“CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF NONFERMENTERS”

By

DR. SHILPA .K. GOKALE

Dissertation

Submitted to the

KLE University

Belgaum, Karnataka

In partial fulfilment

of the requirements for the degree of

DOCTOR OF MEDICINE (M.D)

in

MICROBIOLOGY

Under the Guidance of

DR. (MRS) S. C. METGUD M.D (MICRO)

DEPARTMENT OF MICROBIOLOGY
J.N.MEDICAL COLLEGE, NEHRU NAGAR,
BELGAUM-590010.

MAY 2009

KLE UNIVERSITY BELGAUM, KARNATAKA

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF NONFERMENTERS"** is a bonafide and genuine research work carried out by me, under the guidance of **Dr. (Mrs). S. C. Metgud** **MD (MICRO)**, Professor & Head, Department of Microbiology, J. N. Medical College, Belgaum.

Date:

Place: Belgaum. **(Dr. Shilpa K. Gokale)**

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF NONFERMENTERS"** is a bonafide research work done by **Dr. Shilpa K. Gokale** in partial fulfilment of the requirement for the degree of MD Microbiology.

Date:

Place: Belgaum.

Dr. (Mrs). S. C. Metgud MD (MICRO)

Professor & Head,

Department of Microbiology,

J.N. Medical College,

Belgaum – 590010.

ENDORSEMENT BY THE HOD, PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF NONFERMENTERS"** is a bonafide research work done by **Dr. Shilpa K. Gokale** under the guidance of **Dr. (Mrs). S. C. Metgud** MD (MICRO), Professor & Head, Department of Microbiology, J. N. Medical College, Belgaum.

Dr.S. C. Metgud MD(MICRO)
Professor & Head
Department of Microbiology,
J.N.Medical College,
Nehru Nagar,
Belgaum-590010

Date:
Place: Belgaum.

Dr.V.D.Patil MD, DCH
Principal
J.N.Medical College,
Nehru Nagar,
Belgaum-590010

Date:
Place: Belgaum.

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the KLE University Belgaum, Karnataka shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic/research purpose.

Date:

Place: Belgaum **Dr. Shilpa K. Gokale**

© KLE University Belgaum, Karnataka

ACKNOWLEDGEMENT

At the outset, I would like to express my deep sense of gratitude towards my guide ***Dr. (Mrs) S.C. Metgud M.D.*** *Professor and H.O.D, Department of Microbiology, J.N. Medical College, to whom I am indebted in many ways. Her personal interest and the enthusiasm towards this study and the subject of Microbiology is truly remarkable. Her wealth of knowledge on the subject and experiences with various cases and situations is worth a mention. She has always been very critical and analytical from a wholly constructive view point, always making constructive suggestions to improve not only in this study but also my entire approach to the subject and its practice. She has boosted my morale where required and for her constant willingness and amenability, I am grateful.*

I am thankful to ***Dr. S.G. Karadesai, M.D.*** *Professor in the Department of Microbiology, J.N. Medical College for his ranking advice not only in dissertation work but also in my overall performance in the field of Microbiology.*

I am also very grateful to ***Dr. M.G.Kulkarni, M.D.*** *Professor in the Department of Microbiology, J. N. Medical College Belgaum for his help and guidance during my course.*

My sincere gratitude to ***Dr. (Mrs) J.M. Nagmoti*** *Professor and* ***Dr. Mahantesh Nagmoti*** *Professor, Department of Microbiology J.N.Medical College for their unhesitating, helpable guidance, constant encouragement and motivation.*

I congregate my gratification to ***Dr. Sumati Hogade,*** *Associate Professor,* ***Dr. Manjula*** *Assistant Professor,* ***Dr. Shashank*** *Assistant Professor and* ***Dr. Sheetal*** *Assistant Professor,* ***Dr Madhumati*** *Assistant Professor and* ***Dr. Kavita***

Assistant Professor J.N.Medical College for their help and guidance during my course.

I wish to convey my gratitude to my co-PG Dr. ***Premalatha,*** *who is a great friend and of great help through out my study.*

I would like to say a special thanks to my fellow post graduates and MSc students ***Dr. Narayan, Dr. Basavraj, Dr. Gulnar, Dr. Rashmi, Dr. Swaroopa, Dr. Sheela, Preeti*** *and* ***Shobha*** *for their support.*

I would like to thank Technical Staff and Attainders for their help.

I thank my ***Parents*** *and* ***In-laws*** *for their support and patience.*

I thank my husband ***Shashi*** *for his moral support and help through out the study.*

I thank my sweet little daughter ***Shree*** *for being the constant source of joy.*

I express my thanks to Miss. Veena of ***Sai DTP & Xerox Centre,*** *for her dedicated work and patience in designing and printing of my dissertation.*

Date: ***Signature of the Candidate***

Place: Belgaum

Dr. Shilpa K Gokale

LIST OF ABBREVIATIONS

CDC	Centre for disease control
CHEF	Clamped homogenous electric field
DNA	Deoxyribo nucleic acid
EDTA	Ethylene diamine tetra acetate
ELISA	Enzyme linked immunosorbent assay
FIGE	Field inversion gel electrophoresis
LPS	Lipopolysaccharide
MLEE	Multilocus enzyme electrophoresis
OFPBL	Oxidative fermentative polymyxin-B, Bacitracin, Lactose medium
ONPG	O-nitrophenyl, galactopyranoside
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
RAPD	Random amplified polymorphic DNA finger printing
RNA	Ribo nucleic acid

ABSTRACT

INTRODUCTION :

Nonfermenters are being isolated from various clinical specimens. Although frequently considered as contaminants, the pathogenic potential has been proved beyond doubt by their frequent isolation from clinical material and their association with disease. Most of the Nonfermenters are resistant to routinely used antibiotics. This study has been undertaken with a view to know the incidence of pathogenic nonfermentative gram negative bacilli from various clinical specimens like pus, urine, sputum, blood, aural swabs, nasal swabs, CSF, wounds, endotracheal aspirates etc. during one year period. Sensitivity of the organisms to antimicrobial agents has also been tested and the results reported.

MATERIAL AND METHODS :

The present study was undertaken at the Department of Microbiology, J.N Medical college, Belgaum during the period from 01/01/2007 to 01/01/2008. A total of 130 nonfermenters isolated from clinical samples such as pus, sputum, urine, blood, ear swab, catheter tip and pleural fluid were included in the study. Isolates from clinical samples which gave an alkaline/alkaline (K/K) or alkaline/neutral (K/N) reaction on Triple sugar iron agar. All these were identified by a battery of tests as per standard laboratory techniques. Antimicrobial sensitivity testing was performed on the 130 isolates by Kirby bauer disc diffusion method on Muellor Hinton Agar plate using standard antimicrobial agents.

RESULTS :

Out of 130 isolates, 107(82.3%) were Pseudomonas aeruginosa, 20(15.4%) were Acinetobacter baumanii, 1(0.76%) each of A.lwofii, B.psudomallei and

Moraxella were isolated. The majority of the isolates were isolated from pus/wound discharge (78) followed by blood (30), urine(11), sputum(6) and 2 each from catheter tip and ear swab. Out of 107 isolates of P.aeruginosa 96.2% were sensitive to Meropenem, 50% to Ciprofloxacin, 49.5% to Amikacin. Isolates were least sensitive to Ampicillin, doxycycline, Cefotaxime and Piperacillin. A.baumanii was more sensitive Meropenem(96.2%) followed by Ciprofloxacin (45%), Doxycycline (35%) and Amikacin(25%).

CONCLUSION :

This study shows that Nonfermenters are isolated from clinical specimens frequently and are resistant to most of the routinely used antibiotics showing that these organisms need to be taken seriously and identified and not just regarded as contaminants.

TABLE OF CONTENTS

SL.NO	SECTIONS	PAGE NO.
1	INTRODUCTION	1
2	OBJECTIVES	3
3	REVIEW OF LITERATURE	4
4	MATERIAL AND METHODS	57
5	OBSERVATIONS AND RESULTS	73
6	DISCUSSION	81
7	CONCLUSION	87
8	SUMMARY	88
9	BIBLIOGRAPHY	90
10	ANNEXURES	
	Annexure I: Media	97
	Annexure II: Proforma	103
	Annexure III : Informed Consent	106
	Annexure IV: Master Chart	108

LIST OF TABLES

Sl.No	TABLES	Pg. No.
1	Nonfermenter isolates from different clinical specimens	73
2	Distribution of nonfermenters in different age and sex groups	75
3	Nonfermentative bacilli in clinical specimens n =130	76
4	Biochemical characteristics of nonfermenters	77
5	Antibiotic sensitivity pattern of nonfermenters n=130	78
6	Antibiotic sensitivity pattern of pseudomonas aeruginosa isolates n=107	79
7	Antibiotic sensitivity of acinetobacter species and other nonfermenters	80

LIST OF PHOTOGRAPHS

Sl. No	PHOTOS	Pg. No.
1	Gram's stain showing gram negative bacilli of P. aeruginosa	67
2	Gram's stain showing gram negative coccobacilli of A. baumanii	67
3	Pigmented colonies of P.aeruginosa on Nutrient agar	68
4	Non kactose fermenting colonies of Burkholderia pseudomallei on Mac-Conkey agar	68
5	OF reactions of P.aeruginosa	69
6	OF reactions of B.pseudomallei	69
7	OF reactions of A.baumanii	70
8	OF reactions of A. lwofii	70
9	Oxidase Test	71
10	DNAse Test	71
11	Antibiogram of P.aeruginosa	72

LIST OF GRAPHS

Sl. No.	Graphs	Page No.
1.	Nonfermenter isolates from different clinical specimens	74
2.	Distribution of nonfermenters in different age and sex groups	75
3.	Nonfermentative bacilli in clinical specimens n=130	76
4.	Antibiotic sensitivisty pattern of nonfermenters n=130	78
5.	Antibiotic sensitivity pattern of pseudomonas aeruginosa isolates n=107	79
6.	Antibiotic sensitivity of acinetobacter species and othernonfermenters	80

INTRODUCTION

Nonfermenters are a group of aerobic non-spore forming gram negative bacilli that are either incapable of utilizing carbohydrates as a source of energy or degrade them via oxidative rather than fermentative metabolic pathways[1].

