# CHARACTERISATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON FERMENTING GRAM NEGATIVE BACILLI AND MOLECULAR ANALYSIS OF ACINETOBACTER SPP., FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

***Dissertation submitted to***

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

***in partial fulfillment of the regulations***

***for the award of the degree of***

**M.D. (MICROBIOLOGY)**

**BRANCH – IV**


**CHENGALPATTU MEDICAL COLLEGE,**

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

**CHENNAI – TAMILNADU**

**APRIL 2016**

# CERTIFICATE

This is to certify that this dissertation titled **"CHARACTERISATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON FERMENTING GRAM NEGATIVE BACILLI AND MOLECULAR ANALYSIS OF ACINETOBACTER SPP., FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL ''** is a bonafide record of work done by **DR.M.GOMATHI.,** during the period of her Post graduate study from 2013 to 2016 under guidance and supervision in the Department of Microbiology, Chengalpattu Medical College and Hospital, Chengalpattu – 603 301 in partial fulfillment of the requirement for **M.D. MICROBIOLOGY** degree Examination of The Tamil Nadu Dr. M.G.R. Medical University to be held in April 2016.

**Dr.A.VIJAYALAKSHMI, M.D., M.B.A.,**
**Professor and HOD**
Department of Microbiology,
Chengalpattu Medical College,
Chengalpattu – 603 301

**Dean**
Chengalpattu Medical College & Hospital,
Chengalpattu – 603 301

# DECLARATION

I declare that the dissertation entitled **"CHARACTERISATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON FERMENTING GRAM NEGATIVE BACILLI AND MOLECULAR ANALYSIS OF ACINETOBACTER SPP., FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL "** submitted by me for the degree of M.D. is the record work carried out by me during the period of **April 2014 to April 2015** under the guidance of Professor **DR.V.DILLIRANI,M.D.,D.G.O.,** Department of Microbiology, Chengalpattu Medical College, Chengalpattu. 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) examinations to be held in April 2016.

Place: Chengalpattu **Signature of the Candidate**

Date: **(DR.M.GOMATHI)**

**Signature of the guide**

**Prof.DR.V.DILLIRANI, M.D.,D.G.O.,**

Professor

Department of Microbiology,

Chengalpattu Medical College,

Chengalpattu – 603 301

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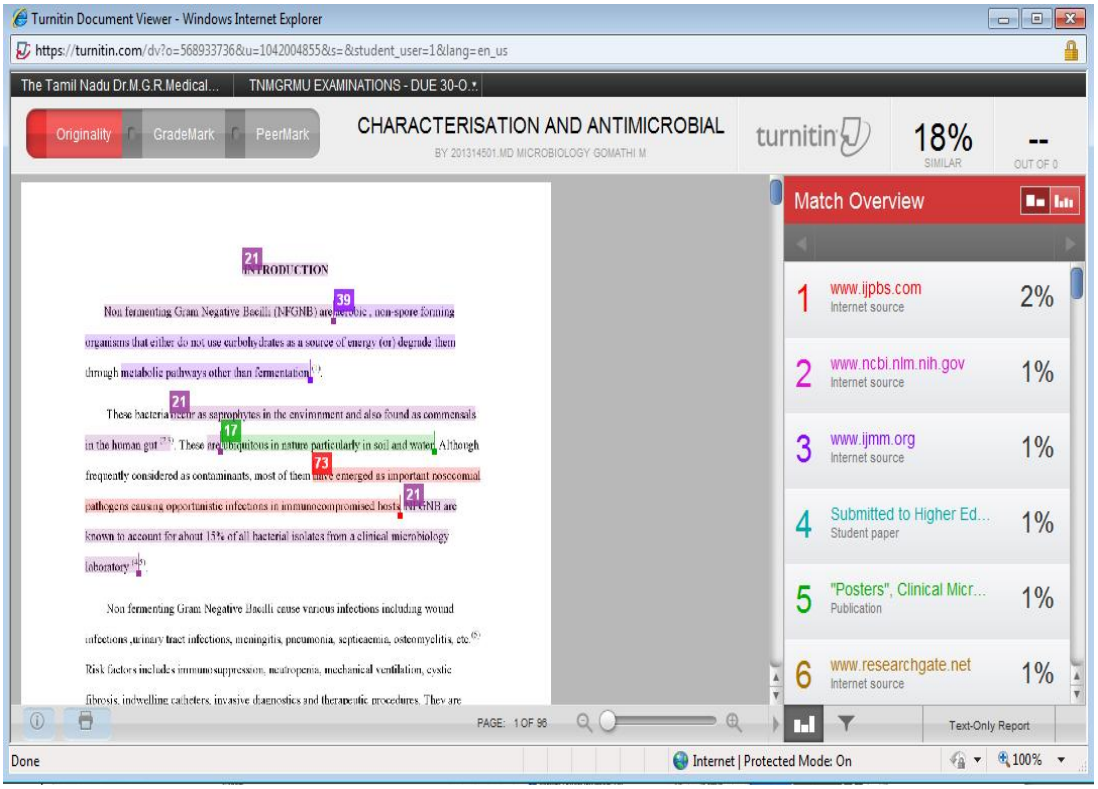
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INTRODUCTION
Non fermenting Gram Negative Bacilli (NFGNB) are aerobic, non-spore forming organisms that either do not use carbohydrates as a source of energy (or) degrade them through metabolic pathways other than fermentation[1].
These bacteria exist as saprophytes in the environment and also found as commensals in the human gut[2]. These are ubiquitous in nature particularly in soil and water. Although frequently considered as contaminants, most of them have emerged as important nosocomial pathogens causing opportunistic infections in immunocompromised hosts. NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory[3].
Non fermenting Gram Negative Bacilli cause various infections including wound infections, urinary tract infections, meningitis, pneumonia, septicaemia, osteomyelitis, etc.[4]. Risk factors includes immunosuppression, neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostics and therapeutic procedures. They are
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# ABSTRACT

**CHARACTERISATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON FERMENTING GRAM NEGATIVE BACILLI AND MOLECULAR ANALYSIS OF ACINETOBACTER SPP., FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL**

**BACKGROUND:**

Non fermenting Gram Negative Bacilli (NFGNB) once considered as contaminants have now emerged as a major cause of life threatening nosocomial infections and as multidrug resistant pathogens.

**AIM:**

To isolate and identify the NFGNB and their antimicrobial susceptibility pattern and to detect the Carbapenem resistant (Oxacillinase`and Metallo Beta Lactamase) *Acinetobacter species* by phenotypic and genotypic methods.

**Materials and Methods:**

This Cross sectional study conducted in Chengalpattu Medical College and Hospital for one year and samples collected like pus, urine, endotracheal aspirates, blood, sputum and body fluids were identified using standard protocol, which includes Grams staining, test for motility, catalase test, oxidase test, OF test and various biochemical reactions. The resistant strains of *Acinetobacter species* are subjected to molecular analysis of OXA-51, VIM and IMP genes.

**Results:**

Out of 110 clinically significant isolates of nonfermenters,54(49%) were *Pseudomonas aeruginosa,* 36(32.7%) *Acinetobacter baumannii*, 8 (7.3%) *Acinetobacter lwoffi,* 6 (5.4%) *S.maltophilia*, and *Pseudomonas stutzeri* and *Burkholderia cepecia* 3(2.8%). The antimicrobial susceptibility pattern revealed maximum resistance to Gentamycin (61.8%), Cotrimoxazole (60%), followed by Ciprofloxacin(50.9%) and Cefotaxime (47.3%).Sensitivity to Polymyxin B (100%) followed by Imipenem and Meropenem (75.5%). ESBL production was 18.18 % and MBL production was 20.5%. Molecular characterization of MBL of Acinetobacter species revealed ,OXA-51 (33.3%), 3 (33.3%) for bla IMP. and 2(22.2%) isolates bla VIM positive .

**CONCLUSION:**

Pseudomonas aeruginosa and Acinetobacter baumannii were the most common NFGNB isolated in this study. Difference in antimicrobial susceptibility by nonfermenters pose a great problem in treating these infections. ESBL and MBL production by these organisms lead to high morbidity and mortality and left with the only option of treating them by potentially toxic drugs like Colistin and Polymyxin B.

# INTRODUCTION

Non fermenting Gram Negative Bacilli (NFGNB) are aerobic, non-spore forming organisms that either do not use carbohydrates as a source of energy (or) degrade them through metabolic pathways other than fermentation [1,2,3].

These bacteria occur as saprophytes in the environment and also found as commensals in the human gut [2]. These are ubiquitous in nature particularly in soil and water. Although frequently considered as contaminants, most of them have emerged as important nosocomial pathogens causing opportunistic infections in immunocompromised hosts. NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory [3].

Non fermenting Gram Negative Bacilli cause various infections including wound infections, urinary tract infections, meningitis, pneumonia, septicaemia, osteomyelitis, etc.,[4] Risk factors includes immunosuppression, neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostics and therapeutic procedures. They are recovered with increasing frequency from clinical specimens. Prolonged hospital stay, broad spectrum antibiotic use and underlying host factors are best predictors of out come [5].

This group includes organisms from diverse genera like *Pseudomonas, Acinetobacter, Stenotrophomonas, Burkholderia, Alcaligenes, Weeksella,* etc., currently; *Pseudomonas aeruginosa and Acinetobacter baumanii* are the most commonly isolated nonfermenters pathogenic for humans whereas infections caused by other species are relatively infrequent [3].

In the National Nosocomial Infection Surveillance (NNIS) survey from the Centres for Disease Control and Prevention (CDC), Infections caused by nonfermenters is the fourth most common cause of nosocomial infection and leading cause of hospital acquired infections. Also it is the most common cause of wound infection among Gram negative bacteria with an isolation rate of up to 62% [6]. Urinary tract infections caused by nonfermenters are mostly hospital acquired and isolations range from 12%-30%. It causes life threatening bacteremia especially in intensive care settings at a rate of 10%. *Ps. aeruginosa* is the leading cause of pneumonia in the ICU patients with a mortality of 80 -100% [7].

*Acinetobacter* species are the opportunistic pathogens with increasing prevalance in the nosocomial infections [8]. Community acquired infections are also common in *Acinetobacter*. It accounts for 10% of all community-acquired bacteremic pneumonias [9].

*Acinetobacter spp.,* have been reported to cause high mortality rate of 32% to 52% in blood stream infections. Similarly mortality rate upto 70% have been reported in ICU acquired pneumonias [9]. Hence the identification of *Acinetobacter spp*., from clinical specimens is very essential.

Members of non fermenting gram negative bacteria show resistance to a wide range of commonly used antibiotics by several mechanisms like antimicrobial inactivating enzymes, reduced access to bacterial targets and point mutations that change targets or cellular functions [10]. The antimicrobial inactivating enzymes are beta lactamases including Extended Spectrum Betalactamases (ESBL), AmpC, Non-metallo beta lactamases and Metallo betalactamases (MBL) [11]. Resistance to carbapenams in nonfermenters can be intrinsic or acquired. Intrinsic resistance is seen in *S.maltophilia* while acquired Class B metallobetalactamases (MBL) and

class D serine carbapenamases are frequently found in *Ps.aeruginosa* and *Acinetobacter* respectively. These acquired MBL genes (IMP, VIM, SPM, GIM types) are usually clustered with other resistance determinants on mobile DNA elements and their presence is virtually constant marker for multidrug résistance. Multidrug resistance (MDR) exhibited by nonfermenters pose a major clinical problem in treating infections caused by them. Therefore early identification and institution of appropriate treatment is necessary to reduce the morbidity and mortality due to these organisms in hospitalised patients [(12)].

Different *Acinetobacter* species have differences in their antimicrobial susceptibility pattern, hence it is important to identify *Acinetobacter* isolates at species level [(13)]. *A.baumannii* is the most common species isolated from clinical specimens and they developed 70% of resistance to third generation cephalosporins, aminoglycosides and quinolones. 87% of *Acinetobacter* isolates were Multidrug resistant [(14)].

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[(11, 15, 16)]. For ESBL and AmpC producers, carbapenem 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 [(17)]. 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 [(18)]. Since oxacillinases are chromosomally mediated, spread of OXA genes to other organisms is less frequent, when compared to MBLgenes, where the spread is plasmid

mediated and hence the propensity of dissemination is multifold. Also metallo beta lactamases are more potent (100-1000 fold) hydrolysers of carbapenems when compared to OXA type carbapenamases which contribute to the carbapenem resistance to a greater extent [16].

Since there are no CLSI guidelines for the detection of Metallobetalactamases, different studies used different methods. Despite PCR being highly accurate and reliable, its accessibility is limited only to reference laboratories. Various non-molecular methods have been studied, all by using the enzyme's zinc dependence activity. Chelating agents, such as 2-mercaptopropionic acid, EDTA and other thiol compounds are used to inhibit its activity [19].

The present study was therefore taken to identify the nonfermenters from various clinical specimens and to determine their antimicrobial susceptibility pattern and to detect the production of Extended Spectrum Betalactamases in nonfermenters (ESBL) and also to detect the carbapenem resistance by different phenotypic methods and molecular detection of resistant genes (blaVIM and IMP and OXA - 51) among *Acinetobacter species*., by PCR in the same isolates. 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

1. To isolate, speciate and determine the antimicrobial susceptibility pattern of the Nonfermenting Gram negative bacilli.

2. To detect Extended Spectrum Betalactamases (ESBL) among the isolated nonfermenters

3. To detect Carbapenem resistance producing *Acinetobacter spp.,* among the isolates

4. To confirm Oxacillanase (OXA) and Metallo Beta Lactamase ( MBL) production by phenotypic & genotypic methods among the *Acinetobacter spp.*,

# REVIEW OF LITERATURE

Nonfermenting Gram Negative Bacilli are a taxonomically diverse group of organisms growing significantly under aerobic conditions. They share common phenotypic feature of failing to acidify the butt of Triple sugar iron agar (TSI) or Kligler iron agar (KIA) agar or oxidative-fermentative (OF) media [20].

Aerobic nonfermenters are cosmopolitan in distribution inhabiting soil, water, plants and animals. Their medical importance is principally from their being opportunistic pathogens and clinical diseases they cause are nosocomial in origin.

Large group of these nonfermenters had undergone confusing taxonomic changes for many years. New definitions of species and genera using modern genotyping analysis, with reliable identification methods resulted in a better knowledge of these bacteria and significantly increased awareness of their pathogenic role in hospitals and in cases of community acquired infections [1,7].

The major genera of nonfermenting Gram negative bacilli have been classified into atleast 15 families (*Alcaligenaceae, Alteromonadaceae, Brucellaceae, Burkholderiaceae, Caulobacteraceae, Comamonadaceae, Flavobacteriaceae, Methylobacteriaceae, Moraxellaceae, Oceanospirillaceae, Pseudomonadaceae, Rhizobiaceae, Sphingobacteriaceae, Sphingomonadaceae and Xanthomonadaceae*). In addition to a number of clinically important nonfermenters which have uncertain taxonomic positions [1].

Medically important nonfermenters can be grouped on the basis of presence or absence of motility and the type of flagella present in strains that are motile: [1]

| MOTILE WITH POLAR FLAGELLA | |
|---|---|
| **Family*Pseudomonadaceae***(rRNA groupI)<br>Genus *Pseudomonas*<br>**Family Burkholderiaceae** (rRNA group II)<br>Genus *Burkholderia*<br>Genus *Cupriavidus*<br>Genus *Lautropia*<br>Genus *Pandoraea*<br>Genus *Ralstonia*<br>**Family Comamonadaceae**<br>(rRNA group III**)**<br>*Genus Comamonas*<br>*Genus Acidovorax*<br>*Genus Delftia*<br>**Family Caulobacteraceae** (rRNA Group IV)<br>*Genus Brevundimonas*<br>**Family Xanthomonadaceae**<br>(rRNA GroupV)<br>*Genus Stenotrophomonas* | **Family Sphingomonadaceae**<br>Genus *Sphingomonas*<br>**Family Oceanospirillaceae**<br>Genus *Balneatrix*<br>**Family Alteromonadaceae**<br>Genus *Alishewanella*<br>Genus *Shewanella*<br>**Family Oxalobacteraceae**<br>Genus *Herbaspirillum*<br>Genus *Massilia*<br>**Family Methylobacteriaceae**<br>Genus *Methylobacterium*<br>Genus *Roseomonas*<br>**Organisms Whose Taxonomlc Position Is Uncertain**<br>CDC Groups Ic, O-l, O-2, O-3, Vb-3 |

| **MOTILE WITH PERITRICHOUS FLAGELLA** | **NONMOTILE,OXIDASE NEGATIVE** |
|---|---|
| **Family *Alcaligenaceae*** | **Family *Moraxellaceae*** |
| Genus *Achromobacter* | Genus *Acinetobacter* |
| Genus *Alcaligenes* | **Family *Alcaligenaceae*** |
| Genus *Bordetella (B. avium, B. hinzii,* | Genus *Bordetella* |
| *B. bronchiseptica, B. trematumatum*) | *(B.pertussis, B.parapertussis,* |
| Genus *Kerstersia* | *B. trematum)* |
| Genus *Oligella* (O. ureolytica) | **Organisms WhoseTaxonomlc Position Is Uncertain** |
| Family *Rhizobiaceae* | CDC group NO-1 |
| Genus *Rhizobium* | CDC group EO-5 |
| **Family *Brucellaceae*** | |
| Genus *Ochrobactrum* | |
| **Family *Halomonadaceae*** | |
| Genus *Halomonas* | |

| NONMOTILE, OXIDASE POSITIVE | |
|---|---|
| **Family Flavobacteriaceae** | **Family Neisseriaceae** |
| Genus *Flavobacterium* | Genus *Neisseria* |
| Genus *Bergeyella* | **Family Alcaligenaceae** |
| Genus *Chryseobacterium* | Genus *Oligella (O. urethralis*) |
| Genus *Empedobacter* | **Family Rhodobacteraceae** |
| Genus *Myroides* | Genus *Paracoccus* (EO-2) |
| Genus *Weeksella* | **OrganismsWhose Taxonomic Position Is Uncertain** |
| **Family Sphingobacteriaceae** | CDC groups EO-3, EO-4, EF-4b |
| Genus *Sphingobacterium* | CDC groups lIc, IIe, Ilg, IIh. IIi, |
| Genus *Pedobacter* | *Gilardi* rod group 1 |
| **Family Moraxellaceae** | |
| Genus *Moraxella* | |
| Genus *Psychrobacter* | |

Among these the most commonly isolated organisms in clinical specimens in descending order of importance are:

1. *Pseudomonas aeruginosa*
2. *Acinetobacter baumannii*
3. *Stenotrophomonas maltophilia*
4. *Burkholderia cepacia*

With species of *Flavobactericeae* family an *Alcaligenes* groups recently been recognized as potential pathogens [(1)].

**RISK FACTORS FOR THE DISEASES CAUSED BY NONFERMENTING GRAM NEGATIVE BACILLI [(6, 7)]**

1. Immunosuppression – Diabetes mellitus, steroids / antibiotic treatment, malignancy and transplantation.

2. Trauma – gunshot, knife wounds, punctures, burns and surgical wounds

3. Foreign body implantation – catheters (urinary / blood stream), Prosthetic devices – corneal implants, contact lenses, joints, valves.

4. Infused fluids – dialysate, saline irrigations.

5. Prolonged hospitalization in Intensive Care Units.

***PSEUDOMONAS AERUGINOSA*:**

*Ps.aeruginosa* is the most common organism isolated among the nonfermenters from clinical specimens, more often than other *Pseudomonas* species especially in the teaching hospitals with more than 500 beds[(6)]. They are ubiquitous organisms distributed widely in nature.

Nosocomial infections are infections causing serious threat to the community and reported in 5-10 percent of hospital admissions throughout the world. In India, the nosocomial infection rate is alarmingly rising and estimated to be about 30-35 percent of all the hospital admissions [(21)].

*Pseudomonas aeruginosa* has become one of the most dreadful causes of nosocomial infections especially in the lung, urinary tract and blood. As a result of its considerable potential to become resistant to many antibiotics multidrug resistant strains are encountered as clinical isolates, leaving physicians with a decreasing armamentarium of effective drugs for treatment.

**TAXONOMY:**

The genus *Pseudomonas* and some closely related genera, many of which were formerly placed in the genus *Pseudomonas,* make up a group often referred to as the pseudomonads. The Pseudomonads are classified into 5 ribosomal RNA homology groups by **Palleroni** [(22)] based on rRNA-DNA homology studies. On the other hand, **Gilardi** classified Pseudomonads into 7 major groups based on the phenotypic characters: *fluorescent, stutzeri, alcaligenes, pseudomallei, acidovorans, facilis - delafieldi and diminuta* [(1)].

Among the Pseudomonads, *Pseudomonas aeruginosa* is the well characterised and most frequently recovered Pseudomonad from the clinical specimens. *P.aeruginosa* infection is especially prevalent among the patients with burns, wounds, acute leukaemia, cystic fibrosis, organ transplants and intravenous drug addiction [(23)].

**HISTORY:**

Before the advent of modern medical microbiology, there was evidence that ***P.aeruginosa*** was a cause of serious wound and surgical infections, as elaborated by **Doggett** .In 1850, it was by **Sedillot** that there were sometimes blue green discharge on surgical dressings were associated with the infection. In 1862**,** **Lucke** first noted rod-shaped organism within the blue green pus. In 1882, **Gessard**

isolated the organism and originally designated them as bacillus pyocyaneus, and other early microbiologists also isolated the organism from infected sites. **Osler** in 1925 thought that the organism to be more of a secondary opportunistic invader of damaged tissues as opposed to a primary cause of infection in healthy tissues [24].

**HABITAT:**

*P.aeruginosa* is an opportunistic patthogen capable of causing infection in immunocompromised patients. In clinical medicine, *P. aeruginosa* has been primarily encountered as a nosocomial pathogen, which reflects its great propensity to grow in a variety of environment with minimal nutritional components [25]. It is usually found in water, soil and plants and is associated with colonisation of healthy humans and animals. Up to 7% of healthy humans are colonised with *P. aeruginosa* in nasal mucosa, throat and on the skin and a high rate of 24% carriage rates in stool are reported [26]. The organism can tolerate temperatures to as high as 45°C to 50°C and can grow in distilled water using dissolved $CO_2$ and residual iron, sulfur, phosphorus, and divalent cations such as carbon which can enhance the growth of *P.aeruginosa* even in antiseptic solutions and other liquids.

Within the health care setting, *P.aeruginosa* colonizes moist surfaces of patients on the ear, axilla and the perineum and is also found in moist inanimate environments including water in sinks, toilets and showers including antiseptic solutions which are used in the wards [27]. Hospital equipments such as respiratory ventilators, cleansing solutions, mops are also the sources of *P.aeruginosa* infection.

**MORPHOLOGY:**

Pseudomonads are gram-negative straight or slightly curved rods arranged singly, in small bundles or short chains, non-sporing, non-acid-fast, strict aerobes, motile by polar flagella

**CULTURAL CHARACTERISTICS:**

*P.aeruginosa* grows readily on ordinary media and can utilize wide range of substrates as carbon and nitrogen sources and is identified by colonial appearances and characteristic grape–like smell of aminoacetophenone [(24)]. *P.aeruginosa* strains exhibit a moth-eaten type of colonial lysis with metallic sheen known as iridescence.

Special media such as Pseudomonas isolation agar, Pseudomonas enrichment broth and Stewart's arginine glucose (AG) medium. And automated systems API 20NE test strips RapID NF Plus system are commercially available [(28)].

**PRODUCTION OF PIGMENTS: [(28)]**

1. Pyocyanin -Demonstration of the presence of blue phenazine pigment is absolute confirmation of P.aerugonosa and is the major diagnostic test.

2. Pyoverdin-The yellow/green pigment produced by most strains, giving the characteristics blue-green appearance of infected pus or cultures.

3. Pyocyanin, pyoverdin pyorubin easily identified on nutrient or sensitivity test agars and pyomelanin require growth medium containing 1%tyrosine.

**IDENTIFICATION:** [1]

*P.aeruginosa* produces large flat colonies with spreading and serrated edges with metallic sheen. Various diffusible pigments are produced like pyoverdin and pyocyanin.. On blood agar it is beta hemolytic. It produces non-lactose fermenting colonies on MacConkey agar. They are motile. It is oxidase positive, catalase positive, indole negative, citrate and urease variable. It oxidizes glucose in OF media, reduces nitrates to nitrites, arginine is decarboxylated, acetamide positive, ONPG negative, sensitive to Polymixin B and grows at 42$^{o}$C, differentiates it from *Pseudomonas fluorescens* and *Pseudomonas putida.*

Minimum requirement for definitive identification of P. aeruginosa are:

1. Gram negative rod
2. Oxidase positive
3. Typical smell (fruity grape-like odor or corn tortilla)
4. Recognizable colony morphology
    a. On blood or chocolate agar appears as large colonies with metallic sheen, mucoid, rough, or pigmented (pyocyanin) and often ß-hemolytic
    b. On MacConkey, appear as lactose-negative with green pigmentation, or metallic sheen

**VIRULENCE FACTORS OF PSEUDOMONAS AERUGINOSA:**

The virulence is multifactorial including loss of host defence mechanisms like immunosuppression, loss of mucosal barrier, cellular factors, toxins elaborated by *Ps.aeruginosa* like endotoxins, exotoxin A, enzymes like elastases, alkaline

protease and hemolysins are responsible for many of the systemic manifestations of *Pseudomonas* disease [1, 6]. In addition, the colonies of the organism form biofilms within which they are protected from host defenses and antimicrobial agents and communicate with each other through complex system of cell to cell signaling called Quorum sensing [28,6]. The production of alginate and epithelial cell tropism in cystic fibrosis is associated with poor prognosis and high mortality [29]

**VIRULENCE FACTORS: [1]**

1. Alginate – Capsular polysaccharide allows the infecting bacteria to adhere to lung epithelial cell surfaces and form biofilms and protect the bacteria from antibiotics and host immune system.
2. Pili –Surface appendages that allows adherence of the organism to GM-1 ganglioside receptoron host epithelial surfaces.
3. Neuraminidase-facilitating binding of pili by removing sialic acid residues from GM-1 ganglioside receptors
4. Lipopolysaccharide-produces endotoxin, sepsis syndrome: fever, shock, oliguria, DIC, leucopenia and metabolic abnormalities
5. Exotoxin A – causes tissue destruction by inhibiting protein synthesis, interrupts cell activity and macrophage response.
6. Enterotoxin – interrupts normal GI activity leading to diarrhoea.
7. Exoenzyme S –Inhibit protein synthesis.
8. PhospholipaseC-Destroys cytoplasmic membrane and pulmonary surfactant, inactivates opsonins
9. Elastase – Cleaves immunoglobulins and compliment components, disrupts neutrophil activity

10. Leukocidin - Inhibits neutrophil and lymphocyte function

11. Pyocyanins – Suppress other bacteria and disrupt respiratory ciliary activity, causes oxidative damage to the tissues.

**PATHOGENICITY:**

*P.aeruginosa* is a highly pathogenic in humans because of multitude, diversity and complexity of its virulence factors. All major classes of bacterial virulence systems are virtually found in this organism including exotoxins, endotoxins, leukocidin, type III secreted toxins, fimbriae, flagella, neuraminidase, elastase, proteases, exoenzymes, phospolipases, iron binding proteins, exo-polysaccharides (alginate) ,bacteriocins, biofilm formation and pigment production such as pyocyanin[(1)].

The primary factor determining the pathogenic potential of *P.aeruginosa* is the immunological status of the human host. One important predisposing factor for community acquired and nosocomial *P.aeruginosa* infection is neutropenia [(30).] Disruption in anatomic barrier functions of skin and mucosal surfaces causes invasive infection.

Other infections caused by *P. aeruginosa* are osteochondritis, chronic suppurativeotitis media, external ear infections, meningitis followed by trauma and surgery, endochondritis and peritonitis [(20, 31)].

Patients with significant burns wound are at high risk for *P.aeruginosa*[(32).] Burn wounds, other types of wounds such as chronic non-healing ulcer, diabetic ulcer, ulcers due to malignancies, use of intravenous or urinay catheters, use of endotracheal tubes are the predisposing factors to acquire infection with *P.aeruginosa.* The healthy eye is highly resistant to *P.aeruginosa* infection, when

the physical integrity of corneal epithelium is lost it becomes a pathogen [33, 34].The organism can also cause dreadful infections of the eye. Pseudomonas keratitis and endophthalmitis are approached as medical emergency that can be fatal and threaten permanent loss of vision [33]. Loss of mucosal barrier is also an important factor in invasive disease. Host factors that have been implicated in high level resistance to *P.aeruginosa* infection include complement proteins [35, 36] lung surfactants [37] and similar members of collectin family, a variety of cytokines and chemokines [36].

The production of mucoid morphotype is due to the production of large amounts of polysaccharide (alginate) [38], which is ultimately responsible for the poor prognosis and high mortality rates among the patients with cystic fibrosis [39].

**QUORUM SENSING - VIRULENCE FACTOR PRODUCTION & BIOFILM FORMATION:**

Recently, small regulatory RNAs have been recognized whose expression is controlled by a repressor protein and these regulator RNAs control production of factors are known as **quorum sensors** that signal the organism how to respond to its environment.

Three important interrelated quorum sensing systems are known and designated as **las, rhl** and **pseudomonas quinolone system**. The molecular mediators of the quorum sensing are known as autoinducers (AI) .They have a role in regulation of gene transcription and virulence factor production which has been linked with PA01 chromosome in *P.aeruginosa* [40].

## ANTIMICROBIAL SUSCEPTIBILITY:

They are sensitive to semisynthetic penicillins like Piperacillin, Ticaricillin, third generation cephalosporins (ceftazidime), carbapenams (imipenam and meropenam), monobactams, aminoglycosides and fluroquinolones [7,41].

It is intrinsically resistant to ampicillin, amoxycillin and amoxicillin-clavulanic acid due to an inducible chromosomal AmpC beta lactamases. Multiple resistance in these organisms is frequent, leading to the development of multidrug and pandrug resistant *P.aeruginosa* strains caused by mutations & or production of betalactamases ranging from extended spectrum of betalactamases to metallobetalactamases [42, 20]

## MULTIDRUG RESISTANCE IN PSEUDOMONAS:

*P. aeruginosa* can develop resistance to antibiotics either through the acquisition of resistance genes on extra chromosomal mobile genetic elements (i.e., plasmids) or through the mutational mechanisms that alter the expression and function of chromosomally mediated mechanisms. Although the availability of certain agents like Doripenem and fourth generation cephalosporin, Cefipime provided the medical community with a certain degree of security, the situation has changed because of the selection strains of *P. aeruginosa* .Various definitions defining multi-drug resistant (MDR), extensively drug resistant (XDR) and pandrug-resistant bacteria (PDR) including *P. aeruginosa* have been recently reported [43]

## PSEUDOMONAS STUTZERI:

The organisms in this group are all soil denitrifiers and can grow anaerobically in nitrate containing media, with production of nitrogen gas. Motile by

polar monotrichous flagella, grow with $NH_4$ as the sole source of nitrogen and acetate as sole source of carbon for energy.

*P.stutzeri* (formerly CDC group Vb-1) is ubiquitous in soil and water and recovered from human s, manure, straw, sewage, stagnant water, baby formula, hospital equipment, eye cosmetics and various clinical specimens [24].

*P.stutzeri* has been associated with infections such as otitis media, conjunctivitis, pneumonia, septic arthritis, endocarditis, meningitis, infections of wounds and osteomyelitis [1].

