISTANCE IN *ACINETOBACTER* ISOLATES BY DISC DIFFUSION METHOD (n=175)

PATTERN OF RESISTANCE	NO OF ISOLATES	PERCENTAGE %
Susceptible	**155**	**88.57%**
Resistant	**20**	**11.43%**

175 isolates of *Acinetobacter species* were screened for meropenem resistance by Kirby -Bauer disc diffusion method, of which 20 isolates (11.43%) were found to be resistant to meropenem.

FIGURE 10: DISTRIBUTION OF MEROPENEM RESISTANCE IN *ACINETOBACTER* ISOLATES

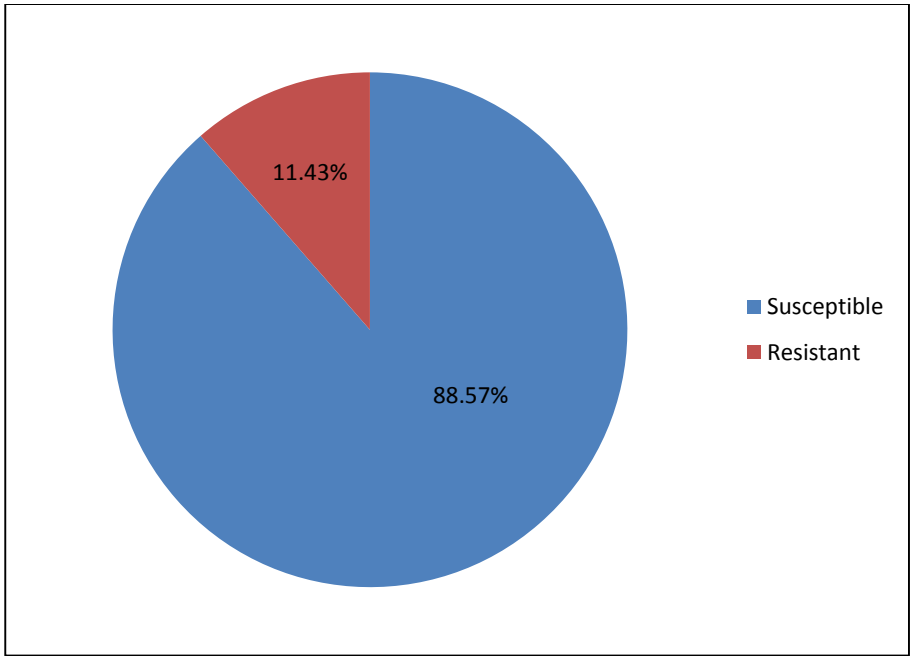


TABLE 14: PROFILE OF MEROPENEM RESISTANT ISOLATES (n =20)

ISOLATE NO.	AGE/ SEX	CLINICAL DIAGNOSIS	WARD	SPECIMEN	RISK FACTORS	SPECIES ISOLATED
1	**45/F**	**Urosepsis**	**SUR**	**urine**	Indwelling catheter	*A.baumannii*
2	**47/M**	**Poisoning**	**ICU**	**urine**	Indwelling catheter	*A.baumannii*
3	**43/M**	**RTA/Head injury**	**NS**	**Blood**	Mechanical ventilation	*A.baumannii*
4	**45/M**	**BB Fracture (left)**	**Ortho**	**urine**	Indwelling catheter	*A.baumannii*
5	**50/M**	**Post TB**	**ICU**	**ET aspirate**	Immuno compromised	*A.baumannii*
6	**34/M**	**Post Renal transplant**	**Nephro**	**urine**	Immuno compromised	*A.baumannii*
7	**48/M**	**Post Renal transplant**	**Nephro**	**urine**	Immuno compromised	*A.baumannii*
8	**35/M**	**Above knee amputation**	**Ortho**	**Wound swab**	Multiple antibiotics	*A.baumannii*
9	**58/M**	**Septicemia**	**ICU**	**Blood**	Mechanical ventilation	*A.baumannii*
10	**60/F**	**Poisoning**	**ICU**	**ET aspirate**	Mechanical ventilation	*A.baumannii*

ISOLATE NO.	AGE/ SEX	CLINICAL DIAGNOSIS	WARD	SPECIMEN	RISK FACTORS	SPECIES ISOLATED
11	**62/F**	**MCTD**	**ICU**	**ET aspirate**	Immuno compromised	*A.baumannii*
12	**58/F**	**Pneumonia**	**ICU**	**ET aspirate**	Immuno compromised	*A.baumannii*
13	**45/M**	**Pneumonia**	**MED**	**Sputum**	Immuno compromised	*A.baumannii*
14	**58/M**	**Aspiration pneumonitis**	**ICU**	**ET aspirate**	Immuno compromised	*A.baumannii*
15	**40/F**	**Poisoning**	**ICU**	**ET aspirate**	Mechanical ventilation	*A.baumannii*
16	**28/M**	**RTA/Head injury**	**NS**	**ET aspirate**	Mechanical ventilation	*A.baumannii*
17	**47/M**	**RTA/Head injury/SDH**	**NS**	**Wound swab**	Mechanical ventilation	*A.baumannii*
18	**20/M**	**RTA/Head injury**	**NS**	**Wound swab**	Mechanical ventilation	*A.baumannii*
19	**20/M**	**CSOM**	**ENT**	**Wound swab**	Multiple antibiotics	*A.baumannii*
20	**20/F**	**Post Renal transplant**	**Nephro**	**Urine**	Immuno compromised	*A.baumannii*

TABLE 15: MIC FOR MEROPENEM RESISTANT ISOLATES

(n =20)

The 20 isolates which were resistant to Meropenem by disc diffusion method were further tested for their meropenem minimum inhibitory concentration.

MIC for Meropenem (µg/ml)	512	256	128	64	32	16	8	4	2	1	0.5	0.25
A.baumannii (*n*=20)	-	9	5	5	1	-	-	-	-	-	-	-
P.aeruginosa ATCC 27853 (control)										1		

All the 20 isolates have their MIC values greater than 8µg/ml, hence they are resistant to meropenem. Among the 20 isolates, 9(45%) isolates have256µg/ml as MIC, 5(25%) isolates have 128 µg/ml as MIC, another 5(25%) isolates have 64 µg/ml as MIC and remaining 1(5%) isolate has 32 µg/ml as MIC.

FIGURE 11: DISTRIBUTION OF MEROPENEM MIC VALUES

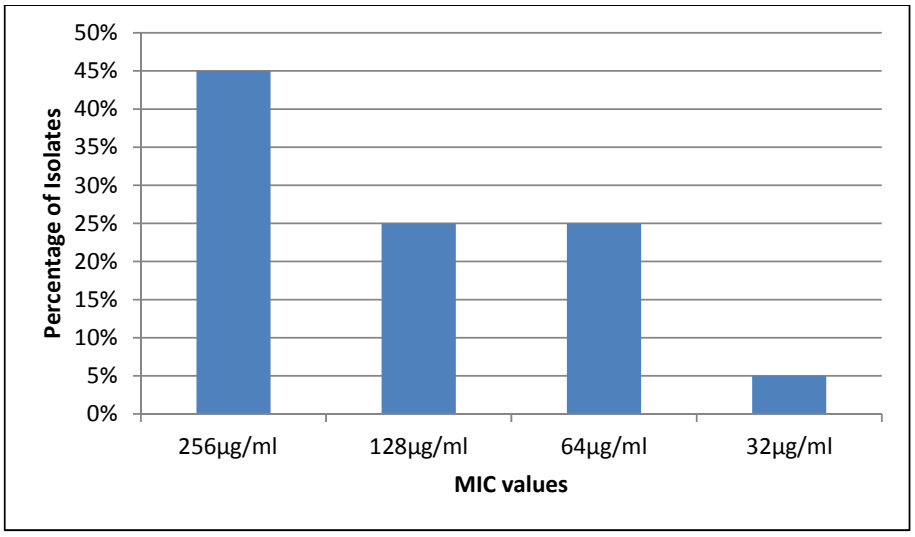


TABLE 16: PHENOTYPIC DETECTION OF RESISTANT MECHANISMS FOR MEROPENEM (n=20)

The meropenem resistance by Kirby -Bauer disc diffusion method was taken as the indicator for carbapenamase production and was further tested for their mechanisms of carbapenam resistance conferred by beta lactamases by phenotypic methods.

PHENOTYPIC TESTS	METHOD	NO. OF ISOLATES	PERCENTAGE %
POSITIVE	Modified Hodge Test (MHT)	9	45%
	Imipenem-EDTA combined disc test (IEDT)	9	45%
	AmpC Disc Test	6	30%
	MHT, IEDT & AmpC Disc Test	3	15%
	MHT & IEDT	7	35%
NEGATIVE	MHT, IEDT and Ampc Disc Test	7	35%

Among the 20 isolates, Modified Hodge test was positive in 9 (45%) isolates, IEDT was positive in 9(45%) isolates, AmpC Disc test was positive in 6(30%) isolates and 7(35%) isolates were negative for all the three phenotypic methods. Out of the 20 isolates, 3(15%) isolates were positive for MHT, IEDT and AmpC disc test and 7(35%) isolates were positive for MHT and IEDT.

TABLE 17: MOLECULAR DETECTION OF RESISTANT MECHANISMS FOR MEROPENEM (n=20)

The Meropenem resistant isolates were tested for most common carbapenamase gene **OXA – 23** and metallo betalactamase genes **blaVIM$_1$** and **blaIMP$_1$** by **PCR**

GENES TESTED	POSITIVE ISOLATES	PERCENTAGE%
OXA – 23	**20**	**100%**
blaVIM$_1$	**9**	**45%**
blaIMP$_1$	**7**	**35%**

Among the 20 meropenem resistant isolates, all the 20 isolates were positive for OXA-23 (100%), 9 (45%) isolates were positive for blaVIM$_1$ and 7 (35%) isolates were positive for blaIMP$_1$.

FIGURE 12: DISTRIBUTION OF VARIOUS GENES AMONG THE MEROPENEM RESISTANT ISOLATES

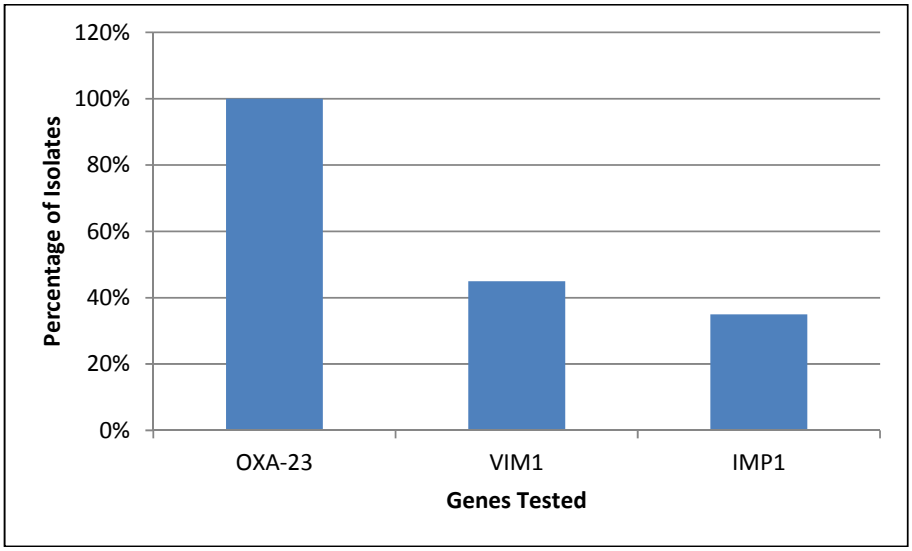


TABLE 18: COMPARISON OF PHENOTYPIC AND GENOTYPIC METHODS IN MEROPENEM RESISTANT ISOLATES

PHENOTYPIC TEST RESULTS	GENES TESTED					
	OXA – 23		bla_{VIM1}		bla_{IMP1}	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive MHT (n = 9)	9	-	2	7	5	4
Positive IEDT (n = 9)	9	-	3	6	6	3
Positive AmpC Disc Test (n=6)	6	-	2	4	3	3
MHT, IEDT & AmpC Disc test Positive (n =3)	3	-	2	1	3	-
Phenotypic tests Negative (n =7)	7	-	3	4	1	6

Among the 9MHT positive isolates, all the 9 isolates were OXA-23 positive, 2 were blaVIM$_1$ positive and 5 were blaIMP$_1$ positive. Similarly among the 9 IEDT positive isolates all the 9 were OXA-23 positive, 3 were blaVIM$_1$ positive and 6 were blaIMP$_1$ positive. Among the 6 AmpC disc test positive isolates, all of them were OXA-23 positive, 2 were blaVIM$_1$ positive and 3 were blaIMP$_1$ positive. Similarly in the 7 phenotypic negative isolates, all were OXA-23 positive, 3 were blaVIM$_1$ positive and 1 isolate was blaIMP$_1$ positive. All the three genes were positive in 2 (10%) isolates, similarly both blaVIM$_1$ and blaIMP$_1$ were positive in 2 (10%) isolates.