The nonfermentative gram negative bacilli and coccobacilli are ubiquitous in environment. They are found in soil, water, plants, decaying vegetation and food stuffs, in hospitals they are isolated from nebulisers, dialysate fluids, saline and catheter devices. These organisms are variably resistant to agents such as chlorhexidine and quarternery ammonium compounds[2,3].

Although frequently considered as commensals or contaminants, the pathogenic potential of nonfermenters has been proved beyond doubt. The nonfermenters accounts for approximately 15% of all isolates of gram negative bacilli found in the clinical microbiology laboratory. Some of the disease manifestations associated with nonfermenting organisms are septicemia, meningitis, osteomyelitis, UTI, pneumonitis and wound infections usually following surgery or trauma[4].

Nonfermenters are being encountered with increasing frequency as agents of opportunistic and very serious infections as well as nosocomial infections. Nonfermenters have a low degree of virulence and most often cause nosocomial infections in patients who are debilitated or immunocomprmised [1,5].

Nonfermenters play an important role in human infections and many of them are resistant to commonly used antibiotics. Early identification of nonfermenters is essential for appropriate treatment[2].

Despite widespread use of antibiotics, the hospital acquired infections still pose great problem. Several factors influence the changing spectrum of ecological agents like increased level of drug resistance, bacterial synergism, environmental risk factors and a higher number of compromised hosts[1]. For these reasons a lot of opportunists such as the nonfermenting gram negative bacilli emerged creating a significant therapeutic challenge[6].

This study has been undertaken with a view to know the incidence of pathogenic nonfermentative gram negative bacilli from various clinical specimens like pus, urine, sputum, blood, aural swabs, nasal swabs, CSF, wounds, endotracheal aspirates etc. during one year period. Sensitivity of the organisms to antimicrobial agents has also been tested and the results reported.

OBJECTIVES

The current study was undertaken

1. To identify and characterise nonfermentative gram negative bacilli isolated from various clinical samples
2. To study the antibiotic susceptibility pattern of clinical isolates

REVIEW OF LITERATURE

CLASSIFICATION OF NONFERMENTERS

The Bergeys manual(9th edition) divides the family Pseudomonadaeceae into four genera. The type species of this family is Pseudomonas, the other three genera are Xanthomonas, Frateuria, Zoogioea[7].

The main body of classification of Pseudomonas species followed the grouping proposed by Palloroni et al based on rRNA/DNA homology studies. The studies of Woese et al on sequence analysis of 16s ribosomal RNA component, confirmed the proposed internal subdivision of Pseudomonas into five rRNA groups, which were further classified on the basis of poly-b-hydroxybutyrate accumulation, arginine dihydrolase, pigment, cytochrome oxidase, utilisation of glucose and denitrification[7.]

Some members of Pseudomonas and Xanthomonas were seen to share a substantial degree of rRNA/DNA homology. Pseudomonas maltophila was oxidase negative and reduced methinine, like xanthomonads. These also had similarities in the enzymes they produced. Therefore in 1983, it was proposed that Pseudomonas maltophilia be reclassified from the genus pseudomonas to the Xanthomonas and renamed as Stenotrophomonas maltophilia.

In 1992 Yabuchi et al proposed that the seven species constituting r-RNA group 2 of genus Pseudomonas were transferred to the genus Burkholderia with Burkholderia cepacia as the type species[8].

In 1986, Bouvet and grimont provided a classification of the genus Acinetobacter into 12 genomospecies based on DNA-DNA hybridization. In 1989, Tjernberg and Ursing added 3 DNA groups 13 through 15 and concurrently Bouvet

and Jean-Jean described 5 groups, 13 through 17. Two groups of Tjernberg and Ursing were phenotypically different from the corresponding groups have the same number. Thus there is still considerable confusion surrounding the subdivision of the species[1].

The family Flavobacteriaeceae includes genus Flavobacterium and initially included several species, F.aquatile (1989), F.balustinum (1929), F.meningosepticum(1959), F.odoratum (1929), F.multivoram (1981)and F.spiritovorum (1982). In 1994 Vandamme et al reported that none of the established species were closely related to the type species F.aquatile. Thus F.balustinum, F.gleum, F.indologenes, F.meningsepticum and others were included in the genus Chryseobacterium and F.spiritivorum and F.multivorum were included in genus Sphingobacterium.

In 1986, the family Flavobacteriaeceae was classified to include seven rRNA clusters[1].

In 1986, Kiredjian et al proposed the new names Alcaligenes xylosoxidans subsp xyosoxidans (formerly Achromobacter xylosoxidans) Alcaligenes xylosoxidans sub sp denitrificans (formerly Alcaligenes dentrificans) based on r RNA studies. They noted that the type species Alcaligenes odorans and introduced another new species Alcaligenes piechaudii[1].

PRESENT CLASSIFICATION

The term nonfermentative gram negative bacilli is used to mean all aerobic gram negative rods that show abundant growth within 24 hours on the surface of TSI

medium, but neither acidify the butt of medium. They comprise 1/5 of all gram negative bacilli from extra intestinal lesions[8].

The major genera of nonfermenters have been classified in to five families Alcaligenaceae, Flavobacteriaeceae, Methylococcaceae, Psuedomonadaceae and Rhizobiaceae. The remaining are not yet assigned to a family and grouped under "organisms whose taxonomic position is uncertain".

Another approach to group nonfermenters is on the basis of motility, type of flagella in motile strains and cytochrome oxidase.

I) Motile with peritrichous flagella:

i) Family psuedomonadaceae:

r RNA group-1:

Fluorescent group : Pseudomonas aeruginosa

Pseudomanas flourescens

Pseudomonas putida

Stuzeri group : Pseudomonas stuzeri

Pseudomonas mendocina

CDC group vb-3

Alkaligenes group : Pseudomonas alkaligenes

Pseudomonas pseudoalkaligenes

r RNA group-2:

Pseudomonas group : Burkholderia mallei

Burkholderia pseudomallei

		Burkholderia cepacia
		Burkholderia gladioli
		Burkholderia pickettii
r RNA group-3:		
Acidovorans group	:	Comamonas acidovorans
		Comamonas terrigena
		Comamonas testosterone
Facillis-delafiedii group	:	Acidovorax delafieldii
		Acidovorax facilis
		Acidovorax temperans
r RNA group-4:		
Diminuta group	:	Brevundimonas diminuta
		Brevundimonas vesicularis
r RNA group-5	:	Stenotrophomonas maltophila.

ii) Unknown nucleic acid homology :

- Chryseomonas luteola
- Flavimonas oryzibalbitans
- Sphingomonas paucimobilis
- Shewanella putrefaciens
- Pseudomonas like group-2

- Methylobacterium
- Roseomonas
- CDC group 1

II) Motile with peritrichate flagella:

i) Family Alcaligeneceae

Genus Alcaligenes

ii) Family Rhizobiaceae

Genus Agrobacterium

iii)Uncertain Taxonomic position

Genus Achromobacter

Ochrobactrum anthropi

Oligella urealytica

CDC Group 4 c-2

III) Non motile, oxidase positive:

i) Family Flavobacteriaceae

Genus Flavobacterium

Genus Chryseobacterium

Genus Empedobacter

Genus Weekesella

Genus Bergeyella

ii) Family Sphingobacteriaceae

Genus Sphingobacterium

iii) Family Moraxellaceae

Genus Moraxella

iv) Family Alcaligenaceae

Genus Oligella

v) Uncertain taxonomic position:

CDC groups EO-3

Gilardi rod group 1

IV) Non motile oxidase negative:

Genus Acinetobacter

Genus Bordetella

CDC Group No-1

CHARACTERISTICS OF NONFERMENTERS

Characteristics of each individual group:

Family Pseudomonadaceae:

Fluorescent group:

The organisms in this group are all characterized by production of awater soluble pigment that fluoresces white to blue-green under long wavelength(400nm) UV light. This pigment is pyoverdin produced by all 3 members of this group.

However, pyocyanin, which is a distinct blue, water soluble pigment, produced only by Pseudomonas aeruginosa.