**IDENTIFICATION:**

Freshly isolated colonies are adherent and have a characteristic wrinkled appearance which may be lost on repeated subculture. They are catalase positive, oxidase positive , motile, oxidises glucose and maltose in OF media, reduces nitrate to nitrogen gas, doesnot decarboxylse lysine, arginine, acetamide negative and sensitive to polymyxin B [1]

**ANTIMICROBIAL SUSCEPTIBILITY**

They are sensitive to semisynthetic penicillins like Piperacillin, Ticarcillin, third generation cephalosporins (ceftazidime), Carbapenems (Imipenem and Meropenem), Monobactams, aminoglycosides and Fluroquinolones.

***ACINETOBACTER SPECIES:***

*Acinetobacter* are strict aerobic, gram negative coccobacillary rods, widely distributed in nature and hospital environments [20]. They are the second most commonly isolated nonfermenters in the clinical specimens next to *Pseudomonas*

*aeruginosa* with a prevalence rate of 10% of all Gram negative isolates[20,6]. They are generally considered as non pathogic but cause serious infections in debilitated patients. The species most frequently isolated is *Acinetobacter baumanii.* It is most often responsible for hospital acquired infections [20]. They are the most common Gram-negative organisms to be isolated from the hands of medical personnel[6].

A study conducted by CDC reported that *A.baumanii* be the cause of 1% nosocomial blood stream infections (CDC). A mortality of 17- 46% is associated with nosocomial bacteremia [44]. Analysis of data from NNIS system showed that the proportion of ICU pneumonia episode ranges from 4% -7% [6].

These organisms have high rate of colonization of trachea. Respiratory tract is the most common site for *A.baumanii* infections in ICU with a mortality rate approaching 70% [45]. Traumatic wounds, burns and postoperative surgical site infections are also common with multidrug resistant strains [3].

**TAXONOMY:**

**HISTORY:**

The Genus *Acinetobacter* has colourful taxonomic history. They were identified in the first decade of the 20$^{th}$ century [9]. *Acinetobacter* was misidentified due to lack of differentiating features.

Genus *Acinetobacter* are a group of Gram negative bacteria belonging to **Gammaproteobacteria** [46]. 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*** [(22)]. 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 [(22)]. 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[(46)]. This family also includes *Moraxella, Psychrobacter* and related organisms. The genus *Acinetobacter* belongs to:-

Phylum - Proteobacteria

Class - Gammaproteobacteria

Order - Pseudomonadales

Family - *Moraxellaceae*

Genus - *Acinetobacter*

**CLASSIFICATION:**

The genus characteristic 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 [47].

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, *A.calcoaceticus* was distinguished into two variants, *A.calcoaceticus var.anitratus* which produces acid from glucose and *A.calcoaceticaus var.lwoffii* which do not produce acid [9].

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 [22].

The Epidemiological identification was done using pulsed field gel electrophoresis, amplified fragment length polymorphism(AFLP), randomly amplified polymorphic DNApolymerase chain reaction(RAPD-PCR), MLST, electrospray ionization mass spectrometry (PCR/ESI-MS) or ribotyping[48], Fluorescent Lactose Denitrification (FLN) was used to identify different species of bacteria in this genus by the amount of acid produced due to metabolism of glucose[49].

In 1986, based on 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 [50].

**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* [(22)].

**MORPHOLOGY:**

Members of the genus *Acinetobacter* are Gram negative rods or coccobacilli. During the exponential phase they appear bacillary to coccobacillary but become more coccoid or diplococcal in the stationary phase and in non selective media [(22)]. Individual cells are 1 to 1.5 by 1.5 to 2.5μm in size.

**CULTURAL CHARACTERISTICS:**

Members of the genus *Acinetobacter* are strict aerobic, catalase positive, oxidase negative, Gram negative rods growing at a wide range of temperatures (often upto 42°C 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 [(22)]. They do not reduce nitrates to nitrites, this distinguishes these organisms from *Enterobacteriaceae* [(9)]. They are not fastidious and most strains grow in defined media containing single carbon and energy source which accounts for its prevalence in nature [(48)].

The colonies are 1-2mm in diameter which is smaller, dome shaped, smooth, slightly mucoid and opaque with grayish white or pale yellow pigmentation. *Acinetobacter* are nonlactose fermenters but it may produce a pinkish hue on MacConkey agar [22]. Hemolytic property on Blood agar is variable.

Some special media like Herellea Agar, Leeds *Acinetobacter* Medium and Liquid Enrichment medium have been used for the isolation of *Acinetobacter* species from various clinical specimens and from environmental samples [50].

**BIOCHEMICAL CHARACTERISTICS:**

| **Organism** | **Genomo species** | **Growthat 37°C** | **Growth at 44 °C** | **Haemo Lysis** | **Of Glucose** | **Arginine hydrolysis** | **Malonate utilisation** |
|---|---|---|---|---|---|---|---|
| A.calcoaceticus | 1 | + | - | - | + | + | + |
| A.baumanii | 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.

## 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.

## EPIDEMIOLOGY:

*Acinetobacter* species are ubiquitous in the environment[22]. *Acinetobacter* species may be isolated from a common source such as computer key boards, Bp cuffs, parenteral nutrition or mechanical ventilator or from the dialysis machine [48].

They are normally isolated from the 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 [9]. *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 [48]. *A.baumannii* colonization is very low in normal individuals but it has higher end during hospitalization.

## BURDEN OF DISEASE:

## WORLDWIDE:

The multidrug resistant *Acinetobacter spp.,* especially *Acinetobacter calcoaceticus baumannii* complex isolates showing a rising trend all over the world. The first carbapenamase enzyme resistance strain was found in Scotland in 1985[51]. Till 2002 carbapenem resistance was not a major health problem in Europe. From 2003 the isolation of resistant strains has been increased.

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 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 The United States. This data suggests that the growing threat of *Acinetobacter* infection in critically ill patients especially in Asia and Europe [(52)].

**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[(11,15,16)].

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.[(9)]. Similarly a study conducted in the same year showed 14.8% of *A.baumannii* isolates were Meropenem resistant [(53)].

An incidence of 14.2% *Acinetobacter* strains were resistant to carbapenem was documented in a study from Christian Medical College, Vellore.[(54)] Similarly a study from All India Institute of Medical Sciences, New Delhi in 2005 has given a prevalence of 34.7% resistance to meropenem, St. John's Medical College, Bangalore showed resistance rate of 14% and from Chandigarh in 2003 resistance rate of 20% have been documented.[(55,18)]

**PATHOGENESIS:**

**VIRULENCE FACTORS [22]**

- **Lipopolysaccharide** – Because of lipopolysaccharide, *Acinetobacter* O antigen display a marked hydrophobicity with the ability to grow on hydrophobic substrates.
- **Capsule** – The presence of polysaccharide capsule protects it against phagocytosis.
- **Fimbriae** – Fimbriae facilitate the adhesion to the 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 butyrate esterase, caprylate esterase and leucine arylamidase potentially involved in damaging tissue lipids.
- **Bacteriocin** production may enhance the survival of *Acinetobacter*.[55]
- **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 the differences in cell physiology in biofilm increases the drug resistance[56].

## CLINICAL MANIFESTATIONS:

The major drawback in the identification of *Acinetobacter* infection is the interpretation in the significance of isolates from the clinical samples [9].

## 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 [9]. Similarly 10% of community acquired pneumonia in adults is due to *Acinetobacter species* and it accounts for 20% of Gram negative pneumonia. 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% [48].

## 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 infections and the mortality rate was 17% to 46%, followed by *A.lwoffii, A. junii and A.parvus.* [48]

## 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*. [9]

**SOFT TISSUE INFECTION:**

The major pathogen in traumatic wounds, postoperative wounds 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 intracranial infections, soft tissue infections, conjunctivitis, endophthalmitis, endocarditis, arthritis, osteomyelitis, pancreatitis and liver abscess.[57]

**RISK FACTORS:**

*Acinetobacters* are generally non pathogenic but it can cause infections in debilitated individuals. It is the second most common nonfermenter isolated from the human specimens next to *P.aeruginosa*. [20]

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

**ANTIMICROBIAL SUSCEPTIBILITY PATTERN:**

Different species of *Acinetobacter* exhibit differences in antimicrobial susceptibility pattern [13]. 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.[58] Nowadays due to the development of various resistance mechanisms, the antibiotic treatment regimen for *Acinetobacter* infection has been very much narrowed.

## MECHANISM OF RESISTANCE:

### Resistance to Carbapenems:

Till the emergence of Imipenem resistant *Acinetobacter* strains Carbapenem remained the only drug to treat severe *Acinetobacter* infections. Due to the emergence of Carbapenem resistant strains, Polymyxins and Tigecycline came to be in use, which also developed resistance in recent years.[53,59,60]

The carbapenem resistance is also mediated by AmpC betalactamases when present along with decreased membrane permeability or due to alterations in penicillin binding proteins.[123,124] 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 [10]:

1. Antimicrobial inactivating enzymes.
2. Reduced access to bacterial targets
3. Point mutations that change targets or cellular functions.

**Treatment of MDR, XDR and PDR *Acinetobacter* species:**

Carbapenem remains the drug of choice for the treatment of MDR *Acinetobacter spp.,* The treatment of XDR *Acinetobacter spp,.*infections include Polymyxins and Tigecycline as the last resort.[61] .

**Combination Therapy:**

The combination therapy including rifampicin, sulbactam, aminoglycosides, carbapenems and colistin can be tried for treating XDR and PDR *Acinetobacter spp.,*[49,60,62].

**CONTROL MEASURES:**

Stringent measures should be taken to control and prevent the spread of MDR *Acinetobacter* infections [63].

**Infection control practices** [63]

1. Standard precautions, environmental cleaning and disinfection.
2. Source control effective during the outbreak.
3. Contact barrier precaution to health care personnel.

4. Cohorting the patients.

5. Cohorting of healthcare personnel.

6. 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.

***STENOTROPHOMONAS MALTOPHILIA:***

Originally classified as *Pseudomonas maltophilia*, it is an obligate aerobe and an ubiquitous organism [(1)]. Ocassionally causes opportunistic infections and is an emerging opportunistic pathogen.[(64)] It is the third most commonly encountered nonfermenter in clinical laboratory next to *Pseudomonas* and *Acinetobacter*[(1,7)].

It is an important nosocomial pathogen associated with substantial morbidity and Mortality rate of 43% especially in immunosuppressed patients' patient in intensive care unit, and pulmonary source of the isolate. The most common site for recovery of *Stenotrophomonas maltophilia* is the respiratory tract. It is one among the most common causes of wound infections due to trauma. It is frequently isolated from patients with ventilator support in ICU [(20)]. It is an important pathogen in cystic fibrosis patients [(1)]. It produces proteolytic enzymes, deoxyribonucleases, ribonucleases, hemolysins, hyaluronidase and mucinase etc. which contribute to its severity in immunosuppressed patients [(7)].

The rate of infections caused by *S.maltophilia* is increased in recent years and are being isolated from wound infections, bacteremia, pneumonia, endocarditis, urinary tract infections, meningitis and peritonitis [(20)]

**IDENTIFICATION:** [1, 64]

*S.maltophilia* is a motile rod, with polar multitrichous flagella and distinguished from pseudomonads by virtue of being lysine and DNAse positive and oxidase negative. *S.maltophilia* is susceptible to colistin and polymyxin. This property is used to distinguish it from *B.cepacia*. Colonies formed are pale yellow / lavender green with good growth on Blood agar and MacConkey agar. It is oxidase negative, motile, catalase positive, indole negative, citrate variable, urease negative. It oxidizes glucose and maltose, decarboxylates lysine, ONPG positive, with variable nitrate reduction.

The following are the characteristics by which presumptive identification of can be made:

- Good growth on blood and MacConkey agars
- Donot produce cytochrome oxidase
- Produce acid in OF maltose but may be negative in OF glucose
- Lysine decarboxylase – positive
- DNase – positive
- Some strains have yellow pigment

**ANTIMICROBIAL SUSCEPTIBILITY:**

The antibiotic susceptibility pattern can be a clue to the identification of *S.maltophilia.* The most active agents are trimethoprim sulphamethoxazole, colistin and quinolones.

Like other nonfermenters it is intrinsically resistant to many common antibiotics like aminoglycosides, carbapenams and many betalactam agents [(1)].

***BURKHOLDERIA CEPACIA:***

It is a motile free living phytopathogen identified as both endemic and epidemic nosocomial pathogen [(64)]. Its detection rates are low, in the range of 1%-16% of clinical samples [(6)]. It belongs to rRNA group Ie. Recent taxonomic advances demonstrated that *B.cepacia* is a cluster of atleast nine closely related genomic species now called *B.cepacia* complex and *includes B.cepacia, B.multivorans, B.cenocepacia, B.stablis, B.vietnamiensis, B.dolosa, B.ambifaria, B.anthina and B.pyrocinia* which can be differentiated on the basis of molecular and biochemical tests.[(1)]

These bacteria are most frequently associated with epidemic spread and with 'Cepacia syndrome' which is manifested by severe progressive respiratory failure and bacterimia. These strains are transmissible between patients and cross infection occur by person-to-person spread.

**VIRULENCE FACTORS:**

- Virulence markers such as cable(cbl) pilus encoded by cable pilin subunit gene(cblA) mediates adherence to mucus glycoproteins and enhances adherence to epithelial cells.
- B.cepacia epidemic strain marker (BCESM) has association with B.cepacia strain types infecting multiple patients with CF
- Virulence factors like proteases, lipases, exopolysaccharides and lipopolysaccharides [(8,1)].

**PATHOGENESIS**:

A few case reports have described serious infections, including severe pneumonia, Invasive otitis and sepsis in cystic fibrosis patients [7] Diabetes mellitus is a potential risk factor for development of infections.by *B.cepacia* [1].

*B.cepacia* is an important pathogen among patients with chronic granulomatous disease and cystic fibrosis. Like other nonfermenters, it can contaminate disinfectant solutions, intravenous solutions, nebulizer solutions, mouth wash and medical equipments.

The major importance of this organism lies in its role as opportunistic agent of pneumonia in cystic fibrosis patients seeded in sputum samples [65].

The spectrum of infections by these organisms includes wound infections, bacteremia, UTI, pneumonia, meningitis, peritonitis, and endocarditis [6].

**IDENTIFICATION:** [1]

Colonies are smooth and glistening, forming non-lactose fermenting colonies on MacConkey agar and yellow pigmented colonies on blood agar. It is weakly oxidase positive, catalase positive, motile, oxidizes all sugars, decarboxylates lysine, ONPG negative, acetamide negative and resistant to Polymixin B. Nitrate reduction is variable.

Selective media:

- Pseudomonas cepacia medium (PCM)-containing crystal violet, polymyxin B and ticarcillin.

- OFPBL medium – containing polymyxin B, bacitracin and lactose
- Burkholderia cepacia selective agar (BCSA) –containing lactose, sucrose, polymyxin B, gentamycin and vancomycin
- BCSA and Mast B.cepacia medium – the most suitable for the growth of all B.cepacia complexes.
- Commercial identification system – RapID NF Plus(Remel), API Rapid NFT(renamed API 20NE biomeriux), VitekGNI (biomerieux), and Uni-N/F Tek (Remel)

**ANTIMICROBIAL SUSCEPTIBILITY:**

As with other nonfermenters, intrinsic antibiotic resistance to aminoglycosides, Polymyxin B typifies *B.cepacia* and greatly complicates treatment. Trimethoprim-sulfamethoxazole has historically been the drug of choice. Most active agents are, Minocycline, ceftazidime, meropenam, Rifampicin, ciprofloxacin and other quinolones [9,1,10].

**MULTIDRUG RESISTANCE IN NONFERMENTING GRAM NEGATIVE BACILLI:**

Nonfermenting Gram Negative Bacilli pose a particular difficulty for healthcare community because they represent the problem of multidrug resistance to the maximum [53] .They are resistant to three or more drugs and important members of this group are *P. aeruginosa, A. baumannii, S.maltophilia* and *B.cepacia* [66]. They use several mechanism of resistance including intrinsic and acquired resistance. Intrinsic resistance is due to relative impermeability of outer membrane proteins compared to that of other gram negative bacteria. Efflux system also contributes to intrinsic resistance.

Acquired resisitance is by mutational changes and acquisition of exogenous genetic material. Lastly resistance may also develop during therapy turning as an initially susceptible into a resistant one.[29]

The increase in multidrug resistant strains suggests therapy with compounds like polymyxin B or colistin must be considered.[53]

## MECHANISMS OF ANTIMICROBIAL RESISTANCE AND BETA LACTAMASES

Bacteria can express more than one mechanism of antibiotic resistance leading to MDR or pandrug resistance. Molecular analysis of nonfermenter isolates from a nosocomial outbreak revealed convergence of several strategies for antibiotic resistance.(i).Over expression of Amp C chromosomal β-lactamases conferring resistance to β-lactams. [67] (ii) Mutational porin loss of OPr D porin, conferring resistance to Imipenem (iii) Upregulation of Mex XY efflux system[68] exports Fluoroquinolones, Tetracyclines, Aminoglycosides and Antipseudomonal β lactam agents.

## BETALACTAMASES – CLASSIFICATION:

β –Lactamases are classes of enzymes that inactivate β –lactam antibiotics by splitting the amide bond of the β lactam ring mediated by either chromosomal genes or by transferrable genes located on plasmids and transposons. In addition, β – lactamases genes bla frequently reside on the integrons, which often carry multiple resistant determinants.

The beta-lactamases are classified by two systems: Ambler's classification – It is a molecular classification based on amino acid sequences. [69]

Bush-Jacoby Medeiros classification – It is a functional classification. [70]

- **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**

| **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 | Carbapenemase | A | Yes |
| 3 | Carbapenemase | B | No |
| 4 | Penicillinase | | No |

The beta-lactamases may be chromosomal or plasmid mediated.

## EXTENDED SPECTRUM OF BETALACTAMASES: [53, 71]

ESBL are a group of betalactamases which share the ability to hydrolyse third generation cephalosporins and are inhibited by clavulanic acid. They are plasmid coded Carbapenams are treatment of choice for serious infections due to ESBL producing organisms. ESBLs in nonfermenters are Ambler class A. These enzymes are SHVtype, TEM type, TEM 1 and 2, CTX-M type, OXA- type, PER-type, VEB, BES – types and others.

Screening tests for ESBL producers are disk diffusion and dilution susceptibility testing methods.

The phenotypic confirmatory tests for ESBL production are: [72]

1. Cephalosporin / clavulanate combination disks.
2. Broth microdilution tests
3. E tests

## CARBAPENAMASES AND METALLOBETALACTAMASES [6,73,74]

Carbapenamases are betalactamases with versatile hydrolytic capacities. They have the ability to hydrolyze penicillins, cephalosporins, monobactams, and carbapenams. Bacteria producing these betalactamases may cause serious infections in which the carbapenamases activity renders many betalactams ineffective. They are members of molecular class A, B and D betalactamases. Class A and D have serine based hydrolytic mechanisms while class B are metallobetalactamases that

contain zinc in the active site. Class D carbapenamases consist of OXA type betalactamases frequently detected in *Acinetobacter baumanii*.

The metallobetalactamases belong to IMP, VIM, SPM, GIM and SIM families and have been detected primarily in *Pseudomonas aeruginosa*. Nonfermenters especially *P.aeruginosa* and *A.baumanii* have acquired metallobetalactamases through genetic elements (plasmids / transposons) and can be transmitted to other bacteria.

These enzymes confer resistance to all carbapenams (Imipenams, Meropenams, Ertapenams), all betalactams, aminoglycosides and quinolones. The dissemination is thought to be driven by regional consumption of ESBLs.

S.maltophilia is naturally resistant to imipenam and meropenam because of chromosomally mediated carbapenamase production [6,11]

The families and subgroups of carbapenamases known till now are IMP-1&2, VIM-1&2, SPM-1, GIM-1, and SIM-1. IMP was first discovered in *Ps.aeruginosa* in Japan and this has spread to other gram negative bacteria and reports show their detection in *A.baumanii, Serratia* and *Klebsiella*. Currently IMP family member number upto 18 in the published literature.

The identification of MBL and other Carbapenemases is of prime importance in choosing the most appropriate antibiotic for the treatment of carbapenem resistant isolates in any health care set up. The severity of infection can be decreased by early and prompt detection and appropriate treatment before the bacteria change to a mucoid phenotype, which extremely difficult to eradicate if once established.

Common antipseudomonal drugs are Carbenecillin, Ceftazidine, Piperacillin-Tazobactum & Cefoperazone – Sulbactum. The Carbapenems are one of the last resorts for the treatment of serious multi-drug resistant infection. Meropenem, Ertapenem and Imipenem/Cilastin are effective against most ESBL and AmpC producing organisms. Though these novel carbapenems are more effective against Multi Drug Resistant infections, resistance is still mediated through OprD deletions and spread of broad-spectrum Carbapenemases and MBLs. In various studies, intravenous Colistin with Rifampin and Imipenem was suggested for the treatment of Carbapenem resistant isolates without MBL production, whereas the combination of Colistin and Rifampicin with or without Tigecycline was suggested for treatment of MBL producing Carbapenem resistant isolates described byVikasmanchanda et al. [12], and Maragakis LL et al.,[75]

The medical community has now started to use drugs like Colistin and Polymyxin B which were once not used due to their toxicity but are now being considered as **''antimicrobials for the 21st century''** [76]. However some researchers reported emergence of Colistin resistant organisms in their study [77], which necessitates the discovery of newer molecules to treat the patients earlier and prevent the development and dissemination of resistance in the future.

**DETECTION OF CARBAPENAMASES: [6]**

1. Raise in MIC of carbapenams of >8 μgm / ml.
2. Microbiological test with inhibitors:
   (i) Disc approximation test with EDTA
   (ii) Combined disc method: Imipenam with EDTA
   (iii) E test strips with Imipenam and Imipenam EDTA combination
   (iv) Modified Hodge test

Of these tests, studies conducted showed that both combined disc test and E test were more sensitive and equally effective for MBL detection

**MOLECULAR METHODS:** [6,78,79]

When the presence of a carbapenamase is suspectd, PCR is the fastest way to determine which family of betalactamase is present.

**Polymerase Chain Reaction (PCR):**

Technique which amplifies a specific DNA target region, to obtain a million or more copies which can be easily visualized by using DNA staining techniques for 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 NFGNB.

# MATERIALS AND METHODS

**Study design:** Cross sectional study

**Study period:** The study period was one year from July 2014 to June 2015.

**Place of Study:** This study was conducted in the Department of Microbiology, Chengalpattu Medical College and Hospital, Chengalpattu.

**Sampling method**: Random sampling

**Study Population:**

Samples were collected from various patients who attended Chengalpattu medical college hospital and satisfied the inclusion criteria. Out of the samples collected, total of 110 isolates of Nonfermenters were isolated and speciation and antimicrobial susceptibilility testing was done according to Clinical Laboratories Standard Institute (CLSI) guidelines. 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:**

- Patients of all age groups coming to the outpatient department of Chengalpattu Medical College Hospital with various illnesses.
- Hospitalised patients undergoing treatment in ICU medical, surgical and paediatric ward.
- Patients affected with burn wounds, Patients with non-healing ulcer
- Diabetic patients with ulcers

- Patients with provisional diagnosis of Septicaemia and Pneumonia
- Patients with indwelling urinary catheter and on ventilators
- Patients with long period of stay in the hospital
- Patients with peritonitis

**EXCLUTION CRITERIA**

- Patients on prior antibiotic therapy
- Isolates of repeated samples from the same patient were not included in the study.
- Patient who do not give consent

**Ethical consideration:**

Approval was obtained from the Institutional ethical committee at Chengalpattu Medical College 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).

**COLLECTION OF SPECIMENS**

- Collection of Blood sample:

Blood samples were collected by strict aseptic technique. The skin over the vene puncture site approximately 5cm diameter was cleaned thoroughly with

70 % ethanol followed by povidone iodine and allowed to dry at least for one minute before collecting the sample. 5 ml of blood was collected in 50 ml of Brain Heart Infusion Broth (B.H.I) in adults. In the paediatric age group 1 to 5 ml of blood was collected in appropriate quantity of B.H.I.

- Collection of Pus sample:

Sterile cotton wool swabs were used to collect the sample from infected sites. The swabs were transported in sterile test tubes to the laboratory. Two swabs were collected from patients and subjected to direct smear and culture.

- Collection of Sputum sample:

The patients were asked to cough deeply before collecting the sputum to avoid mixing of saliva with the sputum. It was collected in the morning before any mouthwash was used and mouth should be rinsed with saline or water just before expectoration. Clean, dry, sterile wide mouthed, screw capped and leak proof containers were used for sputum collection.

- Collection of Urine sample:

Patients were instructed for proper collection of urine samples without any contamination. Male patients were asked to retract the prepuce and clean the urethral meatus with saline and to collect the early morning, mid stream urine. Female patients were asked to clean the genitals with soap and water and to dry the area with sterile gauze pad. The urine was collected with the labia held apart. The specimen was collected in a clean, wide mouthed, screw capped and leak proof container and transported to the laboratory without any delay.

- Collection of wound swab:

In cases of burn wounds and diabetic patients with ulcers, the wound site is cleaned with sterile saline and before application of any topical antibiotic preparation; two swabs were taken with sterile cotton swabs from the edge of the wound with active infection. The swabs are moistened with sterile saline before collecting the specimen to avoid drying of the specimen before processing.

- Collection of Endotracheal aspirate:

In patients with tracheostomies, who were unable to produce sputum, the endotracheal secretions were collected by aspirating the fluid by suctioning. The specimen was collected in a sterile container and transported immediately to the laboratory.

- Collection of Bronchoalveolar Lavage (BAL):

Bronchoscopy assisted bronchial washings or aspirates were obtained by instilling a small amount of sterile physiological saline into the bronchial tree and by withdrawing the fluid. The specimen was collected in a sterile container and transported immediately to the laboratory.

- Collection of ascitic fluid:

The skin over the site of collection was sterilized with 70% alcohol and atleast 10 ml of fluid was aspirated with sterile syringe and needle and collected in a sterile tube or vial and transported to the laboratory.

- The collected specimens were properly labeled with Name, Age, Sex, I.P/ O.P.No. of the patient, Date and Time of collection, Type of sample and Name of the ward and brought to the laboratory and processed immediately.

**PROCESSING OF SAMPLES**:

**Blood:** BHI broth containing Blood samples were incubated at 37˚C for 18-24hours after which the broth which showed turbidity were sub cultured onto the following media using sterile technique.

1. Nutrient Agar
2. MacConkey Agar
3. Blood Agar

The broths which were clear were kept for further incubation for 5-7 days and regarded as negative for growth if it appears clear even after 48 hours of incubation.

**Urine:** The urine specimens were centrifuged at 3000 rotaitons per minute for 5 mts. The sediment was used for direct Gram stain and for inoculating into the culture media and incubation was done at 37˚C for 18-24 hours aerobically.

**Wound swab:** One swab was used for direct Gram staining and the other swab used for inoculating into solid culture media and incubation was done at 37˚C for a period of 18-24 hours aerobically. After this initial processing the swabs were kept in glucose broth and after overnight incubation the glucose broth was observed for turbidity and if the culture plates showed no growth after overnight incubation a repeat subculture was done from the glucose broth which contained the swabs.

**Pus, Sputum, Endotracheal fuid, Ascitic fluid and BAL:** The specimens were processed, first by doing direct Gram staining and then inoculating into culture media and incubation was done at 37˚C for a period of 18-24 hours aerobically.

**CULTURE IDENTIFICATION:**

The organisms were identified by colony morphology on solid media, Gram staining, biochemical reactions and other special identification tests.

**ISOLATION AND IDENTIFICATION:**

Identification is mainly based on the Gram staining, Motility, colony morphology on Nutrient Agar, MacConkey Agar and Blood Agar. All the catalase positive, oxidase positive and negative, nonlactose fermenting colonies on Mac Conkey agar were provisionally identified by colony morphology and pigment production. They were inoculated in Triple sugar iron (TSI) agar slope. The colonies which failed to acidify the TSI agar were considered as nonfermenters and subjected to the following tests. Indole, Citrate, Urease, Nitrate reduction, growth at 42˚C , Sensitivity to Polymyxin B and following special biochemical tests and grouped according to P.C.Schreckenberger scheme[1]

**GRAM STAINING**: From a single isolated colony on Nutrient agar plate, the smear was prepared in clean, dry, grease free slide and it was dried in air and fixed by heating. The smear was then flooded with 0.5% methyl violet and washed with water after 1 minute. Gram's Iodine is added to the smear and washed with water after 1 minute. Then the smear was decolorized with one or two drops of acetone and immediately washed with water. Then counter stain with 1:10 diluted carbol fuschin and washed with water after 1 minute. The smear was then dried with blotting paper and viewed under oil immersion objective. Pink colored bacilli arranged in discrete pattern were identified as Gram Negative Bacilli.

**MOTILITY (HANGING DROP METHOD)** : A clean cover slip was taken and petroleum jelly was applied to all the four corners. A drop of broth culture was

placed on the centre of the cover slip with the sterilized inoculating loop. The cavity slide is taken inverted over the cover slip with the drop so that the drop is placed in the centre. The slide was inverted and focused under 10x and the edge of the drop identified. Then, without changing the field, the focus was shifted to 40x and observed for the motile organisms. Either actively motile and non motile bacilli or coccobacilli were seen.

**BIOCHEMICAL REACTIONS**: With the following tests Non fermenters were identified biochemically.

**Oxidase test (Disc method)**: The oxidase disc was moistened with distilled water and then the colony from nutrient agar was taken with the help of a sterile glass rod and applied over the disc. Deep blue or purple colour in 10 seconds was taken as positive test.

**Catalase test (Tube method):** 2-3 ml of 3% Hydrogen peroxide was taken in a clean test tube. Few colonies of the test organism were taken from the culture plate with a sterile glass rod and immersed in the Hydrogen peroxide solution. Brisk effervescence within ten seconds was considered as catalase positive.

**TSI (Triple sugar iron medium):** An isolated colony from the culture plate was taken with a straight wire loop and stabbed into the butt portion of the TSI medium, withdrawn and streaked in a zig-zag manner over the slant portion and incubation was done at 37˚Cfor 18-24 hours. The observation of alkaline change over the butt portion and alkaline change over slant portion is identified as a non-fermenter.

**Citrate utilization test:** The well isolated colony from the culture plate was taken with a straight wire loop and inoculated into the Simmon's citrate medium and incubation was done at 37˚C for 18-24 hours. The colour change from green to blue

colour or the growth of colonies on the streak line was considered as positive citrate utilization test.

**Oxidation-fermentation (O/F ) test :** Two tubes of O.F medium were inoculated with the organism isolated from Nutrient agar plate by stabbing 3-4 times half way to the bottom of the tube .One tube was promptly covered with a layer of sterile melted paraffin to a depth of 5-10 mm, leaving the other tube open to air. Both the tubes were incubated at 35˚C for upto 30 days. In case of oxidative metabolism, yellow color appears along the upper one fourth of the medium in the tube where no oil overlay was done. In case of fermentative organisms yellow color develops in both the tubes.

**CONTROL**

| | | |
|---|---|---|
| Glucose fermentation | : | *Escherichia coli* |
| Glucose oxidation | : | *Pseudomonas aeruginosa* |
| Non saccharolytic | : | *Alcaligenes species.* |

**Nitrate reduction test:** The nitrate broth was inoculated with a loop full of the test organism isolated in pure culture on agar medium and incubation was done at 35˚C for 18-24 hours. At the end of incubation, add 1 ml of Nitrate A (alpha - Naphthylamine and 5 N 30% Acetic acid) and Nitrate B (Sulphanilic acid and 5 N 30% Acetic acid) reagent in that order. The development of red colour within 30 seconds after adding the reagents indicated a positive nitrate reduction test. If no colour developed after addition of reagents, zinc dust was added to confirm a true negative reaction.