TABLE 19: DETECTION OF EXTENDED SPECTRUM BETA LACTAMASE IN *ACINETOBACTER* ISOLATES (n=175)

ESBL PRODUCTION	NO OF ISOLATES	PERCENTAGE %
Positive	**61**	**34.86%**
Negative	**114**	**65.14%**

175 isolates of *Acinetobacter species* were screened for ESBL production and confirmed by CLSI phenotypic confirmatory method. 61(34.86%) isolates were found to be ESBL producers.

TABLE 20: DISTRIBUTION OF ESBL AMONG *ACINETOBACTER SPP.* (n = 175)

SPECIES	SPECIMEN	NUMBER	TOTAL	PERCENTAGE%
A.baumannii	**Wound swab**	**15**	**56**	**32.00%**
	ET aspirate	**14**		
	Urine	**10**		
	Sputum	**8**		
	Br wash	**4**		
	Body fluids	**4**		
	Blood	**1**		
A.lwoffii	**Wound swab**	**3**	**4**	**2.29%**
	Blood	**1**		
A.junii	**Blood**	**1**	**1**	**0.57%**

The Extended spectrum beta lactamases were common in *A.baumannii* (32.00%), followed by *A.lwoffii* (2.29%) and *A.junii* (0.57%).

FIGURE 13: DISTRIBUTION OF ESBL IN *ACINETOBACTER* ISOLATES

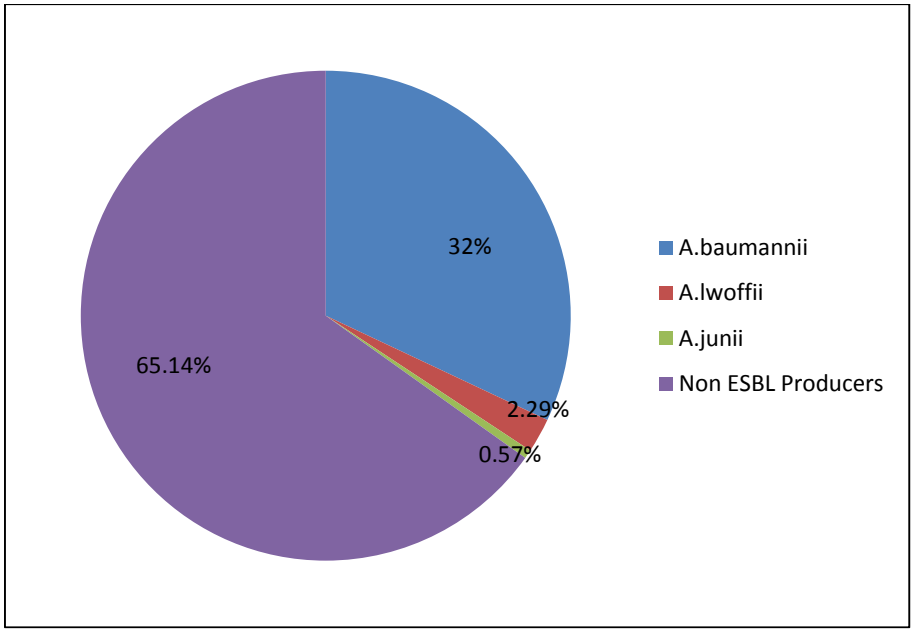


TABLE 21: DETECTION OF AMPC BETA LACTAMASE IN *ACINETOBACTER* ISOLATES BY AMP C DISC TEST (n=175)

AMP C BETA LACTAMASE	NO OF ISOLATES	PERCENTAGE %
Positive	**23**	**13.14%**
Negative	**152**	**86.86 %**

175 isolates of *Acinetobacter species* were screened for Amp C beta lactamases by Disc antagonism test. 23(13.14%) isolates were found to be Amp C screening test positive and confirmed with Amp C disc test.

TABLE 22: DISTRIBUTION OF AMPC AMONG *ACINETOBACTER SPP.* (n=175)

SPECIES	SPECIMEN	NUMBER	TOTAL	PERCENTAGE%
A.baumannii	**ET aspirate**	**8**	**21**	**12%**
	Urine	**7**		
	Blood	**3**		
	Wound swab	**3**		
A.lwoffii	**Blood**	**1**	**2**	**1.14%**
	Urine	**1**		

The AmpC beta lactamase were common in A.baumannii (12%) and A.lwoffii (1.14%).

FIGURE14: DISTRIBUTION OF AMP C IN *ACINETOBACTER* ISOLATES

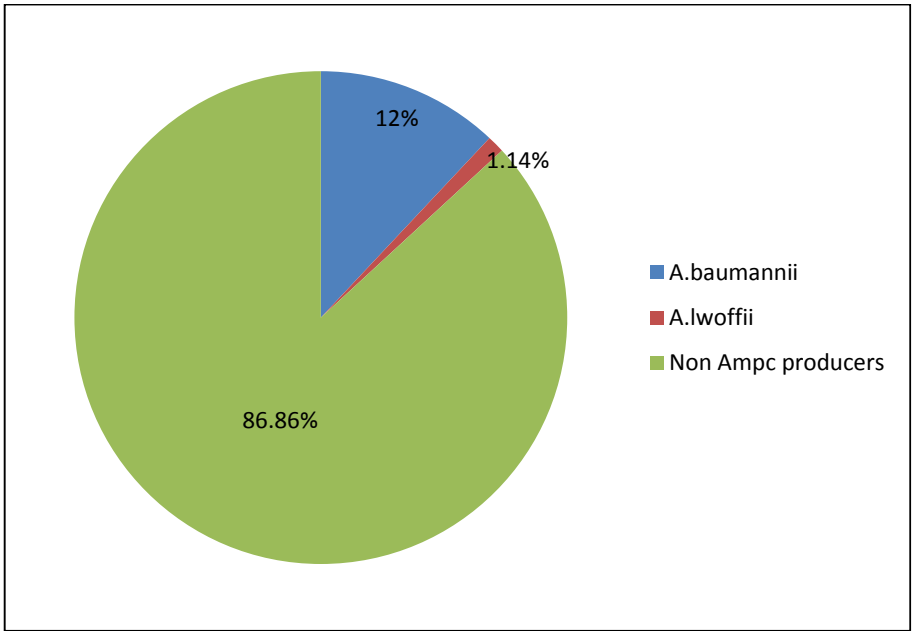


TABLE 23: CLINICAL OUTCOME OF THE PATIENTS WITH MEROPENEM RESISTANCE AND THEIR GENETIC MARKAERS

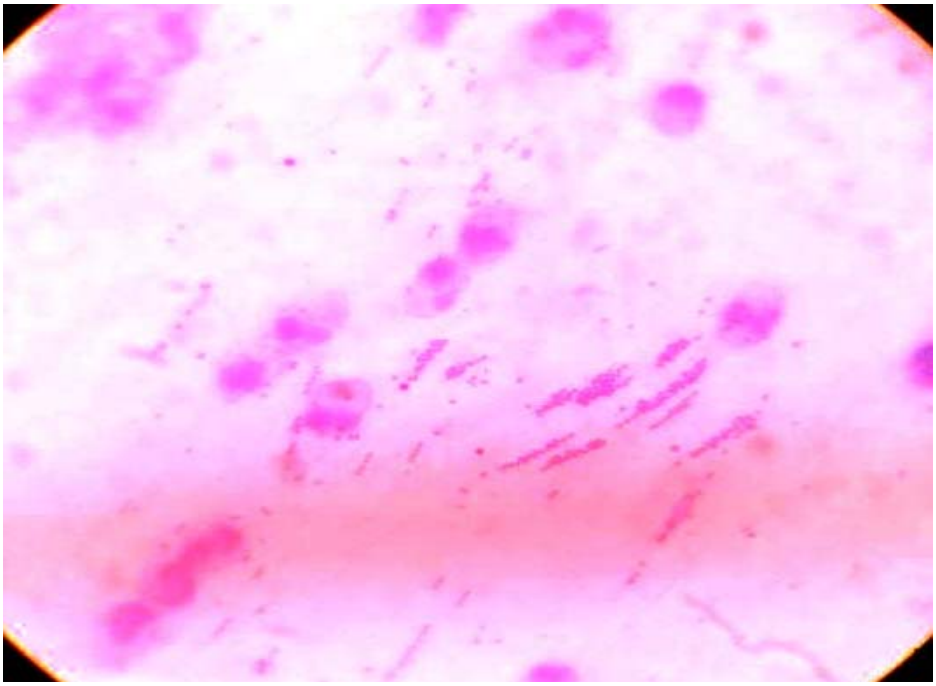
ISOLATE NO.	AGE /SEX	CLINICAL DIAGNOSIS	MIC	GENES TESTED			CLINICAL OUTCOME
				OXA-23	VIM_1	IMP_1	
1	45/F	Urosepsis	256 µg/ml	P	P	N	RECOVERED
2	47/M	Poisoning	256 µg/ml	P	N	P	RECOVERED
3	43/M	RTA/Head injury	64 µg/ml	P	N	N	EXPIRED
4	45/M	BB Fracture (left)	64 µg/ml	P	N	N	RECOVERED
5	50/M	Post TB	32 µg/ml	P	N	P	EXPIRED
6	34/M	Post Renal transplant	64 µg/ml	P	N	N	RECOVERED
7	48/M	Post Renal transplant	256 µg/ml	P	P	N	RECOVERED
8	35/M	Above knee amputation	128 µg/ml	P	N	N	RECOVERED
9	58/M	Septicemia	256 µg/ml	P	N	N	EXPIRED
10	60/F	Poisoning	128 µg/ml	P	P	P	EXPIRED
11	62/F	MCTD	128 µg/ml	P	N	P	EXPIRED

ISOLATE NO.	AGE /SEX	CLINICAL DIAGNOSIS	MIC	GENES TESTED			CLINICAL OUTCOME
				OXA-23	VIM_1	IMP_1	
12	**58/F**	**Pneumonia**	**64 µg/ml**	**P**	**N**	**P**	**RECOVERED**
13	**45/M**	**Pneumonia**	**64 µg/ml**	**P**	**P**	**N**	**RECOVERED**
14	**58/M**	**Aspiration pneumonitis**	**128 µg/ml**	P	P	N	**RECOVERED**
15	**40/F**	**Poisoning**	**128 µg/ml**	P	P	N	**RECOVERED**
16	**28/M**	**RTA/Head injury**	**256 µg/ml**	P	N	P	**RECOVERED**
17	**47/M**	**RTA/Head injury/SDH**	**256 µg/ml**	P	P	P	**RECOVERED**
18	**20/M**	**RTA/Head injury**	**256 µg/ml**	P	P	N	**RECOVERED**
19	**20/M**	**CSOM**	**256 µg/ml**	P	P	N	**RECOVERED**
20	**20/F**	**Post Renal transplant**	**256 µg/ml**	P	N	N	**RECOVERED**

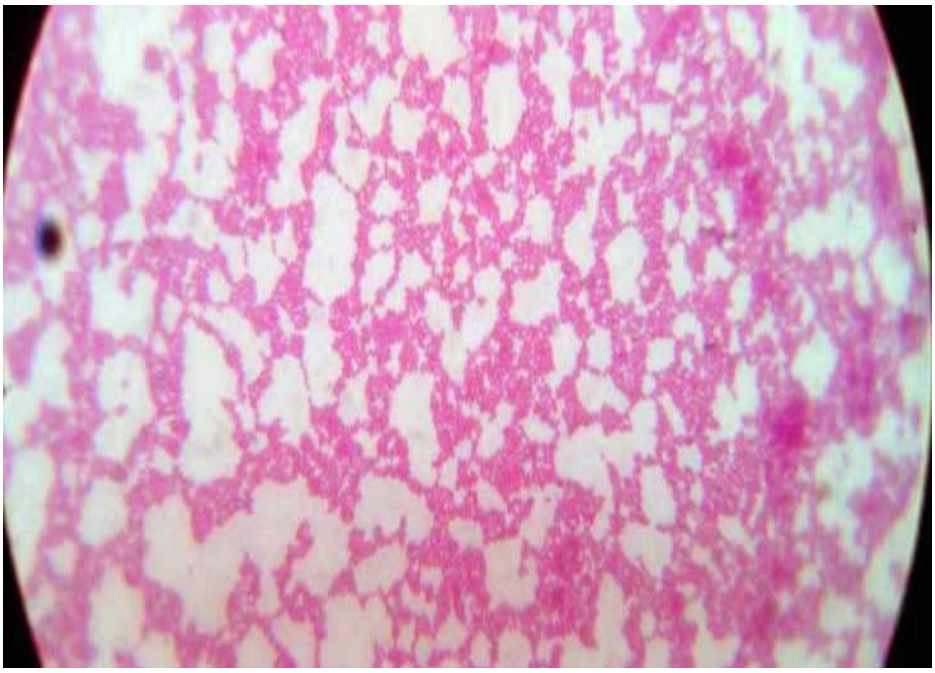
P- Positive, N- Negative

Among the 20 patients with meropenem resistant isolates, 5 patients expired when compared to 100% recovery in the meropenem susceptible group (155 patients).