Pseudomonas aeruginosa:

Morphology: They are rods 1.5-3μ×0.5μ, straight with rounded ends. They are nearly all motile with polar flagella. The normal fimbriae of Pseudomonas aeruginosa differ those of Enterobacteria, in that they do not cause haemagglutination of red cells. Some strains have thicker non polar fimbriae associated with carriage of drug resistant plasmids. Mesosome-like structures have been demonstrated and rearrangement of membranes and mesosomes give rise to neurotubular structures rhapidosomes[9].

Cultural characteristics:

Pseudomonas aeruginosa on nutrient agar forms irregularly round, effuse colonies 2-3mm in diameter, matt surface and butyrous in consistency (type 1). Type 2 colonies are round, smaller, raised and coliform-like. Type 3 colonies are round, smaller, raised and coliform-like. Type 3 colonies are raised and umbonate or rugose and form a granular suspension in saline. Some colonies are mucoid. Many of these strains are non-flagellate and do not produce pyocyanin. Dwarf forms are also seen

visible only after 48-72 hours. The last 2 are seen most often in sputum from patients with cystic fibrosis[9].

Mucoid strains produce an extracellular polysaccharide called alginate which is a polymer of mannuronic and glucoronic acids. This may form a glycocalyx in which the microcolonies are entrapped and protected from host defences.

Non mucoid strains including revertants of mucoid strains produce small amounts of alginate. Two chromosomal genes have been associated with the production of alginate. In-vitro, mucoid variants can be selected by action of bacteriophages and by antibiotics in sublethal concentrations. Bacteriophages that lyse Pseudomonas aeruginosa have been found in sputum of cystic fibrosis patients and phages from mucoid strains were better able to convert non-mucoid strains to mucoid strains[9].

Other extracellular products:

Pseudomonas aeruginosa and related plant pathogens form a heat dialyzable phytotoxin. Also pyocyanin has ability to inhibit mitochondrial enzymes and to cause disruption and cessation of ciliary beat on ciliated nasal epithelium. Extracellular enzymes and hemolysins play a key role in production of local lesions. Several proteolytic enzymes, including a general protease, an alkaline protease and an elastase are produced. These probably contribute to haemorrhages and ecthyema gangrenosum.

Two distinct hemolysins are produced a heat labile enzyme (phospholipase) and a heat stable rhamnolipid. PhospholipaseC causes redness and induration on intradermal injection. The highest frequency of production is amongst urinary isolates

and production is greater from non-mucoid isolates from cystic fibrosis than their mucoid counterparts. Most strains are lipolytic, by an extracellular lipase[9].

A number of substances are elaborated which are inhibitory to other bacteria. These are mainly pigments with their derivatives. An oily substance produced is active against vibrio cholerae. The haemolytic rhamnolipid has action against tubercle bacillus.

Exotoxins- 2 extracellular ADP ribosyl transferases, exotoxins A and S are produced. Exotoxin A inhibits protein synthesis by inhibiting the transfer of ADP-ribose from NAD to elongation factor 2. A proenzyme is formed which is inactive. The toxin causes hypotension, shock, hepatic necrosis and leucopenia in animals. In tissue it causes disruption of collagen, loss of ground substance and death of epithelial and endothelial cells. 90% of strains produce both exotoxin A and S. Exotoxin S is antigenically distinct and acts differently. Specific antibody to them can be demonstrated in serum of patients. The antitoxin protects against mortality.

Other toxin products:

An acidic cytotoxin, a protein that binds pyochelin in a complex with iron, a slime composed of polysaccharide protein and polymerized nucleic acid. Active and passive immunization against slime prevented the death of mice challenged with live organisms.

Exotoxins- A lipopolysacchride(LPS) which causes endotoxic shock and death in mice is produced. Antibody against the O-side chain of chain of LPS is protective to man and acts by enhancing opsonization and bacterial killing. Thus O-side chain gives antigenic specificity while the lipid A moiety is toxic.

Bacteriocins:

R-pyocins, resembling the tails of contractile phages and F- pyocins which are rod shaped and flexuous are produced in 90% strains. 70% strains also produce S-pyocins, which are low molecular weight and diffusible through agar. Pyocins are active only against other members of this species and not against other pseudomonads. Occasionally they may be active against other fluorescent Pseudomonads[8].

Bacteriophages:

Phages active on Pseudomonas aeruginosa can be isolated from sewage and lysogenic strains. Most phages belong to Bradleys type A which is resistant to heating at 60c and exposure to chloroform. They may have DNA or RNA, a latent time of 35-60 minutes and a burst size of 10-200 phages per host cell[9].

Antigenic structure:

LPS contains O-specific polysaccharide side chains linked through a common core oligosaccharide to lipid A. The side chains contain unbranched tri or tetrasaccharides rich in N-acetylated amino sugars. O antigens are heat stable and can be extracted with acid or formamide. They can be detected by precipitation reactions in agar gel, tube or slide agglutination, indirect hemagglutination or ELISA.

Other antigens:

Six major proteins have been identified. Protein F, protein I, Protein H(resolved in to H1 and H2). Fimbrial antigens are not easily differentiable, and fimbrial typing is unreliable.

Pigments:

At least four distinct pigments have been described in Pseudomonas aeruginosa; pyocyanin, fluorescein, pyorubin and pyomelanin. Pyocyanin is a phenazine which is extractable by dissolving in chloroform. Fluorescein(pyoverdin) is insoluble in chloroform but soluble in water. Pyorubin is most often noticed in urinary isolates. The production of pyomelanin is rare, seen in less than 1% of isolates.

Susceptibility to physical and chemical agents:

Pseudomonas aeruginosa is killed at 55^0c in 1 hour, survives for months in water at ambient temperature and multiplies in water containing minimal nutrients such as hospital stocks of distilled water. It is partially resistant to quarternary ammonium compounds, particularly cetrimide and benzalkonium chloride. It has been isolated from antiseptic solutions (hexachlorophane containing creams and solutions and povidone iodine and chlorhexidine solutions). It is sensitive to acid and silver salts. The action of a number of disinfectants is strongly potentiated by addition of ethylenediamine tetra acetate(EDTA).

Susceptibility to Antimicrobial agents:

Some lactams are active carbenicillin, ticarcilin, azlocillin, mezlocillin, and piperacillin; cefoperazone, cefotaxime, cefuldon, ceftazidime, aztreonam, and imipenem. The clinical isolates are more resistant than environmental isolates[10].

Mechanism of resistance of -lactam antibiotics:

- The production of lactamase: A chromosomal and plasmid mediated lactamase are produced. The chromosomal enzyme is more commonly produced. It is normally repressed but iduced by 1st and 2nd generation cephalosporins and by ampicillin. These enzymes are produced transiently,

enzyme production returns to the basal level on removal of the inducer. These depressed mutants can be selected in vivo during therapy. These enzymes cause resistance to almost all - lactams except imipenem, carbenicillin and temocillin. Plasmid mediated -lactamases are present only in minority of the clinical strains.

- The main reason for high intrinsic resistance is the minority of F-porin molecules which form channels across the membrane and cause passage of antibiotics. Less no. of F-porin channels results in low permeability.
- Altered affinity of target penicillin- binding proteins: Pseudomonas aeruginosa alters aminoglycosides by acetylation phosphorylation or adenylation. ciprofloxacin is highly active but resistance may appear during treatment. Administration of pegylated liposomal ciprofloxacin increases the concentration of ciprofloxacin in blood and produces sustained concentration with increase in the area under the plasma concentration time curve(AUC). The AUC/MIC (Minimum inhibitory concentration) ratio is associated with favourable outcome. Mortality is decreased but complete eradication is never observed. chloramphenicol and trimethoprim cross the cell wall poorly. Most strains form a chloramphenicol modifying enzyme. Most aminoglycosides and antipseudomonal penicillins act synergistically against the majority of isolates. Many antibiotics show in vitro inhibition of growth of pseudomonas aeruginosa but only a minority of these show useful activity at in vivo therapeutic level[11].

Habitat:

Pseudomonas aeruginosa has been isolated from water, jet plane fuel, disinfectant solutions[12], hospital sinks and vegetables. Natural carriage by human beings is infrequent. Faecal carriage rates range from 1-15% in healthy subjects, to 60% in hospitalized patients on antibiotics[9].

Clinical infections:

Pseudomonas aeruginosa is the most commonly recovered Pseudomonad in clinical specimens and the sixth most frequent bloodstream isolate in intensive care units. Bacteremia is commoner in burns patients[12].

Predisposing factors for infection include:

- Disruption of cutaneous or mucosal barriers, e.g burns, dermatitis, penetrating trauma, surgery, endotracheal intubation, indwelling venous or urinary catheters and injection drug use[13].
- Disruption of normal bacterial flora, e.g. broad spectrum antibiotic therapy, exposure to the hospital environment.
- Immunosuppression, e.g. neutropenia, qualitative white blood cell defects, hypogammaglobulinemia defective cell mediated immunity, extremes of age, diabetes mellitus, steroid therapy, cystic fibrosis, cancer, AIDS. Infections occur more commonly at sites where moisture tends to collect[8]. Pseudomonas aeruginosa infections are associated with high rates of mortality. factors affecting mortality include[14].