**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 test for *Pseudomonas aeruginosa* and Acinetobacter baumanii.

**Decarboxylation of lysine, ornithine and arginine dihydrolase test [(1)]*:*** Decarboxylases are a group of specific enzymes which react with carboxyl portion of aminoacid forming alkaline reacting amines. The reaction is decarboxylation. Each enzyme is specific for Lysine, Arginine and Ornithine. The lysine and ornithine reactions are truly decarboxylase tests but arginine reaction is now more correctly recognized as dihydrolase test.

**Procedure:** The organism was inoculated in four tubes containing Moller decarboxylase medium. One has the basal medium without aminoacid for control. Other three tubes had Lysine, Arginine and Ornithine each. All tubes were overlaid with liquid paraffin upto 4mm. All were incubated at 37$^{o}$C for 24 hours.

The control tube turned yellow indicating that the organism is viable and the test medium turning blue purple indicating positive result.

**O–nitrophenyl ß – D galactopyranoside:** A dense suspension of the test organism grown in TSI agar was prepared in saline. About 1 drop of toluene was added to the suspension and 0.2ml of ONPG solution was added to the suspension and incubated at 37 $^{o}$C ß-galactosidase producing organism show yellow color after 1 hour or 18-24 hours incubation.

**Acetamide agar*:*** The slant was inoculated with a portion of isolated colony and incubated at 37 $^{o}$C overnight and was observed for color change from green to blue Tubes with negative result were further incubated for 7 days.

**Gelatin liquefaction test:** Gelatin breakdown can be demonstrated by incorporating it in a buffered nutrient agar, growing the culture and then flooding the medium with mercuric chloride that differentially precipitates either gelatin or its breakdown products.causing opacity in the medium with clear zones around gelatin-liquefying colonies.

**Other biochemical tests done are:** Indole production, MR/VP test, Mannitol fermentation, Urea hydrolysis, Aesculin hydrolysis were all negative.

**ANTIMICROBIAL SUSCEPTIBILITY TESTING:**

**Disc diffusion method:**

Antimicrobial susceptibility was performed for all the isolates by modified Kirby -Bauer disc diffusion method using cation adjusted Mueller-Hinton agar plate. Three to four colonies were suspended in peptone water 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 peptone water, visually equivalent to 0.5 McFarland units. Within 15 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 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 used were *E.coli*

ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Overnight broth culture compared to 0.5 McFarland's was used as inoculum. After incubation at 37$^{o}$C for 16-18 hrs, zone of inhibition was noted. Results were interpreted according to CLSI standard.Multidrug resistant (MDR) isolates of the nonfermenters were estimated MDR isolate was defined as resistant to three or more drugs of therapeutic relevance.The panel of drugs used for antimicrobial sensitivity testing was as follows;

| **ANTIBIOTIC DISCS** | **ZONE SIZE (mm)** | | |
|---|---|---|---|
| | **Resistance (mm/less)** | **Intermediate (mm)** | **Sensitive (mm/more)** |
| Cefotaxime(30 µg) | 14 | 15-22 | 23 |
| Ceftazidime (30 µg) | 14 | 15-17 | 18 |
| Amikacin (30µg) | 14 | 15-16 | 17 |
| Gentamycin (10µg) | 12 | 13-14 | 15 |
| Ciprofloxacin(5µg) | 15 | 16-20 | 21 |
| Ofloxacin (5µg) | 12 | 13-15 | 16 |
| 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 |
| Polymyxin B (300U) | 10 | - | 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.[14,81]

**DETECTION OF ANTIMICROBIAL RESISTANCE MECHANISMS:**

**Phenotypic Method:**

All the isolates which were included in this study were subjected to Carbapenemase screening test using Imipenem and Meropenem 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.

**DETECTION OF EXTENDED SPECTRUM ß -LACTAMASES [11,71,72]**

All the nonfermenters that were resistant to cefotaxime and or ceftazidime were tested for Extended Spectrum of ß -lactamases.by the following methods:

**Phenotypic confirmation test with Cephalosporin/clavulanate combination disks.[72]**

This was done as recommended by CLSI. Mueller Hinton Agar plates were swabbed with test organism having the turbidity equivalent to 0.5 Mc Farland's standard. Aseptically cefotaxime disk (30mg), cefotaxime – clavulanic acid (30mg/10mg) ceftazidime (30mg) & ceftazidime clavulanic acid (30mg/10mg) were placed on surface of agar. The plates were incubated at $35^0$C for 16-18 hours

and diameter of zone of inhibition produced was recorded. A 5mm increase in zone diameter for combination disc than that when tested alone confirmed the presence of ESBL production.

ATCC *Escherichia coli* 25922 & *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive control respectively.

**DETECTION OF METALLOBETALACTAMASE PRODUCTION IN *ACINETOBACTER SPP*., BY PHENOTYPIC METHODS**:

**Carbapenamase detection:**

The isolates which were resistant to Imipenem and Meropenem by disc diffusion method as per CLSI guidelines was used as the indicator for carbapenamase production and tested for Oxacillinase, Metallo betalactamases [82,83]

**Screening and confirmation of MBLs**:

The *Acinetobacer* isolates which were found to be resistant to Imipenam, Meropenem by Kirby - Bauer disc diffusion method were selected. The resistant isolates were determined and subjected to various phenotypic detection methods such as Combined disc diffusion Test, Double disc synergy test and Modified Hodge Test and confirmed by genotypic method i.e, PCR.

**Common initial steps – Inoculum preparation:**

1. 3-5 colonies of the strain to be tested were touched from 24 hour culture plate with a straight wire loop and transferred to sterile peptone water and incubation was done at 37˚C and turbidity adjusted to 0.5 McFarland standard.

2. A lawn culture was made on cation balanced Mueller Hinton agar plate with a sterile cotton swab using the inoculum. (Excess broth was expressed by rotating the swab against the inner side of the suspension tube before inoculation.)

3. Inoculum was then allowed to dry for 15 minutes before applying the antibiotic disc.

**Preparation of EDTA solution:** [78]

0.5 M EDTA solution was prepared by dissolving 186.1g of disodium EDTA.2H2O in 1000 ml of distilled water and its pH was adjusted to 8.0 by using NaOH. The mixture was then sterilized by autoclaving. EDTA solution was added on Imipenem and Meropenem discs to obtain a desired concentration of 750 μg per disk.

**Combined Disc Diffusion Test (CDDT):**

- The strain to be tested was inoculated onto MHA plate as suggested by the CLSI. Two (10μg) Imipenem or Meropenem discs were placed on the plate at the distance of 20mm and 10 μl of 0.5 M EDTA solution was added to one of them to obtain the desired concentration (750 μg ).
- After18 hours of incubation, the zone diameter of Imipenem, Meropenem and Imipenem EDTA, Meropenem EDTA discs were compared. The increase in inhibition zone with Imipenem EDTA, Meropenem with EDTA disc ≥5mm than the Imipenem, Meropenem disc alone was considered as MBL positive.

**Double Disc Synergy Test (DDST):**

- Lawn culture of the test organism was prepared over Mueller-Hinton agar plate as per CLSI guidelines.
- A plain sterile disc was kept 20 mm apart from either Imipenem or Meropenem (10µg) disc.
- 5 µl of EDTA was added to plain disc and incubation was done at 37˚C overnight.
- Presence of an extended zone from Imipenem or Meropenem disc towards EDTA was interpreted as positive.

**Modified Hodge Test (MHT):**

- ATCC E.coli 25922 inoculum was prepared in 0.5 Mc Farland standards & lawn culture made on Mueller-Hinton agar plate.
- Meropenem or Ertapenem disc kept in the centre of the lawn.
- Colonies of Imipenem and Meropenem resistant isolates were taken & streaked from edge of the disc to edge of the plate & incubation was done at 37° C over night.
- The length of the streak should be 20 to 25mm.[84,85]

- **Interpretation:**
  - The formation of a clover leaf like indentation along the test strains indicates carbapenemase 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

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

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

The Minimum Inhibitory Concentration is the least amount of antimicrobial that will inhibit visible growth of an organism after overnight incubation.

MIC was determined by using Mueller Hinton broth as the medium in test tubes. Serial dilutions of Meropenam were prepared in distilled water. The concentrations used were 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 μg/ml. A young peptone water culture of the organisms corresponding to the concentration of 5x105/ ml is used as inoculum. A quality control strain of Pseudomonas aeruginosa ATCC 27853 was included. The plate was incubated at 37$^{o}$C for 16-18 hours.

**RESULTS AND INTERPRETATION**

MIC is expressed as the highest dilution which inhibited the growth as judged by the lack of turbidity in the tube.

MIC > 8µgm/ml – Carbapenam resistant, MIC< 8µgm/ml - carbapenam susceptible. Level of resistance to carbapenams is also noted.

**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[41]**:

≤ 2µg/ml - Susceptible

4µg/ml - Intermediate

≥8µg/ml - Resistant

**MOLECULAR METHOD:**

**Polymerase chain reaction:**

The isolates which were resistant to Imipenem and Meropenem by Kirby Bauer Disc diffusion method irrespective of phenotypic methods were subjected to conventional PCR for the detection of Oxacillinase gene OXA-51 and Metallo Beta Lactamases genes bla-IMP1 and bla-VIM1.[78,79]

**Gene Identification**

**Material & Methods:**

DNA purification kit (PureFast® Bacterial Genomic DNA purification kit), PCR Master Mix, Agarose gel electrophoresis consumables and Primers are from HELINI Biomolecules, Chennai, India.

**2X Master Mix**:

It contains 2U of Taq DNA polymerase, 10X Taq reaction buffer, 2mM MgCl2, 1µl of 10mM dNTPs mix and PCR additives.

**Agarose gel electrophoresis:**

Agarose, 50X TAE buffer, 6X gel loading buffer and Ethidium bromide are from HELINI Biomolecules, Chennai.

**Primers**

**IMP gene primer**

Product size = 220bp

Forward Primer - 5'-TTTTGCAGCATTGCTACCGC-3'

Reverse primer - 5'-CACGCTCCACAAACCAAGTG-3'

**VIM gene primer**

Product size = 442bp

Forward Primer - 5'-GTGCTTTGACAACGTTCGCT-3'

Reverse primer - 5'-TCCACGCACTTTCATGACGA-3'

**OXA-51 gene primer**

Product size = 160bp

**Procedure:**

1. Pellet is suspended in 200µl of PBS.
2. Added 50µl of Lysozyme and incubated at 37ºC for 15min.
3. 400□l of Lysis buffer and 40µl of Proteinase K [10mg/ml] is added and gently mixed well.
4. Incubated in water bath at 70°C for 10 min.
5. Transferred whole lysate into PureFast spin column and centrifuged at 10000rpm for 1min.
6. Discard flow through and added 500µl of Wash Buffer and Centrifuge at 10000rpm 1 min.
7. Discard flow through and added 500µl of Wash Buffer-2 and centrifuged at 10000rpm for 1min. Repeated wash one more time.
8. Discarded flow through and Centrifuged column for additional 2 minute to remove any residual ethanol.
9. Eluted DNA by adding 100µl of Elution Buffer and Centrifuged for 1min.
10. Quality and Quantity of extracted DNA is checked by loading in 1% agarose gel and 1µl of extracted DNA is used for PCR amplification.

**PCR Procedure:**

[25µl of Master Mix contains: 10X Taq buffer, 2mM Mgcl2, 0.4mM dNTPs mix, and 2U *Proofreading* Taq DNA polymerase

1. Reactions set up as follows;

| Components | Quantity |
|---|---|
| ***In PCR vial***<br>Master mix | 25µl |
| Primer Mix (10pmoles/µl) | 1µl |
| **Genomic DNA** | **1µl** |
| Water, nuclease free | 22µl |
| Total volume | 50µl |

2. Mixed gently and spin down briefly.

3. Place into PCR machine and program it as follows;

**Initial Denaturation**: 94ºC for 3 min

**Denaturation**: 94ºC for 1 min

**Annealing**: 58ºC for 1min

**Extension**: 72ºC for 1min

} 35 cycles

**Final extension**:72º C for 5 min

**Loading:**

1. Prepare 2% agarose gel. [2gm of agarose in 100ml of 1x TAE buffer]
2. Mix 8µl 6X Gel loading dye to each PCR vial and loaded 5µl of PCR sample.
3. Run electrophoresis at 50V till the dye reaches three fourth distances and observes the bands in UV Transilluminator.

**Agarose gel electrophoresis:**

1. Prepared 2% agarose. (2gm agarose in 100ml of 1X TAE buffer and melted using micro oven)
2. When the agarose gel temperature was around 60ºC, added 5µl of Ethidium bromide.
3. Poured warm agarose solution slowly into the gel platform.
4. Kept the gel set undisturbed till the agarose solidifies.
5. Poured 1XTAE buffer into submarine gel tank.
6. Carefully placed the gel platform into tank. Maintaine the tank buffer level 0.5cm above the gel.
7. PCR Samples are loaded after mixed with gel loading dye along with 10µl HELINI 100bp DNA Ladder. [100bp, 200bp, 300bp, 400bp, 500bp, 600bp, 700bp, 800bp, 900bp, 1000bp]
8. Run electrophoresis at 50V till the dye reaches three fourth distance of the gel.
9. Gel viewed in UV Transilluminator and observed the bands pattern.

**Interpretation:**

The amplified PCR products and 100bp DNA molecular markers were seen as bright fluorescent bands with satisfactory controls. A 160bp corresponds to OXA-51, 442bp corresponds to blaVIM and 220bp corresponds to blaIMP gene.

# RESULTS

A total of 110 clinical isolates of nonfermenters from various clinical samples such as blood, urine, sputum, endotracheal aspirate, broncho-alveolar lavage, wound swab and other body fluids obtained from the patients admitted to various clinical departments of Government Chengalpattu Medical college Hospital, during the period of July 2014 - June15. All the isolates were characterized to the species level and results were analysed.

Table 1 Age distribution (n=110)

| Age in years | Number of patients | Percentage (%) |
|---|---|---|
| < 10 | 23 | 20.91 |
| 11-20 | 5 | 4.54 |
| 21-30 | 21 | 19.09 |
| 31-40 | 16 | 14.54 |
| 41-50 | 14 | 12.73 |
| 51-60 | 10 | 9.09 |
| >60 | 21 | 19.09 |
| TOTAL | 110 | 100 |

Age distribution of nonfermenters was analysed which showed, majority of the patients were from the age group of less than 10 years of age 23(20.91%), followed by 21-30 years 21(19.09%) and elderly patients 21(19.09), >60 years of age.

**FIGURE 1: AGE DISTRIBUTION (N=110)**

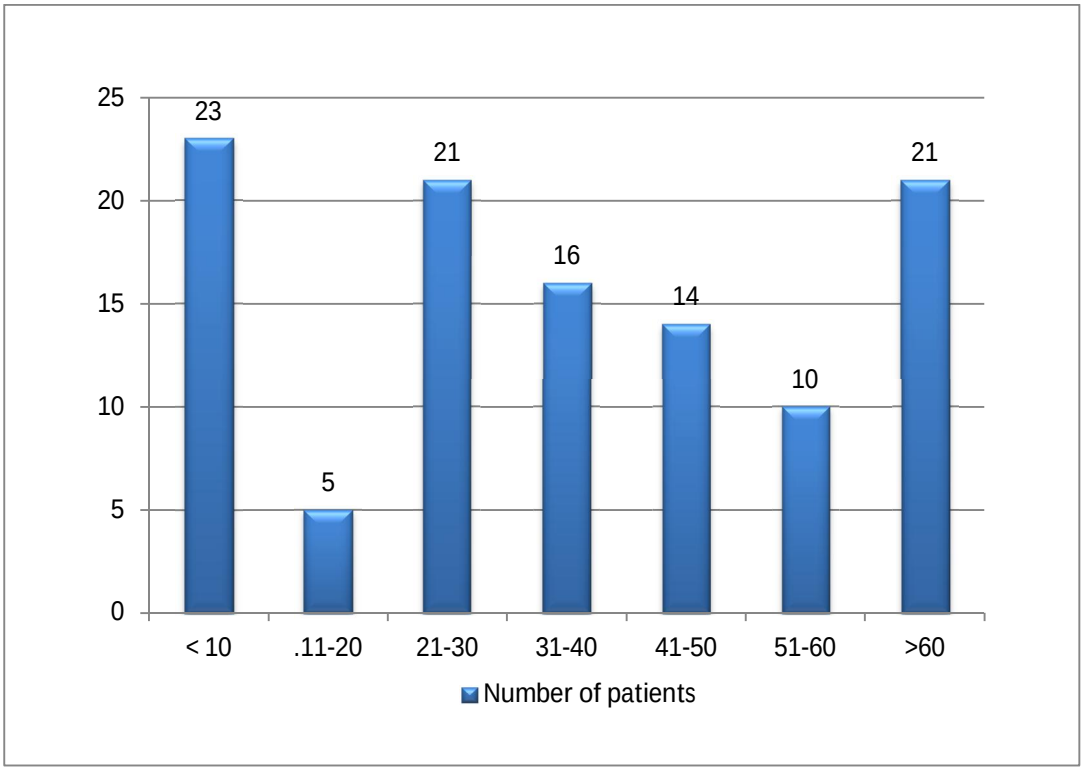


**TABLE 2.GENDER DISTRIBUTION ( n = 110)**

| Sex | Number | Percentage (%) |
|---|---|---|
| Male | 72 | 65.4 |
| Female | 38 | 34.5 |

Of the 110 isolates of 72(65.4%) were males and 38 (34.5%) were females.

**FIGURE 2: GENDER DISTRIBUTION (n=110)**

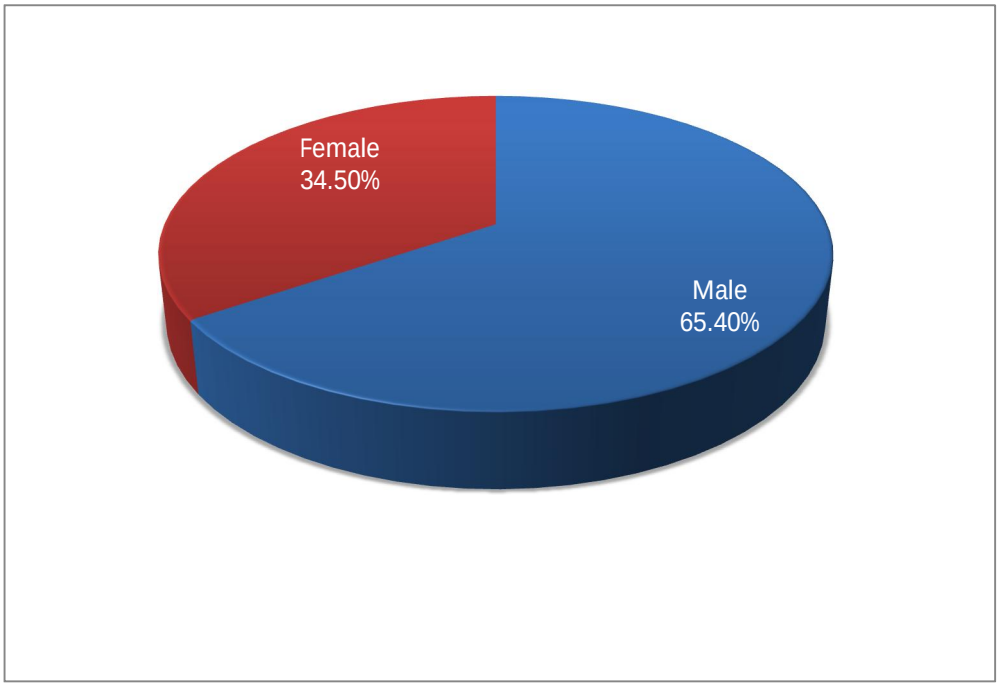


**TABLE 3: DISTRIBUTION OF SAMPLES ( n =110)**

| Clinical sample | Isolates | Percentage (%) |
|---|---|---|
| Pus | 43 | 39 |
| Urine | 20 | 18.1 |
| Wound swab | 19 | 17.2 |
| Blood | 11 | 10 |
| Sputum | 9 | 8.1 |
| Endotracheal aspirate | 5 | 4.5 |
| Body fluids | 3 | 2.7 |

Among the nonfermenters , 43 (39%) were isolated from pus, 20 (18.1%) were from urine, 19 (17.2%) from wound swab, 11 (10%) from blood, 9 (8.1%) from sputum, , 5 (4.5%) from endotracheal aspirate and 3 (2.7%) from body fluids

**FIGURE 3: DISTRIBUTION OF SAMPLES (n=110)**

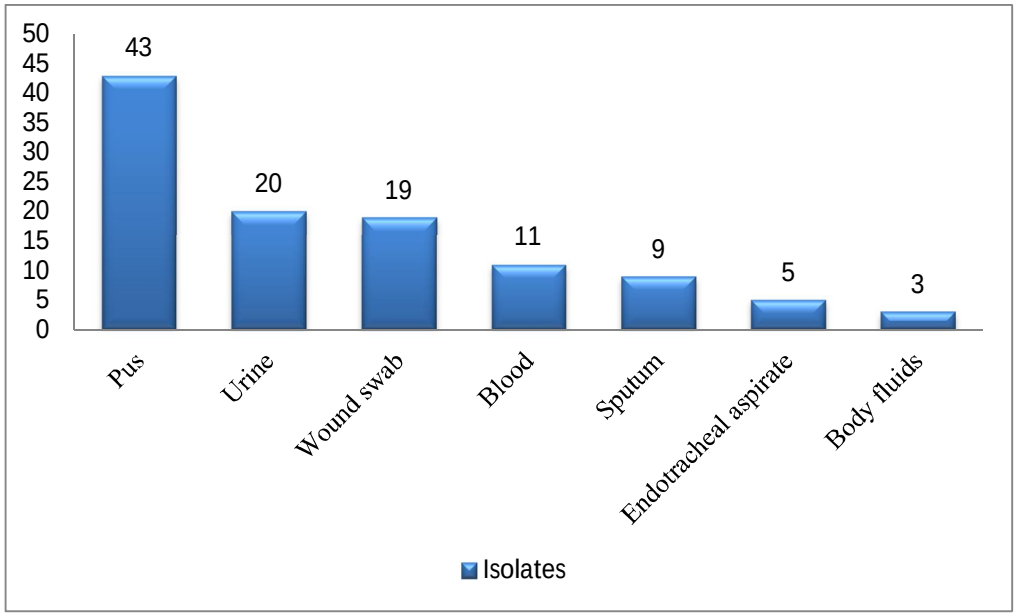


**TABLE 4: DISTRIBUTION OF CLINICAL ISOLATES (n=110)**

| Speciality | Clinical isolates | Percentage (%) |
|---|---|---|
| Surgery | 32 | 29.09 |
| Intensive care unit | 22 | 20.01 |
| Medicine | 16 | 14.55 |
| OG | 11 | 10 |
| Urology | 9 | 8.18 |
| Burns | 6 | 5.45 |
| Ortho | 5 | 4.55 |
| Paediatrics | 4 | 3.64 |
| Otorhinolaryngology | 3 | 2.73 |
| TB ward | 1 | 0.91 |
| Dermatology | 1 | 0.91 |
| TOTAL | 110 | 100 |

Majority of isolates of nonfermenters were from Surgical ward (40%) followed by ICU (20%).

**FIGURE 4: DISTRIBUTION OF CLINICAL ISOLATES (n=110)**

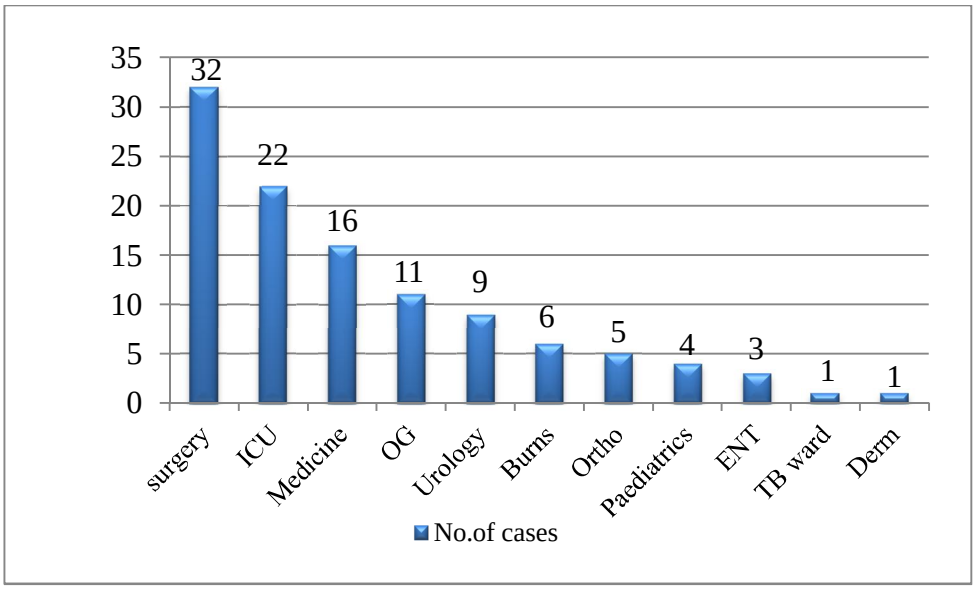


**Table 5: RISK FACTORS ASSOCIATED WITH INFECTIONS BY NONFERMENTERS (n=110)**

| Risk Factors | Clinical isolates | Percentage (%) |
|---|---|---|
| Surgery/Trauma | 44 | 40 |
| ICU stay | 22 | 20 |
| Prolonged antibiotic therapy | 10 | 9.9 |
| Catheter and Instrumentation | 9 | 8.18 |
| Diabetes Mellitus | 7 | 6.36 |
| Burns | 6 | 9.09 |
| Malignancy | 1 | 0.91 |
| Ventillator associated pneumonia | 1 | 0.91 |
| TOTAL | 100 | 95.35 |

Wound swab following surgery/trauma (40%) was the major risk factor contributes to the infection with nonfermenters.

**FIGURE 5: RISK FACTORS**

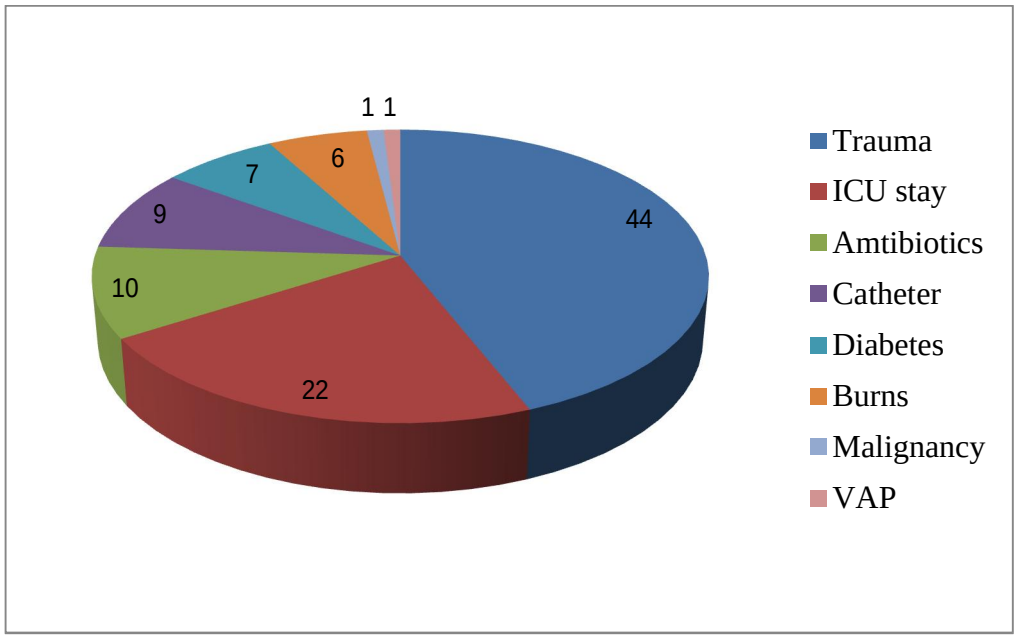


**TABLE 6: SPECIATION OF NONFERMENTERS (n = 110)**

| Clinical isolates | Number | Percentage (%) |
|---|---|---|
| Pseudomonas aeruginosa | 54 | 49 |
| Acinetobacter baumanii | 36 | 32.7 |
| Acinetobacter lwoffi | 8 | 7.3 |
| Stenotrophomonas maltophilia | 6 | 5.4 |
| Pseudomonas stutzeri | 3 | 2.8 |
| Burkholderia cepacia | 3 | 2.8 |
| TOTAL | 110 | 100 |

Among the nonfermenters Pseudomonas aeruginosa (49%) was the predominant isolate followed by Acinetobacter baumanii (32.7%), Acinetobacter lwoffi (7.3), S. maltophilia (5.4%), Pseudomonas stutzeri and Burkholderia cepecia (2.8%)

**FIGURE 6: SPECIATION OF NONFERMENTERS (n=110)**

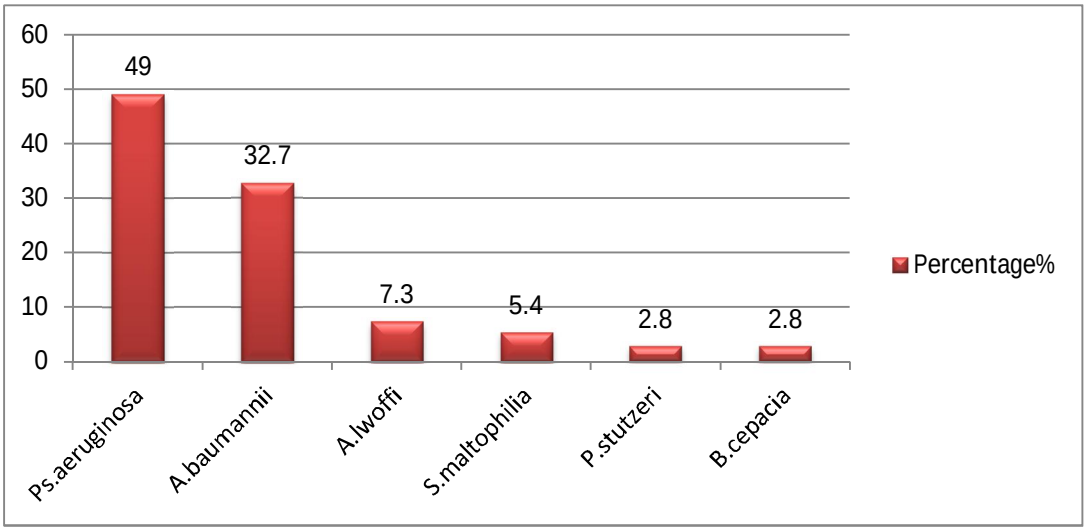


**TABLE 7. SAMPLE WISE ORGANISM ISOLATION (n=110)**

| Specimen | P.aeruginosa. (n=54) | | P.stutzeri (n=3) | | B.cepacia (n=3) | | A.baumannii (n=36) | | A.lwoffi (n=8) | | S.maltophilia (n=6) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Pus | 19 | 35.2 | 2 | 66.7 | 0 | 0 | 19 | 52.8 | 0 | 0 | 3 | 50 |
| sputum | 2 | 3.7 | 0 | 0 | 3 | 100 | 2 | 5.6 | 2 | 25 | 0 | 0 |
| urine | 14 | 25.9 | 0 | 0 | 0 | 0 | 2 | 5.6 | 4 | 50 | 0 | 0 |
| WS | 13 | 24.1 | 0 | 0 | 0 | 0 | 4 | 11.1 | 1 | 12.5 | 1 | 16.7 |
| Blood | 4 | 7.4 | 1 | 33.3 | 0 | 0 | 4 | 11.1 | 1 | 12.5 | 1 | 16.7 |
| As.fluid | 1 | 1.85 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 16.7 |
| ET swab | 1 | 1.85 | 0 | 0 | 0 | 0 | 4 | 11.1 | 0 | 0 | 0 | 0 |
| Pl.fluid | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2.8 | 0 | 0 | 0 | 0 |
| TOTAL | 54 | | 3 | | 3 | | 36 | | 8 | | 6 | |

*Pseudomonas aeruginosa* was predominantly isolated from pus, (35.2%), also *Acinetobacter baumanni* (52.8%), *Acinetobacter lwoffi* (25%), *Stenotrophomonas maltophilia* (50%), *Pseudomonas stutzeri* from pus (66.7%) and *Burkholderia cepacia* from sputum (100%).