DIRECT GRAM STAIN SHOWING GRAM NEGATIVE COCCO BACILLI



GRAM NEGATIVE COCCO BACILLI IN CULTURE SMEAR



BIOCHEMICAL REACTIONS OF *ACINETOBACTER BAUMANNII*

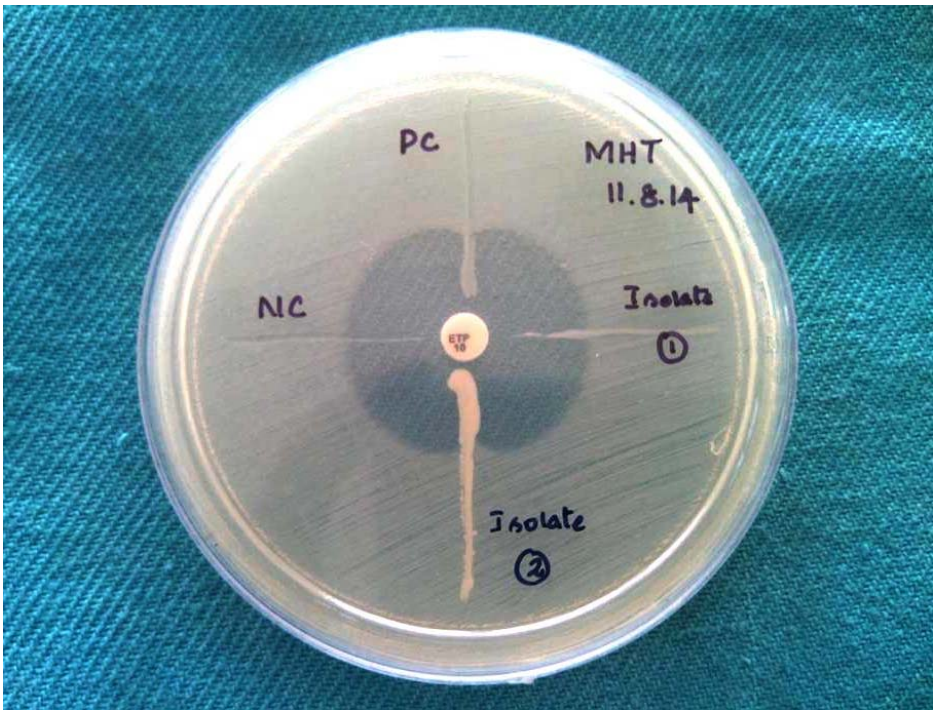
BIOCHEMICAL REACTIONS OF *ACINETOBACTER CALCOACETICUS*

BIOCHEMICAL REACTIONS OF *ACINETOBACTER LWOFFII*

BIOCHEMICAL REACTIONS OF *ACINETOBACTER JUNII*

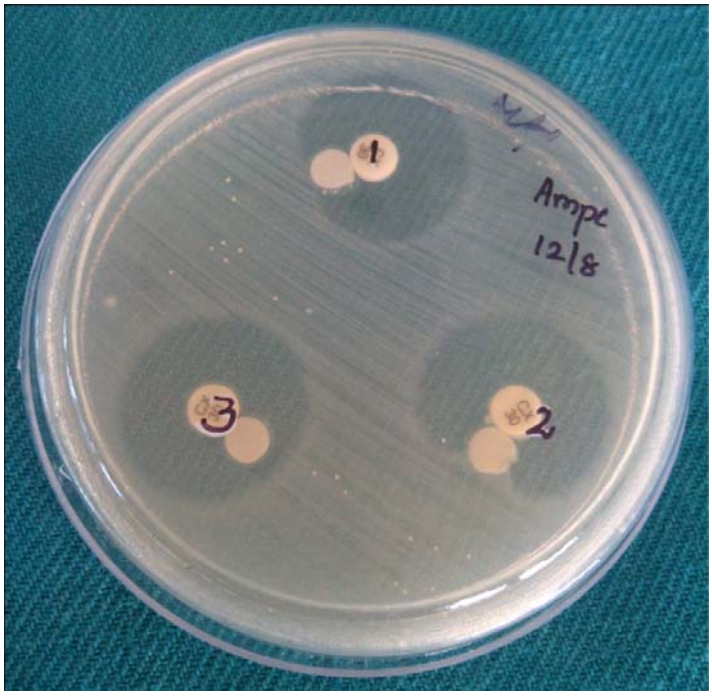
MEROPENEM MIC- MACROBROTH DILUTION METHOD

MODIFIED HODGE TEST FOR OXACILLINASE DETECTION



Meropenem resistant Isolate 2 – MHT Positive, Isolate 1 - Negative

AMPC DISC TEST FOR AMPC BETA LACTAMASE DETECTION

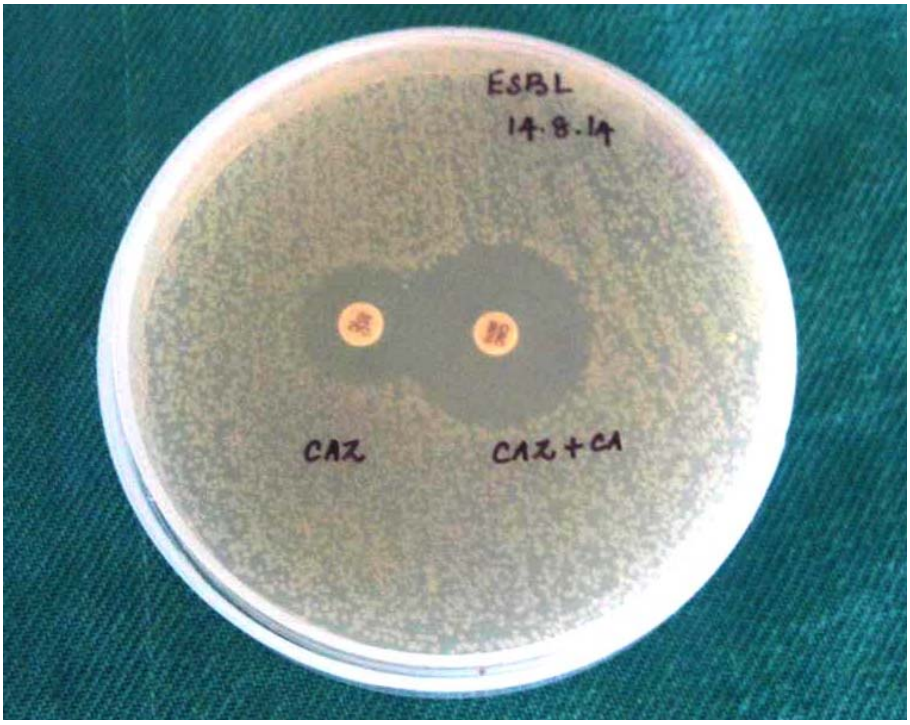


Isolate 1&2 - Positive, 3 – Negative

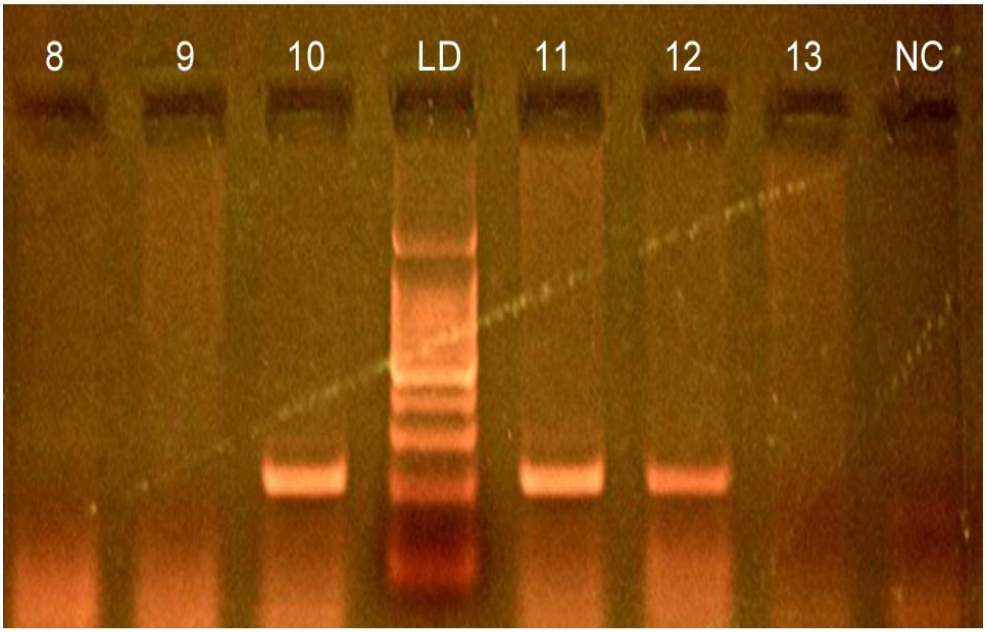
IMIPENEM-EDTA COMBINED DISC TEST FOR MBL DETECTION