- Hospitalization in ICU
- Coagulopathy
- Septic shock
- Age 65 years

Pseudomonas aeruginosa is commonly isolated from pus and wounds[30]. The exudation from the wound of a bluish pus with grape-like odour is characteristic. The organism also causes urinary tract and lower respiratory tract infections, eye infections including keratitis, corneal ulcer infections and endophthalmitis, endocarditis,meningitis, brain abscess and infections of bone and joint. Secondarily it also infects patients with burn wound, crush injury, penetrating trauma, endotracheal intubation, indwelling catheter and prolonged antibiotic therapy.

Pseudomonas fluorescens and putidas:

These exist in water and may be present in the water sources in the hospital. Pseudomonas fluorescens has been found to grow at 4^0c. Both may exist as pharyngeal flora and cause rare opportunistic infections. Pseudomonas putidas has been reported to cause septic arthritis and catheter related sepsis in cancer patients, as well as bacteremia[6]. Both species have been identified with bacteremia from transfused blood[7]. Pseudomonas fluorescens psuedobacteremia has been reported to be due to contamination of lithium heparin tubes which were being used for blood collection[9].

Stuzeri Group:

Pseudomonas stuzeri is present in soil and water and has been recovered from humans, manure, straw, sewage, stagnant water, baby formula, hospital equipment, cosmetics and various clinical specimens. It has rarely been associated with infections of the ear, conjuctiva, pneumonia, septic arthritis, endocarditis, meningitis and wound and synthetic graft infections. it has been isolated from blood culture[8].

The organisms in this group are all soil elenitrifiers and can grow anaerobically in nitrate containing media, with production of nitrogen gas.Freshly isolated colonies have a characteristic wrinkled appearance. At least 7 genomic groups (called genomovars) have been identified. Pseudomonas stuzeri has a wider pattern of antibiotic sensitivity than other pseudomonads. It is sensitive to ampicillin, carbenicillin, colistin and gentamicin but is often resistant to first generation cephalosporins. It is more sensitive than Psuedomonas aeruginosa to quarternary ammonium compounds[9].

Pseudomonas mendocina and CDC Group vb-3 are rarely isolated.group vb-3 resembles Psuedomonas stuzeri except it is arginine positive. A case of vb-3 septicemia has been reported in a case of multiple myeloma[8].

Alcaligenes group:

The members of this group are asaccharolytic or weakly saccharolytic in OF glucose medium. There have been reports of eye infections, empyema and one case of endocarditis caused by Pseudomonas alcaligenes. Pseudomonas alcaligenes is sensitive to carbenicillin and sulphonamides[9].

Pseudomallei group:

Burkholderia mallei causes respiratory infection called Glanders. Burkholderia psuedomallei causes melioidosis. Burkholderia cepacia is a slender multitrichous rod that stains irregularly [9]. Colonies on nutrient agar are diffuse greyish and may produce a purple pigment. Selective media include Psuedomonas cepacia medium (PCM), Oxidative Fermentative Polymyxin-B, Bacitracin, Lactose medium (OF PBL) [15] and TB-T medium with trypan blue and tetracycline[9]. It is a low grade human pathogen, but can cause sepsis in hospital patients[1].

Protease and lipase production has been demonstrated. The organisms can contaminate disinfectant solutions including chlorhexidine and cetsimide and can survive in distilled water for upto a year. The organism is highly susceptible to cotrimoxazole[12]. Typing by means of O and H antigens or by susceptibility to and production of bacteriocins can be done.

B.cepacia has been reported to colonise the respiratory tract of cystic fibrosis patients[15]. pseudobacteremias have been reported due to use of B.cepacia contaminated disinfectant solutions.

Pneumonia and pneumonitis can develop following use of contaminated anaesthetic, urinary tract infection following contaminated irrigation fluids, septicemia following heart surgery, endocarditis caused by contaminated valves, brain abscess, conjunctivitis and septic arthritis have been reported. Infection in a hydrocephalic child whose Holter value was colonized due to contaminated chlorhexidine has been reported. Cohorting of patients is essential to prevent spread of infection[15].

Thus B.cepacia has emerged as a cause of opportunistic human infections. B.gladioli is primarily a plant pathogen causing flower rot. It is cytochrome oxidase negative and produces yellow colonies on blood agar in 48-72 hours. It has been reported to cause pneumonia and septicemia in patients with chronic granulomatous disease, malignancies and burns[12].

Burkholderia (Ralstonia) picketii:

It is a non pigmented pseudomonad that is oxidase positive. The species is devided into 3 variants (biovar Va-1, biovar Va-2 also called B.thomasii). This organism is slow growing and produces pinpoint colonies on blood agar. It differs from B.cepacia in its failure to acidify several sugars, dulcitol, inositol and sorbitol; and to decarboxylate lysine and ornithine[16].

This organism is rarely associated with human infection but has been reported to cause nosocomial infections[8]. Colonization with B.picketii following suction with contaminated saline in patients on endotracheal tubes has been reported. These organisms are generally resistant aminoglycosides, ampicillin and colistin carbenicillin sensitivity is variable[17].

Acidovorans group:

These organisms are normally found in soil. These belong to the alkaline pseudomonads. C.acidovorans has the unique feature of producing an orange indole reaction. The organism has been isolated from many clinical specimens including blood and is usually considered to be non-pathogenic[8].

C.testosteroni is rarely isolated, being most often isolated in association with anatomic anomalies of the gastrointestinal tract. C.terrigena is not considered a human pathogen[8].

Facilis-Delafieldi group:

These are oxidase positive and motile. Acidovorax delafieldii and Acidovorax.temperans have been isolated from clinical isolates. The pathogenicity and clinical significance is not known[8].

Diminuta group:

These monotrichate organisms require pantothenate, biotin and cyanocobalamin for growth. Brevundimonas diminuta also requires cysteine or methionine.

B.vesicularis has orange or yellow colonies due to carotenoid pigment, and is distinguished by a strong esculin hydrolysis reaction. B.vesicularis has been isolated from dialysate fluid, dialysate machine, oral abscess and scalp wound[8]. B.diminuta has been recovered from blood cultures[17].

Genus Stenotrophomonas:

This is the 2nd most common nonfermenters isolated from clinical specimens. It is found in raw milk, fish and sewage. The organism requires methionine for growth. It is characterized by its ability to decarboxylate lysine and by its DNAase activity[18].

Though more frequently isolated as a colonizer than as a pathogen[16] .S.maltophilia is emerging as a major pathogen causing hospital infection and is associated with high morbidity and mortality . The organism causes opportunistic infections, especially in cancer patients[18].

S.maltophilia has been isolated from blood[18]. Pneumonia, endocarditis, cholangitis, UTI, meningitis and wound infections have also been reported[8]. Risk factors for S.maltophilia bacteremia include age over 40 years, requirement for care in

an ICU, pulmonary source of the isolate[18], malignancies, neutropenia, treatment with broad spectrum antibiotics and indwelling vascular catheters[19].

Sources of hospital infection may include chlorhexidine/cetrimide, EDTA in blood collection tubes, cardiopulmonary pump, transducer dome etc[18]. S.maltophilia is resistant to multiple antibiotics[18] especially aminoglycosides and beta-lactams, but shows good sensitivity to cotrimoxazole.

Apart from cotrimoxazole, quinolones and minocycline are the effective, though cotrimoxazole and minocycline are not bactericidal. Ticarcillin-clavulanic acid is an effective combination[19]. A combination of antibiotics is recommended for the treatment of serious infections with S.maltophilia.

Genus chryseomonas:

This genus consists of only Chryseomonas luteola. This group of organisms was previously known as CDC group Ve-1/Chromobacterium typhiflavus[20]. It is motile and oxidase negative. Chryseomonas has been recovered from a variety of clinical specimens. It is often isolated with other organisms and is not considered to be clinically significant. Bacteremia[20], endocarditis, osteomyelitis and peritonitis have been reported[8].

Genus Flavimonas:

This genus comprises of a single species, F.orbyzihabitans was previously grouped as CDC group Ve-2. This organism is motile and oxidase negative and is distinguished by yellow colonies on blood agar, negative esculin hydrolysis and ONPG and single polar flagellum[8].

F.oryzihabitans has been recovered from wounds, sputum, ear, eye, urine, peritoneal fluid, blood and inhalation therapy equipment. It also appears to be the

emerging pathogen in peritonitis associated with continuous ambulatory peritoneal dialysis(CAPD). Artificial grafts, IV drug abuse, severe head trauma and bone marrow transplantation are predisposing factors[8]. Infections are typically related to presence of intravascular catheter[21].

Genus sphingomonas:

Sphingomonas paucimobilis previously CDC group k-1 is the most common species in human specimens[24]. Motility occurs at 18-22^{0}c but not at 37^{0}c, oxidase reaction is positive. Colonies are yellow pigmented, but slow to appear. Thus it can be confused with flavobacteria. The pathogenecity of S.paucimobilis is demonstrated by presence of an atypical glycolipid bound to outer membrane, corresponding to Lipid-A. endotoxin activity has been demonstrated by limulus amoebocyte tests[22].