**FIGURE 7: SAMPLE WISE ORGANISM ISOLATION (n=110)**

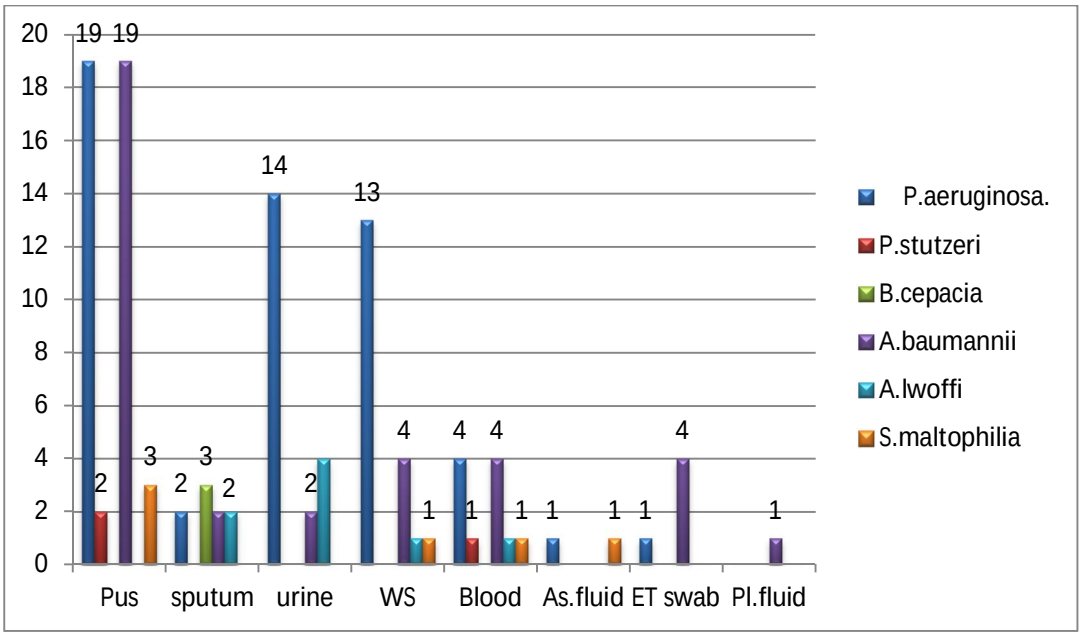


**TABLE 8: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NONFERMENTERS (n=110)**

| Drugs | Ps.aeruginosa (n=54) | | A.baumaniii (n=36) | | A.lwoffii (n=8 | | P.stutzeri (n=3) | | S.maltophilia (n=6) | | B.cepaciaa (n=3) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | % | S | % | S | % | S | % | S | % | S | % |
| Gentamycin | 22 | 40.7 | 18 | 50 | 4 | 50 | 1 | 33.3 | - | - | - | - |
| Amikacin | 32 | 59.3 | 26 | 72.2 | 6 | 80 | 2 | 66.7 | 2 | 33.3 | - | - |
| Ciprofloxacin | 23 | 42.6 | 18 | 50 | 4 | 50 | 2 | 66.7 | 6 | 100 | 1 | 33.3 |
| Ofloxacin | 23 | 42.6 | 18 | 50 | 4 | 50 | 2 | 66.7 | 6 | 100 | 1 | 33.3 |
| Ceftazidime | 30 | 56 | 20 | 55.6 | 6 | 80 | 2 | 66.7 | - | - | 1 | 33.3 |
| Cefotaxime | - | - | 20 | 55.6 | 6 | 80 | - | - | - | - | 1 | 33.3 |
| Pip - Taz | 39 | 72 | 26 | 72.2 | 8 | 100 | 3 | 100 | 1 | 16.7 | 1 | 33.3 |
| Cotrimoxazole | - | - | 20 | 55.6 | 4 | 50 | - | - | 6 | 100 | 3 | 100 |
| Imipenem | 43 | 79.6 | 27 | 75 | 8 | 100 | 3 | 100 | - | - | 2 | 66.7 |
| Meropenem | 43 | 79.6 | 27 | 75 | 8 | 100 | 3 | 100 | - | - | 2 | 66.7 |
| Polymyxin - B | 54 | 100 | 36 | 100 | 8 | 100 | 3 | 100 | 6 | 100 | - | - |

The disk diffusion susceptibility testing of the isolates shows the percentage of sensitivity and resistance of the isolates. Among all the isolates maximum resistance was recorded for Gentamycin (61.8%), Cotrimoxazole (60%), followed by Ciprofloxacin (50.9%) and Cefotaxime (47.3%).

**FIGURE 8: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NONFERMENTERS (n=110)**

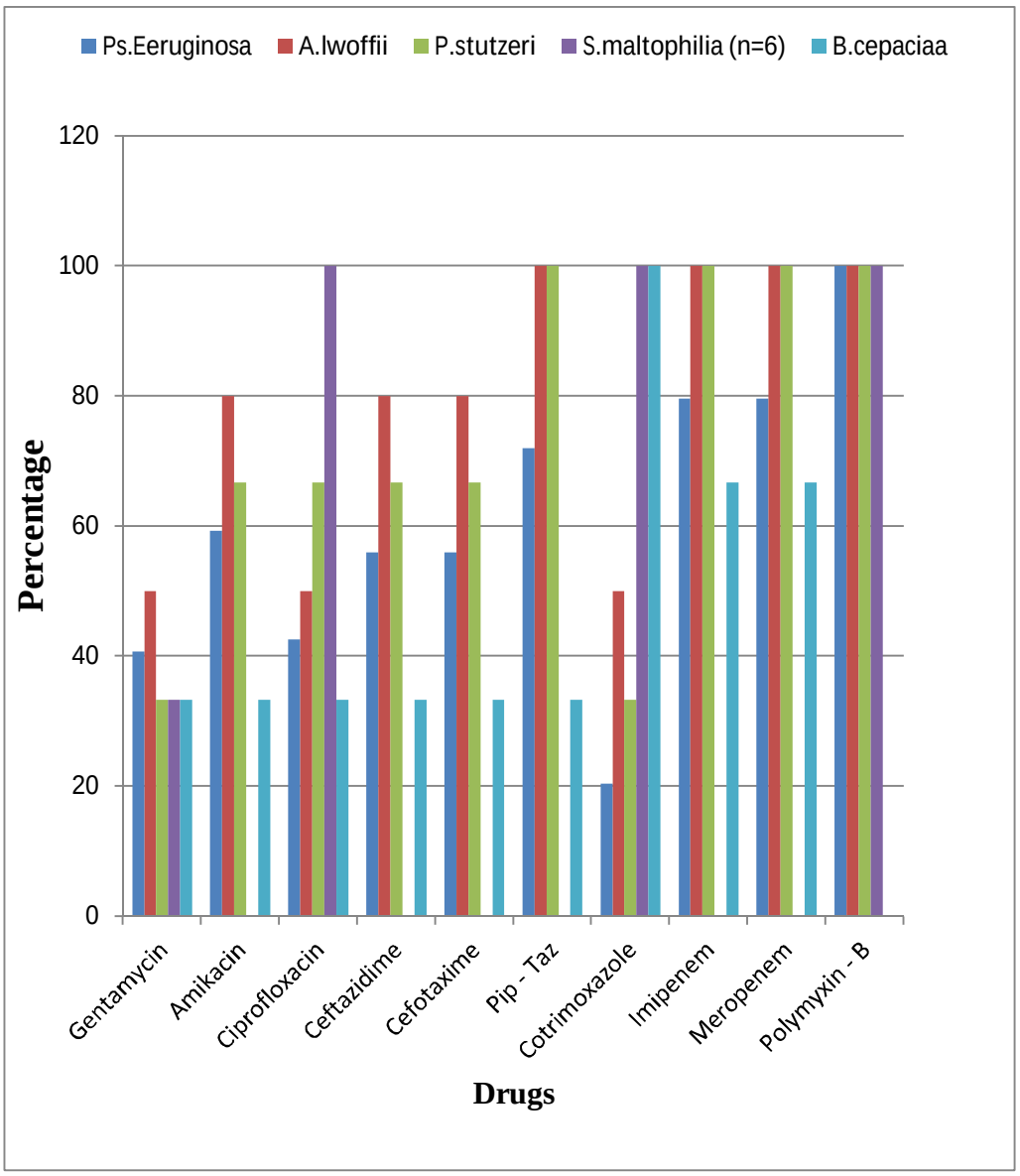


**TABLE 9: DETECTION OF EXTENDED SPECTRUM BETA LACTAMASE IN NONFERMENTERS (n=110)**

| ESBL production | No. of isolates | Percentage % |
|---|---|---|
| Positive | 20 | 18.18 |
| Negative | 90 | 81.82 |

Among the 110 Nonfermenter isolates screened for ESBL production and confirmed by CLSI phenotypic confirmatory method. 20(18.18%) isolates were found to be ESBL producers.(p value-0.0001 as per one proportion Z-test).ESBL detection is significant.

**FIGURE 9: ESBL DETECTION IN NONFERMENTERS (n=110)**

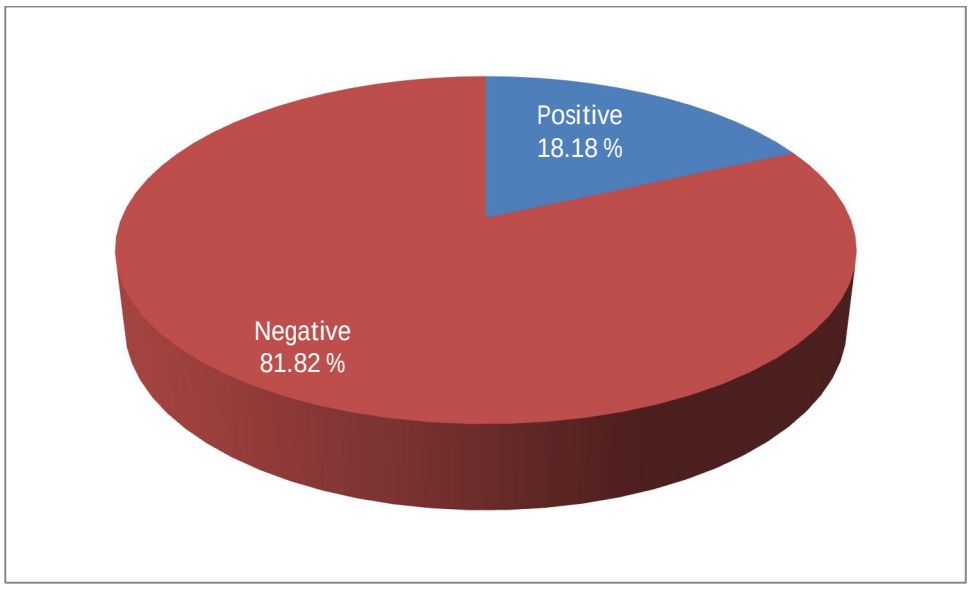


**TABLE 10: ESBL PRODUCTION IN NONFERMENTERS n=110**

| Organism | Total No. | ESBL producers | Percentage (%) | One proportion Z-Test |
|---|---|---|---|---|
| Ps.aeruginosa | 54 | 9 | 16.7 | 0.0001 |
| A.baumanii | 36 | 6 | 16.7 | 0.0009 |
| A.lwoffi | 8 | 2 | 25.0 | 0..7516 |
| Ps.stutzeri | 3 | 1 | 33.3 | 0.9885 |
| S.maltophilia | 6 | 1 | 16.7 | 0.2420 |
| B.cepacia | 3 | 1 | 33.3 | 0.9885 |
| Total | 110 | 20 | 18.18 | |

Among the isolated nonfermenters 20(18.18) were ESBL producers, majority 9(16.7%) were *Pseudomonas aeruginosa*, followed by *A.baumannii* 6 (16.7%), *A.lwoffi* 2(25%), *P.stutzeri* and *B.cepacia* 1(33.3%) and *S.maltophilia* 1(16.7%). ESBL production in *Pseudomonas aeruginosa* and *A. baumannii* is statistically significant (p value-0.0001 &0.0009).

**FIGURE 10: ESBL PRODUCERS IN NONFERMENTERS (n=20)**

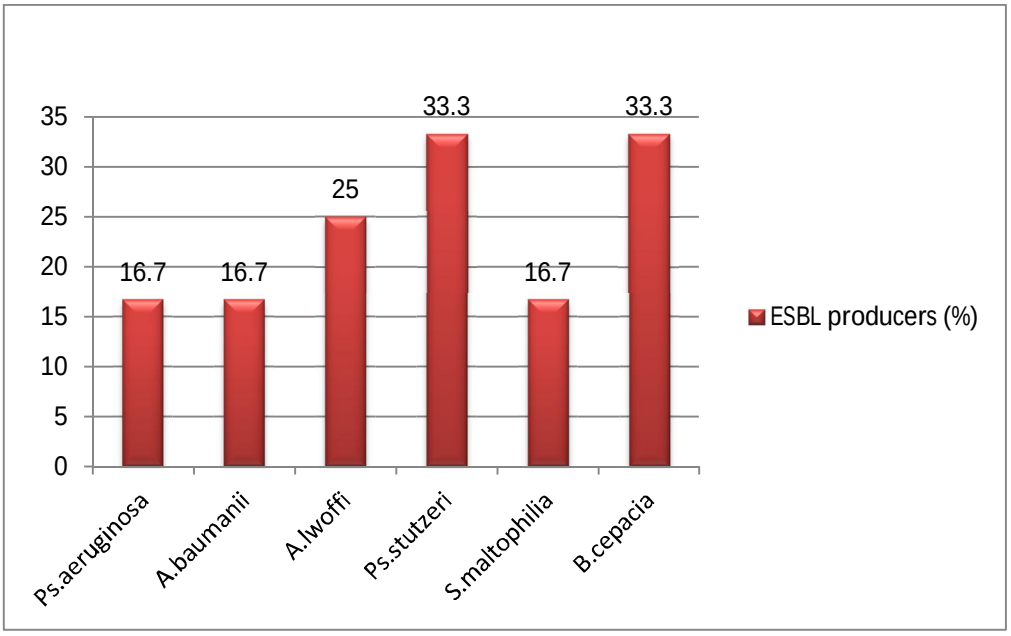


**TABLE 11: SAMPLE DISTRIBUTION OF ESBL ( n=20)**

| Clinical Sample | Total No. | ESBL producers | Percentage (%) |
|---|---|---|---|
| Sputum | 9 | 4 | 44.4 |
| ET swab | 5 | 2 | 40.0 |
| Body fluid | 3 | 1 | 33.3 |
| Blood | 11 | 3 | 27.3 |
| Wound swab | 19 | 3 | 15.8 |
| Pus | 43 | 6 | 14.0 |
| Urine | 20 | 1 | 5.0 |
| Total | 110 | 20 | 18.18 |

Patient with nosocomial pneumonia of sputum of clinical sample accounts to 44.4% and on ventilator of ETswab 40%. Isolates collected from patients with wound infection showed results for ESBL production of (29.8%).

**FIGURE 11: SAMPLE DISTRIBUTION IN ESBL PRODUCERS (n=20)**

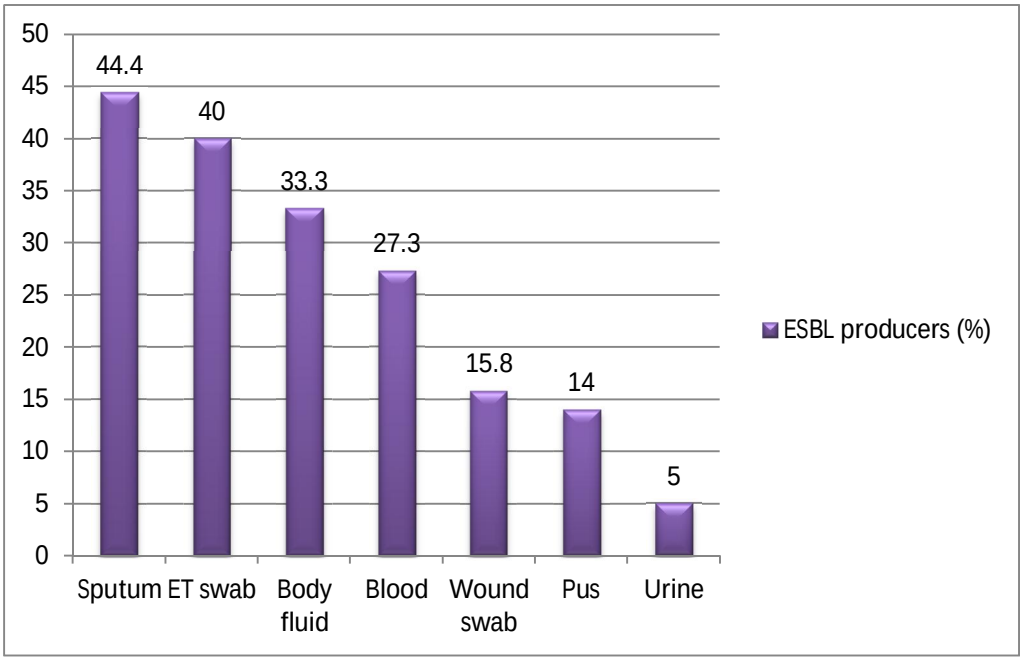


**TABLE 12: DETECTION OF MEROPENEM RESISTANCE IN *ACINETOBACTER* ISOLATES BY DISC DIFFUSION METHOD (n=44)**

| Pattern of susceptibility | No of isolates | Percentage % |
|---|---|---|
| Susceptible | 35 | 79.5 |
| Resistant | 9 | 20.5 |

Among the 44 isolates of *Acinetobacter species* screened for Meropenem resistance by Kirby -Bauer disc diffusion method, of which 9 isolates (20.5%) were found to be resistant to Meropenem which is significant. (p value – 0.0015 as per one proportion Z-test).

**FIGURE 12: MBL DETECTION IN ACINETOBACTER spp., (n=44)**

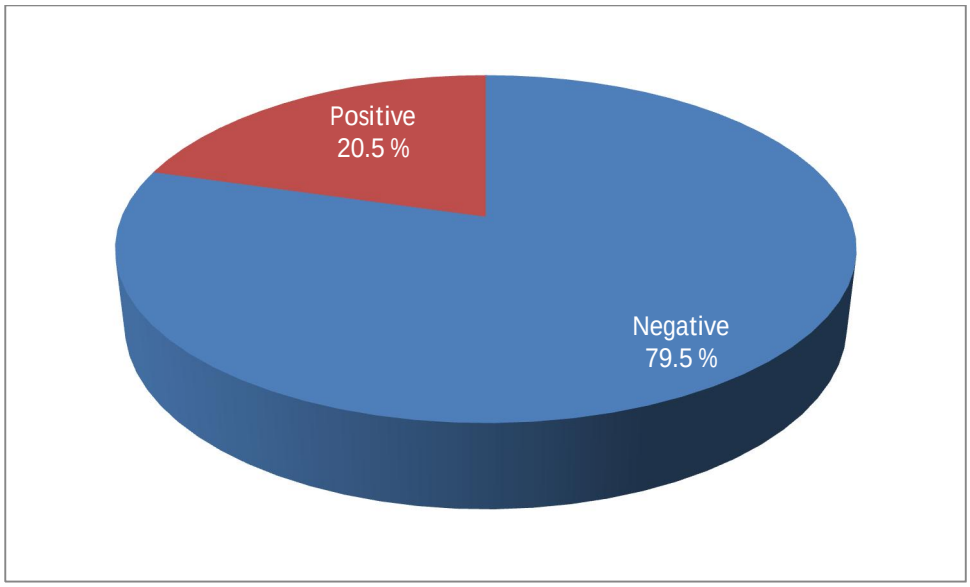


**TABLE 13: DETECTION OF MBL PRODUCTION IN ACINETOBACTER SPECIES (n=44)**

| Organism | MBL producers | Percentage |
|---|---|---|
| A.baumanii (36) | 9 | 20.5 |
| A.lwoffi (8) | - | - |
| TOTAL | 9 | 20.5 |

Out of 44 isolates of *Acinetobacter species* 9(20.5%) isolates of *A.baumanii.* were MBL producers. None of the isolates of *A.lwoffi* were MBL producers.

**TABLE 14: SAMPLE DISTRIBUTION OF MBL ISOLATES (n=9)**

| Clinical samples | No. of MBL | Percentage (%) |
|---|---|---|
| Pus | 3 | 33.33 |
| ET swab | 2 | 22.22 |
| Sputum | 1 | 11.11 |
| Blood | 3 | 33.33 |
| Total | 9 | 100 |

Maximum MBL producers were from pus and blood (33.3%) samples respectively.

**FIGURE 13: SAMPLE DISTRIBUTION OF MBL ISOLATES (n=9)**

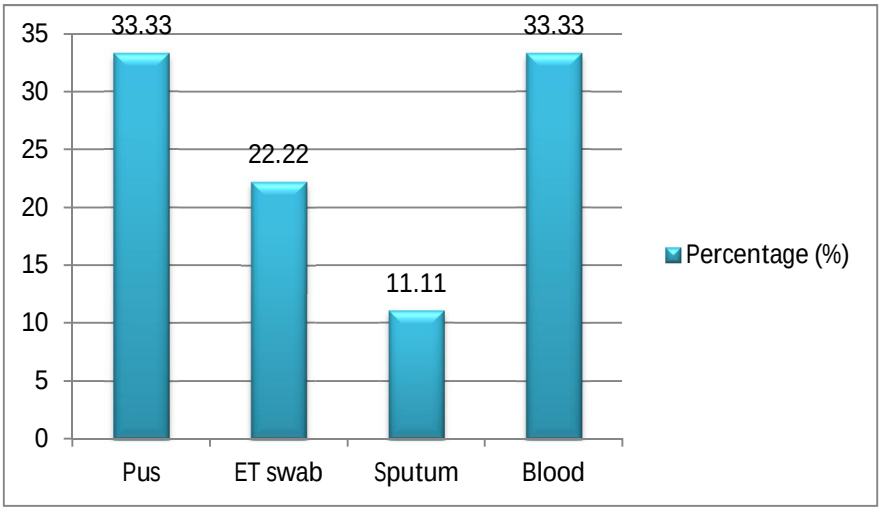


**TABLE 15: MBL DETECTION BY DIFFERENT PHENOTYPIC METHODS (n=9)**

| Phenotypic tests | Method | No. of isolates | Percentage% |
|---|---|---|---|
| Positive | Modified Hodge Test (MHT) | 3 | 33.3 |
| | Double Disc synergy Test(DDST) | 4 | 44.4 |
| | Combined Disc Diffusion Test(CDDT) | 5 | 55.6 |
| | MHT,DDST,CDDT | 3 | 33.3 |
| | CDDT,DDST | 4 | 44.4 |
| | CDDT | 1 | 11.1 |
| Negative | MHT,CDDT,DDST | 4 | 44.4 |

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 phenotypic methods.

Among the 9 isolates, Modified Hodge test was positive in 3 (33.3%) isolates, CDDT was positive in 5(55.6%) isolates, DDST ws positive in 4 (44.4%), 4(44.4%) isolates were negative for all the three phenotypic methods. Out of the 9 isolates CDDT,DDST was positive in 4 (44.4%) isolates, MHT, CDDT, DDST was positive in 3 (33.3%) isolates and CDDT alone was positive in 1(11.1) isolates and all were negative in 4 (44.4) isolate.

**TABLE 16: MIC FOR MEROPENEM BY MACROBROTH DILUTION METHOD (n =9)**

| MIC for Meropenem (µg/ml) | 512 | 256 | 128 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0.25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A.baumannii (n=9) | - | 3 | 2 | 2 | 2 | - | - | - | - | - | - | - |
| Percentage (%) | - | 33.3 | 22.2 | 22.2 | 22.2 | - | - | - | - | - | - | - |

Among the 9 Meropenem resistant isolates further tested for their meropenem minimum inhibitory concentration, All the 9 isolates have their MIC values greater than 8µg/ml, hence they are resistant to meropenem. Among the 9 isolates, 3(33.3%) isolates have MIC 256µg/ml, 2(22.2% isolates 128 µg/ml, another 2(22.2%) isolates 64 µg/ml and the remaining 2(22.2%) isolates have 32 µg/ml.

**FIGURE 14: DISTRIBUTION OF MIC VALUES FOR MEROPENEM (n=9)**

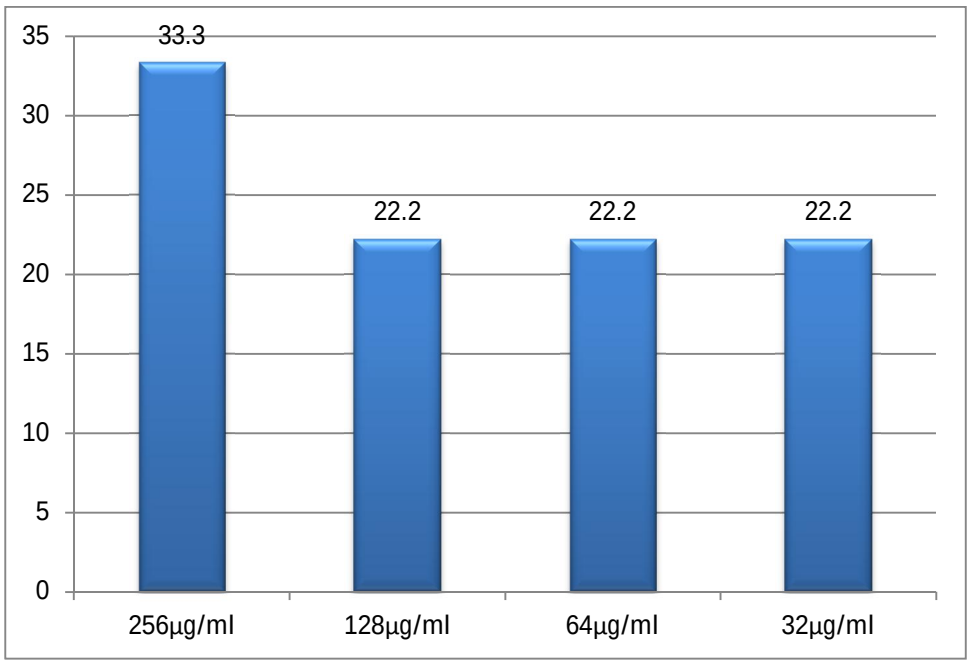


**TABLE 17: MOLECULAR DETECTION OF MBL RESISTANT GENES IN ACINETOBACTER BAUMANNII (n=9)**

| GENES TESTED | POSITIVE | PERCENTAGE % |
|---|---|---|
| OXA-51 | 3 | 33.3 |
| bla VIM | 2 | 22.2 |
| bla IMP | 3 | 33.3 |
| TOTAL | 8 | 88.8 |

The Meropenem resistant isolates were tested for most common carbapenamase gene, OXA – 51 and metallo betalactamase genes VIM and IMP by PCR. Among the 9 Meropenem resistant isolates, 3 isolates were positive for OXA-51 (33.3%), 2(22.2%) isolates VIM and 3 (33.3%) isolates were positive for IMP

**FIGURE 15: DISTRIBUTION OF RESISTANT GENES IN ACINETOBACTER SPP., (n=9)**

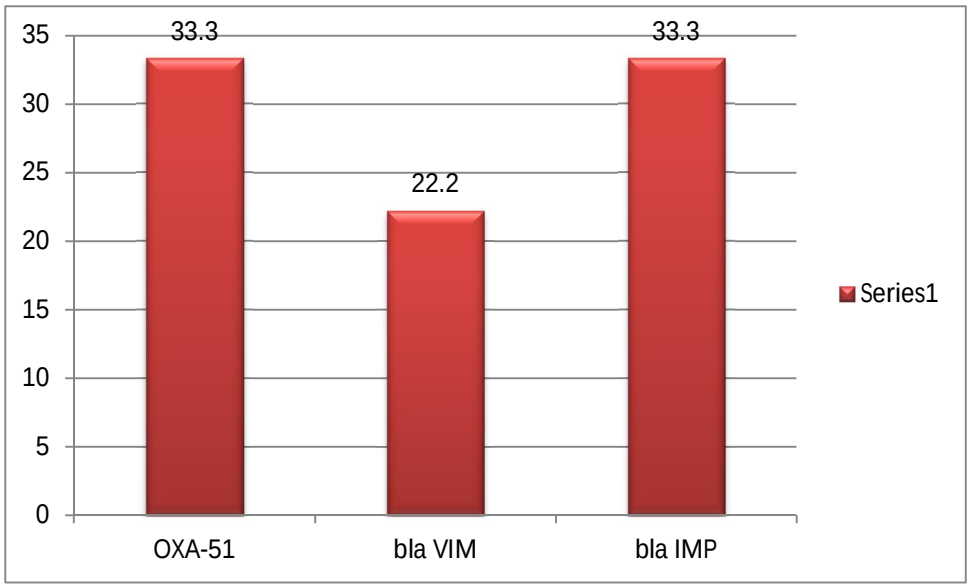


**TABLE 18: COMPARISON OF MBL DETECTION BY DIFFERENT METHODS**

| Organism | No. | Modified Hodgetest | | Double disc Synergy test | | Combined disc test | | PCR | |
|---|---|---|---|---|---|---|---|---|---|
| | | +ve | -ve | +ve | -ve | + ve | - ve | +ve | -ve |
| A.baumanii | 9 | 3 | 6 | 4 | 5 | 5 | 4 | 4 | 5 |
| Total | 9 | 3 | 6 | 4 | 5 | 5 | 4 | 4 | 5 |

Sensitivity and specificity of Modified Hodge test for *A.baumannii* was 75% and 100% respectively and for the EDTA – disk synergy test sensitivity was 100% and specificity 80% whereas for the EDTA- combined disc test both were 100% The differences in sensitivity and specificity between these tests were statistically significant ($p<0.01$ by chi-square test).