I – Imipenem IE – Imipenem EDTA

CLSI PHENOTYPIC CONFIRMATORY METHOD FOR ESBL

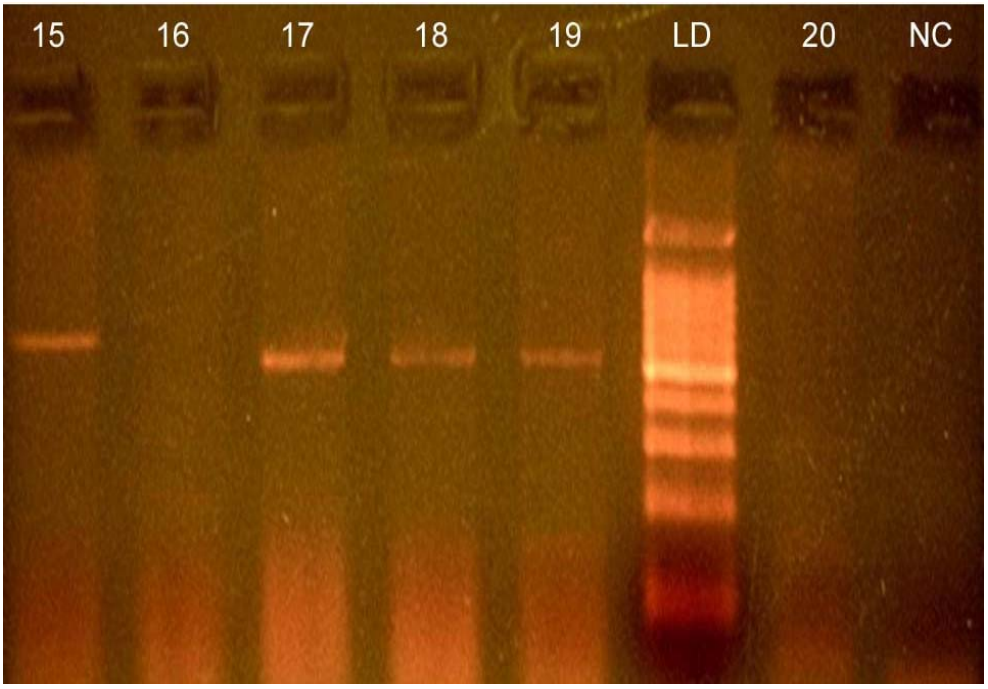


PCR FOR BLA-IMP$_1$ GENE



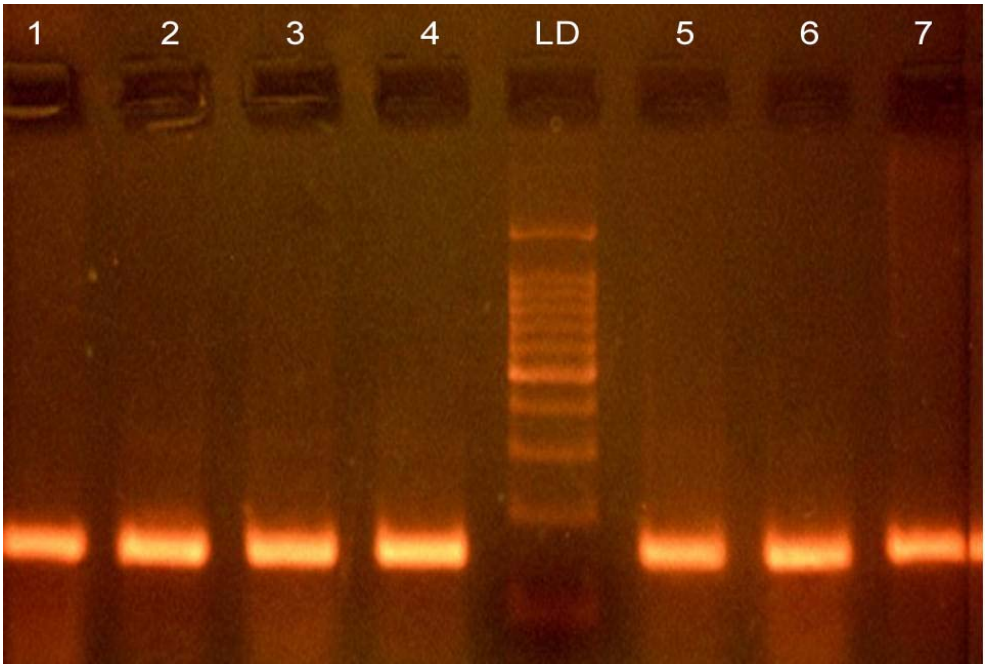
Isolate No. – 10,11,12 - Positive LD – DNA ladder NC – Negative control

PCR FOR BLA-VIM$_1$ GENE



Isolate No.–15, 17, 18, 19 - Positive LD- DNA ladder NC–Negative control

PCR FOR OXA-23 GENE



Isolate No. – 1,2,3,4,5,6,7-Positive LD- DNA ladder

Discussion

DISCUSSION

Acinetobacter infections presents a global medical challenge because it is an important opportunistic GNB in health care institutions. It has gained importance because of its ability to survive under a wide range of environmental conditions, having numerous intrinsic and acquired drug resistance mechanisms and the emergence of multidrug and pandrug resistant strains.[75] The isolation and identification of resistance pattern of *Acinetobacter* infections helps in selection of appropriate antibiotics, reducing the morbidity and mortality of the patients and in reducing the spread of resistant strains in the community.

This cross sectional study was conducted in the **Institute of Microbiology, Madras Medical College, Chennai** during the period from Sep 2013 to Aug 2014.

The present study includes **175** clinically significant, consecutive, non-duplicate *Acinetobacter* isolates.

In the present study, among the 175 *Acinetobacter* isolates, 120(68.57%) isolates were from male patients and remaining 55(31.43%) isolates were from female patients (Table-1). The male to female ratio was 2.18:1which is high when compared to study done by Muktikesh

Dash et al. where he reported male to female ratio of 1.08:1.[76] Out of the 175isolates, 84 isolates(48.0%) were from the patients in the age group of 41 – 60 years followed by 48 isolates (27.43%) in 21 – 40 years age group, 33 isolates (18.86%) from patients aged more than 60 years and 10 isolates (5.71%) in 18-20 years age group (Table-2).

In the present study, the distribution of *Acinetobacter* species in various clinical specimens was in the following order, urine specimen 45 (25.71%), endotracheal aspirate 41 (23.43%), wound swab 41 (23.43%), blood 17 (9.71%), sputum (6.86%), bronchial wash (4.00%), PD fluid (2.86%), CSF (1.71%), pleural fluid (1.14%) and ascitic fluid (1.14%) (Table-3). The maximum number of *Acinetobacter* isolates were from respiratory samples 63(36.00%). This is very similar to the study conducted by Apoorva Tripathi et al. where 35.78% of isolates were from respiratory specimens[78] where as Muktikesh Dash et al. in his study reported that *Acinetobacter* isolates were common from pus sample 56.9%.[76]

In this study, *Acinetobacter* isolates were predominantly from patients in Intensive care units 20.57%, followed by Medicine unit 17.71%, Neuro surgery 14.86%, Surgery unit 13.7%, Nephrology 12%, orthopaedics ward 8.6%, thoracic medicine 8% and from other wards 4.57% (Table-4). This is similar to the study conducted by Muktikesh

Dash et al. where 45.2% of the isolates were from patients admitted in Intensive care units, 26.3% and 19% from patients admitted in surgery and medical wards respectively.[76] Similarly Apoorva Tripathi et al. and Namita jaggi et al. in their studies documented 61.2% and 76.7% of the *Acinetobacter* isolates were from Intensive care units respectively.[77],[78] This implies that *Acinetobacter* infections are more common in critically ill patients in Intensive care units.

In the present study, four *Acinetobacter species* were isolated, of which ***A.baumannii*** 142 (81.14%) was the most common species followed by ***A.lwoffii*** 18(10.29%), ***A.calcoaceticus*** 8 (4.57%) and ***A.junii*** 7(4%) (Table-5). This is similar to the study conducted by Muktikesh Dash et al. where he documented that 79.6% isolates were *A.baumannii*, 12.4% were *A.lwoffii* and 8% were other species.[76] Similarly Apoorva Tripathi et al. have reported that 74.50% isolates were *A.baumannii* and 24.50% were *A.lwoffii*.[78] Like many other studies, the species most commonly isolated in this study was *A.baumannii* because of its natural habitat in the environment when compared to other species.

With regard to the species distribution of *Acinetobacter* isolates in various clinical infections in this study, *A.baumannii* was isolated from respiratory tract infections, wound infections, urinary tract infections and septicemia. *A.lwoffii* was isolated from wound infections and UTI.

A.calcoaceticus was isolated from wound infections. *A.junii* was isolated from wound infections and septicemia (Table-6). This implies that *Acinetobacter* infections commonly affects the patients with breaches in airway and skin integrity.[94]

The **antimicrobial sensitivity pattern** of Acinetobacter *spp.*was studied. It differs among the different species. In the present study **A.baumannii** has higher percentage of resistance to various classes of antibiotics and the percentage of resistance was as follows, cefotaxime (86.62%), ciprofloxacin (80.99%), cotrimoxazole (80.99%), ceftazidime (79.58%), gentamicin (73.24%), cefepime (66.91%) and amikacin (57.04%), piperacillin tazobactum (38.02%), Imipenem (11.97%), Meropenem (14.08%), Tigecycline (16.90%) and polymyxin B (3.52%). All the *A.baumannii* isolates (100%) in this study were sensitive to colistin (Table-8). The low resistance pattern of *A.baumannii* to imipenem and meropenem indicate that they can be used as effective drugs in the treatment of MDR *Acinetobacter* infections.[103]

The sensitivity pattern of *A.baumannii* was similar to the studies done by Neelam taneja et al., Prasanth et al. and Gomty mahajan et al. in which the authors have documented more than 70% resistance to cephalosporins, aminoglycosides and quinolones.[49],[51],[59] Similarly

Sohaila Mushtaq et al. have reported more than 60% resistance to cephalosporins and 50% resistance to aminoglycosides and quinolones.[79]

Imipenem (11.97%) and Meropenem (14.08%) resistance in *A.baumannii* was low when compared to other studies done by Manu chaudhary and Anurag payasi where they have reported 35% resistance towards carbapenem drugs.[12] Gladstone et al. Lee et al., Sinha et al., Purti tripathi et al. and Apoorva Tripathi et al. have reported 14.2%, 21.18%, 35%, 43% and 52.63% of imipenem resistance respectively.[17],[74],[78],[80] Similarly very high percentage resistance of imipenem (89.6%) and meropenem (99%) was reported by Namita jaggi et al. and John et al. respectively.[77],[84] The very high resistance may be due to the ability of the organism to produce more than one hydrolyzing enzyme or showing modifications in more than one outer membrane porin channels or may be due to selection pressure.[98]

In the present study, **Tigecycline** resistance in *A.baumannii* isolates was 16.90%. However tigecycline resistance varies between 8.3% to 74.8% as documented by Gomty Mahajan et al. and Namita jaggi et al. respectively.[51],[59],[77] The wide variation among the reported studies with regard to tigecycline resistance may be due to varying degree of mutations in efflux pumps.[104]

Polymyxin B resistance in *A.baumannii* isolates was (3.52%) and all the *A.baumannii* isolates (100%) were sensitive to colistin. Namita jaggi et al. have documented 1.9% resistance to polymyxin-B and 1.2% resistance to colistin.[77] This shows that *A.baumannii* isolates that were included in this study have not developed resistance mechanisms like efflux pumps or modification of cell membrane lipopolysaccharides for the above mentioned drugs.[37],[38]

The percentage of resistance for ***A.lwoffii*** isolates in this study was, cotrimoxazole (83.33%), ciprofloxacin (72.22%), cefotaxime (66.67%), gentamicin (55.56%), ceftazidime (44.44%), amikacin (38.89%), cefepime (27.78%), piperacillin tazobactum (16.67%) and tigecycline (5.55%). All the *A.lwoffii* isolates (100%) were sensitive to imipenem, meropenem, polymyxin B and colistin (Table-9). This antimicrobial susceptibility pattern is similar to the study done by Apoorva Tripathi et al. [78]

The percentage of resistance for ***A.calcoaceticus*** isolates in this study was, ciprofloxacin (62.50%), cotrimoxazole (62.50%), cefotaxime (25%), gentamicin (25%), amikacin (12.50%) and tigecycline (12.50%). All the eight isolates (100%) were sensitive to ceftazidime, cefepime, piperacillin tazobactum, imipenem, meropenem, polymyxin B and colistin (Table-10).

The percentage of resistance for ***A.junii*** isolates in this study was ciprofloxacin (85.71%), cotrimoxazole (85.71%), gentamicin (85.71%), amikacin (57.14%) and cefotaxime (42.86%), ceftazidime (28.57%), cefepime (28.57%), piperacillin tazobactum (14.29%) and tigecycline (14.29%). All the seven isolates (100%) were sensitive to imipenem, meropenem, polymyxin B and colistin (Table-11).

In the present study, *A.baumannii* had higher percentage of resistance to third and fourth generation cephalosporins, aminoglycosides and quinolones when compared to *A.lwoffii, A.calcoaceticus and A.junii* which exhibited lesser resistance to cephalosporins, aminoglycosides and quinolones. Whereas *A.baumannii* exhibited relatively lesser percentage of resistance to carbapenems when compared to other species which exhibited no resistance or 100% susceptibility to carbapenems. This is because of multiple virulence factors of *A.baumannii* like biofilm formation, production of capsule and efflux pumps. Hence there was a significant difference (p value <0.05) between the antimicrobial sensitivity pattern of *A.baumannii* and other species (Table-12).

Multidrug resistance among *Acinetobacter spp*. is common because of its potential to respond quickly to the changes in selective environmental pressure and inherent property of having chromosomally

encoded AmpC cephalosporinases (ADCs).[94] In the present study Multi drug resistance was 60%. This is similar to the study done by Muktikesh Dash et al. where he documented 54.7% of MDR.[76] However Gomty Mahajan et al. and Purti Tripathi et al. has reported multidrug resistance of 70% and 89.71% respectively.[59],[80] MDR was more common in *A.baumannii* (92.38%) when compared to other species in this study. This is similar to the study conducted by Seifert et al. and Prashanth et al.[81],[82]

Extended drug resistance was found to be 11.43% and there were no pan drug resistant isolate in this study. Gaynes et al. have documented 17% of XDR and Jyoti Sharma et al. have documented 22.38% of PDR *Acinetobacter spp.*[95],[96]

Acinetobacter species are very notorious for their ability to acquire **antibiotic resistance** by beta lactamase production, reduced access to bacterial targets and mutations that change targets or cellular functions.[18] All the 175 isolates included in this study were screened for production of beta lactamases like carbapenamase, extended spectrum beta lactamase and Amp C beta lactamase.