The organism is isolated from environment and water or saline used for irrigation of wounds. The organism has been isolated from blood[22], CSF, urine, wounds, vagina and cervix. Nosocomial infections from contamination of saline, hemodialysis fluids or contamination of transplanted bone marrow during in vitro processing has been reported. The source of infection may be endogenous(skin flora of patient). Catheter related infection has been reported mostly in immunocompromised patients.

S.paucimobilis is sensitive to erythromycin, tetracycline, chloramphenicol, cotrimoxazole, ceftazidime, ceftriaxone and aminoglycosides, but resistant to ureidopenicillins and 1st generation and 2nd generation cephalosporins[22].

Genus shewanella:

These were first isolated from butter. Three biovars are present biovar 1 (CDCgroup b-1), biovar 2(CDC group b-2) and biovar 3. S. putrefaciens is the

common species isolated from human clinical specimens. These are oxidase positive and motile. Orange to tan pigment is produced on blood agar. These are the only non-fermenters which produce hydrogen sulfide in KIA and TSI media.

Biovar 1 is mainly responsible for spoilage of refrigerated protein rich food. Biovars 2 and 3 are isolates from skin ulcers, ear infections, osteomyelitis, bacteremia[23], peritonitis in patients undergoing CAPD. It has also been isolated from the environment, but actual infection caused by the organism should be confirmed by repeated isolation.

Pseudomonas-like Group2:

This was previously included in CDC group 4-d. this organism has been isolated from blood, CSF, stool, urine, dialysate and respiratory tract[8].

CDC Group WO-1 (weakly oxidative):

These are similar to commamonas and B.mallei. They are weak or late oxidizers and if positive completely reduce nitrates to nitrites. They are arginine and xylose negative[24].

These have been isolated from blood, CSF, lung, wound and environment. They may produce yellow, green, ambar, tan or brown pigment[8].

Family Methylococcaceae:

Genus methylobacterium:

These are oxidase positive, catalase positive, urease and amylase positive motile organisms. They have been isolated from blood and wounds.

Genus Roseomonas:

These are pink pigmented bacteria which are phenotypically and genotypically similar to Genus Methylobacterium. Isolates have been recovered from blood, wounds, exudates and abscesses and genitourinary sites.

Apart from blood agar and nutrient agar, those organisms grow well in Sabourauds agar and do not appear black under UV light[8].

Family Alcaligenaceae:

Genus Alcaligenes:

Alcaligenes faecalis is the most common isolate of this genus. These organisms produce strong alkaline reactions in all carbohydrate media. They reduce nitrite but not nitrate. Some strains (previously A.odorans) produce a fruity odour.

A.faecalis has been isolated from blood, sputum and urine. Most infections are opportunistic and acquired from moist items such as nebulizers, respirators and lavage fluids[8].

A.xylosoxidans subsp.xylosoxidans:

Acidifies glucose and xylose. It has been isolated from blood, CSF, bronchial washings, pus, urine and wounds. The organism colonizes respiratory tract of intubated patients.

A.xylosoxidans subsp.dentrificans is isolated from blood collection tubes, blood[25], ear, CSF and urine. Septicemia often concomitant with other organisms[25].

A.bronchiseptiea produces a strong urease positive reaction. Alcaligenes have been shown to produce -lactamase (cephalosporinase) both plasmid and chromosome mediated. Penicillinase has also been demonstrated[25].

Genus bordetella:

The 3 species B.pertussis, B.parapertussis and B.bronchiseptica are genotypically homologous, but phenotypically different. B.bronchiseptica has been isolated from sinusitis, tracheobronchitis, pneumonia, septicemia and whooping cough and endocarditis. B.bronchiseptica can cause fatal infection with bacteremia especially in immunosuppressed patients[26].

Bordetella holmesii is a new species associated with septicemia which has been isolated from the blood of human being. It is the only bordetella species not associated with respiratory infections. It produces a brown soluble pigment, is non motile and oxidase negative. This organism was classified previously as CDC group NO-2. Opportunistic and postoperative blood infections have been reported.

Family Rhizobiaceae:

Genus Agrobacterium (CDC Group Vd-3):

These are plant pathogens occurring in soils. Agrobacterium radiobacter and Agrobacterium tumefaciens have been isolated from human clinical specimens. Genetically these two species are same.

Colonies on MacConkey agar are moist and may be mucoid. Biovar-2 does not grow at 35^0c, but growth has been reported indicating thermal adaptation[27]. These organisms give a rapid urease reaction, esculin hydrolysis and ONPG test positive and a positive test for phenylalanine deaminase. They produce 3-ketolactose from lactose.

A.radiobacter is isolated from blood[1], peritoneal dialysate, urine and ascitic fluid[8]. Isolates are more common in patients with transcutaneous catheters or artificial implants or immunocompromised patients[28]. However, isolation of these organisms is rarely linked with infection[8].

Genus Achromobacter:

This is a genus without any named species. Six groups (A-F) were identified, of which groups A, C and D were found identical to Ochrobacterium anthropi. Groups B and E constitute a single unnamed species and Group F is distinct. Group B has been isolated from blood, Groups Eand F also have been recovered from blood[8].

Genus Ochrobacterium:

These belong to CDC Group vd 1 and 2. Ochrobacterium anthropi are distinguished by ability to hydrolyse urea, negative esculin hydrolysis and negative ONPG test. They may produce H_2s they appear mucoid on MacConkey agar. Strains have been isolated from blood[1,21], wounds, urine, genital tract, ears, faeces, eye and CSF[24]. They are especially emerging as cause of infection in patients with central venous catheter (CVC) related sepsis and in patients after surgical manipulations such as genitourinary manipulation. They are sensitive to aminoglycosides, carbenicillin, quinolones, tetracycline and cotrimoxazole.

Genus Oligella:

These are coccobacillary oxidase positive and non motile. They grow better in media containing blood or serum. Oligella urealytica is motile, hydrolyses urea, reduce nitrates and nitrites and are resistant to penicillin. Oligella urethralis are non motile, does not hydrolyse urea, reduces only nitrites and are susceptible to penicillin. Most isolates are from human urine samples and from the urogenital tarct[24].

CDC Group 4c-2:

These are oxidase positive motile and nonsaccharolytic. Septicemia, peritonitis and wound infection following cat bite have been reported[8].

Family Flavobacteriaceae:

Genus Flavobacterium:

None of the organisms originally incuded in this genus are related to the type species F.aquatile. Therefore F.balustinum, F.gleum, F.indologenes, F.meningosepticum and others have all been shifted to genus Chryseobacterium. None of the newly classified Flavobacterium species are found in human clinical specimens[8].

Chryseobacterium is naturally present in soil water, plants and foodstuffs. It is distinguished by positive indole reaction. An unknown, unclassified bacillus was isolated from blood of six patients with pneumonia of which one had meningitis, in France. These patients were from the same hot spring area. The organism resembled Flavobacterium, was differentiated by biochemical and protein profiles and was similar to Chryseobacterium in protein profile but different chemically.

C.meningosepticum is associated with neonatal meningitis, bacteremia and pneumonia in adults.

Weeksella and Bergeyella:

These are oxidase positive, indole positive, nonsacharolytic, susceptible to penicillin and do not grow on MacConkey agar. Weeksella virosa gives slimy colonies on blood agar which can be confused with Klebsiella[8].

W.virosa has been recovered from the urogenital tract of women. There is no evidence for its pathogenic role. B.zoohelcum is part of normal oral and nasal flora of dogs. This organism gives an intense urease positive reaction.

Flavobacterium odoratum:

This is generically misnamed. Colonies are effuse and spreading and may be confused with those of Bacillus species. The organism is isolated from urine most commonly, also from wound, sputum, blood and ear specimens[8].

Genus Sphingobacterium:

These are yellow pigmented, oxidase positive, non-motile, indole negative and urease positive. S.multivorum and S.spiritivoram are most frequently isolated from human clinical specimens. They are similar to Sphingomonas pancimobilis and differ by lack of motility and resistance to polymyxin, carbon substrate assimilation and cellular fatty acid composition.

Blood and urine are most common sources for S.multivorum[8]. S.multivorum is isolated from various specimens, but rarely associated with serious infection. It has been isolated from blood.

Genus Moraxella:

These are characterized by poor growth on MacConkey agar and pinpoint colonies on blood agar.

Many species have been identified of which M.osloensis and M.phenylpyruvica commonly do not require growth supplements. Many of the species are fastidious and therefore biochemical reactions are often negative or equivocal.

One report of bacteremia by M.atlantae is documented. M.nonliquefaciens is common in the normal flora of the upper respiratory tract. One isolate has been reported in blood. M.osloensis has been reported in blood[1].

M.lacunata, M.nonliquefaciens, M.osloensis, M.phenylpyruvica (CDC group M-2) and M.atlantae (CDCgroup M-3) are of medical importance. Groups M-5 and M-6 are placed in Genus Neisseria (M-5 is N.weaveri and M-6 is N.elongata subsp.nitroreducens). N.elongata consists of 3 subspecies, nitroreducens, glycolytica and elongata of which N.elongata subsp.glycolytica has been isolated from blood of a patient with subacute bacterial endocarditis.