**FIGURE 1: INFECTIONS CAUSED BY NONFERMENTING GRAM NEGATIVE BACILLI**

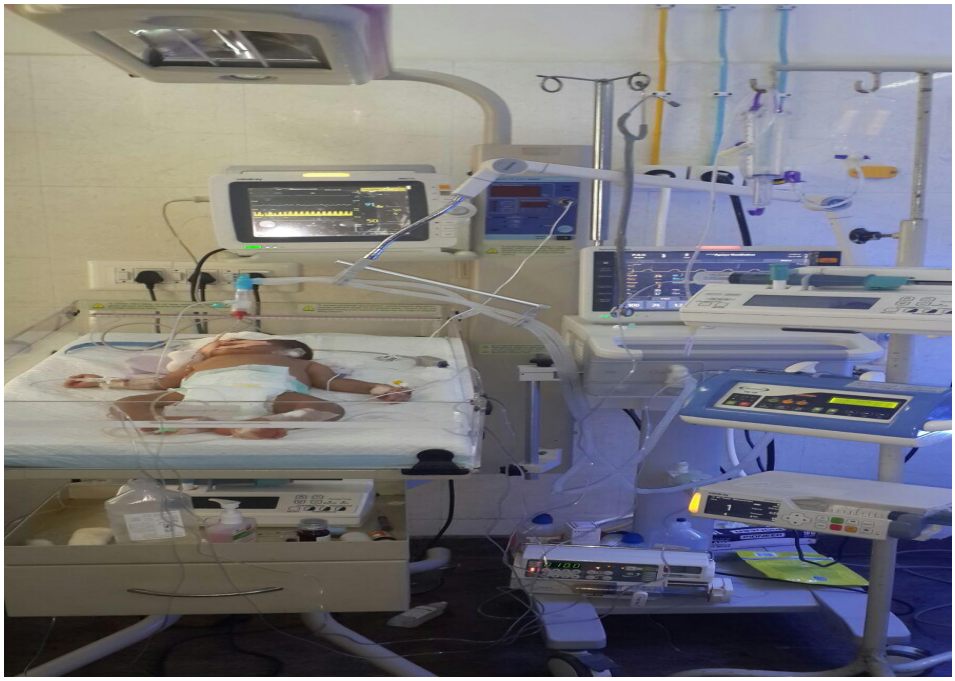


**FIGURE 2: DIRECT GRAM STAIN SHOWING PLENTY OF GRAM NEGATIVE BACILLI WITH PUS CELLS**

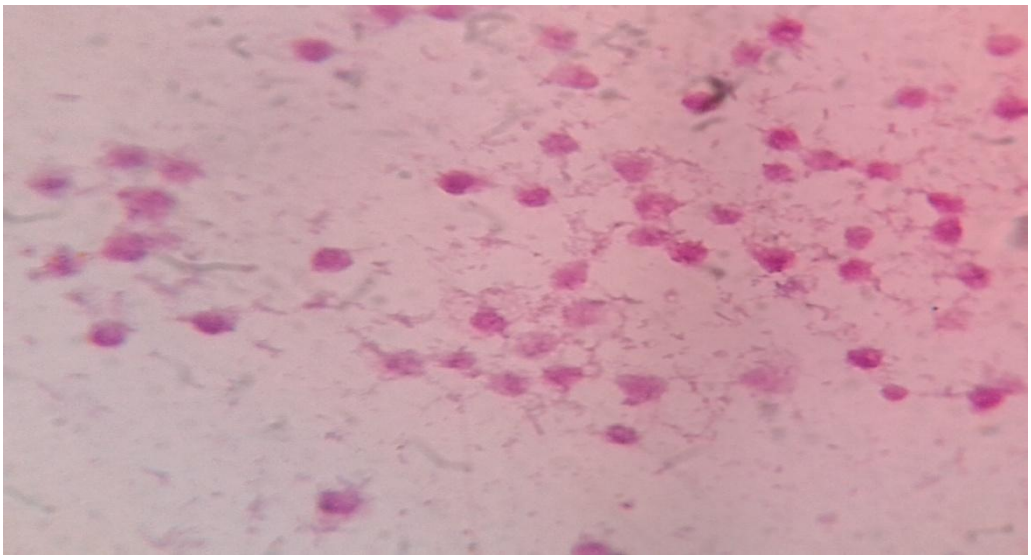


**FIGURE 3: GRAM STAINING SHOWING GRAM NEGATIVE BACILLI**

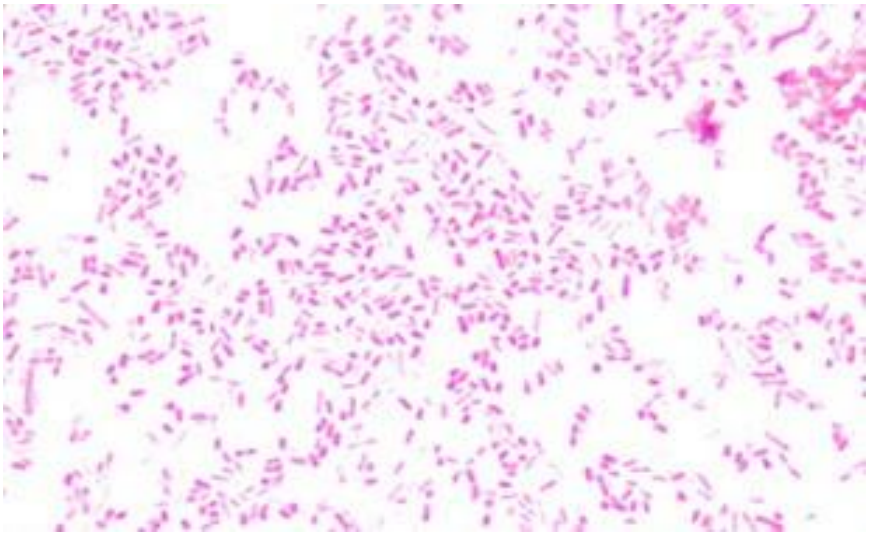


**FIGURE 4: MACCONKEY AGAR SHOWING GROWTH OF PSEUDOMONAS AERUGINOSA**

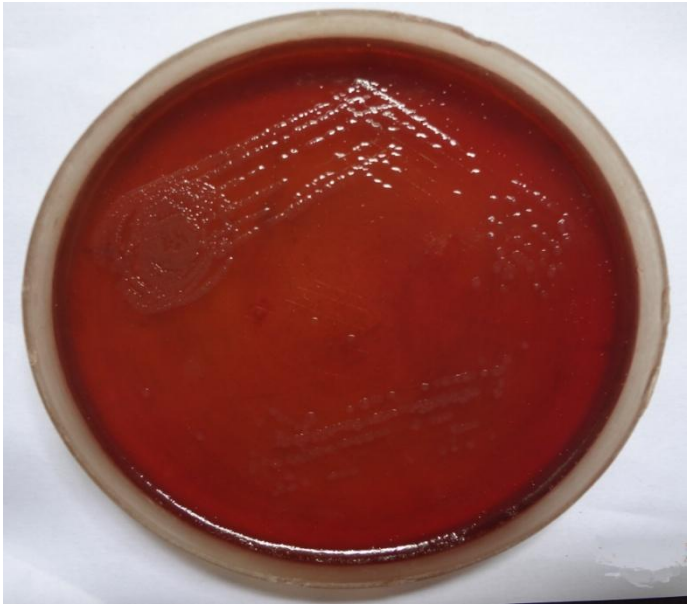


**FIGURE 5: BLOOD AGAR SHOWING GROWTH OF PSEUDOMONAS AERUGINOSA**

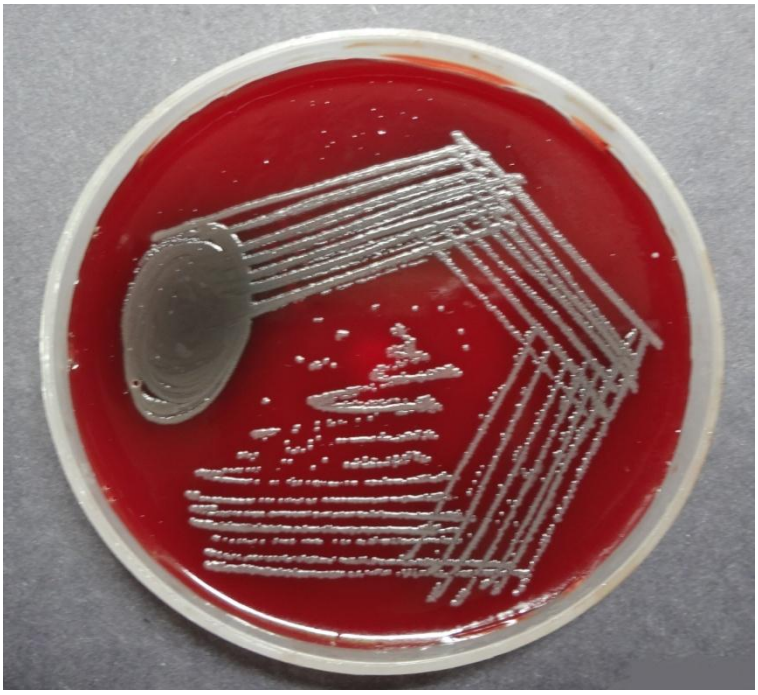


**FIGURE 6: CETRIMIDE AGAR SHOWING GROWTH OF PSEUDOMONAS AERUGINOSA**

**FIGURE 7: CATALASE TEST – POSITIVE**

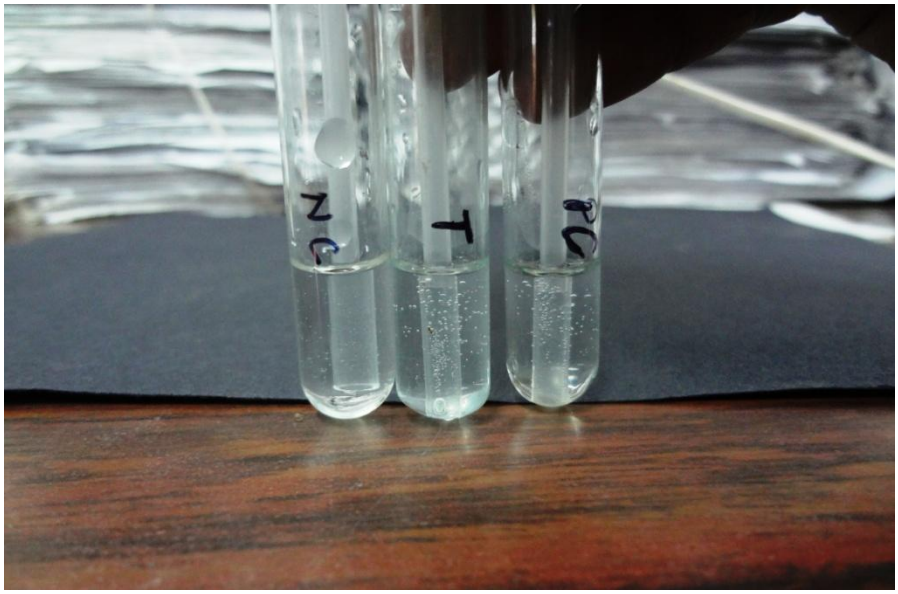



**FIGURE 8: OXIDASE TEST – POSITIVE (DISC METHOD)**

**FIGURE 9: BIOCHEMICAL REACTIONS OF PSEUDOMONAS AERUGINOSA**

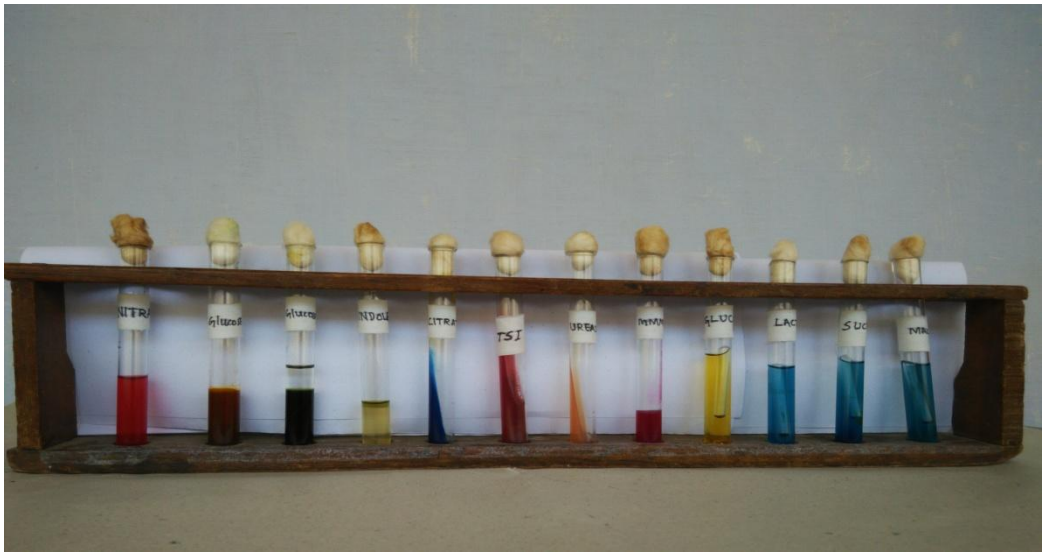



**FIGURE 10: ANTIBIOGRAM OF PSEUDOMONAS AERUGINOSA**

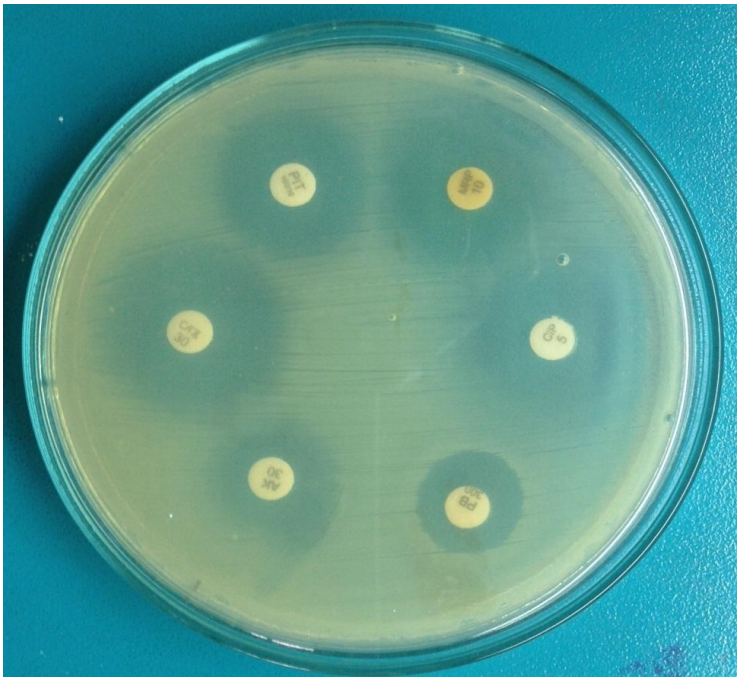


**FIGURE 11: GROWTH OF PSEUDOMONAS STUTZERI ON MAC CONKEY AGAR**

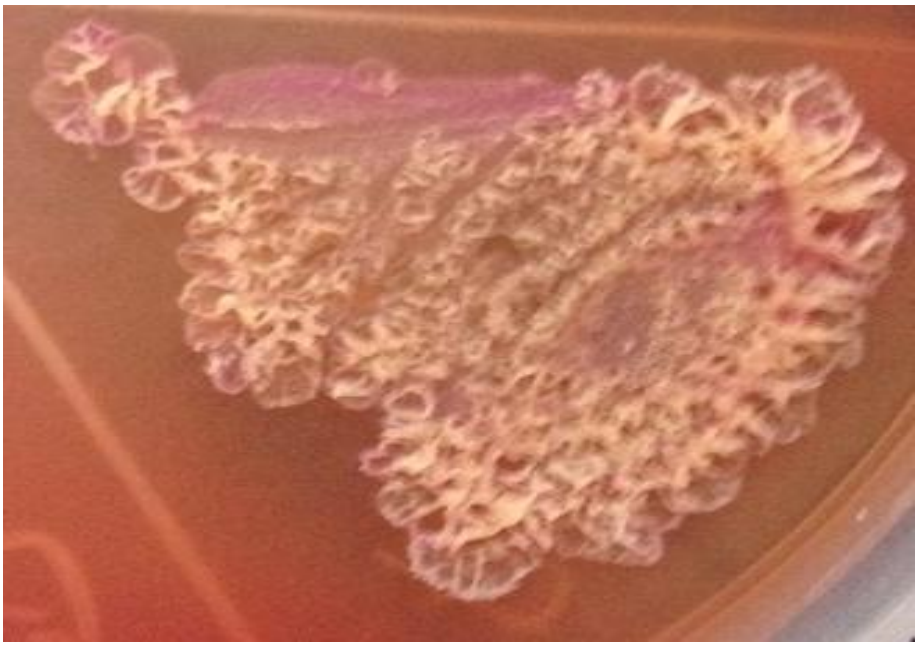


**FIGURE 12: BIOCHEMICAL REACTIONS OF PSEUDOMONAS STUTZERI**

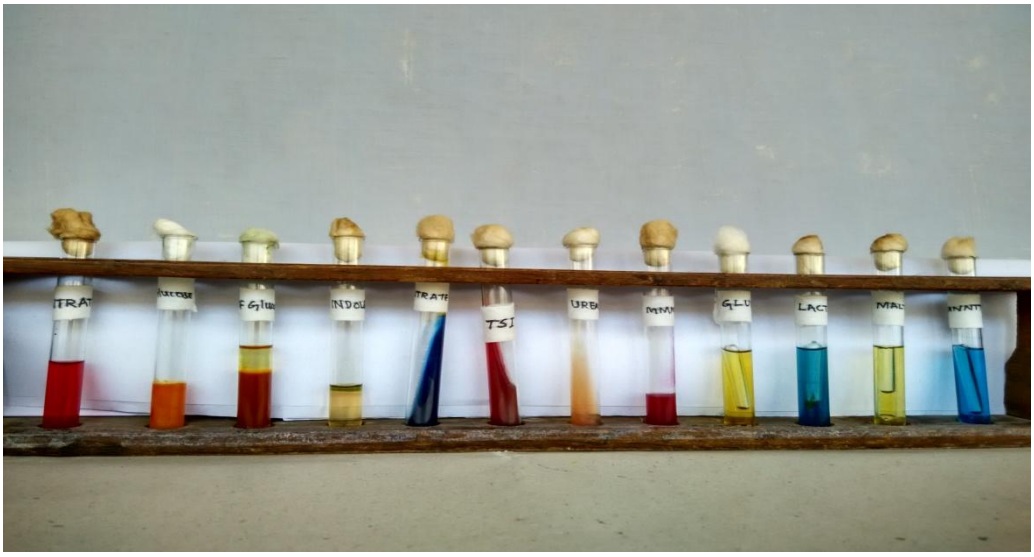


**FIGURE 13: ANTIBIOGRAM OF PSEUDOMONAS STUTZERI**

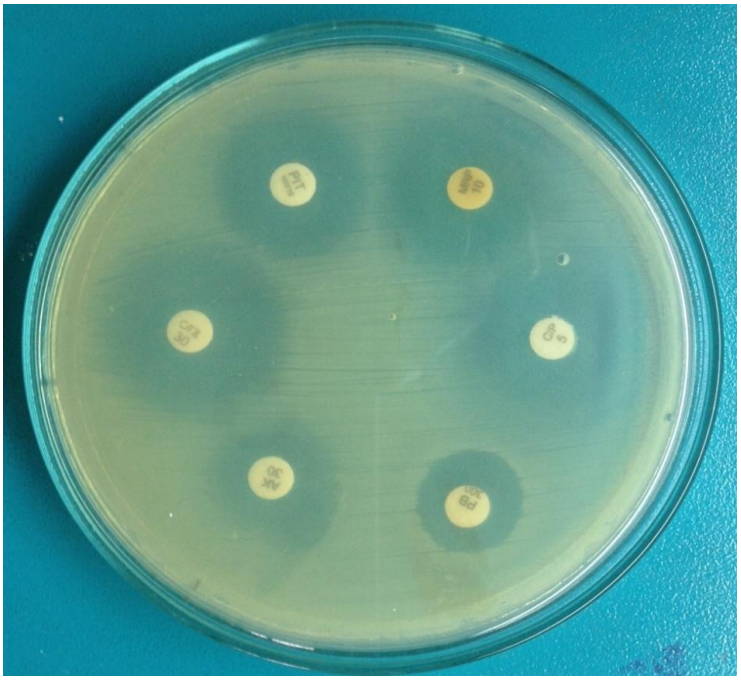


**FIGURE 14: BIOCHEMICAL REACTIONS OF STENOTROPHOMONAS MALTOPHILIA**

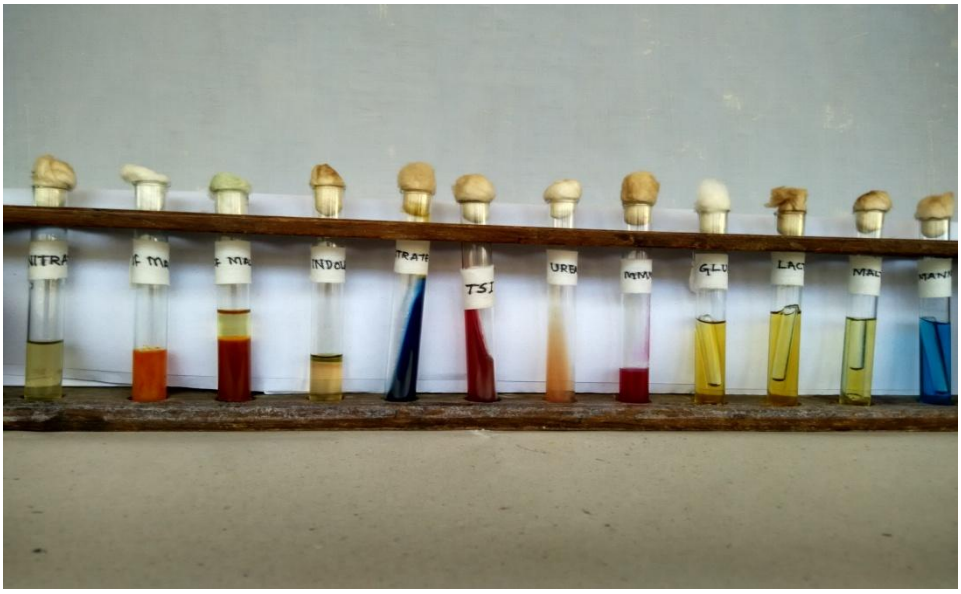


**FIGURE 15: ANTIBIOGRAM OF STENOTROPHOMONAS MALTOPHILIA**

**FIGURE 16: GRAM STAIN SHOWING BURKHOLDERIA CEPACIA**

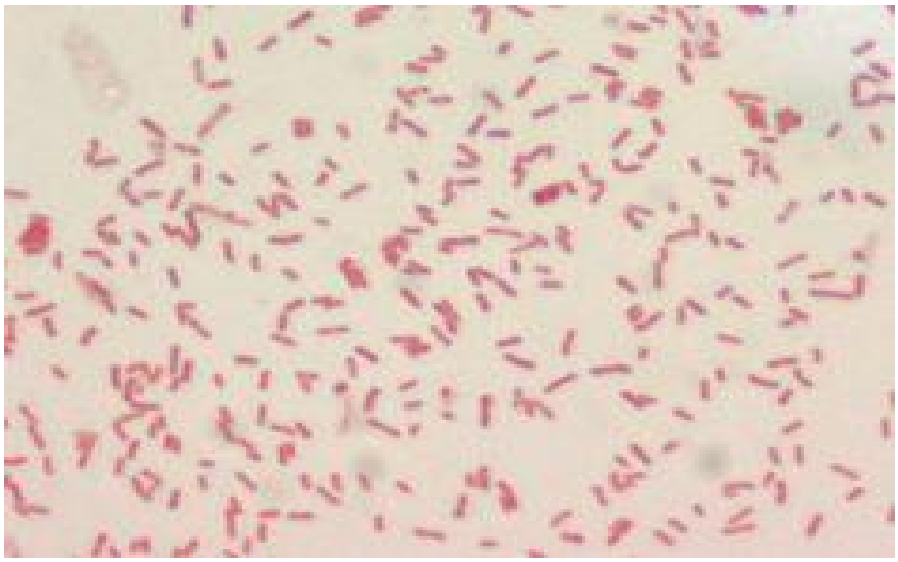


**FIGURE 17: BIOCHEMICAL REACTIONS OF BURKHOLDERIA CEPACIA**

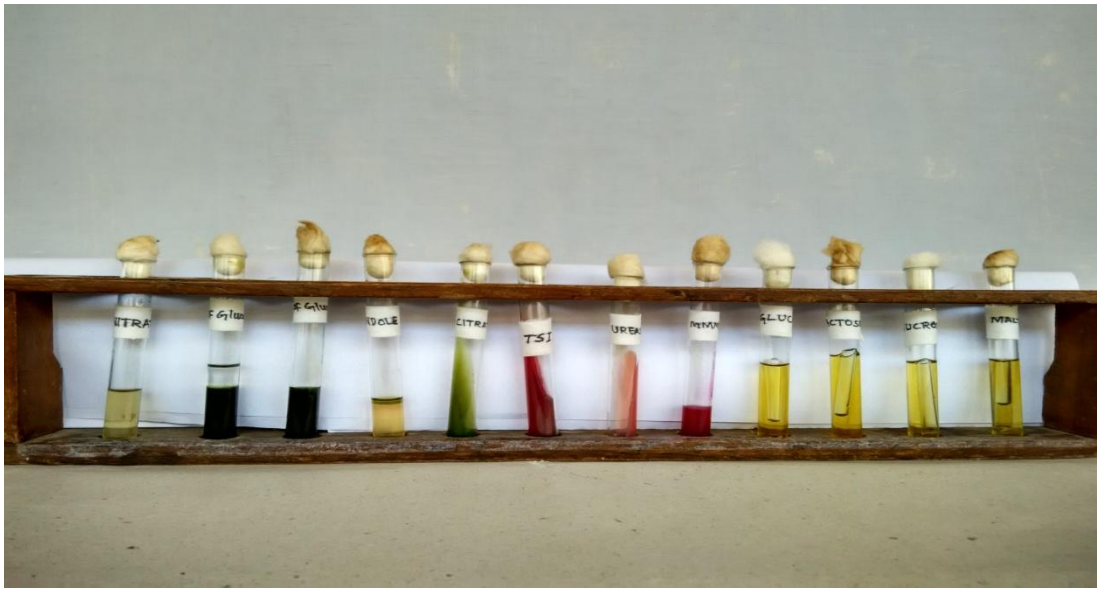



**FIGURE 18: ANTIBIOGRAM OF BURKHOLDERIA CEPACIA**

**FIGURE 19: DIRECT GRAM STAIN SHOWING GRAM NEGATIVE COCCO BACILLI**

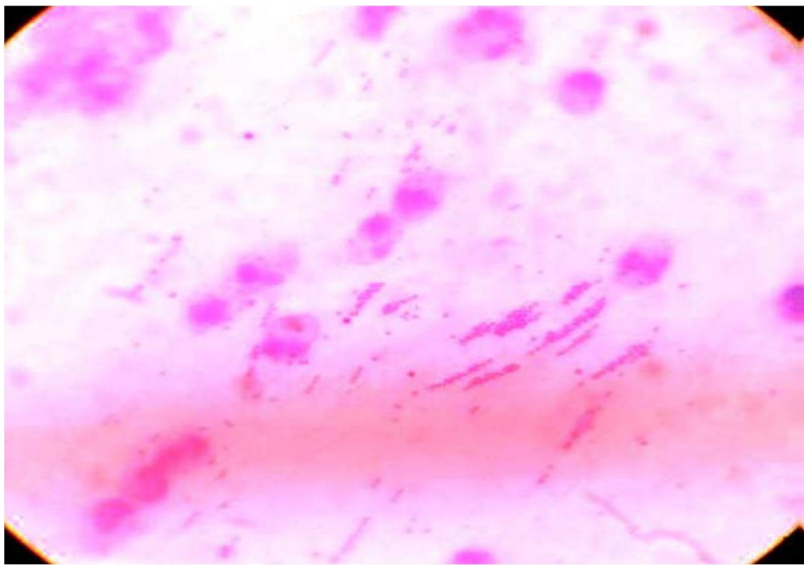


**FIGURE 20: GRAM NEGATIVE COCCO BACILLI IN CULTURE SMEAR**

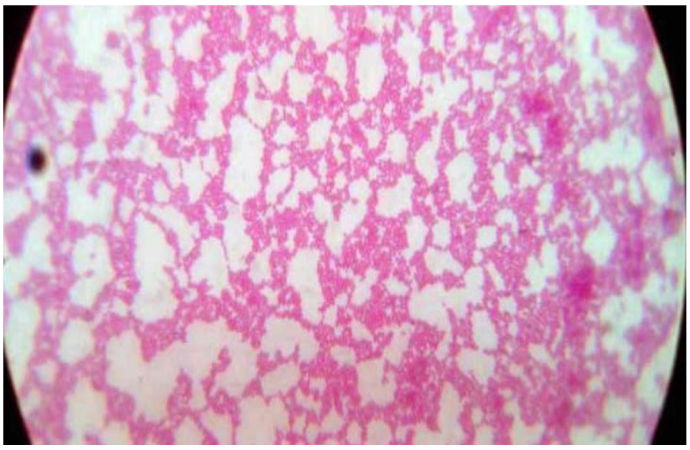


**FIGURE 21: GROWTH OF ACINETOBACTER SPECIES ON MAC CONKEY AGAR**

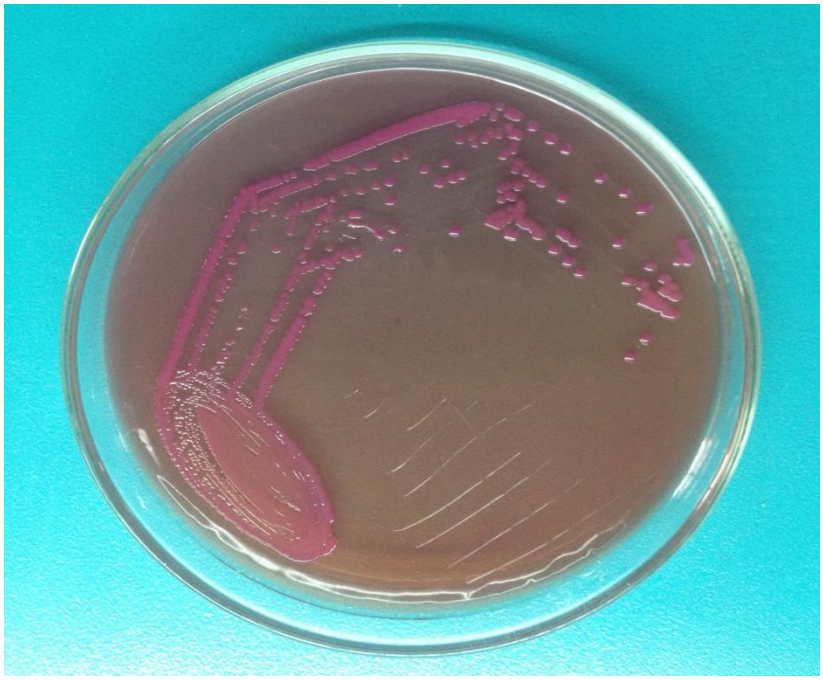


**FIGURE 22: GROWTH OF ACINETOBACTER SPECIES ON BLOOD AGAR**

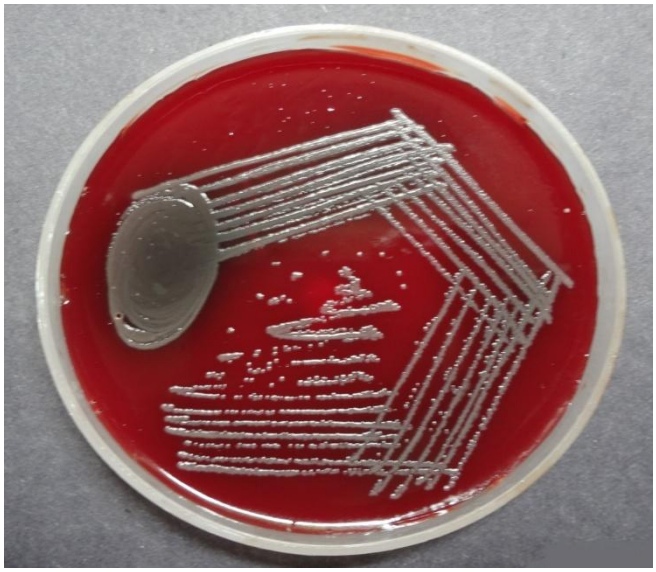


**FIGURE 23: GROWTH OF ACINETOBACTER SPECIES ON CHOCOLATE AGAR**

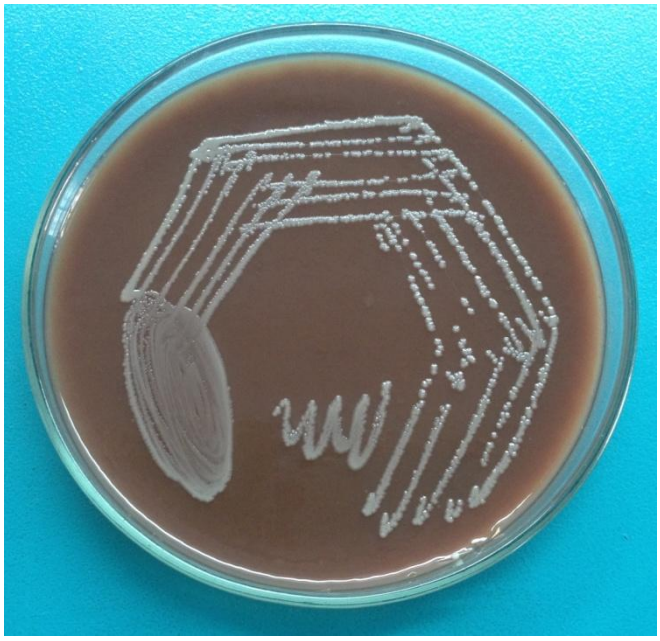


**FIGURE 24: BIOCHEMICAL REACTIONS OF *ACINETOBACTER BAUMANNII***

**FIGURE 25: BIOCHEMICAL REACTIONS OF *ACINETOBACTER LWOFFII***


**FIGURE 26: ANTIBIOGRAM OF ACINETOBACTER SPECIES**

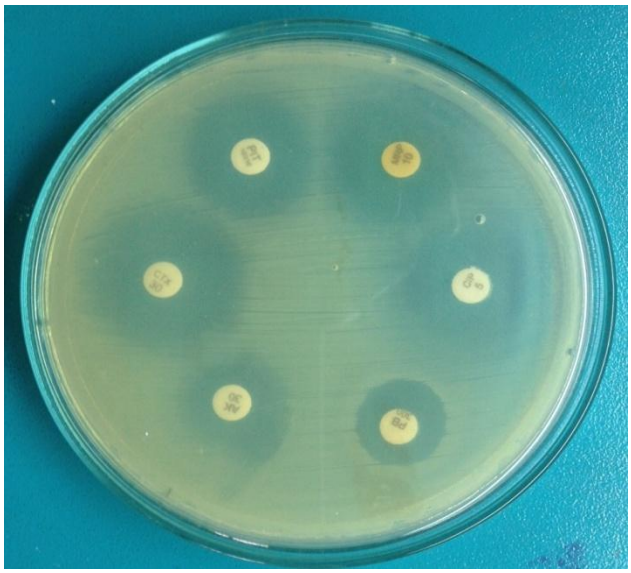
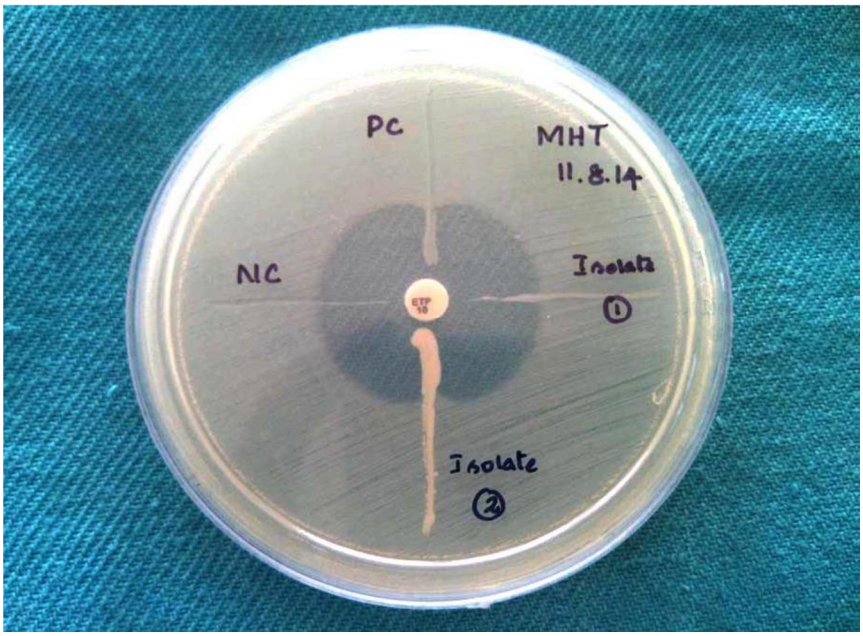


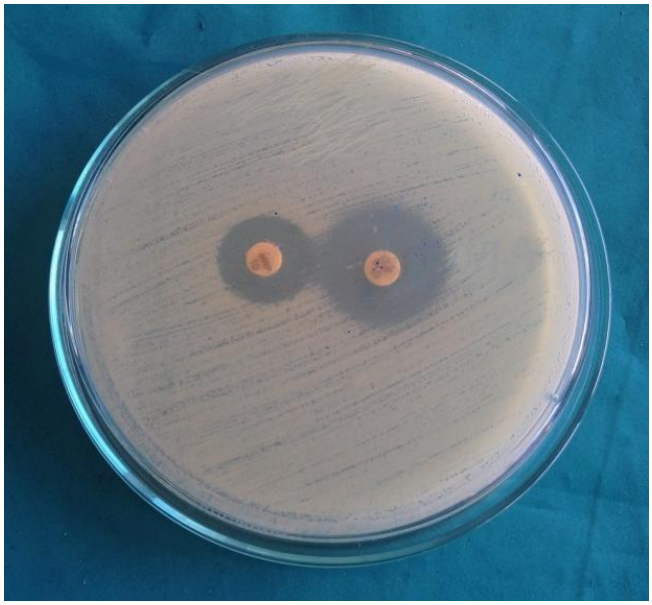
**FIGURE 27: MIC OF MEROPENEM - MACROBROTH DILUTION METHOD**


**FIGURE 28: HODGE TEST FOR OXACILLINASE DETECTION**




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

**FIGURE 29: IMIPENEM-EDTA COMBINED DISC TEST FOR MBL DETECTION**



**I – Imipenem IE – Imipenem EDTA**

**FIGURE 30: DOUBLE DISK SYNERGY TEST FOR MBL DETECTION**

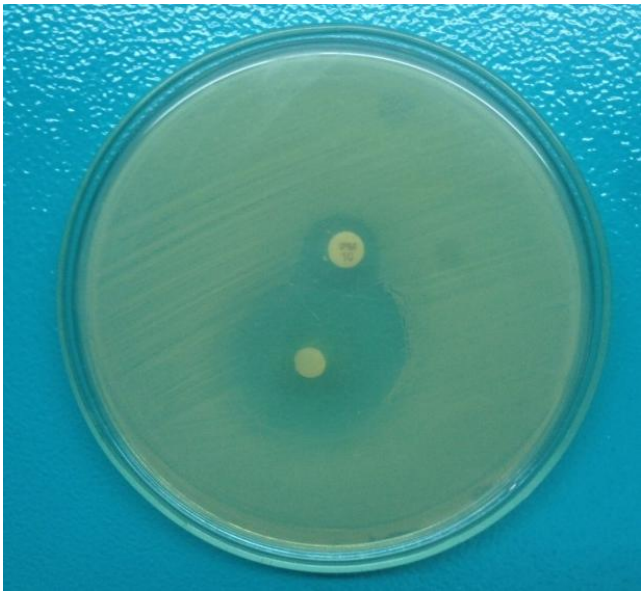


**FIGURE 31: PHENOTYPIC CONFIRMATORY METHOD FOR ESBL**

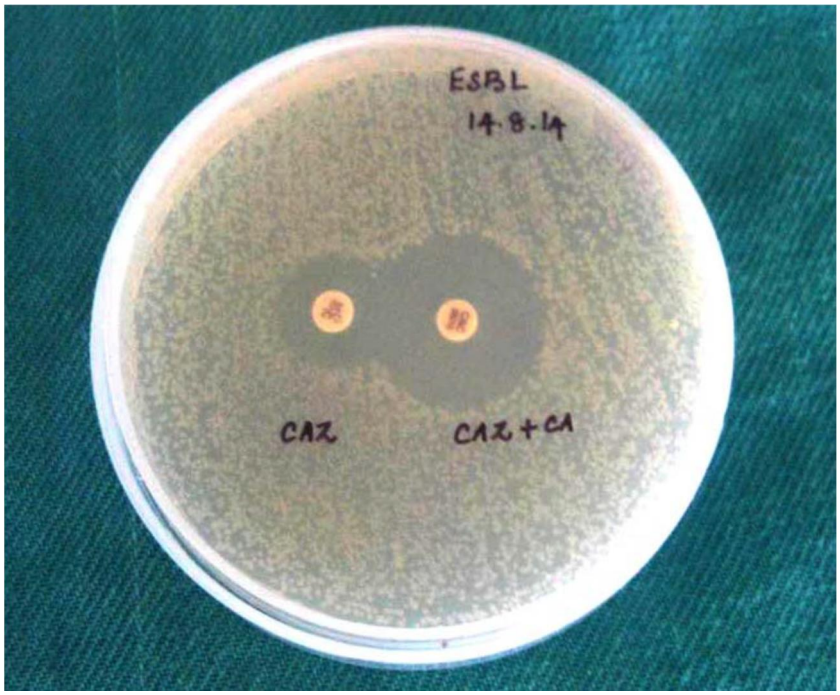
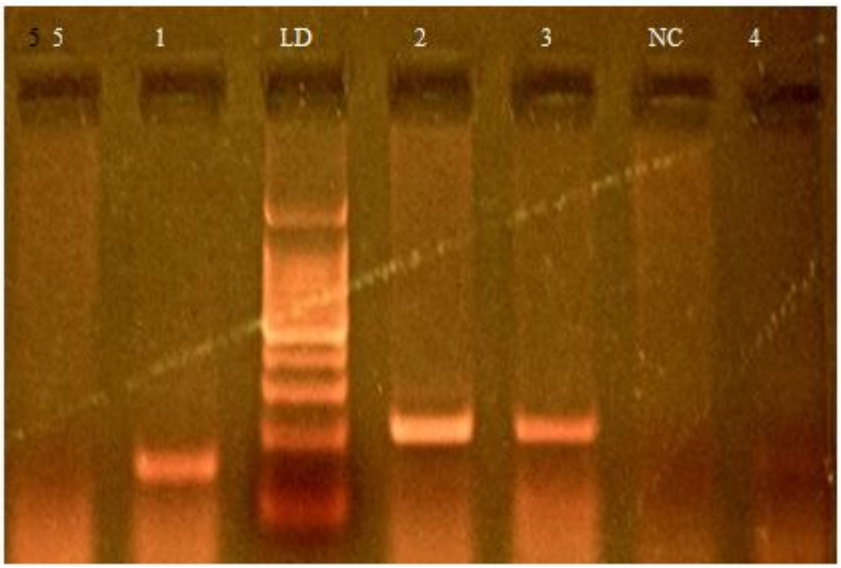
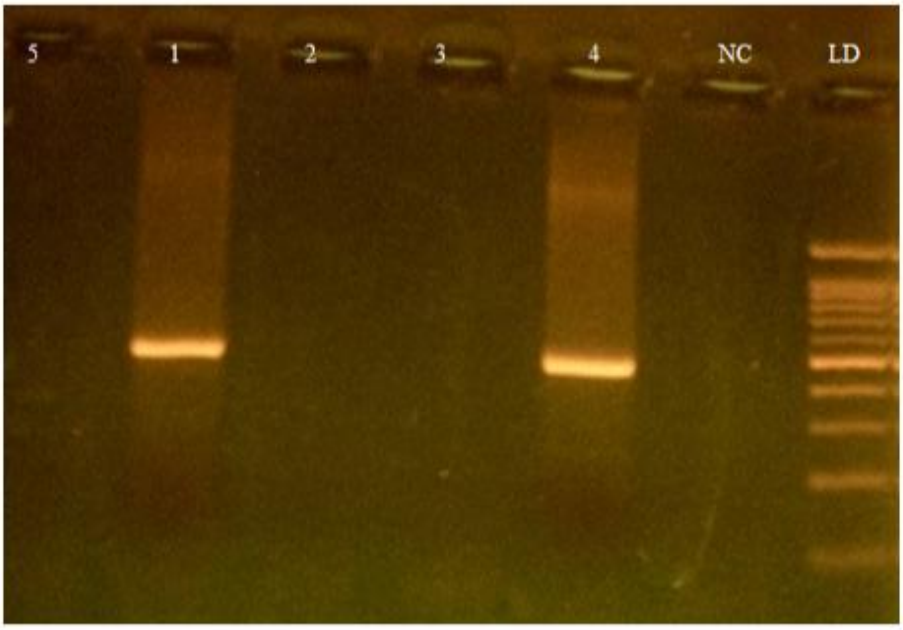



**FIGURE 32: PCR FOR IMPGENE**



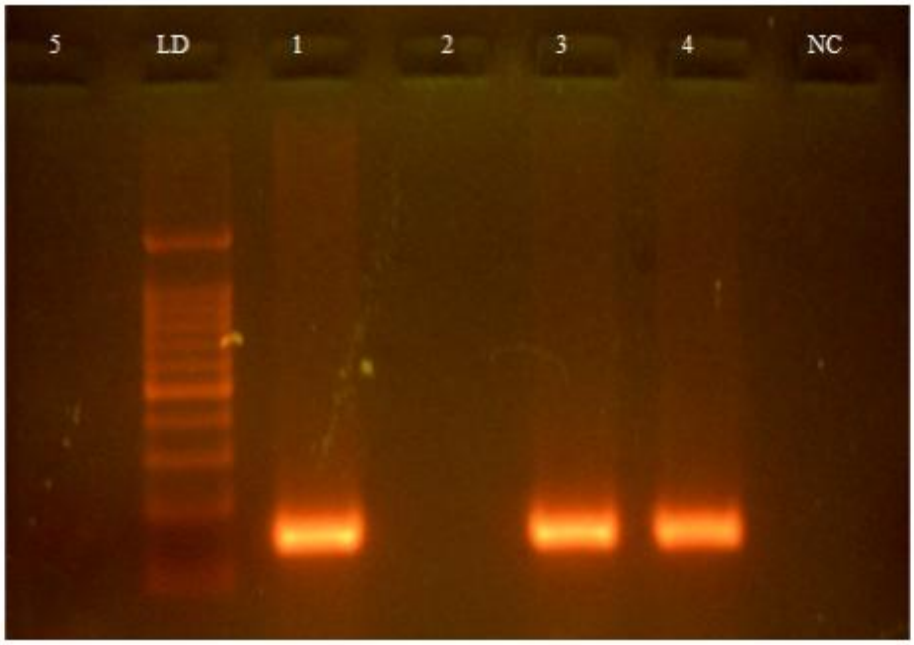

**Isolate No.-1, 2, 3 Positive LD-DNA Ladder NC- Negative Control**

**FIGURE 33: PCR FOR VIM GENE**




**Isolate No.-2, 4 Positive LD-DNA Ladder NC- Negative Control**

**FIGURE 34: PCR FOR OXA-51 GENE**




**Isolate No.-1, 3, 4 Positive LD-DNA Ladder NC- Negative Control**

# DISCUSSION

Non fermenting Gram Negative bacilli (NFGNB) are being isolated with increasing frequency from clinical specimens and treatment failure due to their multidrug resistance in recent years has led to the interest in the present study. 110 clinical isolates of non fermenting Gram negative bacilli isolated from various clinical samples like pus, urine, endotracheal aspirates, blood, sputum, body fluids and were evaluated for their role in infections in hospitalised patients including the characteristics of their drug resistance.

This Cross Sectional study was conducted in the Department of Microbiology, Chengalpattu Medical College and hospital, Chengalpattu during the period of July 2014 to June 2015.The present study includes 110 clinically significant, consecutive, nonfermenter isolates.

In this present study, observed that infections with nonfermenters were common in the patients of the age group < 10 years 23(20.91%), followed by between the age group 21-30 years 21(19.09%) and patient in advanced age of > 60 years 21(19.09%) **(Table 1).** This would be due to weekened immune system and chronic disease associated with advanced age and hormonal abnormalities in (21-30) yrs. of age group.

Out of 110 isolates in this study, maximum number of isolates were from males (65.4%) as compared to females ( 34.5%) **(Table 2)** with male to female ratio of 1:1.9. Similar observation was made by Kirtilaxmi Benachinmardi *et al.* who observed 68 were males and 32 were females with male to female ratio of 1:2.1 with

maximum number of cases observed from the age group of 21-30 yrs and 41-50yrs.[3]

In the present study out of 110 nonfermenters, 43(39%) were isolated from pus, 20 (18.1%) from urine, 19(17.2%) wound swab, 11(10%) blood, 9(8.1%) sputum, 5 (4.5%) from endotracheal aspirate and 3(2.7%) from body fluids **(Table 3**). Gokale K.Shilpa *et al.* from Belgaum reported ,58.4% nonfermenters isolated from pus/wound discharge followed by blood 23%, 8.2% from urine, 4.5% sputum and 2.3% pleural fluid.[2] . Indian study conducted .by Kirtilaxmi *et al*. also stated the isolation rate of 21% from pus, 11% from urine, 7% from blood and 17% from tracheal aspirate.[3] Kalidas Rit *et al.* from Kolkatta observed 27.86% from pus sample, 18.4%,from tracheal aspirate, 16.41% sputum and blood each and15.92% from urine [86].

In this present study maximum number of isolates were from Surgical wards (28.18%) followed by Intensive care unit (20.01%) and Medicine (14.55%) ward. **(Table 4).** Similar findings were reported by Anupurba *et al*. from India, higher prevalence rate of 29.9% in surgery wards. The second highest source observed in the present study was intensive care units (20.01%) followed by Medical ward (14.55%). These data clearly state the importance of the infections caused by NFGNB in the intensive care settings. Keertilaxmi Benachinmardi *et al.* also reported the isolation of 37% of NFGNB from intensive care units[3]. Outbreaks of Burkholderia cepacia complex septicaemia have been documented worldwide in intensive care units (ICUs), oncology units and renal failure patients [64].

In this present study, risk factors were present in 95% of the infections caused by Nonfermenters and the commonest risk factor associated was Surgery/Trauma (40%) followed by ICU stay (20%), prolonged antibiotic

therapy(9.9%), Catheter and Instrumentation (8.18%) followed by Diabetes mellitus (6.36%), Burns (9.09%) , Malignancy and Ventillator associated pneumonia (0.91%). **(Table 5)**. In contrast to the study from Keertilaxmi Benachinmardi *et al.,* reported, the isolation of 37% NFGNB from intensive care units is identified as a risk factor due to prolonged stay [(3)]. The study is from Muktikesh Dash *et al.* reported the significant risk factors for infection were age ≥55 years (13.5%), admission in the hospital as inpatients, longer (≥7 days) duration of stay in the hospital (6.5%), having undergone any invasive procedures like catheterization (63.5%), intubation, and mechanical ventilation, and with co-morbid conditions, i.e., diabetes mellitus, chronic obstructive pulmonary disease, asthma, neurologic impairment, congestive cardiac failure, end-stage renal disease, cancer, hepatitis and human immunodeficiency virus [(87)]. A longer hospital stay in a high-risk unit, use of mechanical ventilation, admission as inpatient into the ICUs, and underlying co-morbid conditions have been identified as the risk factors.

In this present study, the commonest isolates were *Pseudomonas aeruginosa* 54(49%) followed by *Acinetobacter baumanii* 36(32.7%), *Acinetobacter lwoffi* 8(7.3), *S.maltophilia* 6 (5.4%), *Pseudomonas stutzeri* and *Burkholderia cepecia* 3(2.8%) among the NFGNB **(Table 6**). Kalidas Rit *et al*. also reported, *Pseudomonas aeruginosa* (50.24%) as the predominant isolate followed by *A.baumannii* (24.87%), *A.lwoffi* (5.47%) *S.maltophilia* (2.98%), *Pseudomonas stutzeri* (1.99%) and *Burkholderia cepacia* (6.96%) [(86)]. In the present study, Pseudomonas spp., and Acinetobacter spp., were the commonest NFGNB isolated which correlates with the study done by Kirtilaxmi Benachinmardi *et al*.,[(2)].

In this present study, Among the 54 *Pseudomonas aeruginosa* isolated, 19 (35.2%) were from pus, 2(3.7%) from sputum, 14(25.9%) were from Urine, 13 (24.1%) from Wound swab, 4(7.4%) from blood, 1(1.85%) from Ascitic fluid and

ET swab **(Table 7).** Similarly Kirtilaxmi Benachinmardi *et al.* showed isolation rate of *Pseudomonas aeruginosa*, 15(25%) from pus, sputum and tracheal aspirate 7(11.7%), urine 8(13.3%), and blood 1(1.7%) respectively [(2)]. Study from Kalidas Rit *et al.* showed similar isolation of *Pseudomonas aeruginosa* 31(30.7%) from pus, 20(19.8%) from sputum,18 (17.8%) tracheal aspirate,14(13.9%) from blood, and 16(15.8%) from urine[(86)].