Among the 175 isolates, 20 isolates (11.43%) were found to be resistant to meropenem by Kirby Bauer disc diffusion method and all

these 20 *Acinetobacter* isolates belong to *baumannii* species (Table-13). The meropenem resistance in this study was low when compared to the study done by Gladstone et al. and Sinha et al. where they have documented 14.2% and 28% of meropenem resistance respectively.[5],[17] Very high level of meropenem resistance 89.6% was reported by Namita jaggi et al.[77]

In the present study, among the 20 meropenem resistant isolates, 17 isolates were resistant to imipenem and the remaining 3 isolates were sensitive to imipenem. This is because of the difference in the pharmacodynamic property among the carbapenem drugs.[97] This is similar to the study done by Muktikesh Dash et al. where 19% resistance to imipenem and 22% resistance to meropenem was documented.[76]

The **meropenem resistant** isolates by Kirby Bauer disc diffusion method were further confirmed for their meropenem resistance by **MIC**. All the 20 isolates have their MIC in the resistant range (≥8 µg/ml), hence disc diffusion method correlates with MIC.[48] This shows that the regular screening for meropenem resistance can be done using disc diffusion method and further confirmed by MIC.

Among the 20 isolates, 9(45%) isolates have meropenem MIC of 256µg/ml, 5(25%) isolates have 128 µg/ml, another 5(25%) isolates have

64 µg/ml and remaining 1(5%) isolate has 32 µg/ml as MIC (Table-15). Amudhan et al. has documented MIC values ranging from 8µg/ml to 128µg/ml.[83] Maryam Noori et al. have reported meropenem MIC as high as 256µg/ml in Iran.[99] In this study 9 isolates have 256µg/ml as MIC, indicating the prevalence of high level resistant strains among the isolates.

The 20 meropenem resistant isolates tested by the indicator method (i.e) meropenem disc diffusion were proceeded for the detection of carbapenamase production.[5] The mechanism of carbapenam resistance by beta lactamases were tested by the following **phenotypic methods** – Modified Hodge test for oxacillinase, Imipenem-EDTA combined disc test for metallo beta lactamase and AmpC disc test for AmpC beta lactamase (Table-16).

Among the 20 meropenem resistant isolates, **Modified Hodge test** was positive in 9/20 (45%) isolates. This was similar to the study done by Gomty mahajan et al. (47.6%)[59]. But MHT positivity varies between 2.2% to 71% in other studies. This may be due to lack of standardization of phenotypic procedures for detection of carbapenemase in NFGNBs, as there are no standard procedures described in CLSI and other similar guidelines.

Imipenem-EDTA combined disc test was positive in 9/20(45%) isolates. Sinha et al. and Uma et al. have documented 60.71% and 71% of MBL respectively.[20],[23] Similarly Gupta et al., Lee et al. and Franklin et al. have documented 7.5%, 14% and 16% MBL respectively.[85],[86],[87] The reason for the variations in MBL detection may be due to lack of standard procedures and variations in the expression of MBL gene.[98]

AmpC Disc test was positive in 6/20(30%) isolates which was low when compared to Sinha et al. and Deepa et al. where they reported 60.71% and 73% of AmpC beta lactamases in carbapenem resistant isolates respectively.[11],[54] AmpC beta lactamases alone may not be the cause for carbapenem resistance but when it is present along with decreased membrane permeability, it is capable of conferring carbapenem resistance.[5]

Among the 20 meropenem resistant isolates, 7/20(35%) isolates were negative for all the three phenotypic methods. This shows that carbapenem resistance in those isolates may be due to non-expression of carbapenem resistant genes, altered porin channels and/or efflux pump mechanisms. 3/20 (15%) isolates were positive for all the three tests and 7/20 (35%) isolates were positive for both oxacillinase and metallo beta lactamase. This implies that combination of several mechanisms may exist in the same isolate to confer carbapenem resistance.[18]

In this study, the 20 isolates which were resistant to Meropenem by Disc diffusion method were subjected to **PCR** for the detection of the most common Oxacillinase gene OXA-23 and Metallo Beta Lactamases genes bla-IMP_1 and bla-VIM_1.[72],[73]

All the 20 meropenem resistant isolates (100%) were **OXA-23** positive. This is similar to the study done by Yang soon Lee et al. in Korea, in the year 2011, where all carbapenem non susceptible isolates collected from 9 hospitals were OXA-23 positive (100%).[90] Similarly in Iran, Nasrollah Sohrabi et al. have documented 88.7% of OXA-23 positive isolates.[91] Amongst the Indian studies, the maximum OXA-23 positive isolates was reported as 81.89% in the study done by Amudhan et al.[83] Oxacillinases would also inactivate carbapenems, though they are less efficient hydrolyzers of carbapenems invitro than MBLs.[59] Hence the occurrence of OXA-23 positive isolates also poses therapeutic failure .

Bla-IMP_1 was positive in 7 isolates (35%) of the 20 meropenem resistant isolates. This is marginally high when compared to the study done by Sinha et al. and Lee et al. where they have reported 32.14% and 28.9% of MBL respectively.[5],[86]

Bla-VIM$_1$ was positive in 9 isolates (45%) (Table-17). This is low when compared to the studies done by Manu chaudhary and Lee et al. where they have documented 59% and 71.1% of VIM$_1$ respectively.

All the three genes were positive in 2 (10%) isolates and both blaVIM$_1$ and blaIMP$_1$ were positive in 2 (10%) isolates. Amudhan et al. have documented 0.86% of all three genes positive isolates.[83] Similarly Sinha et al. have documented 7.14% of both VIM$_1$ and IMP$_1$ positive isolates.[5]

In this study, OXA-23 was positive in all the 20 meropenem resistant isolates. Amongst the 20 meropenem resistant isolates MHT was positive only in 9 isolates. Similarly amongst 14 MBL genes (blaVIM$_1$ & IMP$_1$) positive isolates, only 9 isolates tested positive in Imipenem-EDTA combined disc test. Hence the detection of various carbapenemases by genotypic method was more sensitive when compared to phenotypic methods.[105]

The occurrence of MBL has tremendous therapeutic consequences because they also carry other multidrug resistance genes. Although MBLs are less in Acinetobacters than OXA type carbapenamases, they have 100-1000 fold high hydrolytic activities towards carbapenems and also they have the ability to participate in horizontal gene transfer among

other GNBs.[59] Hence the identification of MBL and oxacillinase producing isolate is also essential for the infection control management.

Extended spectrum beta lactamases continues to be a major challenge in health care institutions, hence the knowledge about their prevalence is an essential guide towards appropriate antibiotic treatment. In the present study all the 175 isolates were screened for ESBL production. 61(34.86%) isolates were found to be ESBL producers. The Extended spectrum beta lactamases were common in *A.baumannii* (91.80%), followed by *A.lwoffii* (6.56%) and *A.junii* (1.64%). Sinha et al. have reported 28% of ESBL in Acinetobacter spp. and 69.04% of it was due to A.baumannii and 30.96% was due to A.lwoffii.[74] Vahaboglu et al, Yong et al. and Manu chaudhary et al. have documented ESBL production of 46%, 54.63% and 83.6% respectively.[12],[88][89] None of the meropenem resistant isolates were ESBL producers.

The 175 isolates in the present study were screened for **AmpC beta lactamases**. 23(13.14%) isolates were found to be AmpC producers. AmpC beta lactamases were also common in A.baumannii (91.30%) followed by A.lwoffii (8.70%). This was low when compared to the study done by Singhal et al where she reported 28.57% of AmpC beta lactamases in Acinetobacter spp.[60]

The **treatment** of Acinetobacter infections remains a great challenge because resistance to aminoglycosides, cephalosporins and quinolones has substantially increased worldwide. Carbapenems are the drug of choice for MDR Acinetobacter infections, for ESBL and AmpC producing isolates, but resistance to carbapenems by the production of carbapenamases and various other mechanisms has limited the therapeutic options. The recently approved tigecycline for multi drug resistant isolates have limited clinical applications because of its toxicities and its unavailability in most of the countries. Because of increasing carbapenem resistance and limited therapeutic options available, the old antibiotic colistin is being used more extensively nowadays, but resistance to colistin has also been reported. But in my study no resistance was detected for colistin among the isolates.[51],[77],[92] Hence currently combination therapy like meropenem with tigecycline and colistin with sulbactum or rifampicin are being tried in the treatment of *Acinetobacter* infections.[38],[44],[46]

The **clinical outcome** of the patients with meropenem resistance was determined in this study. Among the 20 patients with meropenem resistant isolates, 5 patients expired when compared to 100% recovery in the meropenem susceptible group (155 patients). OXA-23 was positive in all the five patients, blaIMP$_1$ was positive in 3 patients and blaVIM$_1$ was

positive in a single patient. All the three genes were positive in one patient. The mortality rate in patients with MBL gene was 21.43%, this is low when compared to the mortality rate of 44.44% as documented by Sinha et al.[20]

Hence the prevalence of *Acinetobacter* infections emphasizes the need for early detection of various beta lactamases, which would help in selection of appropriate antibiotic regimen and prevention of emergence and dissemination of MDR strains.

Summary

SUMMARY

- Clinically significant, consecutive, non duplicate ***Acinetobacter* isolates (n=175)** from various clinical specimens were included in this study.

- There was a male predominance among the cases (68.57%).

- Maximum number of cases occurred in the age group of 41 – 60 years (48%).

- Majority of the isolates were from urine (25.71%), followed by endotracheal aspirate (23.43%) and wound swab (23.43%). Blood accounts for 9.71% of the isolates. Remaining isolates were from sputum (6.86%), bronchial wash (4.00%), PD fluid (2.86%), CSF (1.71%), pleural fluid (1.14%) and ascitic fluid (1.14%).

- *Acinetobacter* isolates were predominantly from patients in Intensive care units 20.57%, followed by Medicine unit 17.71%, Neuro surgery 14.86%, Surgery unit 13.7%, Nephrology 12%, orthopaedics ward 8.6%, thoracic medicine 8% and from other wards 4.57%.

- *Acinetobacter baumannii* (81.14%) was the most common species isolated followed by *A.lwoffii* (10.29%), *A.calcoaceticus* (4.57%) and *A.junii* (4%).

- *A.baumannii* was isolated from respiratory tract infections, wound infections, urinary tract infections and septicemia. *A.lwoffii* was isolated from wound infections and UTI. *A.calcoaceticus* was isolated from wound infections. *A.junii* was isolated from wound infections and septicemia.

- In the present study, *A.baumannii* had higher percentage of resistance to third and fourth generation cephalosporins, aminoglycosides and quinolones when compared to *A.lwoffii, A.calcoaceticus and A.junii* which exhibited lesser resistance to cephalosporins, aminoglycosides and quinolones. Whereas *A.baumannii* exhibited relatively lesser percentage of resistance to carbapenems when compared to other species which exhibited no resistance (0%) or 100% susceptibility to carbapenems. Hence there was a significant difference (p value <0.05) between the antimicrobial sensitivity pattern of *A.baumannii* and other species.

- **Multidrug resistant** in *Acinetobacter spp*. was found to be 60%. Higher percentage of MDR was found in *A.baumannii* (92.38%) when compared to other species.

- **Extended drug resistance** was found to be 11.43% and there was no pan drug resistant isolate in this study.

- 20 isolates (11.43%) were found to be resistant to meropenem by Kirby Bauer disc diffusion method and all these 20 isolates were *A.baumannii.*

- All the 20 isolates have their MIC above 8 µg/ml, hence disc diffusion method correlates with MIC.

- Among the 20 isolates, Modified Hodge test was positive in 9 (45%) isolates, IEDT was positive in 9(45%) isolates, AmpC Disc test was positive in 6(30%) isolates and 7(35%) isolates were negative for all the three phenotypic methods. Of the 20 isolates, 3 isolates were positive for MHT, IEDT and AmpC disc test and 7 isolates were positive for MHT and IEDT.

- Among the 20 meropenem resistant isolates, all the 20 isolates were positive for OXA-23 (100%), 9 (30%) isolates were positive for blaVIM$_1$ and 7 (35%) isolates were positive for blaIMP$_1$.