CDC Group EO-2 and EO-3:(Eugonic oxidizers)

These have been recovered from urine, eye, blood, brain, CSF, throat, vagina and wounds. EO-2 have a characteristic O shaped morphology. EO-3 have a yellow nondiffusible pigment[8].

Psychrobacter immobilis:

These grow best at 20^0c and do not grow at 35^0c. It has been recovered from blood, brain, tissue, urethra, poultry, wound, CSF, vagina and eye[8].

Gilardi Rod –Group 1:

These are distinguished by a positive phenylalanine deaminase reaction. They have been recovered from wounds, blood and urine. Their pathogenic potential has not been established[8].

Genus Acinetobacter:

Acinetobacter species play an important role in the colonization and infection of patients admitted to hospitals. They have been implicated in bacteremia, urinary tract infection and meningitis and particularly in nosocomial pneumonia especially ventilator associated pneumonia in ICU's. New molecular and typing methods have now been developed for this genus.

Classification:

Originally the genus included both oxidase positive and negative non-motile strains. In 1971, it was decided that Acinetobacter comprise of only oxidase negative strains. Only 2 species, A.calcoaceticus and A.lwofii were listed in the approved lists of bacterial names and only one species in bergey's manual of systematic bacteriology.

On the basis of DNA related criteria, 19 homology groups (genomic species) have now been recognized.

Three separate numbering systems, by Bouvet et al, Tjernberg and Ursing and Nishimura et al have been proposed. Of these common numbers are genomic species 1 – Acinetobacter calcoaceticus, species 2 – A.baumanii, species 3 – unnamed, species 4 - A.haemolyticus, species 5 – A.junii, species 6 – unnamed, species 7 – A.johnsonii, species 8 – A.lwofii and species 12 – A. radioresistens. Species 9-11 and 13- 19 are unnamed [3,8].

A.baumanii and A.calcoaceticus are glucose oxidizing and previously grouped together under A.calcoaceticus var anitratus. This is the commonest isolate from blood. A.baumanii is the main genomic species associated with nosocomial infection outbreaks and also the commonest isolate from clinical specimens[3].

Non-glucose oxidizing species commonly isolated include A.lwofii, A.jhonsonii and Acinetobacter genomic species 12. A.lwofii is the 2nd most common non-fermenters from human specimens[1].

A.johnsonii has been associated with catheter related bacteremia. Isolation of species other than A.baumanii should be correlated with clinical features and repeated

isolation of the same strain from single patient will indicate infection by the species isolated. These species are natural inhabitants of human skin.

Morphology:

Acinetobacter are short stout gram negative coccobacilli, with a DNA G+C content of 39-47mol%[29]. they are strictly aerobic, nonmotile and frequently capsulate.

Cultivation:

These organisms grow on common media. A mineral base, with ammonium salt as nitrogen source and a single carbon source are useful[3]. Leeds selective medium is an antibiotic containing selective medium and is useful for recovery from clinical and environmental sources.

Liquid enrichment cultivations in a mineral medium with single carbon and energy source and ammonium salt as nitrogen source, at pH 5.5-6 are inoculated with vigorous shaking during incubation. Shaking allows Acinetobacters to outgrow any Pseudomonads. Subculture from this broth onto solid media is useful for recovery of Acinetobacter from various specimens.

A.baumanii can grow at 37^0c or higher, but some species grow only at lower temperatures. Therefore cultivation temperature of 30^0c is recommended[3].

Cultural characteristics:

Acinetobacters are non-motile, short rods which become coccoid in the stationary growth phase. They are gram-negative and may show gram variability. Some environmental strains show a diffusible brown pigment.

Identification:

All species are oxidase negative nonfermenters and most strains do not reduce nitrates to nitrites. A battery of biochemical tests must be performed including ability to utilize various carbohydrates as single carbon source. Oxidation of glucose, gelatin hydrolysis, growth at 42^0c and 44^0c and hemolysis. A study by Gerner Smidt et al was able to identify only 78% of Acinetobacter species by phenotypic tests, 19.8% not identified and 2.2% were wrongly identified. Problems were in identifying differences between Group 1 and 3, 2 and 13, 8 and 15. Simple biochemical tests do not differentiate between A.baumanii, Group 3 and Group 13.

DNA-DNA hybridization is the most reliable method of species identification. Phenotypic identification methods for Acinetobacter species are not reliable Ribotyping and PCR following restriction analysis of 16s rRNA genes or PCR of the 16S-23S spacer regions can be done[3].

Clinical infections:

A.baumanii is the cause of most nosocomial infections. A.lwofii, A.johnsonii and other species are normal inhabitants of normal skin, oropharynx and vagina. Isolation of Acinetobacter species may not necessarily indicate infection with these organisms[3].

Acinetobacter species may occur singly or as part of polymicrobial flora. Risk factors for Acinetobacter septicemias include immunocompromised patients[3],cancer, burns, trauma, surgery[3], use of transducers, ventilation and hyperalimentation.

Central venous catheters are the main source of bacteremia, though exit site infection is often absent in patients with catheter related bacteremia. Risk factors for

neonatal septicemia, usually late onset septicemias are low birth weight, previous antibiotic therapy, mechanical ventilation and presence of neonatal convulsions[3].

A.baumanii is more often associated with clinical sepsis than A.lwofii[2]. Nosocomial pneumonia is mostly associated with ventilated patients in ICU's. Risk factors are advanced age, chronic lung disease, surgery, immunosuppression, use of antimicrobial agents, invasive devices and respiratory equipment[3].

Meningitis may occur following trauma or neurosurgical procedures. Other infections are native valve endocarditis, peritonitis in patients on CAPD, biliary infection, osteomyelitis and eye infection following surgery or fitting of contact lens[3].

Pathogenicity:

Acinetobacter species are considered to be low grade pathogens.

Characeristics of Acinetobacter species that enhace pathogenesis are:

- Polysaccharide capsule formed of L-rhamnose, D-glucose, D-glucoronic acid and D-mannose which renders the strains more hydrophilic.
- Property of adhesion to human epithelial cells in presence of fimbriae or capsular polysaccharide.
- Production of lipolytic enzymes.
- Toxic role of the lipopolysaccharide component of cell wall and presence of lipid.
- Production of endotoxin may be responsible for symptoms observed in Acinetobacter septicemia.

Acinetobacter strains have been shown to produce siderophores such as aerobactin and iron repressible outer membrane receptor proteins.

Human carriage and colonization:

Acinetobacters are part of the bacterial flora of the skin, in axilla, groin, toe webs and other moist sites. The carriage rate is higher in hospital patients as compared to outpatients. Digestive tract colonization has been reported to be a major reservoir of resistant strains. The higher carriage rate in hospital environmental sources and cross transmission may be responsible for colonization rather than endogenous sources. Patients colonized with Acinetobacter have a higher rate of infection than non-colonized[3].

Environmental sources:

Acinetobacter species have been found in air, floor, bed linen, mattresses and pillows of patients colonized with these organisms. Even after the source of outbreaks eliminated, the problem may persist for several months due to colonization of patients.

The organisms have also been recovered from telephone handles, door knobs, patient charts, table tops and other surfaces from an ICU. They are present in sewage, soil, drinking and surface waters and foodstuffs. Contaminated materials have been reported as the cause for many outbreaks e.g. air humidifiers, mattresses, dialysis fluid, inadequately sterilized reusable needles, respiratory monitoring devices and resuscitation.

Radiation resistance has been demonstrated for Acinetobacter species. So ethylene oxide is the preferred mode of sterilization of such contaminated equipment. Health personnel with skin lesions such as dermatitis may be colonized and act as the sorce of the outbreak. A seasonal increase in incidence of Acinetobacter infections in summer has been reported in tropical countries and colonization of hands and skin of

normal population may increase in summer leding to increased infections in general wards outside the ICU.

Antibiotic resistance:

Acinetobacter species develop antibiotic resistance rapidly, probably due to long term evolutionary exposure to antibiotic producing bacteria in the soil[3]. Only sconjugation has so far been shown to play a role in transfer of antibiotic resistant genes. Transposons may play an important role in conjugation with integrons. High proportional of the strains are resistant to all commonly used antimicrobials, including aminopenicillins, ureidopenicillins, cephalosporins, aminoglycosides, chloramphenicol and tetracyclines.

Broad spectrum cephalosporins imipenem, tobramycin, amikacin and fluoroquinolones are partly effective but minimum inhibitory concentration for these drugs have increased in the last decade[3].

Imipenem resistance is seen mostly in A.baumanii, but imipenem remains the most active drug. A.lwofii, A.johnsonii and A.junii are less associated with nosocomial infections and more susceptible to antibiotics. A.baumanii is generally more resistant and multi drug resistance is common in this species.

-lactam resistance is mostly due to -lactamases, including TEM-1, TEM-2,cephalosporinases ACE-1 to ACE-4 and a new enzyme ARI-1 which is a carbapenemase, which is plasmid encoded and hydrolyses imipenem and azlocillin. Ampicillin-sulbactam is a more effective combination than amoxicillin-clavulanic acid.