Because of high intrinsic resistance of different NFGNB to different antimicrobial agents, the value of proper identification and resistance testing is foremost important in a given setup to guide appropriate selection of empiric therapy. In this study, the antimicrobial susceptibility pattern of *Ps.aeruginosa* showed 43(79.6%) sensitivity to Imipenem and Meropenem followed by Piperacillin tazobactum 39 (72%), Amikacin 32(59.3%), Ceftazidime 30 (56%), Ciprofloxacin and Ofloxacin 23(42.6%) and Gentamycin 22 (40.7%) and Polymyxin B 54(100%). **(Table 8).**

Study from Gokale K.Shilpa *et al.* from Belgaum reported which is consistent with our results, that most of the isolates of *Pseudomonas aeruginosa* were sensitive to Meropenem(96.2%), followed by Ciprofloxacin (50.4%) and Amikacin (49.5%).[(2)]

A study conducted by Nautiyal *et al*., reported all the isolated *Pseudomonas aeruginosa* were sensitive to polymyxin B.[(88)] Kirtilaxmi Benachinmardi *et al.*, reported the sensitivity of *Pseudomonas aeruginosa* were Ceftazidime(60%), Gentamycin(65%), Piperacillin-tazobactum(73.3%), Imipenem (80%), Amikacin (83.3%), Ciprofloxacin (58.3%)[(3)] which is similar to the present study .

In the present study, *Acinetobacter spp.,* were the second most common isolate among the nonfermenters, contributes to (40%). Study by Kirtilaxmi Benachinmardi *et al.* also reported *Acinetobacterspp.,* as the second most common isolate among the NFGNB.[3] In the present study, among the 36 isolated *Acinetobacter baumannii* 19(52.8%) were predominantly isolated from pus, 2(5.6%) from sputum and urine, 4(11.1%) from wound swab, Blood and ET swab and 1(2.8%) from Pleural fluid **(Table 7).** Muktikesh Dash *et al.* reported similarly in his study that *Acinetobacter* isolates were common from pus sample (56.9%) [87]. Kalidas Rit *et al.* showed, 10 (20%) isolation were from pus and blood each, 9 (18%) from sputum, 8 (16%) urine , (22%)11 tracheal aspirates[86].

In the present study, the isolates of *A.baumannii* (n=36) showed 27(75%) sensitivity to Meropenem and Imipenem followed by Amikacin and Piperacillin tazobactum 26(72.2%), Ceftazidime and Cotrimoxazole 20(55.6%) , Gentamycin and Ciprofloxacin and Ofloxacin 18(50%) each respectively. **(Table 8).** All the isolates were sensitive to polymyxin B 36 (100%), similar results reported by Nautiyal *et al.,*[88]

Similar to the present study, Sohaila Mushtaq *et al.*, have reported high (60%) resistance to cephalosporins and 50% resistance to aminoglycosides and quinolones.[89]. Gokale K.Shilpa *et al.* also reported same sensitivity pattern of Meropenem 90% and Ciprofloxacin 45% [2]

Among the 8 *Acinetobacter lwoffi* isolates, 2(25%) were isolated from pus, 4(50%) from urine and each 1(12.5%) from wound swab and blood.**(Table 7).** Similarly, study by Kalidas Rit *et al.* showed that among 11(5.5%) isolated *A.lwoffi,* 3(27.3%) were from pus and tracheal aspirate, 2 (18.2%) sputum sample, 1(9.1%) blood and urine[86].

In this present study, all the isolates of *A.lwoffi* were sensitive to Imipenem, Meropenem and Piperacillin tazobactum 8(100%) followed by Cefotaxime, Ceftazidime and Amikacin 6(80%), Gentamycin , Cotrimoxazole and Ciprofloxacin and Ofloxacin 4(50%) ,Polymyxin B(100%).**(Table 8).** Similar to the present study, a study conducted by Nautiyal *et al.*, reported all the isolated *A.lwoffi* were 100% sensitive to polymyxin B .[88]

In contrast with the present study, Kirtilaxmi Benachinmardi *et al.*, showed only 66.7% sensitivity to Imipenem, Piperacillin tazobactum, Ciprofloxacin, Gentamycin and Amikacin followed by (50%) sensitive to Cotrimoxazole and (33.3%) to Cefotaxime and Ceftazidime [3]. Also similarly reported by study from Malini *et al.*, showed that 100% sensitivity to Imipenem. [90]

Among the 6 *Stenotrophomonas maltophilia* isolated, 3(50%) were isolated from pus,1 (12.5%) from wound swab, blood and Ascitic fluid**.(Table 7).** Similar study from Deepak juyal *et al.* showed that out of 6(2.4%) *Stenotrophomonas maltophilia* isolated 4 (66.7%) were from pus, each 1(16.7%) from ear swab and urine [91].

In this present study, the antimicrobial susceptibility among the isolated *S.maltophilia*, majority were sensitive to Cotrimoxazole, Ciprofloxacin and Ofloxacin and Polymyxin B 6(100%), followed by Amikacin 2(33.3%) and Piperacillin tazobactum1 (16.7%).**(Table 8).**

Similar to the present study, a study conducted by Nautiyal *et al.*, reported all the isolated *S.maltophilia* were 100% sensitive to polymyxin B .[88]

Similar report was shown in a study conducted by Deepak juyal *et al*., showed 100% sensitivity to Ciprofloxacin and Cotrimoxazole and 33.3% to piperacillin tazobactum and 16.67% to Gentamycin [(91)].

*S. maltophilia* is intrinsically resistant to most β-lactams, including carbapenems.[(64)].

Among the 3 *Pseudomonas stutzeri* isolated, 2(66.7%) were from pus, and 1(33.3%) from blood **(Table 7).** Similar study from Kalidas Rit *et al*., showed isolation of *Pseudomonas stutzeri* of 2 (50%) from pus,1 (25%)from blood and urine.[(86 )]

Among the isolated *P.stutzeri* (n=3), all were sensitive to Imipenem, Meropenem and piperacillin tazobactum and Polymyxin B 3(100%), followed by Amikacin, Ciprofloxacin, Ofloxacin and Ceftazidime 2(66.7%), Gentamycin 1(33.3%). **(Table 8).**Similar to the present study, study conducted by Nautiyal *et al.*, reported all the isolated *P.stutzeri* were 100% sensitive to polymyxin B.[(88)]

Similar study from Kirtilaxmi Benachinmardi *et al.* showed 100% sensitivity to Imipenem, Piperacillin tazobactum [(3).]

In this present study, among the 3 isolated *Burkholderia cepacia,* all were (100%) from sputum sample **(Table 7).** In contrast to present study, Kalidas Rit *et al.* showed isolation of 5 (35.7%) from pus and, 2(1%) from sputum, Tracheal aspirate, Blood and Urine [(86)].

In this present study, among the isolated *Burkholderia cepacia* (n=3), all were sensitive to Cotrimoxazole 3(100%), followed by Imipenem and Meropenem 2(66.7%), Ciprofloxacin, Ofloxacin, Cefotaxime and Ceftazidime 1(33.3%)

**(Table8).** In contrast to the present study, Kalidas *et al*. showed the sensitivity pattern of 92.8% to Imipenem and Cotrimoxazole, 85% to Ceftazidime and Ciprofloxacin, Piperacillin tazobactum 57%.

*Burkholderia cepacia* is intrinsically resistant to aminoglycosides and polymyxins and often develops resistance to β-lactams due to the presence of inducible chromosomal β-lactamases and altered penicillin-binding proteins. Antibiotic efflux pumps in Burkholderia mediate resistance to chloramphenicol, trimethoprim and fluoroquinolones [64)].

Multidrug resistance is a major problem with non fermenting gram negative bacilli and so the infections caused by them are very difficult to be treated. Polymyxins are the remaining antimicrobial drug class with fairly consistent activity against multidrug resistant strains of nonfermenters.

ESBL continued to be a major challenge in healthcare institutions, hence knowledge about their prevalence is very essential to initiate appropriate antimicrobial therapy. In the present study, all the 110 isolates were screened for ESBL production and confirmed by CLSI phenotypic confirmatory method. 20(18.18%) isolates were found to be ESBL producers. (p value – 0.0001 as per one proportion Z-Test) which is statistically significant.**(Table 9).**

In the present study, 9(16.7%) *Pseudomonas aeruginosa*, followed by *A.baumannii* 6 (16.7%), *A.lwoffi* 2(25%), *P.stutzeri* 1(33.3%) and *B.cepacia* and *S.maltophilia* 1(16.7%) were ESBL producers.**(Table 10).** While *S.maltophilia* and *B.cepacia* show intrinsic resistance to ß-lactams, ESBL production by *P.aeruginosa* and *A.baumannii* is significant. (P value of *P.aeruginosa* – 0.0001 and *A.baumannii* – 0.0009) which is statistically significant. In the present study, maximum sensitivity

among ESBL producers was seen with Piperacillin-tazobactum (100%) followed by Imipenem and Meropenem (90.5%)

Various studies showed difference in prevalence of ESBL in different areas. Sinha *et al.,* has 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*.[53] Loveena Oberoi *et al.,* reported (18.75%) *Pseudomonas aeruginosa* was ESBL producer. [11]

In the present study, the maximum of ESBL positive isolates were from sputum sample (44.4%), followed by (40%) from ET swab, (33.3%) from body fluids, (27.3%) from Blood, wound swab (15.8%), pus (14%), and urine (5%). **(Table 11).**

In the study conducted by Loveena Oberoi *et al*., reported the prevalence of ESBL production was (35.16%) found to be maximum as compared to the other β lactamases. Similar findings were reported in a study done by Bandekar *et al.* which showed a high prevalence of the ESBL producers (39.8%) in burns patients [13]. These observation showed an increase in the prevalence of ESBL producing organisms in India.