- 61(34.86%) isolates were found to be ESBL producers and *A.baumannii* was the predominant species (91.80%).

- 23(13.14%) isolates were found to be AmpC beta lactamase producers and *A.baumannii* was the predominant species (91.30%).

- The **clinical outcome** of the patients with meropenem resistance was determined in this study. Among the 20 patients with meropenem resistant isolates, 5 patients expired when compared to 100% recovery in the meropenem susceptible group.

- The mortality rate in patients with MBL gene was 21.43%.

Conclusion

CONCLUSION

Acinetobacter species are the second most common nonfermenter isolated from clinical specimens next to *Pseudomonas species*. The infections caused by MDR *Acinetobacter* that are capable of producing various beta lactamases are associated with significant morbidity and mortality. Hence *Acinetobacter* has been added to the list of significant microbial challenges of current era.

Although *A.baumannii* was the most common species isolated from patients with various clinical diseases, other species like *A.lwoffii, A.calcoaceticus* and *A.junii* were also isolated in a proportion of clinical infections. *A.baumannii* was the most resistant when compared to other *Acinetobacter* species and there was a significant difference in their antimicrobial sensitivity pattern.

MDR *Acinetobacter* infections were predominant and XDR *Acinetobacter* infections have also been recorded but no PDR *Acinetobacter* was isolated in this study. Extended spectrum beta lactamases and AmpC beta lactamases were also detected in a significant number of *Acinetobacter* isolates, implifying their contribution to multidrug resistance.

Carbapenems remain the drug of choice for the MDR acinetobacter infections. But resistance to carbapenems due to production of various beta lactamases is of great concern as they are encoded by genes which are horizontally transmissible. There is difference between phenotypic and genotypic methods in the sensitivity of detection of carbapenamases where genotypic methods are more sensitive and remain the gold standard.

In this study, gene coding for oxacillinase was positive in 100% and MBL genes in 70% of meropenem resistant isolates. Among the MBL genes, $blaVIM_1$ (45%) was more common than $blaIMP_1$ (35%). Oxacillinases also confer resistance to carbapenems, although they are less efficient hydrolyzers of carbapenems when compared to MBLs. The occurrence of MBL is not only a therapeutic issue, but poses a serious concern for infection control as well. Hence the treatment option is left with the polymyxin-B and colistin which are highly nephrotoxic and neurotoxic. In this study 3.52% of isolates were resistant to polymyxin-B and all the isolates (100%) were susceptible to colistin.

The high prevalence of *Acinetobacter* infections emphasizes the need for early detection of various beta lactamases, which would help in selection of appropriate antibiotic regimen and prevention of emergence and dissemination of MDR strains. Hence, methods for detection of

carbapenamases have to be standardized by formulating guidelines and be routinely used.

The present need is that all the health care institutions should have a coordinated effort to curtail inappropriate use of antibiotics, their own antimicrobial stewardship program, vigilant detection of resistant *Acinetobacters*, regular surveillance and infection control protocols to control the increasing incidence of highly resistant *Acinetobacters*.

Appendix

APPENDIX-I

ABBREVIATIONS

GNB	-	Gram Negative Bacilli
NFGNF	-	Non fermentative Gram negative bacilli
A.calcoaceticus	-	Acinetobacter calcoaceticus
A.baumannii	-	Acinetobacter baumannii
A.haemolyticus	-	Acinetobacter haemolyticus
A.lwoffii	-	Acinetobacter lwoffii
A.junii	-	Acinetobacter junii
A.johnsonii	-	Acinetobacter johnsonii
PD fluid	-	Peritoneal Dialysate fluid
CSF	-	Cerebro spinal fluid
ET aspirate	-	Enditracheal aspirate
Br Wash	-	Bronchial Wash
CLED	-	Cysteine Lactose Electrolyte Defecient Medium
MH broth	-	Mueller Hinton broth
MHA	-	Mueller Hinton Agar
ATCC	-	American Type Culture Collection
CLSI	-	Clinical & Laboratory Standards Institute
MIC	-	Minimum Inhibitory Concentration
PCR	-	Polymerase chain reaction
bp	-	base pair
ESBL	-	Extended spectrum beta lactamase
MBL	-	Metallo beta lactamase
IEDT	-	mipenem-EDTA combined disc test

MHT	-	Modified Hodge Test
EDTA	-	thylene Diamine Tetra acetic Acid
OF	-	Oxidation Fermentation
OXA-23	-	Oxacillinase beta lactamase
bla-IMP	-	Imipenamase metallo beta lactamase
bla-VIM	-	Verona integron encoded metallo beta lactamase
ICU	-	Intensive care unit
NS	-	Neuro Surgery
MED	-	Medicine
SUR	-	Surgery
UTI	-	Urinary tract infection
SEN	-	Sensitive
RES	-	Resistant

APPENDIX - II

A. STAINS AND REAGENTS

Gram staining:

Methyl violet (2%)	l0g Methyl violet in 100ml Absolute alcohol in 1 litre of Distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolorizing agent
Carbol fuchsin (1%)	Secondary stain

B. MEDIA USED

1. Mac Conkey agar:

Peptone	20 g
Sodium taurocholate	5 g
Distilled Water	1 ltr
Agar	20 g
2% neutral red in 50% ethanol	3.5 ml
10% lactose solution	l00 ml

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix. Heat in free steam (100°C) for 1 hour then autoclave at 115°C for 15 minutes.

2. Blood agar (5% sheep blood agar):

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	l0g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood (sterile) at 55°C adjust pH to 7.4.

3. Mueller- Hinton Agar:

Ingredients	Gms / Litre
Beef infusion	300ml
Caesein hydrolysate	17.5g
Starch	1.5g
Agar	l0g
Distilled water	l Ltr
pH = 7.4	

22 grams of media is suspended in 1000 ml of distilled water. Dissolve the medium completely. Dispense and sterilize by autoclaving at 115-121°C for 10 minutes. DO NOT OVERHEAT.

MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION:

1. Catalase Test:

3% hydrogen peroxide.

2.Oxidase Reagent:

Tetra methyl p-phenylene diamine dihyrochloride- 1%aqueous solution.

3.Indole test:

Kovac's reagent

Amyl or isoamyl alcohol	150ml
Para dimethyl amino benzaldehyde	10g
Concentrated hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4. Simmon's Citrate Medium:

Koser's medium	1 ltr
Agar	20g
Bromothymol blue 0.2%	40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes.

5.Triple Sugar Iron medium:

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	1g
Lactose	10g
Sucrose	10g
Ferric citrate	0.3g
Sodium chloride	5g
Sodum thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	12ml
Distilled water	1 Lt

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

9. Mannitol motility medium

Agar	5g
Peptone	1g
Potassium nitrate	1g
Mannitol	2g
Phenol red indicator	
Distilled water	l000ml
pH 7.2	

8.Decarboxylase media:

8a.Moller decarboxylase broth base:

Ingredients	**gms/L**
Peptone	5
Beef extract	5
Bromocresol purple	0.01
Cresol red	0.005

Glucose	0.5
Pyridoxal	0.005
Final pH 6	

8b. Aminoacid:

Add 10 g of the levo form of the aminoacid for 1000ml.mix and dispense in sterile tubes.

9. Hugh & Leifson's Oxidation –Fermentation test:

Peptone	2g
Sodium chloride	5g
D-glucose	10g
Bromothymol blue	0.03g
Agar	3.0g
Dipotassium phosphate	0.30g
Distilled water	1Lt
pH =7.1	

Basal medium is autoclaved.1% of sterile sugar solutions is added to the basal medium. Dispense into sterile test tubes without slant.

10. **Malonate Utilization test:**

Yeast Extract	1 g
Ammonium sulphate	2 g
Dipotassium phosphate	0.6 g
Potassium phosphate	0.4 g
Sodium chloride	2 g
Sodium malonate	3 g
Bromothymol blue	0.025g
Distilled water	1 Lt

Adjust the pH to 7.4. Sterilize by autoclaving at 121C for 15min.

11. EDTA solution:

0.5M EDTA solution was prepared by adding 186.1gm of disodium EDTA in 1000ml of distilled water. pH was 8. Sterilized by autoclaving at 121°C for 15 min.

Annexure

ANNEXURE-I - CERTIFICATE OF APPROVAL

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To

Dr. J.Thiriveni.,
Post Graduate in MD Microbiology,
Institute of Microbiology,
Madras Medical College, Chennai-3.

Dear **Dr. J.Thiriveni.,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled ***"Speciation of Acinetobacter isolates and determination of resistance pattern by phenotypic and genotypic methods" "*** No.12122013

The following members of Ethics Committee were present in the meeting held on 11.12.2013 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2. Prof. B. Kalaiselvi, MD Vice Principal, MMC, Ch-3	-- Member Secretary
3. Prof. Ramadevi, Director i/c, Instt. of Biochemistry, Chennai.	-- Member
4. Prof. P. Karkuzhali, MD for Dr. V. Ramamoorthy Prof. Instt. of Pathology, MMC, Ch-3	-- Member
5. Thiru. S. Govindasamy, BABL	-- Lawyer
6. Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

20/12/13

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE-II - PROFORMA

- Isolate No :
- Name : IP no :
- Age : Ward :
- Sex : D.O.A :
- Address : D.O.D :

- Source of the
  Specimen :
  Invasive procedures :
  (catheterization,IV line,Mechanical intubation).

  Clinical Diagnosis :

  Treatment History :

Microbiological investigation:

1. Confirmation of the Isolate :

2. Speciation :

Growth at		Hemolysis on BAP	OF dextrose test	Arginine	Malonate utilisation	Gelatin liquefaction	Chloramphenicol Sensitivity
37 ºC	42 ºC						

3. Name of the species identified :

4. Antimicrobial sensitivity pattern:

   Sensitive to :

   Resistance to :

5. Resistance pattern identified :

6. MBL gene detection by PCR :

ANNEXURE-III - CONSENT FORM

STUDY TITLE : "**Speciation of *Acinetobacter* isolates and Detection of Resistance patterns by Phenotypic & Genotypic Method".**

I……, hereby give consent to participate in the study conducted by Dr.J.Thiriveni, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of study, I also give consent to give my clinical Specimen (urine,blood,pus,sputum,CSF,Asciticfluid, Plueral fluid, Tracheal swab and Bronchial wash.) for further investigations.I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression Place Date

Of the patient/ relative

Patient Name & Address:

Signature of the investigator:

Signature of the Guide :

சுய ஒப்புதல் படிவம்

ஆய்வு தலைப்பு : ஹைபர்லிபிடெமியா நோய் சிகிச்சையில் லிகோபீன் பங்கு வழக்கமான சிகிச்சை முறையுடன் ஓர் திறந்தநிலை ஒப்பீடு ஆய்வு.

பெயர் : வயது : தேதி : வெளி நோயாளி எண் :

..... என்பவராகிய நான் இந்த ஆய்வின் விவரங்களும் அதன் நோக்கங்களும் முறையாக அறிந்து கொண்டேன். எனது சந்தேகங்கள் அனைத்திற்கும் தகுந்த விளக்கம் அளிக்கப்பட்டது. இந்த ஆய்வில் முழு சுதந்திரத்துடன் மற்றும் சுயநினைவுடன் பங்கு கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன். இச் சுய ஒப்புதல் படிவத்தை பற்றி எனக்கு விளக்கப்பட்டது.

இந்த ஆய்வினை பற்றிய அனைத்து தகவல்களும் எனக்கு தெரிவிக்கப்பட்டது. இந்த ஆய்வில் எனது உரிமை மற்றும் பங்கினை பற்றி அறிந்து கொண்டேன்.

இந்த ஆய்வில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின் வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

இந்த ஆய்வில் கலந்து கொள்வதன் மூலம் என்னிடம் பெறப்படும் தகவலை ஆய்வாளர் இன்ஸ்டிட்யூசனல் எத்திக்ஸ் கமிட்டியினரிடமோ, அரசு நிறுவனத்திடமோ தேவைப்பட்டால் பகிர்ந்து கொள்ளலாம் என சம்மதிக்கிறேன்.

இந்த ஆய்வின் முடிவுகளை வெளியிடும்போது எனது பெயரோ, அடையாளமோ வெளியிடப்படாது என அறிந்து கொண்டேன். இந்த ஆய்வின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன். இந்த ஆய்விற்காக இரத்தப் பரிசோதனை செய்துக் கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் பங்கேற்கும் பொழுது ஏதேனும் சந்தேகம் ஏற்பட்டால், உடனே ஆய்வாளரை தொடர்பு கொள்ள வேண்டும் என் அறிந்து கொண்டேன்.