All three types of aminoglycoside modifying enzymes have been demonstrated (acetylating, adenylating and phosphorylating). Geographic variations

in distribution of these genes is seen[3]. amikacin is very effective[4]. The gene aac(6)-Ig is responsible for amikacin resistance and present only in A.haemolyticus[3]. Both plasmid and transposon locations for aminoglycoside resistance genes have been demonstrated.

4-fluoroquinolones resistance can develop by mutations in the gyr-1 gene or by outer membrane changes which result in decreased uptake. Selection of resistance by 4-fluoroquinolones can result in cross resistance to -lactams. Clinafloxin is more effective than ciprofloxacin, levofloxacin, moxifloxacin, nalidixic acid, sparfloxacin and trovafloxacin against A.baumanii. A single mutation in gry-A gene does not confer resistance clivafloxacin and a second mutation is required for organism to express resistance.

CDC Group No.1 (Non oxidase):

This is a fastidious rod isolated from dog and cat bite wounds. It gives a positive nitrate reduction test, but do not reduce nitrite.

ISOLATION AND IDENTIFICATION OF NONFERMENTERS

Most of the nonfermenters isolated from clinical specimens are nonfastidious and grow on common media used in the laboratory like nutrient agar, blood agar (BA) and chocolate agar. Some of them do not grow or grow slowly on MacConkey agar (MA)[8].

Selective media can be used for Pseudomonas aeruginosa (cetrimide agar) for Burkholderia cepacia (OFPBL agar and TB-T medium and PC medium) for Burkholderia pseudomallei (Ashdown medium) and for Bordetella parapertusis (Regan-Lowe medium).

Two media, King's A and King's B medium have been described for the demonstration of pyocyanin and fluorescein pigments respectively produced by Pseudomonas aeruginosa.

On King's A medium, after incubation for 5 days at 37^0c, around 80% of Pseudomonas aeruginosa strains form pyocyanin. The intensity of pigmentation often increases on removal of cultures from the incubator to room temperature (18-22^0c) for 3-4 hours. Around 70% of clinical isolates produced fluorescein pigment on King's B agar[9].

All colonies of nonfermenters on MacConkey agar are non-lactose fermenting colonies.

The following nonfermenters grow well in blood agar except Methylobacterium species which do not grow well on BA. But do not grow or grow slowly on MacConkey agar[8,16].

- B.vesicularis (60% strains grow on MA).

- CDC group No.1 (only 20% of the atrains grow on MA).
- Sphingomonas paucimobilis.
- Moraxella species grow slowly on MA. M.lacunata does not grow at all. M.osloensis, Neisseria elongate and N.weaveri may grow as non-lactose fermenters.
- Weeksella virosa.
- Bergeyella zoohelcum.
- Methylobacterium species.

Incubation:

Incubation of blood agar plates should be at 35^0c in ambient air or 5% carbondioxide for 24 hours and longer for slow growing organisms. Incubation of MacConkey agar plates should be at 35^0c in ambient air[8,16].

Some Acinetobacter species e.g. A.johnsonii do not grow at 35^0c incubation therefore at 30^0c is also recommended[3]. Psychrobacter immobilis will not grow better at room temperature than at 37^0c, all further tests should be incubated at room temperature.

Following are slow growing non-fermenters:

- B.picketii which take 72 hours to produce visible colonies.
- B.holmesii may take 3 days to show visible growth.
- B.parapertusis may take 3 days to show visible growth.

Pigment producing nonfermenters:

A) Nonfermenters which produce yellow pigment on blood agar are as follows'[8,16]

- B.vesicularis (orange)
- B.gladioli produces pigment on BA,MA and OFPBL agar.
- Chryseobacterium species
- Sphingobacterium species
- Chryseomonas luteola
- Flavimonas oryzihabitans
- Sphingomonas paucimobilis
- CDC group O-1 and O-2

B) Nonfermenters which produce brownish pigment with metallic sheen are:

- Pseudomonas mendocina,Shewanella putrefaciens.
- Bergeyella zoohelcum
- Weeksella virosa
- Brodetella holmesii
- Some environmental species of Acinetobacter.

C) Roseomonas species produces pink pigmented colonies.

D) Stenotrophomonas maltophilia may produce lavender-green to light purple pigment

E) Pseudomonas aeruginosa usually produces bluish green pigmentation due to production of pyocyanin

Colony characteristics of predominant nonfermenters:

Pseudomonas aeruginosa: Spreading and flat colonies with serrated edges, confluent growth, often with a metallic sheen and with bluish green pigmentation. Colonies are often beta-haemolytic with a grape like or corn taco-like odour. Mucoid colonies are seen in patients with cystic fibrosis'[8,16]..

Pseudomonas mendocina: Smooth non wrinkled flat colonies with brownish yellow pigment.

Pseudomonas stuzeri and B.pseudomallei: Dry wrinkled colonies.

B.pseudomallei produces violet-purple colonies on Ashdown medium.

B.cepacia: Smooth colonies on blood agar and OFPBL.

Achromobacter group Produce smooth glistening colonies with entire margin.

Alcaligenes species form glistening colonies. A.faecalis gives feather edged colonies usually surrounded by a zone of green discoloration

Alcaligenes piechaudii produces non-pigmented colonies usually surrounded by a zone of discolouration

Oligella species forms small opaque whitish colonies

Roseomonas species forms pink pigmented colonies

Shewanella putrefaciens gives convex, smooth circular colonies with brown pigment which may be mucoid and show a green discolouration of the medium

On BA Acinetobacter species usually produce smooth , pale yellow to grayish white colonies. Some environmental strains may produce a diffusible brown pigment. Some isolates may show haemolysis on BA, especially strains of A.haemolyticus. On MA, the colonies exhibit a purplish line which may be mistaken from lactose fermentation

Stenotrophomonas maltophilia produces large colonies with uneven edges and lavender green to light purple pigment, with an ammonium smell

Moraxella atlantae, M.lacunata and M.nonliquefaciens form colonies which may be spreading and may pit the agar

Bergeyella zoohelcum and Weeksella virosa usually do not grow well on blood agar. Colonies are sticky and may produce tan pigment

Methods for identifying nonfermenters:

- The contents of many differential test media are not supportive for the growth of many fastidious species of nonfermenters. Therefore, by using a highly nutritious medium like Kliglers iron agar or Triple sugar iron agar to propagate the colonies, the time required for growth to take place in the test substrates can be reduced[8].
- Thus a heavy inoculum from overnight growth of bacteria (log phase of growth) should be used to perform the tests.
- Nonfermenters may not give reliable sugar reactions on peptone- containing media and should be grown on media with ammonium salt as the sole nitrogen source. Snell and Lapage found that for nonfermenters, Ammonium salt sugars medium of Smith, Gordon and Clark (1952) gave the most positive results.

- Clinically important nonfermenters should be subcategorized into several groups based on visual observations, Gram stain characteristics and result of rapid biochemical test[8].

MINIMUM TESTS FOR IDENTIFICATION OF COMMON NON FERMENTERS

Pseudomonas aeruginosa[8]:

- Large colonies, grape like odour
- Pyocyanin produced
- Oxidase positive colonies
- Growth at 42^0c
- Alkalinization of acetamide
- Motile with polar, monotrichous flagellum

Acinetobacter baumanii[8]:

- Appear as cocci or coccobacilli on gram stain
- Grow well on MacConkey agar
- Oxidase negative
- Exhibit rapid utilization of glucose with production of acid
- Non motile
- Penicillin resistant

<u>Stenotrophomonas maltophilia[8]:</u>

- Good growth on BA and MA
- Oxidase negative
- Produce acid in OF maltose, but may be negative in OF glucose
- Lysine decarboxylase positive
- Some strains have yellow pigment

Various schemes for identification of nonfermenters have been proposed.

- The Weaver-Hollis scheme – provides an identification key, 12 identification tables and a numerical code book [8]. This schedule has preliminary tests as oxidase and growth in MacConkey agar, based on which further tests are done.
- The Gilardi scheme– This scheme identifies nonfermenters on the basis of motility, oxidase activity and morphology.

Three major groups are recognized:

A) Motile oxidase positive bacillus group (Pseudomonas and Alcaligenes)

B) Non-motile oxidase negative diplococcus group (Acinetobacter) and oxidase positive diplobacillus group (Moraxella)

C) Non-motile oxidase positive, yellow pigmented bacillus group (Flavobacterium)

- The Pickett scheme: This is a viable scheme with identification tables similar to the Weaver-Hollis scheme. This scheme in addition to oxidase and motility, also uses the acidification of glucose as a preliminary test.

- Oberhofer's scheme: In this scheme nonfermenters are divided in to two major groups, oxidative and non-oxidative. The oxidative group is further subdivided in to fluorescent, nonfluorescent, peritrichous and yellow-pigmented oxidizers[8].

- Romeo has published dichotomous keys using 22 tests and media to construct 12 keys, representing various subgroups of 100 species of bacteria[8].