In the present study, 44 isolates of *Acinetobacter species* were screened for Meropenem resistance by modified Kirby -Bauer disc diffusion method and MBLproduction by phenotypic method of which 9 isolates *A.baumannii*, (20.5%) were found to be resistant to Meropenem as well as MBL producers (p value 0.0015 as per one proportion Z-Test) which is significant.**(Table-12&13).**

Due to difference in antimicrobial susceptibility pattern in different hospitals, frequent studies are valuable in deciding most adequate therapy. In this present study, the meropenem resistance was low (20.5%) when compared to the

study done by Sinha *et al*., where they have documented 28% of meropenem resistance. [18], All the isolates were resistant to both Imipenem and Meropenem (Gladston*e et al*.,) [54] Kyungwon Lee *et al.* from Korea reported 14.2% of MBL resistance in *Acinetobacter spp*.,[73] Gomty Mahajan *et al.* reported 31.8% isolates were meropenem resistant[16]. But the study conducted by Richa Hans *et al.,* high resistance of 68% was observed against meropenem [74]. In India MBL production among *A.baumannii* isolates has been reported as 42% (Amudhan *et al*.,).[78]

In this present study, the sample wise distribution of MBL in *Acinetobacter species* was analysed and among the MBL positive isolates, 3 (33.33%) were in pus and blood, 2(22.22%) from ET swab, 1(11.11%) from sputum and prevalence of MBL is 20.5%. **(Table14)**. In contrast to the present study, Kyungwon Lee *et al.* reported MBL producing isolates were mainly obtained from specimens of sputum (50.0%) and urine (29.3%) followed by blood, body fluids and venous catheter tip.[73]

Similarly, study by Sinha *et al.* reported maximum number of *Acinetobacter* isolates were from pus (37.14%) followed by blood (22.85%), and Urine (13.57%). Highest percentage of *Acinetobacte*r was isolated from ICU (22.14%), followed by paediatrics (20.71%), neurosurgery (15.71%) and general surgery wards (12.87%).[9]

The 9 meropenem resistant isolates were tested by the indicator method (i.e) meropenem disc diffusion were proceeded for the detection of carbapenamase production.[9]

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 and double disc synergy test for metallo beta lactamase **(Table-15).**

Among the 9 meropenem resistant isolates, **Modified Hodge test** was positive in 3 (33.3%) isolates. This was similar to the study done by Gomty mahajan *et al.* (47.6%)[16]. 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 NFGNB, as there are no standard procedures described in CLSI and other similar guidelines. In contrast to the present study, Shanthi Amudhan *et al*., reported MHT positivity in 94.4% of the isolates.[79] And also study from Amudhan *et al*., reported modified Hodge test was positive in 97.4% isolates of *A.baumannii* [78]

In this present study**, Imipenem-EDTA combined disc test (**CDDT) was positive in 5(55.6%) isolates, **Double disc synergy test** (DDST ) was positive in 4 (44.4%) , isolates. Similar to the present study, Sinha *et al*., and Uma *et al*., have documented 60.71% and 71% of MBL respectively.[16] by CDDT**.** In contrast to the present study, Gupta *et al.,* Lee *et al*., and Franklin *et al*. have documented 7.5%, 14% and 16% MBL respectively.[16,73] Study from Shanthi Amudhan *et al*. reported MBL screening with EDTA was positive in 80.4%[79]. Study from Amudhan *et al*., reported the metallo-beta-lactamase screening test with EDTA was positive in 79.3% isolates.[78] Study from Gomty mahajan *et al*. reported 19% of MBL producers by EDS test.[16]

The reason for the variations in MBL detection may be due to lack of standard procedures and variations in the expression of MBL gene.[16]

Among the 9 meropenem resistant isolates, 4/9(44.4%) 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. 3/9 (33.3%) isolates were positive for all the three tests and 3/9 (33.3%) isolates were positive for both oxacillinase and metallo betalactamase. Out of the 9 isolates CDDT,DDST was positive in 4 (44.4%) isolates, MHT, CDDT, DDST was positive in 3 (33.3%) isolates and CDDT alone was positive in 1(11.1%) isolates and all were negative in 4 (44.4%) isolate. Study from Richa Hans *et al.*, reported co existence of Carbapenemase and MBL production was observed in 16% isolates. [74]

This implies that combination of several mechanisms may exist in the same isolate to confer carbapenem resistance.[12]

The **meropenem resistant** isolates were further confirmed by MIC. All the 9 isolates have their MIC in the resistant range (>8µg/ml), hence disc diffusion method correlates with MIC.[41] This shows that regular screening for meropenem resistance can be done using disc diffusion method and further confirmed by MIC .

In this present study, among the 9 MBL isolates, MIC value of 3(33.3%) isolates were 256µg/ml, followed by 128 µg/ml for 2(22.2%), 2(22.2%) isolates have 64 µg/ml and the remaining 2(22.2%) isolate has 32 µg/ml. **(Table-16).**

Study from *Sinha et al*. reported MIC range of 8 and 64 µg/ml[9]. Amudhan *et al*. has documented MIC values ranging from 8µg/ml to128µg/ml[78].

Maryam Noori *et al*. have reported meropenem MIC as high as 256µg/ml in Iran.[92] In this study, out of 9 resistant isolates 3 isolates have MIC value of

256µg/ml , indicating the prevalence of high level resistant strains among the isolates, which is similar to the present study.

In this study, the 9 isolates of *A.baumannii* which were resistant to Meropenem by Disc diffusion method were subjected to PCR for the detection of most common Oxicillinase gene OXA-51 and Metallo Beta Lactamases genes bla-IMP and bla-VIM.**(Table 17).**

In this study, among the 9 Meropenem resistant isolates, all the 3 isolates were positive for OXA-51 (33.3%), In Indian studies, in contrast to the present study, the maximum OXA-51 positive isolates was reported as 83.96% in the study done by Amudhan *et al.*[17] Oxacillinases would also inactivate carbapenems, though they are less efficient hydrolyzers of carbapenems invitro than MBLs.[8]

In this present study, 2(22.2%) isolates were positive for blaVIM1 and 3 (33.3%) isolates were positive for blaIMP1. Among the 9 isolates, one isolate showed positivity of IMP,VIM and OXA-51 and two isolates showed positivity of IMP,OXA-51 and OXA-51 and VIM**. (Table 17).**

Study from Shanthi Amudhan *et al.*, reported MBL genes IMP and IMP were detected in 51.4% of the isolates. Among these MBL producers 89 isolates carried VIM alone, two carried IMP alone and 1 carried both .[79] Study from sinha *et al.*, reported nine *Acinetobacter* isolates carried bla-IMP gene and two of these also carried bla-VIM.[9]

In the study by K.Lee *et al*. from Korea reported 28.9% of MBL positive isolates of *Acinetobacter spp*. were IMP producers, showed prevalence of 71.1% of VIM -2 and 28.9% IMP-1of *Acinetobacter spp.,*[73] Similar to the present study, Amudhan *et al.* have documented 0.86% of all three genes positive isolates.[78]

Similarly Sinh*a et al*. have documented 7.14% of both VIM1 and IMP1 positive isolates.[9]

In the present study by comparing the various MBL detection methods with the gold standard PCR, the sensitivity and specificity of Modified Hodge test was 75% and 100% , Disc synergy test was 100% sensitve and 80% specific and for combined disc test both were 100%. The differences in both sensitivity and specificity between these tests were statistically significant. (**Table 18).**

**MANAGEMENT OF INFECTIONS BY NFGNB**

The prevalence and sensitivity of nonfermenters often varies between communities, in the same community and hospitals, among different patient populations in the same hospital. Faced these variations, the physician in clinical practice has the responsibility of making clinical judgments and should access to recent data on the prevalence and antimicrobial resistance pattern of commonly encountered pathogens.

It is therefore important to institute a system for the surveillance of antimicrobial resistance that will involve the collection of both clinical and microbiological data.

The present study observed highest resistance of NFGNB against Gentamicin & Cefotaxime antibiotics which are commonly used drugs .This necessitates the judicious use of these antibiotics in empirical therapy. Maximum sensitivity was observed with newer agents like carbapenams and pipercillin-tazobactum and Polymyxin.

Moderatly sensitive to Aminoglycosides and Fluroquinolones. Major risk of using monotherapy is the emergence of antibiotic resistance as observed in the present study which showed high rate of multidrug resistance and ESBL producers.

Carbapenamase resistance, though not high was still observed as an emerging drug resistant mechanisms in the NFGNB from this hospital. Antibiotic therapy either empirical or documented is based upon antibiotic combination supplemented by the knowledge of local epidemiology of susceptibility pattern in choosing a suitable combination.

Therefore combination therapy such as piperacillin-tazobactum, quinolones amikacin,imipenam-amikacin would be an ideal choice of therapy on the basis of antimicrobial susceptibility testing as observed in this study along with an adequate infection control measures especially in the surgical and ICU units.[93,94]

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 producing isolates, but resistance to carbapenems by the production of carbapenamases and various other mechanisms has limited the therapeutic options.[95]

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.[96]. In my study all the isolates were sensitive to Polymyxin B.

Hence currently combination therapy like meropenem with tigecycline and colistin with sulbactum or rifampicin are being tried in the treatment of *Acinetobacter spp.,* infection[97].

# SUMMARY

This study was conducted at the Department of Microbiology, Chengalpattu Medical college and Hospital aimed at isolation, speciation and determination of antimicrobial susceptibility among the nonfermenters with molecular analysis of resistant genes of Acinetobacter spp., revealed the following findings:

- A total of 110 clinical isolates of NFGNB from various clinical specimens were included in this study
- Majority of isolated nonfermenters were from the age group of less than 10 years (20.91%) with preponderance to males (65.4%).
- Among the nonfermenters isolated , (39%) were from pus, (18.1%) from urine, (17.2%) from wound swab, (10%) blood, (8.1%) sputum, (4.5%) endotracheal aspirate and (2.7%) from body fluids
- Majority of isolates of nonfermenters were from Surgical ward (40%) followed by ICU (20%).
- The major risk factor contributes to infection with nonfermenters was surgery/trauma (40%)
- Among the isolated nonfermenters *Pseudomonas aeruginosa* (49%) was the predominant isolate followed by *Acinetobacter baumanii* (32.7%), *Acinetobacter lwoffi* (7.3%), *S. maltophilia* (5.4%), *Pseudomonas stutzeri* and *Burkholderia cepecia* (2.8%).
- *Pseudomonas aeruginosa* (35.2%). *Acinetobacter baumanni* (52.8%), *Acinetobacter lwoffi* (25%), *Stenotrophomonas maltophilia* (50%), *Pseudomonas stutzeri* (66.7%) were isolated predominantly from pus and *Burkholderia cepacia* from sputum (100%).

- The antimicrobial susceptibility pattern of the isolated nonfermenters revealed maximum resistance was recorded for Gentamycin (61.8%), Cotrimoxazole (60%), followed by Ciprofloxacin (50.9%) and Cefotaxime (47.3%).

- All the isolates were sensitive to Polymyxin B (100%) followed by Imipenem and Meropenem (75.5%).

- Extended spectrum betalactamase (ESBL) was observed in 20(18.18%) isolated nonfermenters. (16.7%) *pseudomonas aeruginosa*, followed by *A.baumannii* (16.7%), *A.lwoffi* (25%), *P.stutzeri* (33.3%) and *B.cepacia* and *S.maltophilia* (16.7%) were the ESBL producers.

- Maximum of ESBL positive isolates were from sputum (44.4%), followed by (40%) ET swab, wound swab (29.8%).

- Among the 44 isolated *Acinetobacter species* screened for Meropenem resistance by modified Kirby -Bauer disc diffusion method, 9 isolates of A.baumannii (20.5%) were found to be resistant to Meropenem.

- All the 9 Meropenem resistant isolates of *A.baumannii* had their MIC above 8 μg/ml. (≥8μg/ml –Resistant)

- Maximum MBL producers were from pus and blood (33.3%) samples respectively

- Combined disc diffusion test was a better method of detection of metallobetalactamase production phenotypically with a sensitivity and specificity of 100%.

- Molecular characterization of 9 MBL Acinetobacter baumannii revealed 3 (33.3%) OXA-51 gene, 3 (33.3%) blaIMP gene and 2(22.2%) blaVIM gene positivity.
- There is multiple gene expression for 3 (60%) isolates indicating the occurrence of increasing MBL resistance among Acinetobacter baumannii.

# CONCLUSION

Observations from this present study showed that the aerobic NFGNB which are usually considered as contaminants are now emerging as important nosocomial pathogens.The various clinical samples from which they were isolated proved their existence in all the sites leading to a range of diseases. Different antimicrobial susceptibility pattern and multidrug resistance exhibited by nonfermenters pose a great problems in treating these infections. ESBL and MBL production by these organisms lead to high morbidity and mortality and we were left with the only option of treating them by potentially toxic drugs like Colistin and Polymyxin B. Care in detection, evaluation of effective antibiotic option, judicious use of antibiotics by instituting antibiotic policy and infection control measures will help to fight against these multidrug resistant nonfermenters in the effective managementof patients**.**

***Acinetobacter species*** are the second most common nonfermenter isolated from clinical specimens next to *Pseudomonas species*. The infections caused by multidrug resistant *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 in the current era.

*A.baumannii* was the most common species isolated and most resistant when compared to other *Acinetobacter* species and there was a significant difference in their antimicrobial susceptibility pattern.

Carbapenems remain the drug of choice for the MDR Acinetobacter infections. But resistance to carbapenems occur 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.

The occurrence of MBL is not only a therapeutic issue, but poses 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, all the isolates were sensitive to polymyxin-B.

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, and vigilant detection of resistant non fermenters, regular surveillance and infection control protocols to control the increasing incidence of highly resistant nonfermenters.

# APPENDIX-I

## ABBREVIATIONS

| | | |
|---|---|---|
| NFGNB | - | Nonfermenting gram negative bacilli |
| *Ps.aeruginosa* | - | *Pseudomonas aeruginosa* |
| *Ps.stutzeri* | - | *Pseudomonas stutzeri* |
| *A.baumanii* | - | *Acinetobacter baumanii* |
| *A.lwoffii* | - | *Acinetobacter lwoffii* |
| *S.maltophilia* | - | *Stenotrophomonas maltophilia* |
| *B.cepacia* | - | *Burkholderia cepacia* |
| Mac plate | - | MacConkey plate |
| MHA | - | Mueller Hinton Agar |
| MDR | - | Multidrug resistant |
| ESBL | - | Extended Spectrum Of Betalactamases |
| MBL | - | Metallobetalactamases |
| ATCC | - | American Type Culture Collection |
| CLSI | - | Clinical & Laboratory Standards Institute |
| MIC | - | Minimum Inhibitory Concentration |
| PCR | - | Polymerase chain reaction |
| CDDT | - | combined discdiffusion test |
| DDST | - | Double disc synergy test |

| | | |
|---|---|---|
| MHT | - | Modified Hodge Test |
| EDTA | - | Ethylene Diamine Tetra acetic Acid |
| OXA-23 | - | Oxacillinase beta lactamase |
| bla-IMP | - | Imipenamase metallo beta lactamase |
| bla-VIM | - | Verona integron encoded metallo beta lactamase |

# APPENDIX II

## PREPARATION OF GRAM STAIN

## GRAM STAIN REAGENTS

1. Methyl violet - Primary stain

   Methyl violet 10 g

   95% ethyl alcohol 100 ml

   Distilled water 1 L

2. Gram's Iodine – Mordant

   Iodine 10 g

   Potassium iodide 20g

   Distilled water 1 L

3. Acetone - Decolouriser

4. Dilute Carbol Fuchsin - Counter stain

   Basic fuchsin 0.3g

   95% Ethyl alcohol 10 ml

   Phenol crystals, melted 5 ml

   Distilled water 95 ml

Basic fuchsin was dissolved in alcohol.5 % phenol solution was added and was allowed to stand overnight. Then the solution filtered through coarse filter paper.

**MEDIA USED:**

**PREPARATION OF MAC KONKEY AGAR**

Contents:

Sodium taurocholate 5.0 g

Peptone 20.0 gm

Sodium chloride 5.0 g

Lactose 10.0 g

Agar 15.0 g

Distilled water 1000 ml

Neutral red (2% solution in 50% ethanol) 3.5 ml

- 5 g sodium taurocholate or bile salts, 20 g of peptone, 5 g sodium chloride and 15 g agar were mixed with 1000 ml water.
- Steamed until the solids were dissolved.
- Cooled to about 50˚C, and at this temperature the reaction was adjusted to pH 7.5 to 7.8. Autoclaved at 121˚C for 15 minutes and filtered while hot through a good grade of filter paper, or a plug of cotton wrapped in gauze placed in the funnel.
- The reaction of the filtrate was adjusted to pH 7.3 at 50˚C or pH 7.5 at room temperature. 10 gm lactose and 3.5 ml of 2% solution of neutral red in 50% ethanol were added. Mixed thoroughly and distributed in flasks and sterilized in the autoclave at 121˚C for 15 minutes.
- For use, melted in the steamer, poured into sterile petri dishes and allowed to set.

**PREPARARION OF BLOOD AGAR**

Nutrient agar 100 ml

Sheep blood (defibrinated) 10 ml

- The sterile nutrient agar was melted by steaming and cooled to 45˚C.
- 5% -10% sheep blood was added aseptically with constant shaking.
- The blood was mixed with molten nutrient agar thoroughly but gently, to avoid froth formation.To remove the bubbles, media was flamed.
- Immediately poured into petri dishes and allowed to set.

**PREPARATION OF MUELLER – HINTON AGAR**

Contents:

Beef extract 2.0 gm

Acidicase Peptone 17.5 gm

Starch 1.5 gm

Agar 17.0 gm

Distilled water 1000 ml

Final pH 7.4 + 0.2

Dissolve the ingredients in one liter of distilled water. Mix thoroughly. Heated with frequent agitation and boiled for one minute. Dispensed and sterilized by autoclaving at 121˚C for 15 minutes. Should not be over heated.

## ACETAMIDE AGAR

**Ingredients:**

Magnesium Sulphate : 0.2g

Ammonium dihydrogen Phosphate : 1g

Pottasium monohydrogen phosphate : 1g

Sodium Chloride : 5g

Acetamide : 10g

Bromothymol blue solution : 6.4ml

Agar : 15g

Final pH : 6.9

Distilled water : 1 litre

The ingredients are mixed and pH adjusted to 6.9, dispensed into screw cap tubes and sterilized at 1210C for 15 min. The medium was allowed to cool in a slant. The slant was inoculated with a portion of isolated colony and incubated at 370C overnight and was observed for color change. Tubes with negative result were further incubated for 7 days.

**Control**

Positive control : Pseudomonas aeruginosa

Negative Control : Stenotrophomonas maltophilia

## 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. riple 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.

**6. Mannitol motility medium**

Agar 5g

Peptone 1g

Potassium nitrate 1g

Mannitol 2g

Phenol red indicator

Distilled water l000ml

pH 7.2

**7. Nitrate medium:**

Potassium nitrate 0.2g

Peptone 5g

Distilled water 1000ml

The above contents were mixed and tubed in 5 ml amounts and autoclaved at 121˚c for 15 minutes.

**Test reagent**: Solution A: 8 g of sulphanilic acid was dissolved in 1 L of acetic acid 5 mol/litre Solution B:5 g of alpha-naphthlamine in 1 L of acetic acid 5 mol/litre. Immediately before use, equal volumes of solutions A and B were mixed to get the test reagent.

**8. Decarboxylase media:**

**Moller decarboxylase broth base:**

Peptone 5 g

Beef extract 5 g

Bromocresol purple 0.01 g

Cresol red 0.005 g

Glucose 0.5 g

Pyridoxal 0.005 g

Distilled water 1 lit.

Final pH 6

**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 1lit.

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 lit.

Adjust the pH to 7.4. Sterilize by autoclaving at 121$^{o}$C for 15min.

## ANNEXURE I

**INSTITUTIONAL ETHICS COMMITTEE**

**CHENGALPATTU MEDICAL COLLEGE , CHENGALPATTU**

**APPROVAL OF ETHICAL COMMITTEE**

To

Dr.M.Gomathi
MD Microbiology
(2nd Year),
Dept of Microbiology,
Chengelpattu Medical College ,
Chengelpattu

Dear Dr.

The Institutional Ethical Committee of Chengalpattu Medical College reviewed and discussed your application to conduct the clinical / dissertation work entitled

CHARECTERISATION AND ANTIBIOTIC SENSITIVITY PATTERN OF NON FERMENTING GRAM NEGATIVE BACILLI AND MOLECULAR ANALYSIS OF ACINETOBACTER SPP.,FROM VARIOUS CLINICAL SAMPLES

ON 11.06.2014

The following documents reviewed

1. Trial protocol, dated _____version no
2. Patient information sheet and informed consent form in English and / or
   vernacular language.
3. Investigators Brochure, dated _____version
4. Principal Investigators current CV
5. Investigators undertaking

The following members of the Ethics committee were present at the meeting held on

Date 11.06.2014 Time 11.30 Noon Place Chengalpattu Medical College

Approved _____ Chairman Ethics Committee

_____ Member secretary of Ethics Committee.

11/6/14

Name of each member with designation:-

Clinical Members

1. Dr.R.Muthuselvan MD.,
   Prof & HOD of Medicine, CHMC

2. Dr.C.Srinivasan MD.,
   Prof & HOD of Surgery, CHMC

Biological Scientist

3. Dr.K.Baskaran MD.,
   Asso Prof of Pharmacology, CHMC

Non Clinical Member

4. Dr.P.Parasakthi MD
   Prof & HOD of Forensic Medicine,CHMC

5. Member from Nongovernmental
   Voluntary Organisation : Mr.P.Durairaj

6. Philosopher : Mr.K.S.Ramprasad

7. Lawyer : Lr. I. M. Karimala Basha

8. Layperson : Mr.Dilli

We approve the clinical trial to be conducted in its presented form

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

Yours sincerely

11/6/14

Member secretary, Ethics Committee

# ANNEXURE II

## DATA COLLECTION PROFORMA

1. Case No. :
2. Name and address of the patient :
3. Age & sex :
4. OP & IP No :
5. Ward and Unit :
6. Date of admission :
7. Occupation and income :
8. Clinical diagnosis :
9. Relevant co-existing clinical conditions/illnesses :
10. History of presenting illness :
11. Past history :
12. Personal history :
13. Treatment History :

    (Antibiotics taken if any)

14. Sample collected :
15. Date of sample collection :

**Microbiological investigation:**

1. Confirmation of the Isolate :

2. Speciation :

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

## PATIENT CONSENT FORM

**STUDY DETAIL:**

**"CHARACTERISATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON FERMENTING GRAM NEGATIVE BACILLI AND MOLECULAR ANALYSIS OF ACINETOBACTER SPP., FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL "**

**STUDY CENTER: CHENGALPATTU MEDICAL COLLEGE & HOSPITAL, CHENGALPATTU**

**PATIENT NAME:** **PATIENT AGE:**

**IDENTIFICATION NUMBER:**

**PATIENT TO TICK ( )THESE BOXES**

**I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask the question and all my questions and doubts have been answered to my satisfaction.**

**I understand that investigator, regulatory authorities and the ethics committee will not need my permission to look at my health records both in respect to the current study and any further research that may be conducted in relation to it, even if withdraw from the study, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from the study.**

**I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperative with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or wellbeing or any unexpected or unusual symptoms.**

**I hereby give consent to participate in this study.**

**Signature/Thumb impression:**

**Patient name and address:** **Date:**

**Signature of the investigator:** **Place:**

# சுய ஒப்புதல் படிவம்

**ஆய்வு செய்யப்படும் தலைப்பு: "CHARACTERISATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON FERMENTING GRAM NEGATIVE BACILLI AND MOLECULAR ANALYSIS OF ACINETOBACTER SPP., FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL " .**

ஆய்வு செய்யப்படும் இடம் :

பங்கு பெறுபவரின் பெயர் :

பங்கு பெறுபவரின் வயது :

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கபட்டுள்ளது. நான் இவ்வாய்வில் தன்னிச்சையாக பங்கேற்கின்றேன். எந்த காரணத்தினாலோ, எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகிக் கொள்ளலாம் என்றும் அறிந்துகொண்டேன்.

இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்து மேலும் ஆய்வு மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவை இல்லை என அறிந்து கொள்கிறேன். இந்த ஆய்வின் மூலம் கிடைக்கும் தகவலையோ,முடிவையோ பயன்படுத்திக் கொள்ள மறுக்கமாட்டேன்.

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன். இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம்: இடம்:

பங்கேற்பவரின் பெயர் மற்றும் விலாசம்: தேதி:

ஆய்வாளரின் கையொப்பம்:

# ANNEXURE – IV MASTER CHART

| S.No | Micro No. | Age | sex | ward | Diagnosis | specimen | organism | Gentamycin | Amikacin | Pip-taz | Cefataxime | Ceftazidime | Imipenem | Meropenem | PolymyxinB | Ofloxacin | Ciprofloxacin | Cotrimoxazole | Resistance pattern | MRP(MIC) | MHT | CDDT | CDST | IMP | VIM | OXA-51 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1618 | 2/12 | Fch | NICU | Sepsis | Blood | A.bau | S | S | S | S | S | S | S | S | S | S | S | | | | | | | | |
| 2 | 3181 | 27 | F | OG | UTI | Urine | A.lwofi | S | S | S | S | S | S | S | S | S | S | S | | | | | | | | |
| 3 | 3086 | 70 | M | Urology | UTI | Urine | A.lwofi | S | S | S | S | S | S | S | S | S | S | S | | | | | | | | |
| 4 | 1103 | 5 | Fch | Paed.sur | WI | Pus | Ps.stut | R | S | S | R | R | S | S | S | S | S | R | ESBL | | | | | | | |
| 5 | 3406 | 24 | M | Medicine | UTI | Urine | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | | |
| 6 | 1086 | 30 | M | Surgery | WI | WS | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | | |
| 7 | 1603 | 3/12 | Fch | NICU | PUO | Blood | A.bau | R | S | R | R | R | R | R | S | R | R | R | MBL | 256 | + | + | + | + | + | + |
| 8 | 1787 | 5/365 | Mch | NICU | Sepsis | Blood | A.bau | R | S | R | R | R | R | R | S | R | R | R | MBL | 128 | | + | + | + | | |
| 9 | 3545 | 28 | F | Medicine | UTI | Urine | A.lwofi | S | S | S | S | S | S | S | S | R | R | S | | | | | | | | |
| 10 | 1205 | 5/365 | Fch | NICU | Sepsis | ET swab | A.bau | R | R | R | R | R | R | R | S | R | R | R | MBL | 256 | + | + | + | + | | + |
| 11 | 1221 | 70 | M | Medicine | Pneumonia | Sputum | A.bau | S | S | S | R | R | S | S | S | S | S | S | ESBL | | | | | | | |
| 12 | 1219 | 6/365 | Mch | NICU | Sepsis | ET swab | A.bau | R | R | R | R | R | R | R | S | R | R | R | MBL | 256 | + | + | + | | + | + |
| 13 | 1243 | 78 | M | Surgery | Pyothorax | Pus | S.malt | R | R | R | R | R | R | R | S | S | S | S | | | | | | | | |
| 14 | 3944 | 30 | F | Urology | UTI | Urine | A.lwofi | R | R | S | R | S | S | S | S | R | R | R | ESBL | | | | | | | |
| 15 | 3981 | 15 | M | Medicine | UTI | Urine | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | | |
| 16 | 3486 | 40 | M | Medicine | Burns | WS | A.lwofi | S | S | S | S | S | S | S | S | S | S | S | | | | | | | | |
| 17 | 1469 | 58 | M | Surgery | WI | Pus | A.bau | R | R | R | R | R | R | R | S | R | R | R | MBL | 128 | | + | | | | |
| 18 | 1481 | 52 | M | Surgery | WI | WS | Ps.aeru | R | S | S | R | R | R | R | S | R | R | R | ESBL | | | | | | | |
| 19 | 1595 | 82 | M | Surgery | SSI | Pus | Ps.aeru | R | R | R | R | R | R | R | S | S | S | R | | | | | | | | |
| 20 | 1603 | 24 | F | OG | SSI | Pus | Ps.aeru | R | S | S | R | S | S | S | S | R | R | R | | | | | | | | |
| 21 | 1605 | 40 | M | Ortho | #BBLeg | Pus | A.bau | R | S | R | R | R | R | R | S | R | R | R | MBL | 64 | | | | | | |
| 22 | 1641 | 82 | M | Burns | Burns | WS | A.bau | R | R | S | R | R | S | S | S | R | R | R | ESBL | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | 4161 | 24 | M | Urology | UTI | Urine | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |
| 24 | 4417 | 86 | M | Urology | UTI | Urine | Ps.aeru | S | S | S | R | S | S | S | S | R | R | R | | | | | | | |
| 25 | 4425 | 35 | F | Urology | UTI | Urine | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |
| 26 | 4497 | 20 | M | Urology | Pyelo | Urine | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |
| 27 | 4551 | 58 | M | Surgery | UTI | Urine | Ps.aeru | R | R | S | R | S | S | S | S | S | S | R | | | | | | | |
| 28 | 1694 | 1 | F | NICU | Burns | WS | A.bau | S | S | S | S | S | S | | S | S | S | S | | | | | | | |
| 29 | 4631 | 48 | F | Medicine | UTI | Urine | A.bau | S | S | S | S | S | S | S | S | S | S | S | | | | | | | |
| 30 | 4815 | 21 | F | OG | UTI | Urine | A.bau | R | S | S | S | S | S | S | S | S | S | R | | | | | | | |
| 31 | 5181 | 23 | F | OG | UTI | Urine | Ps.aeru | R | R | S | R | S | S | S | S | R | R | R | | | | | | | |
| 32 | 1783 | 70 | M | Ortho | Osteo | Pus | A.bau | R | S | S | R | R | S | S | S | S | S | R | ESBL | | | | | | |
| 33 | 1784 | 58 | F | Burns | Burns | WS | S.malt | R | R | S | R | R | R | R | S | S | S | S | ESBL | | | | | | |
| 34 | 1760 | 20 | F | OG | SSI | Pus | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |
| 35 | 1735 | 82 | M | Surgery | WI | Pus | A.bau | S | S | S | S | S | S | S | S | S | S | S | | | | | | | |
| 36 | 1727 | 30 | M | Surgery | WI | Pus | Ps.aeru | R | S | S | R | R | S | S | S | S | S | R | ESBL | | | | | | |
| 37 | 1694 | 1 | F | NICU | Burns | WS | A.bau | R | S | S | S | S | S | S | S | R | R | R | | | | | | | |
| 38 | 1684 | 30 | M | Ortho | WI | Pus | Ps.aeru | R | S | R | R | R | S | S | S | S | S | R | | | | | | | |
| 39 | 1665 | 1 | M | NICU | Asphyxia | ET swab | A.bau | R | R | S | R | R | S | S | S | S | S | R | ESBL | | | | | | |
| 40 | 1641 | 82 | M | Surgery | DM foot | Pus | A.bau | S | S | S | S | S | S | S | S | S | S | S | | | | | | | |
| 41 | 2465 | 1 | F | NICU | Sepsis | Blood | Ps.aeru | R | R | S | R | R | S | S | S | S | S | R | ESBL | | | | | | |
| 42 | 2412 | 1 | M | NICU | Sepsis | Blood | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |
| 43 | 2355 | 10 | F | Paed | PUO | Blood | S.malt | R | R | R | R | R | R | R | S | S | S | S | | | | | | | |
| 44 | 2345 | 1 | M | PICU | PUO | Blood | Ps.aeru | R | R | S | R | R | S | S | S | R | R | R | ESBL | | | | | | |
| 45 | 2486 | 1 | M | NICU | Sepsis | Blood | Ps.stut | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |
| 46 | 5324 | 6 | M | PICU | UTI | Urine | Ps.aeru | S | S | S | R | S | S | S | S | R | R | R | | | | | | | |
| 47 | 1838 | 70 | F | Surgery | DM foot | Pus | A.bau | S | S | S | R | R | S | S | S | S | S | S | ESBL | | | | | | |
| 48 | 1840 | 24 | F | Surgery | WI | Pus | A.bau | S | S | S | S | S | S | S | S | R | R | S | | | | | | | |
| 49 | 1844 | 33 | M | Surgery | SSI | Pus | A.bau | S | S | S | S | S | S | S | S | S | S | S | | | | | | | |
| 50 | 1846 | 54 | M | Medicine | Ascites | As.fluid | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |
| 51 | 1889 | 1 | F | NICU | WI | WS | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |
| 52 | 1902 | 50 | F | Surgery | WI | WS | Ps.aeru | S | S | S | R | S | S | S | S | R | R | R | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 53 | 1910 | 32 | M | NICU | VAP | ET swab | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |
| 54 | 1918 | 54 | M | Surgery | SSI | Pus | A.bau | S | S | S | S | S | S | S | S | R | R | S | | | | | | | |
| 55 | 1924 | 57 | F | Surgery | WI | Pus | A.bau | R | R | S | S | S | S | S | S | S | S | S | | | | | | | |
| 56 | 1926 | 50 | M | Surgery | WI | Pus | S.malt | R | R | R | R | R | R | R | S | S | S | S | | | | | | | |
| 57 | 1936 | 45 | M | Surgery | DM foot | Pus | S.malt | R | R | R | R | R | R | R | S | S | S | S | | | | | | | |
| 58 | 1940 | 39 | M | ENT | OE | Pus | Ps.aeru | R | R | S | R | S | S | S | S | R | R | R | | | | | | | |
| 59 | 1944 | 50 | M | Burns | Burns | WS | Ps.aeru | R | S | R | R | R | S | S | S | R | R | R | | | | | | | |
| 60 | 2018 | 25 | F | OG | WI | Pus | Ps.aeru | R | R | R | R | R | S | S | S | R | R | R | | | | | | | |
| 61 | 2031 | 38 | M | Ortho | OM | Pus | A.bau | S | S | S | S | S | S | S | S | S | S | S | | | | | | | |
| 62 | 2036 | 45 | F | Surgery | WI | Pus | Ps.aeru | R | R | S | R | S | S | S | S | R | R | R | | | | | | | |
| 63 | 2046 | 30 | F | OG | WI | Pus | S.malt | R | R | R | R | R | R | R | S | S | S | R | | | | | | | |
| 64 | 2049 | 70 | F | Medicine | Ascites | As.fluid | Ps.aeru | R | S | S | R | R | S | S | S | R | R | S | ESBL | | | | | | |
| 65 | 2068 | 65 | M | Burns | Burns | WS | A.bau | S | S | S | S | S | S | S | S | S | S | S | | | | | | | |
| 66 | 2590 | 1 | F | NICU | Sepsis | Blood | Ps.aeru | R | R | S | R | S | S | S | S | R | R | R | | | | | | | |
| 67 | 2514 | 6 | M | Paed | PUO | Blood | A.lwofi | R | S | S | R | R | S | S | S | S | S | R | ESBL | | | | | | |
| 68 | 2001 | 40 | M | Medicine | DM foot | Pus | A.bau | S | S | S | S | S | S | S | S | S | S | S | | | | | | | |
| 69 | 2005 | 25 | M | Medicine | Pneumonia | Sputum | Ps.aeru | R | R | S | R | S | S | S | S | R | R | R | | | | | | | |
| 70 | 2008 | 24 | M | ENT | OE | Pus | Ps.stut | R | R | S | R | S | S | S | S | R | R | R | | | | | | | |
| 71 | 2574 | 6 | M | ENT | CSOM | Pus | A.bau | R | R | S | S | S | S | S | S | R | R | R | | | | | | | |
| 72 | 936 | 2 | M | PICU | Pneumonia | Sputum | A.lwofi | R | R | S | S | S | S | S | S | R | R | R | | | | | | | |
| 73 | 941 | 40 | M | Medicine | DM foot | Pus | Ps.aeru | S | S | S | R | S | S | S | S | R | R | R | | | | | | | |
| 74 | 942 | 50 | M | Burns | Burns | WS | Ps.aeru | R | R | S | R | S | R | S | S | R | R | R | | | | | | | |
| 75 | 961 | 66 | M | Surgery | Pyothorax | Pus | A.bau | R | R | R | R | R | S | S | S | R | R | R | | | | | | | |
| 76 | 985 | 26 | F | Surgery | DMfoot | Pus | Ps.aeru | R | S | R | R | R | R | R | S | R | R | R | | | | | | | |
| 77 | 1003 | 17 | M | Ortho | #BBLL | WS | Ps.aeru | R | S | R | R | R | R | R | S | R | R | R | | | | | | | |
| 78 | 1005 | 65 | M | Surgery | DMCllulitis | Pus | A.bau | S | S | S | S | S | S | S | S | S | S | S | | | | | | | |
| 79 | 1036 | 70 | M | Surgery | Malig,SSI | Pus | A.bau | S | S | S | S | S | S | S | S | S | S | S | | | | | | | |
| 80 | 1089 | 65 | M | Surgery | SSI | Pus | A.bau | R | S | S | S | S | S | S | S | R | R | S | | | | | | | |
| 81 | 2016 | 40 | M | IMCU | Pl.effusion | Pl.fluid | A.bau | R | S | S | S | S | S | S | S | R | R | S | | | | | | | |
| 82 | 2073 | 54 | M | Surgery | SSI | WS | Ps.aeru | R | R | S | R | S | R | R | S | R | R | R | | | | | | | |