நான் இந்த ஆய்வில் இரத்த மாதிரிகள் எடுக்க அனுமதி தருகிறேன்.

இச்சுய ஒப்புதல் படிவத்தில் கையெழுத்திடுவதன் மூலம் இதிலுள்ள அனைத்து விஷயங்களும் எனக்கு தெளிவாக விளக்கப்பட்டது என்று தெரிவிக்கிறேன் என்று புரிந்து கொண்டேன். இச்சுய ஒப்புதல் படிவத்தின் ஒரு நகல் எனக்கு கொடுக்கப்படும் என்று தெரிந்து கொண்டேன்.

பங்கேற்பாளர் கையொப்பம் தேதி :

ஆய்வாளர் கையொப்பம் தேதி :

ANNEXURE – IV MASTER CHART

S. NO.	AGE	SEX	IP NO.	WARD	DIAGNOSIS	SPECIMEN	SPECIES IDENTIFIED	CEFOTAXIME	CEFTAZIDIME	CEFEPIME	AMIKACIN	GENTAMYCIN	CIPRO	PIPTAZ	COTRI	IMIPENEM	MEROPENEM	TIGECYCLINE	POLY B	COLISTIN	RES PATTERN
1	70	M	19788	TM	COPD	SPUTUM	A.baumannii	R	R	R	S	S	S	S	S	S	S	S	S	S	ESBL
2	60	M	20378	SUR	CHRONIC ULCER	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	
3	72	F	24291	SUR	DIAB FOOT	WOUNDSWAB	A.baumanni	R	R	R	S	R	R	R	S	S	S	S	S	S	ESBL
4	32	F	23400	MED	SLE	BLOOD	A.lwoffii	R	S	S	S	S	S	S	S	S	S	S	S	S	
5	45	M	14057	NEPHRO	POST TRANS	SPUTUM	A.baumanni	R	S	S	S	S	S	S	R	S	S	S	S	S	
6	39	F	10861	NEPHRO	CKD	PD FLUID	A.baumanni	S	S	S	S	S	S	S	S	S	S	S	S	S	
7	66	M	14425	SUR	BED SORE	WOUNDSWAB	A.calcoaceticus	R	S	S	S	R	R	S	R	S	S	S	S	S	
8	32	M	24941	NS	HEADINJURY	URINE	A.baumanni	R	S	S	R	R	R	S	R	S	S	S	S	S	
9	20	M	27197	NEPHRO	NEPHROTICSY	URINE	A.baumanni	R	S	S	R	R	R	S	R	S	S	S	S	S	
10	30	M	27036	SUR	HAEMANGIOMA	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
11	35	M	23654	SUR	DIAB FOOT	WOUNDSWAB	A.lwoffii	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
12	24	M	26636	SUR	CHRONIC ULCER	WOUNDSWAB	A.baumanni	R	S	S	R	R	R	S	R	S	S	S	S	S	
13	55	F	4297	NEPHRO	REN TRANS	URINE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	
14	35	M	28009	MED	FFE	URINE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	
15	63	M	19002	ICU	COLANGITIS	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
16	35	M	26085	ORTHO	AK AMPUTATION	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	R	R	S	R	S	S	S	MBL
17	30	M	13929	NS	HEADINJURY	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
18	70	M	15006	ICU	SEPTICAEMIA	BLOOD	A.junii	R	R	R	R	R	R	R	R	S	S	R	S	S	
19	85	M	16541	ICU	ASPIRATION PNEUMONIA	ET ASPIRATE	A.baumanni	S	S	S	S	S	S	S	S	S	S	S	S	S	
20	42	F	11455	NS	HYDROCEPHALUS	CSF	A.baumanni	S	S	S	R	R	R	S	R	S	S	S	S	S	
21	38	M	13661	SGE	POSTCOLOSTOMY	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
22	54	M	20487	NS	ANNEURYSM	ET ASPIRATE	A.baumanni	R	R	S	R	R	R	R	R	S	S	S	S	S	AMPC
23	58	F	23180	ICU	LRIETINF	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	R	R	S	S	S	MBL
24	58	M	21950	ICU	LRIETINF	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	R	R	R	R	S	MBL
25	23	M	30222	NS	POLYTRAUMA	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	
26	65	M	30581	MED	UROSEPSIS	URINE	A.lwoffii	R	R	S	R	R	R	R	R	S	S	S	S	S	AMPC
27	45	M	29158	NS	HEADINJURY	WOUNDSWAB	A.baumanni	R	R	R	R	R	S	R	R	S	S	R	S	S	
28	48	M	20923	MED	SHT/CVA	URINE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
29	50	M	22398	ICU	PTB	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	R	R	R	S	S	MBL
30	52	M	63192	ORTHO	INF IMP	WOUNDSWAB	A.lwoffii	R	R	R	S	S	R	S	R	S	S	S	S	S	ESBL
31	56	M	7846	ORTHO	BB FRACTURE	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	R	S	S	
32	45	M	15758	SUR	DIAB FOOT	WOUNDSWAB	A.junii	S	S	S	R	R	R	S	R	S	S	S	S	S	
33	55	F	19564	MED	PNEUMONIA	SPUTUM	A.baumanni	R	R	R	S	S	S	S	S	S	S	S	S	S	ESBL
34	24	F	18217	NEPHRO	SLE	URINE	A.baumanni	R	R	R	R	R	R	R	R	S	S	R	S	S	
35	60	F	39500	ICU	POISONING	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	R	R	R	S	S	MBL
36	56	M	19348	ICU	DCLD	URINE	A.baumanni	R	R	R	S	S	S	S	S	S	S	S	S	S	ESBL
37	85	M	16541	ICU	ASPIRATION PNEUMONIA	ET ASPIRATE	A.baumanni	R	R	S	R	R	R	R	R	S	S	S	S	S	AMPC
38	32	M	15533	URO	UROSEPSIS	URINE	A.baumanni	R	R	S	S	R	R	R	R	S	S	S	S	S	AMPC
39	38	F	14359	MED	AML	URINE	A.baumanni	R	R	S	S	R	R	R	R	S	S	S	S	S	AMPC
40	65	M	14821	MED	FFE	BLOOD	A.lwoffii	R	S	S	S	S	S	S	R	S	S	S	S	S	
41	20	F	4302	NEPHRO	POST TRANS	URINE	A.baumanni	R	R	R	R	R	R	R	R	R	R	R	R	S	MBL
42	44	M	2188	ORTHO	DEGLOVING INJURY	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
43	43	M	12094	NS	HEADINJURY	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	S	R	S	S	S	MBL
44	66	M	4271	ICU	SEPTICAEMIA	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
45	30	F	6407	NEPHRO	SEPTICAEMIA	BLOOD	A.junii	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
46	35	M	124802	NEPHRO	CKD	PD FLUID	A.baumanni	S	S	S	S	S	S	S	R	S	S	S	S	S	
47	50	M	4805	ICU	ARDS	SPUTUM	A.baumanni	S	S	S	S	S	S	S	S	S	S	S	S	S	
48	61	M	7705	TM	ULMASS	BR WASH	A.baumanni	S	S	S	S	S	S	S	S	S	S	S	S	S	
49	34	F	3254	NEPHRO	SEPTICAEMIA	BLOOD	A.baumanni	R	R	R	S	R	R	R	R	S	S	R	S	S	
50	62	F	43742	ICU	MCTD	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	R	R	R	S	S	MBL

S. NO.	AGE	SEX	IP NO.	WARD	DIAGNOSIS	SPECIMEN	SPECIES IDENTIFIED	CEFOTAXIME	CEFTAZIDIME	CEFEPIME	AMIKACIN	GENTAMYCIN	CIPRO	PIPTAZ	COTRI	IMIPENEM	MEROPENEM	TIGECYCLINE	POLY B	COLISTIN	RES PATTERN
51	68	F	41652	NS	SAH	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	S	S	S	S	S	AMPC
52	46	M	2665	NEPHRO	CATHETERSEPSIS	BLOOD	A.baumanni	S	S	S	S	S	S	S	S	S	S	S	S	S	
53	60	M	14826	NEPHRO	CKD	URINE	A.calcoaceticus	S	S	S	S	S	R	S	R	S	S	S	S	S	
54	61	M	12346	NEPHRO	CKD	PDFLUID	A.baumanni	S	S	S	S	S	S	S	S	S	S	S	S	S	
55	45	F	113889	NS	HYDROCEPHALUS	CSF	A.baumanni	R	R	R	R	R	R	S	S	S	S	S	S	S	ESBL
56	60	M	11615	SUR	DIAB FOOT	WOUNDSWAB	A.junii	S	S	S	S	S	S	S	S	S	S	S	S	S	
57	47	M	7229	NS	HEADINJURY	URINE	A.baumanni	R	R	S	S	R	R	R	R	S	S	S	S	S	AMPC
58	45	F	42126	SUR	UROSEPSIS	URINE	A.baumanni	R	R	R	R	R	R	R	R	R	R	S	S	S	MBL
59	55	F	21978	ICU	RHD	BLOOD	A.baumanni	R	R	S	S	R	R	S	R	S	S	S	S	S	
60	45	M	40656	ORTHO	BB FRACTURE	URINE	A.baumanni	R	R	R	R	R	R	R	R	S	R	S	S	S	MBL
61	20	M	118983	ORTHO	OSTEOSARCOMA	WOUNDSWAB	A.lwoffii	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
62	21	F	120646	SUR	UROSEPSIS	URINE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
63	34	M	123960	NEPHRO	POST TRANS	URINE	A.baumanni	R	R	R	R	R	R	R	R	R	R	S	R	S	MBL
64	25	M	122796	ORTHO	ACETABULAR FRACTURE	URINE	A.baumanni	R	R	R	R	R	R	R	R	S	S	R	S	S	
65	48	M	45017	NEPHRO	POST TRANS	URINE	A.baumanni	R	R	R	R	R	R	R	R	R	R	R	S	S	MBL
66	33	M	107115	NS	HEADINJURY	CSF	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
67	38	F	8879	CT	POST TVR	BLOOD	A.lwoffii	R	R	S	R	R	R	R	R	S	S	S	S	S	AMPC
68	65	M	114597	ORTHO	INF IMP	WOUNDSWAB	A.calcoaceticus	S	S	S	S	S	S	S	S	S	S	S	S	S	
69	47	M	31121	ICU	POISONING	URINE	A.baumanni	R	R	R	R	R	R	R	R	R	R	R	R	S	MBL-2
70	60	M	6547	ICU	MYASTHENIAGRAVIS	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
71	48	M	20923	MED	CVA	URINE	A.lwoffii	R	R	R	R	R	R	R	R	S	S	R	S	S	
72	33	M	5073	SUR	DIAB FOOT	WOUNDSWAB	A.junii	R	S	S	S	R	R	S	R	S	S	S	S	S	
73	55	F	7284	MED	CIRRHOSIS	ASCITICFLUID	A.baumanni	S	S	S	S	S	S	S	R	S	S	S	S	S	
74	65	M	27850	MED	HYDROCEPHALUS	BLOOD	A.baumanni	R	R	S	S	S	R	R	S	S	S	S	S	S	AMPC
75	20	M	1241	NS	HEADINJURY	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	S	S	R	S	S	
76	45	M	54683	TM	COPD	SPUTUM	A.baumanni	R	R	R	R	R	R	R	R	R	R	S	S	S	MBL
77	58	M	17620	MED	UTI	URINE	A.calcoaceticus	S	S	S	S	S	R	S	S	S	S	S	S	S	
78	45	F	120525	SUR	DIAB FOOT	WOUNDSWAB	A.baumanni	R	R	S	R	R	R	R	S	S	S	S	S	S	AMPC
79	35	M	122718	ICU	POISONING	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
80	58	M	55993	ICU	ASPIRATION PNEUMONIA	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	R	R	S	S	S	MBL
81	72	M	27842	ICU	SEPTICAEMIA	BLOOD	A.baumanni	R	R	S	S	S	R	R	S	S	S	S	S	S	AMPC
82	50	M	6452	NS	SEPTICAEMIA	ET ASPIRATE	A.baumanni	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
83	32	M	24941	NS	HEADINJURY	URINE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	
84	70	M	5878	ICU	SEPTICAEMIA	ET ASPIRATE	A.baumanni	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
85	30	M	22906	NEPHRO	SEPTICAEMIA	BLOOD	A.baumanni	R	R	S	R	R	R	R	R	S	S	S	S	S	AMPC
86	45	M	14057	NEPHRO	POST TRANS	SPUTUM	A.baumanni	R	R	R	R	R	R	S	R	S	S	R	S	S	
87	40	F	54983	ICU	POISONING	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	R	R	R	S	S	MBL
88	45	M	13300	NS	SDH	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
89	40	F	13691	ICU	POISONING	ET ASPIRATE	A.baumanni	R	R	R	R	R	S	S	R	S	S	S	S	S	ESBL
90	43	F	12628	MED	RHD	SPUTUM	A.baumanni	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
91	48	M	11870	NS	SEIZURE DISORDER	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	S	S	R	S	S	
92	45	M	13723	NS	SDH	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
93	28	M	54067	NS	HEADINJURY	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	R	R	R	S	S	MBL
94	45	M	38623	NEPHRO	CKD	BLOOD	A.lwoffii	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
95	23	F	22756	NEPHRO	NEPHRITIS	URINE	A.baumanni	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
96	35	F	6295	TM	CA BRONCHUS	BR WASH	A.baumanni	S	S	S	S	S	R	S	S	S	S	S	S	S	
97	75	F	11368	TM	AECOPD	SPUTUM	A.baumanni	R	R	R	S	S	S	S	S	S	S	S	S	S	ESBL
98	65	M	24146	MED	UTI	URINE	A.calcoaceticus	R	S	S	R	R	R	S	R	S	S	R	S	S	
99	20	M	118983	ORTHO	OSTEOMYELITIS	WOUNDSWAB	A.lwoffii	R	R	S	S	R	R	S	R	S	S	S	S	S	
100	76	M	8404	ORTHO	INFECTEDWOUND	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL

S. NO.	AGE	SEX	IP NO.	WARD	DIAGNOSIS	SPECIMEN	SPECIES IDENTIFIED	CEFOTAXIME	CEFTAZIDIME	CEFEPIME	AMIKACIN	GENTAMYCIN	CIPRO	PIPTAZ	COTRI	IMIPENEM	MEROPENEM	TIGECYCLINE	POLY B	COLISTIN	RES PATTERN
101	40	F	13691	ICU	POISONING	ET ASPIRATE	A.baumanni	R	R	S	R	R	S	R	R	S	S	S	S	S	AMPC
102	60	M	7964	SUR	DIAB FOOT	WOUNDSWAB	A.junii	S	S	S	R	R	R	S	R	S	S	S	S	S	
103	47	M	35113	MED	UTI	URINE	A.baumanni	R	R	R	S	S	R	S	R	S	S	S	S	S	ESBL
104	47	M	11870	NS	SDH	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	R	R	R	R	R	R	S	MBL
105	55	F	34512	MED	FFE	BLOOD	A.baumanni	R	S	S	S	S	R	S	R	S	S	S	S	S	
106	75	F	10976	TM	RMLCONSOLIDATION	BR WASH	A.baumanni	R	R	R	S	R	R	S	S	S	S	S	S	S	ESBL
107	20	M	14066	NS	HEADINJURY	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	R	R	R	S	S	MBL
108	40	F	36089	MED	FFE	URINE	A.lwoffii	S	S	S	S	S	R	S	R	S	S	S	S	S	
109	60	M	11968	TM	PNEUMONITIS	BR WASH	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
110	75	M	37452	MGE	CIRRHOSIS	URINE	A.calcoaceticus	S	S	S	S	S	R	S	R	S	S	S	S	S	
111	37	M	5735	ORTHO	MULTIPLE FRACTURE	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	S	S	S	S	S	S	ESBL
112	46	M	33629	NS	SDH	ET ASPIRATE	A.baumanni	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
113	24	M	12337	NS	CRANIOTOMY	ET ASPIRATE	A.baumanni	R	R	S	S	S	R	S	R	S	S	S	S	S	
114	25	F	39186	ICU	POISONING	URINE	A.baumanni	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
115	42	F	16654	TM	PNEOMONIA	BR WASH	A.baumanni	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
116	55	M	16752	TM	RLLMASS	BR WASH	A.baumanni	S	S	S	S	S	R	S	S	S	S	S	S	S	
117	33	F	7036	SUR	POSTOP	URINE	A.baumanni	R	S	S	R	R	R	S	R	S	S	S	S	S	
118	65	M	13694	SUR	INFECTEDWOUND	WOUNDSWAB	A.lwoffii	S	S	S	S	S	S	S	S	S	S	S	S	S	
119	51	M	14940	ORTHO	MULTIPLE FRACTURE	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	R	S	S	
120	75	F	39158	MED	FFE	URINE	A.junii	S	S	S	S	R	R	S	R	S	S	S	S	S	
121	43	M	39846	MED	FFE	URINE	A.baumanni	R	R	S	S	R	R	R	R	S	S	S	S	S	AMP
122	60	F	123532	ORTHO	OSTEOMYELITIS	WOUNDSWAB	A.lwoffii	S	S	S	R	R	R	S	S	S	S	S	S	S	
123	45	F	17635	TM	BRONCHIECTASIS	SPUTUM	A.calcoaceticus	S	S	S	S	S	S	S	S	S	S	S	S	S	
124	40	F	15472	SUR	DIAB FOOT	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	R	S	S	
125	50	M	36361	NS	HEADINJURY	ET ASPIRATE	A.baumanni	R	S	S	R	S	R	S	S	S	S	S	S	S	
126	50	F	41306	MED	FFE	URINE	A.baumanni	S	S	S	S	S	S	S	S	S	S	S	S	S	
127	85	M	16541	ICU	ASPIRATION PNEUMONIA	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	S	S	R	S	S	
128	55	M	2737	ORTHO	INFECTEDWOUND	WOUNDSWAB	A.baumanni	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
129	45	M	20368	MED	CIRRHOSIS	ASCITICFLUID	A.baumanni	R	R	S	S	S	S	S	S	S	S	S	S	S	
130	45	M	18490	NS	SDH	ET ASPIRATE	A.baumanni	R	R	S	S	R	R	R	R	S	S	S	S	S	AMPC
131	42	M	32088	URO	UROLITHIASIS	URINE	A.lwoffii	S	S	S	S	S	R	S	R	S	S	S	S	S	
132	50	M	36419	MED	FFE	BLOOD	A.baumanni	S	S	S	S	S	R	S	S	S	S	S	S	S	
133	38	M	20648	MED	PNEUMONIA	PLUERALFLUID	A.baumanni	S	S	S	S	S	S	S	S	S	S	S	S	S	
134	49	M	20635	TM	COPD	SPUTUM	A.baumanni	R	R	R	S	S	S	S	S	S	S	S	S	S	ESBL
135	43	M	16520	ORTHO	INFECTEDWOUND	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
136	52	F	32170	MED	FFE	URINE	A.baumanni	S	S	S	S	S	R	S	R	S	S	S	S	S	
137	45	M	15758	SUR	DIAB FOOT	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
138	38	M	21205	MED	PNEUMONIA	PLUERALFLUID	A.baumanni	R	R	R	S	R	R	S	S	S	S	S	S	S	ESBL
139	35	M	21249	NS	HEADINJURY	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
140	50	M	34915	ICU	CVA	ET ASPIRATE	A.baumanni	R	R	S	R	R	R	R	R	S	S	S	S	S	AMPC
141	52	F	32714	MED	UTI	URINE	A.lwoffii	S	S	S	S	S	R	S	R	S	S	S	S	S	
142	75	M	45554	TM	COPD	SPUTUM	A.baumanni	S	S	S	S	S	R	S	R	S	S	S	S	S	
143	60	M	26750	ICU	SEPTICAEMIA	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	S	S	R	S	S	
144	80	M	20487	NS	HEADINJURY	ET ASPIRATE	A.baumanni	R	R	S	S	R	R	R	R	S	S	S	S	S	AMPC
145	58	F	23180	ICU	SEPTICAEMIA	ET ASPIRATE	A.baumanni	R	R	R	S	R	R	R	R	S	S	S	S	S	
146	56	M	78461	SUR	DIAB FOOT	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
147	80	M	44378	NEPHRO	CKD	URINE	A.baumanni	R	R	R	S	S	R	R	R	S	S	S	S	S	
148	58	M	29150	ICU	CVA	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
149	25	F	30577	ICU	POISONING	ET ASPIRATE	A.baumanni	R	R	R	S	S	S	R	R	S	S	S	S	S	ESBL
150	70	M	34702	TM	COPD	SPUTUM	A.baumanni	R	R	R	S	S	S	R	R	S	S	S	S	S	

S. NO.	AGE	SEX	IP NO.	WARD	DIAGNOSIS	SPECIMEN	SPECIES IDENTIFIED	CEFOTAXIME	CEFTAZIDIME	CEFEPIME	AMIKACIN	GENTAMYCIN	CIPRO	PIPTAZ	COTRI	IMIPENEM	MEROPENEM	TIGECYCLINE	POLY B	COLISTIN	RES PATTERN
151	43	M	39812	MGE	CIRRHOSIS	URINE	A.baumanni	R	R	S	R	R	R	R	R	S	S	S	S	S	
152	35	M	45277	MED	CIRRHOSIS	BLOOD	A.lwoffii	R	S	S	R	R	S	S	R	S	S	S	S	S	
153	65	M	21813	SUR	INFECTEDWOUND	WOUNDSWAB	A.calcoaceticus	S	S	S	S	S	S	S	R	S	S	S	S	S	
154	45	M	40656	MED	CKD	URINE	A.baumanni	R	R	R	S	S	R	R	R	S	S	S	S	S	
155	60	F	10021	SUR	DIAB FOOT	WOUNDSWAB	A.baumanni	R	R	R	S	S	S	S	S	S	S	S	S	S	ESBL
156	24	F	29007	ICU	POISONING	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	S	S	S	S	S	ESBL
157	50	F	35955	NEPHRO	CKD	PDFLUID	A.baumanni	R	R	R	S	S	S	R	R	S	S	S	S	S	ESBL
158	35	M	33236	ICU	POISONING	ET ASPIRATE	A.baumanni	S	S	S	S	S	S	S	R	S	S	S	S	S	
159	23	M	21482	SUR	INFECTEDWOUND	WOUNDSWAB	A.lwoffii	S	S	S	S	S	S	S	R	S	S	S	S	S	
160	48	F	47174	MGE	CKD/PHT	BLOOD	A.baumanni	R	R	R	S	R	R	R	R	S	S	S	S	S	
161	75	F	47539	MED	FFE	URINE	A.baumanni	R	S	S	S	R	R	S	R	S	S	S	S	S	
162	19	M	34489	TM	PNEUMONIA	BR WASH	A.baumanni	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
163	59	F	34550	ICU	ASPIRATION PNEUMONIA	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	R	R	S	S	S	S	S	
164	19	F	43323	ICU	POISONING	URINE	A.baumanni	R	R	R	R	R	R	R	R	S	S	R	S	S	
165	60	M	20378	SUR	DIAB FOOT	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
166	47	M	50661	MED	UTI	URINE	A.baumanni	R	S	S	S	S	R	S	R	S	S	S	S	S	
167	44	M	35008	ICU	POISONING	ET ASPIRATE	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
168	48	M	90821	SUR	DIAB FOOT	WOUNDSWAB	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
169	70	M	39591	NEPHRO	CKD	PDFLUID	A.baumanni	S	S	S	S	S	S	S	R	S	S	S	S	S	
170	34	M	51793	SUR	SEPSIS	URINE	A.baumanni	R	R	R	S	R	R	S	R	S	S	S	S	S	ESBL
171	47	F	31121	ICU	GBS	BLOOD	A.baumanni	R	R	R	R	R	R	S	R	S	S	S	S	S	ESBL
172	60	F	5333	MED	UTI	URINE	A.lwoffii	R	S	S	R	R	R	S	R	S	S	S	S	S	
173	20	M	16101	ENT		WOUNDSWAB	A.baumanni	R	R	R	R	R	R	R	R	R	R	S	S	S	MBL
174	20	F	52384	MED	FFE	URINE	A.baumanni	R	S	S	R	R	R	S	R	S	S	S	S	S	
175	38	F	44045	ICU	POISONING	ET ASPIRATE	A.baumanni	S	S	S	S	S	S	S	R	S	S	S	S	S	

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