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| 83 | 6149 | 49 | M | Urology | Pyelo | Urine | Ps.aeru | R | S | R | R | R | R | R | S | S | S | R | | | | | | | |
| 84 | 2124 | 40 | M | Surgery | Cellilitis | Pus | Ps.aeru | R | R | S | R | S | S | S | S | R | R | R | | | | | | | |
| 85 | 3 | 65 | M | Surgery | SSI | Pus | Ps.aeru | R | R | S | R | S | S | S | S | R | R | R | | | | | | | |
| 86 | 15 | 65 | M | Surgery | Dmfoot | Pus | Ps.aeru | R | R | S | R | R | S | S | S | S | S | R | ESBL | | | | | | |
| 87 | 17 | 1 | F | PICU | Pneumonia | ET swab | A.bau | R | R | S | R | R | S | S | S | S | S | R | ESBL | | | | | | |
| 88 | 1924 | 57 | F | Surgery | Abscess | Pus | A.bau | R | R | S | S | S | S | S | S | R | R | R | | | | | | | |
| 89 | 1614 | 19 | F | Urology | UTI | Urine | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |
| 90 | 449 | 47 | M | IMCU | Empyema | Pus | Ps.aeru | R | R | R | R | R | S | S | S | S | S | R | | | | | | | |
| 91 | 520 | 45 | M | Surgery | WI | WS | Ps.aeru | R | R | S | R | S | S | S | S | R | R | R | | | | | | | |
| 92 | 7315 | 60 | F | SICU | SSI | Pus | A.bau | R | S | R | R | R | R | R | S | R | R | R | MBL | 64 | | | | | |
| 93 | 542 | 22 | F | OG | WI | WS | Ps.aeru | S | S | S | R | S | S | S | S | R | R | R | | | | | | | |
| 94 | 559 | 68 | M | Surgery | SSI | Pus | Ps.aeru | R | S | S | R | R | S | S | S | R | R | R | ESBL | | | | | | |
| 95 | 569 | 45 | F | Surgery | Crush injury | Pus | Ps.aeru | R | S | R | R | R | S | S | S | R | R | R | | | | | | | |
| 96 | 595 | 21 | M | Medicine | COPD | Sputum | Ps.aeru | R | R | S | R | R | S | S | S | R | R | R | ESBL | | | | | | |
| 97 | 597 | 34 | M | TB ward | Pneumonia | Sputum | A.lwofi | S | S | S | S | R | S | S | S | R | R | R | ESBL | | | | | | |
| 98 | 1713 | 47 | F | Derm | UTI | Urine | Ps.aeru | S | S | S | R | R | S | S | S | S | S | R | ESBL | | | | | | |
| 99 | 505 | 1 | M | NICU | Sepsis | Blood | A.bau | R | S | R | R | R | R | R | S | R | R | R | MBL | 32 | | | | | |
| 100 | 1819 | 24 | M | Urology | Pyelo | Urine | Ps.aeru | R | R | R | R | R | R | R | S | R | R | R | | | | | | | |
| 101 | 616 | 37 | F | OG | WI | Pus | Ps.aeru | S | S | R | R | R | R | R | S | S | S | R | | | | | | | |
| 102 | 2076 | 55 | M | IMCU | Empyema | Sputum | A.bau | R | S | R | R | R | R | R | S | R | R | R | MBL | 32 | | | | | |
| 103 | 1125 | 50 | M | TB ward | UL Cons. | Sputum | B.cep | R | R | R | R | R | S | S | R | R | R | S | | | | | | | |
| 104 | 910 | 31 | M | Burns | Burns | WS | Ps.aeru | R | R | S | R | S | S | S | S | R | R | R | | | | | | | |
| 105 | 913 | 27 | F | Surgery | SSI | WS | Ps.aeru | R | R | R | R | R | R | R | S | R | R | R | | | | | | | |
| 106 | 4848 | 3 | M | Paed | UTI | Urine | Ps.aeru | R | R | R | R | R | R | R | S | R | R | R | | | | | | | |
| 107 | 914 | 37 | M | Burns | Burns | WS | Ps.aeru | R | S | R | R | R | R | R | R | R | R | R | | | | | | | |
| 108 | 927 | 65 | M | Medicine | Pneumonia | Sputum | B.cep | R | R | R | R | R | R | R | R | R | R | S | | | | | | | |
| 109 | 1207 | 45 | M | Medicine | COPD | Sputum | B.cep | R | R | S | R | R | S | S | R | S | S | S | ESBL | | | | | | |
| 110 | 1109 | 40 | F | Surgery | Abscess | Pus | Ps.aeru | S | S | S | R | S | S | S | S | S | S | R | | | | | | | |

# BIBLIOGRAPHY


1. Koneman EW,Allen SD. Colour atlas and Text book of diagnostic Microbiology, 6th edition, Philadelphia, Lippincott-Williams& Wilkins Publishers,2006:p .624-662.

2. Gokhale S, Metgud SC. Characterization and antibiotic sensitivity pattern of nonfermenting Gram negative bacilli from various clinical samples in a tertiary care hospital. Belgaum J Pharm Biomed Sci 2012;17:1-5.

3. Kirtilaxmi K. Benachinmardi, Padmavathy M, Malini J, Naveneeth B. V Prevalence of non-fermenting Gram-negative bacilli and their in vitro susceptibility pattern at a tertiary care teaching hospital .Department of Microbiology, Employees'State Insurance Corporation Medical College and PGIMSR, Bengaluru,Karnataka, India

4. Nagoba BS, Deshmukh SR, Ulka G Gude et al. Invitro susceptibility of Pseudomonas aeruginosa to different antibiotics. Indian J Med Microbiol 1997,15:185-186.

5. Mishra B, Bhujwala RA, Shrinivas, Non fermenters in human infections. Indian J Med Res 1986 Jun; 83:561-6.

6. Quinn JP . 1998 Clinical problems posed by multiresistant nonfermenting gramnegative pathogens. Clin Infect Dis. Suppl 1:S117-24.

7. Shigeki Fujitani and Victor L. Yu 2006 Diagnosis of Ventilator-Associated Pneumonia: Focus on Nonbronchoscopic Techniques and Blinded Bronchial Sampling and Endotracheal Aspirates Nonbronchoscopic Bronchoalveolar Lavage, Including Mini-BAL, Blinded Protected Specimen Brush *J Intensive Care Med*; 21; 17.

8. Mandell, Douglas and Bannett's. Principles & Practice of Infectious Diseases. 7thEdition, p. 2881-84.

9. Sinha N, Agarwal J, Srivastava S, Singh M.Analysis of Carbapenem-resistant *Acinetobacter* from a tertiary care setting in North India. Indian J Med Microbiol.2013;31:p.60-3.

10. Fellpe Fernandez, Luis Martinez, M Carman, Juan A. Relationship Between Betalactamase production, Outer membrane proteins and Penicillin binding proteins profiles on the activity of Carbapenems against clinical isolates of *A.baumannii*. J. Antimicrob. Chemo. 2003;51(3):p.565-74.

11. Loveena Oberoi, Nachhatarjit Singh, Poonam Sharma, Aruna Agarwal. ESBL, AmpC and MBL beta lactamases producing superbugs. Havoc in the ICU of Punjab,India.J of Clinical Diagnostic Reseach.2013;7(1):p. 70-73.

12. Vikas Manchanda, Sinha Sanchalta and NP Singh. Multi Drug Resistant *Acinetobacter*. J Glob Infect Dis 2010;2(3):291-304.

13. The Genus *Acinetobacter* Prokaryotes, 2006(6), Towner

14. Gales AC, Reis AO, Jones RN. Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines. J Clin Microbiol. 2001 Jan;39(1):183-90.

15. Manu Chaudhary and Anurag Payasi. Molecular Characterisation and Antimicrobial Susceptibility Study of *A.baumannii* Clinical Isolates from Middle East, African and Indian Patients. J Proteomics Bioinform 2012;5(11):p.265-69.

16. Gomathy Mahajan, Sheevani Sheemar, Shashi Chopra et al.Carbapenem Resistance and Phenotypic Detection of Carbapenamases in Clinical isolates of *A.baumannii*.Indian J of Med sciences 2011;65(1):18-25.

17. Taneja N, singh G, Singh M, Sharma M. Emergence of Tigecycline and colistin resistant *A.baumannii* in patients with complicated UTI in North India. Indian J Med res 2011;133:p.681-684.

18. M Sinha H Srinivasa. Mechanisms of resistance to carbapenems in meropenem resistant *Acinetobacter* isolates from clinical samples. Indian J Med Microbiol. 2007;25(2):121-5.

19. Franklin C, liolios L, Peleg AY. Phenotypic detection of Carbapenem-Susceptible metallo-β-lactamase - producing gram –negative bacilli in the clinical laboratory.J clin Microbiol 2006; 44: 3139-44.

20. Patrick R Murray, Ellen jo Baron, James H Jorgensen, Marie Louise Landry, Michael A Pfaller. Manual of Clinical Microbiology.

21. Frost and Sullivan Market Insight. Nosocomial infections in India: Assuming Dangerous proportions.Date published 20 july 2001.By Vasudha mukherjee.

22. Palleroni NJ. Family I. Pseudomonadaceae. In : krieg NR, Holt JG, eds. Bergey's Manual of systematic bacteriology.Vol.1.Baltimore : Williams & Wilkins, 1984 :141-219.

23. Bodey GP, Bolivar R, Fainstein V, et al .Infections caused by pseudomonas aeruginosa. Rev Infect Dis 1983;5:279-313

24. Topley Wilson's Microbiology & Microbial Infections 10th edition:p. 1301-1311.

25. National Nosocomial Infections Surveillance (NNIS ) System Report, data summary from January 1992 to June 2002 , issued August 2002. Am J infect control .2002; 30:458-475.

26. Cornaglia G, Riccio ML, Mazzariot A, Lauretti L, Fontana R, Rosslini GM. Appearnce of IMP-1 metallo-β-lactamase in Europe. Lancet 1999; 353: 899-900

27. Molina DN, Colon M, Bermudex RH et al.Unusual presentation of Pseudomonas aeruginosa infection : a review. Bol Asoc Med PR. 1991; 83: 160-163.

28. J.G.Collee/A.G.Fraser, B.P.Marmion/A.Simmons,14th edition by Mackie & McCarney Practical Medical Microbiology, 413-423.

29. Nicolas Troillet, Matthew H. Samore, and Yehuda Carmeli 1997 Imipenem-Resistant *Pseudomonas aeruginosa:* Risk Factors and Antibiotic Susceptibility Patterns *Clinical Infectious Diseases*;25:1094-8

30. Raje Ns, Rao SR et al . Infection analysis in acute lymphoblastic leukemia: a report of 499 consecutive episodes in India. Pediatr Hematol oncol. 1994;11:271-280.

31. Wang H, Chen MJ, 2003 Changes of antimicrobial resistance among nonfermenting gram-negative bacilli isolated from intensive care units from 1994 to 2001 in China *Zhonghua Yi Xue Za Zhi*. 10;83(5):385-90

32. Seema Bose et al. Incidence of Metallo betalactamases producing pseudomonas aeruginosa in burn ward of a tertiary care rural hospital. 2012. IJBR 3 [05];233-238.

33. Eifrig Cw, Scott IU et al . Endophthalmitis caused by Pseudomonas aeruginosa. Ophthalmology.2003; 110: 1714-1717.

34. Cole.N.Krockenbeger M, Bao s, et al. Effect of exogenous IL-6 during Pscudomonas aeruginosa concentration in experimental contact lens related microbial keratitis . Cornea.1993; 12: 10-18

35. Yonger JG, Shankar – Sinha S, et al. Murine complement interactions with Pseudomonas aeruginosa and their consequences during pneumonia . Am J Respir cell Mol Biol .2003; 29 : 432-438.

36. Pier GB, Grout M. Complement deposition by antibodies to Pseudomonas aeruginosa mucoid expody secharide (MEP) and by non-MEP specific opsonise. J Immunol. 1991;147:1869-1876.

37. Ni.M, Evans DJ , Hawgood et al. Surfactant protein D is present in human tear fluid the cornea and inhibits epithelial cell invasion by Pseudomonas aeurginosa .Infect Immune . 2005; 3:2147-2156.

38. Parad RB, Gerard CJ et al. Pulmonary outcomes in cystic fibrosis is influenced primarily by mucoid Pseudomonas aeruginosa and immune status and only modestly by genotype. Infect Immun. 1999; 67: 4744-4750.

39. MayTb, Shina barger D, Maharaj R,et al.Alginate synthesis by Pseudomonas aeruginosa, a key pathogenic factor in chronic pulmonary infections of patients with cystic fibrosis .Clin Microbiol Rev 1991; 4: 191-206.

40. Everett et al . Regulation of las and rhl Quorum sensing in Pseudomonas aeruginosa. Journal of Bacteriology 1997.3127-3132. 49. Prakash S. 2006. Carbapenam sensitivity profile amongst bacterial isolates from clinical specimens in Kanpur city. *Indian J Crit Care Med* 10:250-3

41. CLSI document M100-S 24.vol.34 No.1 . Performance standards for Antimicrobial Susceptility Testing;24 informational supplements.Jan14, Interpretive standards for *Pseudomonas spp. Acinetobacter spp.,S.maltophilia,B.cepacia.*

42. Fitzroy A, Orrett MD. 2004 Antimicrobial susceptibility survey of *P.aeruginosa* strains isolated from clinical sources. *J Nat Med Assoc*; 96:1065-9.

43. Magiorakos AP,Srinivasan A et al.Multidrug resistant,extensively drug-resistant and pan drug resistant bacteria:an international expert proposal for interim standard definitions for acquired resistance.Clin Microbiol Infect.2012;18:268-81.

44. Elizabeth T.S. Houang,Y.W .Chu. C.M. et al 2001. Epidemiology and Infection Control Implications Of *Acinetbacter* spp in Hong Kong *Journal Of ClinicalMicrobiology,* p228-234.

45. Mandell G, Bennett JE, Dolin R. 2000 Principles and practice of infectious diseases. Churchill Livingstone 5th ed;2:239-4

46. Frost and Sullivan Market Insight. Nosocomial infections in India: Assuming Dangerous proportions.Date published 20 july 2001.By Vasudha mukherjee.

47. Bouvet and Grimont. Taxonomy of the genus *Acinetobacter* with the recognition of *A.baumannii sp.nov., A.haemolyticus sp.nov., A.johnsonii sp.nov., A.junii sp.nov.,* and emended descriptions of *A.calcoaceticus* and *A.lwoffii.* Int J Syst. Bacteriol.1986;36:p.228-240.

48. Jonathan Cohen, William G Powderly, Steven M.Opal Infectious Diseases 3rd edition, Mosby Elsevier:p.1719-1721.

49. James Versalovic, Karen C Carroll, Guido Funke, James H Jorgensen, Marie Louise Landry, David W Warnock. Manual of Clinical Microbiology.10th edition, ASM press, Washington, DC:p.714-720.

50. MAA Sarhan, AA Osman, WO Haimour, MN Mohamed, TH Taha, NM Abdalla. Identification of *Acinetobacter* clinical isolates by PCR analysis of 16-23S ribosomal ribonucleic acid internal transcribed spacer. Indian J Med Microbiol.2014;32(2):p.143-147.

51. Jubelle K, Valenzuelal, Lee Thomas, Sally R. Partridgel.Horizontal Gene Transfer In a Polyclonal Outbreak of Carbapenem.American Society for Microbiology Journal of Clinical Microbiology.

52. M.E.Falagas, E.A.Karveli, I.I.Siempos & K.Z. Vardakas. Epidemiol Infect.2008 August 136(8):p.1009-101922.

53. Sinha M, srinivasa H, Macaden R. Antibiotic resistance profile & Extanded Spectrum Beta-lactamase production in *Acinetobacter species*.Indian J Med Res 2007;126:63-67

54. P Gladstone, P Rajendran, K N Brahmadathan. Incidence of carbapenem resistant Non fermentative gram negative bacteria from patients with

respiratory infections in the intensive care units.Indian J Med Microbio.2005;23(3):189-191.

55. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in north India. Indian J Med Res. 2006 Jul;124(1):95-8.

56. R.Srinivasa Rao, R Uma Karthika, SP Singh, P Shashikala, R Kanungo, S Jayachandran, K Prasanth.Correlation between Biofilm Production and Multiple Drug resistance in Imipenem resistance clinical isolates of *A.baumannii*. Indian J of Medical Microbiology 2008 ,26(4):p.333-337.

57. Wand M, Olive GM et al. Corneal perforation and Iris prolapse due to *Mima polymorpha*.Arch ophthalmol.1975;93:239-41.

58. K Prashanth and S Badrinath. Invitro susceptibility pattern of *Acinetobacter species* to commonly used Cephalosporins, Quinolones and Aminoglycosides. Indian J Med Microbiol2004;22(2):p.97-103.

59. Li J, Nation RL, Milne RW, Turnidge JD, Coulthard K et al. Colistin the Reemerging antibiotic for MDR gram negative bacterial infections. Lancet Infect Dis 2006;6:p.581-601.

60. V Gupta, N Bansal, S Palta, S Gombar, J Chander. Comparative Invitro activity of polymyxins against Carbapenem susceptible and resistant non-fermenters from critically ill patients.Indian J Med Microbiol 2014; 32(2): p.203.

61. Rice LB. Challenges in Identifying New Antimicrobial Agents effective for treating infections with *A.baumannii & P.aeruginosa*. Clin Infect Dis 2006; 43: p.100-5.

62. F Simsek, H Gedik, M T Yildirmak, NE Iris. Colistin against colistin-onlysusceptible *A.baumannii* related infections. Monotherapy or Combination

63. Lisa LMaragakis and Trish M Perl. *Acinetobacter baumannii* epidemiology, Antimicrobial resistance and Treatment options. Clinical Inf D 2008; 46: p.1254-63.

64. Gautam.V, Ray.P, Vandamme.P, Sharma M et al 2009 Identification of lysine positive non-fermenting gram negative bacilli *Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex *Indian J Med Microbiol* 27(2)128-3

65. Pavel Drevinek, Hana Hrbackova, Ondrej Cinek,. et al 2002 Direct PCR Detection of *Burkholderia cepacia* Complex and Identification of Its Genomovars by Using Sputum as Source of DNA *J. Clin. Microbiol*40: 3485-3488.

66. David L. Paterson 2006 The Epidemiological Profile Of Infections with Multidrug Resistant *Pseudomonas aeruginosa and Acinetobacter* species. *Clinical Infectious Diseases,* 43:S43-8

67. Livermore, DM: Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? Clin Infect Dis 2002; 34: 634-640.

68. Kohler, T.,M. Carbapenem activities against Pseudomonas aeruginosa: respective contributions of opr D and efflux systems. Antimicrob. Agents chemother.43:424-427.

69. Ambler RP.The structure of beta-lactamases, Phil Trans R Soc lond B1980; 289: 321-331

70. Bush K, Jacoby Ga, Medeiros AA. A functional Classification scheme for betalactamases and its correlation with molecular structure. Antimicrob. Agents Chemother 1995;39:1211-33.

71. David L. Paterson and Robert A. Bonomo 2005 Extended-Spectrum ß-Lactamases: a Clinical Update *Clinical Microbiology Reviews,* p. 657-686, Vol.18, No. 4

72. CLSI document M100-S 24.vol.34 No.1 Tab-2B-2, Performance standards for Antimicrobial Susceptility Testing;24 informational supplements.Jan14, Interpretive standards for *Pseudomonasspp.,Acinetobacter spp.*

73. Lee K, lee WG, Uh Y, Ha GV, Cho J, Chong Y. VIM and IMP type MBL producing *Pseudomonas species and Acinetobacter spp.* in Korean Hospitals. Emerg Infect Dis 2003;9:868-71.

74. Richa Hans, Dakshina Bisht, Ritu Agarwal, M.Irfan.Phenotypic detection of MBL, AMPC Beta-lactamase and Carbapenemases in Multidrug resistant isolates of Acinetobacter baumannii, IJMRHS vol.4 Issue 2 23rd Jan 2915. ISSN: 2319-5886.

75. Maragakis Lh, Pcrl TM. Acinetobacter baumanni:epidemiology, antimicrobial resistance, and treatment options. Clin Infect dis 2008., 46: 1254-63.

76. Michalo poulas A,Falagus ME. Colistin and polymyxin B in critical care. Crit care clin 2008;24:377-391

77. Antoni adou A, kontopidou F, Poulakou G. colistin resistant isolates of klebsiella pneumonia emerging in intensive care unit patient: first report of a multiclonal cluster. J Antimicrobe chemother 2007; 59:786 -790

78. SM Amudhan, U Sekar, K Arunagiri, B Sekar.OXA beta lactamase mediated carbapenem resistance in *A.baumannii*.Indian J of Med Microbiol 2011; 29(3): 269-74.

79. M.Shanthi Amudhan,U Sekar K Arunagiri, B Sekar.bla $_{IMP}$ bla$_{VIM}$ mediated carbapenem resistance in Pseudomonas and Acinetobacter species in India. J Infect Dev Ctries 2012;6(11):757-762.

80. Lee K, lee WG, Uh Y, Ha GV, Cho J, Chong Y. VIM and IMP type MBL producing *Pseudomonas species and Acinetobacter spp.* in Korean Hospitals. Emerg Infect Dis 2003;9:868-71.

81. Galani I, Kontopidou F, Souli M, Rekatsina PD et al. Colistin susceptibility testing by E test & Disc Diffusion methods. Int J Antimicrobial Agents 2008; 31: 434-9.

82. Kumar AV, Pillai VS, Dinesh KR, Karim S. The phenotypic detection of carbapenamase in meropenem resistant *Acinetobacter calcoaceticus-baumannii* complex in a TCH in South india. J Clin Diagn Res 2011;5:223-6.

83. Noyal MJ, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple Screening tests for detection of carbapenamases in clinical isolates of nonfermentative gram-negative bacteria. Indian J Med Res 2009;129:707-12.

84. Anil VK, Vishnu SP, Kavitha R, Dinesh, Shamsul. The Phenotypic Detection of Carbapenamases in the Meropenem resistant *A.calcoaceticus baumannii complex* in a Tertiary care Hospital in a south India.J of Clin Diagnostic Research 2011;5:p.22-26.

85. Dheepa Muthusamy & Appalraju Boppe. Phenotypic Methods for the detection of various Betalactamases in Carbapenamase resistant isolates of *A.baumannii* in a Tertiary care Hospital in a south India. India.J of Clin Diagnostic Research. 2012;6(6):p.970-73

86. Kalidas Rit*, Falguni Nag**, Hirak Jyoti Raj, PK Maity ,Prevalence and Susceptibility Profiles of Nonfermentative Gram-negative Bacilli Infection in a Tertiary Care Hospital of Eastern India Indian Journal of Clinical Practice, Vol. 24, No. 5, October 2013

87. Muktikesh Dash, Sanghamitra Padhi, Swetlana Pattnaik, Indrani Mohanty, Pooja Misra. Frequency, risk factors, and antibiogram of *Acinetobacter* species isolated from various clinical samples in a tertiary care hospital in Odisha, India. Avicenna journal of Medicine 2013;3(4):97-102

88. Nautiyal S,JauhariS,GoelN,Mahawal BS, Current trend of Nonfermenting Gram Negative Bacilli in a Tertiary Care Hospital In Dehradun, Uttarkhand ,International Journal of Advanced Research (2014),Vol.2,Issue 2,322-328.

89. Sohaila Mushtaq et al. Antimicrobial Susceptibility Pattern of *Acinetobacter* species isolated from Clinical specimens in a Tertiary care hospital. Biomedica 2013;29:23-26.

90. Malini A, Deepa EK, Gokul BN, Prasad SR. Nonfermenting gram‐negative bacilli infections in a tertiary care hospital in Kolar, Karnataka. J Lab Physicians 2009;1:62‐6.

91. Deepak Juyal, Rajat Prakash, Shamanth A.Shankarnarayan,, Munesh Sharma, Vikrant Negi, Neelam Sharma. Prevalence of non-fermenting gram negative bacilli and their in vitro susceptibility pattern in a tertiary care hospital of Uttarkhand: A study from foothills of Himalayas.Saudi journal for Health Sciences-vol2, issue2, May-August 2013.

92. Mariyam Noori et al. High prevalence of MBL producing *A.baumannii* isolated from Two hospitals of Tehran, Iran. Paediatric Infec Disease 2014; 3(1).

93. Veenu, Rama S, Arora DR. 1999 Isolation and susceptibility pattern of non fermenting Gram negative bacilli from clinical samples.Indian J Med Microbiol;17(1):14-7.

94. Vijaya D, K.Bavani S, Veena M, 2000 Prevalence of nonfermenters in clinical specimens:54:87-91 Indian journal Of medical sciences volume54, Issue 3, p87-91.

95. Yang Soon Lee , Young Ree Kim et al. Increasing prevalence of blaOXA-23 carrying *A.baumannii* and the emergence of blaOXA-182 carrying A.nosocomialis in Korea. Diag. Microbiol & Infectious Disease2013; 7(2): 160- 63.

96. Nasrollah Sohrabi et al. Prevalence of OXA type beta lactamases among *A.baumannii* isolates from North west of Iran. Microbiol Drug Resistance 2012;18(4):385-9.

97. Roxanne J Owen, Jian Li, Roger L Nation, Devis Sperman. Invitro Pharmacodynamics of Colistin against *A.baumannii* clinical isolates. J of Antimicrobial Chemotherapy.2006;59(3):473-77.