- Schreckenberger P C has proposed a scheme of classifying non-fermenters in to four groups[8]:

  A) Oxidase positive, motile

  B) Oxidase positive, non-motile

  C) Oxidase negative, motile

  D) Oxidase negative, non-motile

Tables A-E of biochemical characteristics of important non fermenters are given on the ensuing pages:

Table A: Biochemical characteristics of important oxidase positive, motile Psuedomonads[8]

	Pyoverdin	Glucose	Maltose	Lactose	Mannitol	Arginine	Lysine	NO3-NO2	NO3-N2	Urea	ONPG	DNAase	Acetamide	Esculin	Polymyxin
P.aeruginosa	+	+	V	-	V	+	-	+	V	V	-	-	+	-	S
P.fluorescens	+	+	V	-	+	+	-	V	-	-	-	-	-	-	S
P.putida	+	+	V	-	V	+	-	-	-	V	-	-	-	-	S
P.stuzeri	-	+	+	-	V	-	-	+	+	V	-	-	-	-	S
B.cepacia	-	+	+	+	+	-	+	V	-	V	V	-	V	V	R
C.acidovorans	-	-	-	-	+	-	-	+	-	-	-	-	+	-	V
B.diminuta	-	V	-	-	-	-	-	-	-	-	-	V	-	-	V
S.putrefaciens	-	+	V	-	-	-	-	+	-	-	-	+	-	-	S
S.paucimobilis	-	+	+	+	-	-	-	-	-	-	+	-	-	+	S

V – 11-89% Results positive   + – Positive   - – Negative   S – Sensitive   S – Resistant

Table B: Biochemical characteristics of other important oxidase positive nonfermenters[8]

	Oxidase	Growth on MA	Growth at 420c	Motility	OF glucose	Nitrate reduction	Urea	OF xylose	Gelatin	Indole
Methylobacterium	+	-	-	+	V	V	+	+	N	N
Roseomonas	+	+	+	V	V	V	+	N	N	N
Alcaligenes faecalis	+	+	V	+	ALK	-	-	ALK	V	-
Alcaligenes xylosoxidans	+	+	V	+	A	+	-	A	-	-
Alcaligenes piechaudii	+	+	N	+	ALK	+	-	ALK	-	-
Bordetella bronchiseptica	+	+	N	+	ALK	+	++	ALK	-	-
Oligella urealytica	+	V	N	V	ALK	+	++	ALK	-	-
Agrobacterium radiobacter	+	+	V	+	A	V	+	A	-	-
Achromobacter	+	+	N	+	A	+	+	A	N	N

++ - strong positive V - 11-89% results positive ALK - Alkaline reaction + – Positive - – Negative

A - Acid reaction N - Results not found

Table C: Biochemical characteristics of important oxidase positive non-motile non-fermenters[8]

	Oxidase	Growth on MA	OFglucose	OFmannitol	Indole	Starch	Esculin	ONPG	DNAase	Urea	Penicillin	Polymyxin	Gelatin	Morphology	NO3 reduction
Chryseobacterium meningosepticum	+	+	A	A	+	-	+	+	+	-	R	R	+	b	-
Sphinobacterium multivorum	+	V	A	-	-	V	+	+	-	+	R	R	-	b	-
Weeksella spp.	+	-	-	-	+	-	-	-	-	-	S	S	+	b	-
Bergeyella spp.	+	-	-	-	+	-	-	-	-	++	S	R	+	b	-
Moraxella nonliquefaciens	+	V	-	-	-	-	-	N	-	-	S	S	+	cb	+
Moraxella osloensis	+	V	-	-	-	-	-	N	-	-	S	S	-	cb	-
Moraxella atlantae	+	+	-	-	-	-	-	N	-	-	S	N	-	cb	-
Neisseria elongata	+	V	-	-	N	N	N	N	-	-	S	N	-	b	+

b : Bacilli A : Acid produced cb : Coccobacilii ++ : Strong positive S : Sensitive

R : Resistant V : 11-89% results positive N – Results not found

Table D: Biochemical characteristics of important oxidase negative motile Nonfermenters[8]

	Glucose	Maltose	Lactose	Mannitol	Arginine	Lysine	NO3-NO2	NO3-N2	Urea	ONPG	DNAase	Acetamide	Esculin	polymyxin
Burkholderia gladioli	+	+	+	+	-	+	V	-	+	+	-	-	-	R
Stenotrophomonas maltophilia	+	++	+	-	-	+	V	-	-	+	+	-	+	S
Chryseomonas luteola	+	+	-	+	V	-	V	-	V	+	-	-	+	S
Flavimonas oryzihabitans	+	+	-	+	-	-	-	-	V	-	-	-	-	S

++ - Strong positive V - 11-89% results positive + – Positive - – Negative R - Resistant S - Sensitive

Table E: Biochemical characteristics of important oxidase negative, non-motile nonfermenters[8]

	Growth on MA	Acid from glucose	Growth at 440c	Growth at 420c	Gelatinase	Hemolysis	Growth in Lactate	Growth in Maltose	Growth in Citrate	Growth Aspartate	Nitrate Reduced
Acinetobacter calcoaceticus	+	+	-	-	-	-	+	+	+	+	-
Acinetobacter baumanii	+	+	+	+	-	-	+	+	+	+	-
Acinetobacter junii	+	-	V	V	-	V	+	-	V	-	-
Acinetobacter lwofii	+	-	-	-	-	-	+	-	-	-	-
CDC Group No.1	V	-	V	V	-	-	N	N	-	N	+

V - 11-89% results positive + – Positive - – Negative

N - Results not found

COMMERCIAL KIT SYSTEMS:

Packaged kit systems have been designed for, or adapted to identification of the nonfermentative bacilli. These are convenient to use, have a long shelf life and preclude the need for fresh supplies of media and reagents. They also provide standardized techniques that are accurate and give reproducible results[8].

Problems in the use of the kits include the tendency for organisms which exhibit weak or delayed activity to give a false negative result and inclusion of some tests that may not be applicable to identification of nonfermenters. The following are some of the kits available:

1. Oxi/Ferm Tube: This system identifies the commonly encountered species Pseudomonas aeruginosa, S.maltophilia and Acinetobacter species, more accurately than conventional methods. However, all nonfermenters could not be detected as accurately. Slow growing or weakly reactive organisms gave false negative results.

Correct identification - 50-62%.

Incorrect identification - 24%

Not identified - 3-12%

2. The API 20E system: (biomerieux vitex, Inc., Hazelwood MO) This system was originally designed for identification of Enterbacteriaeceae and has been extended to include nonfermenters. Common nonfermenters are identified with upto 99% accuracy, but performance with less common nonfermenters is not as accurate[8].

3. The API-NFT/ API NE (non enteric): This is a modification of the API 20E strip. Overall correct identification[30] 75-79%.

Not identified - 21% after 24 hours.

Incorrect identification - 13.8% (twice that of the crystal enteric NF system).

4. The REMEL N/F system: (Remel, Lenex KS) This_system gives good results for Pseudomonas aeruginosa and a high rate of correct identification of B.cepacia[15,31].

5. The CRYSTAL ENTERIC/ NONFERMENTER SYSTEM: (Becton Dickinson Microbiology Systems Cockeysville MD) This system includes some medically relevant nonfermenters which are difficult to distinguish. It includes Alcaligenes, Burkholderia, Comamonas, Moraxella, Ochrobacterium, Oligella and Pseudomonas.

Correct identification - 75.9%.

Incorrect identification - 6.3%

6. The RAPID NF PLUS SYSTEM: (Innovative Diagnostic Systems. Inc., Norcross GA).

   In this system all Acinetobacter species are identified as A.calcoaceticus.

   Correct identification - 90.1% without additional tests.

   - 6.1% with additional tests.

7. The BIOLOG SYSTEM: (Biolog Inc., Hayward CA) This system identifies 275 species and biogroups of nonfermenters. No other commercial identification system has as many taxa in a single database, however the large number of taxa included may make the number of tests available, inadequate in practice[8].

AUTOMATED IDENTIFICATION SYSTEMS[8]:

1) The Vitek system. (bio Merieux Vitek Inc, Hazelwood MO) was first introduced to perform antimicrobial susceptibility tests and was later modified to improve accuracy. Smith et al correctly identified 89.3% species of nonfermenters, compared with conventional methods.

2) The Microscan Walkaway - 96, Walkaway - 40 and Autoscan - 4 (Dade Microscan Inc, West Sacremento, CA) is a fully aytomated instrument. It incubates upto 96 panels simultaneously, automatically, adds reagents when required, reads and interprets results and prints reports without operator intervention. Walkaway 96-92.3% correct identification (pfaller et al).

3) The sensitive AP 80 system. (Accumed International, Westlake, OH) uses fluorescent technology to detect bacterial growth and enzyme activity.It can be inoculated and incubated offline and then read in the sensititre Autoreader, or it can be inoculated and placed in the Automated Reading and Incubation system(ARIS). Staneck et al, reported 99.2% correct identification for P.aeruginosa, Acinetobacter and S.maltophilia and 95.1% for all nonfermenters tested.

TYPING METHODS:

Serotyping:

Pseudomonas aeruginosa: Indirect haemagglutination and ELISA are suitable for detection of O antigen. The International Antigen Typing Scheme (IATS) identifies 17 O serotypes and a further 3 serotypes have been proposed but not validated internationally.

Serotypes 06 and 011 have been found to predominate in clinical specimens.012-017 are rare and account for less than 1% of isolates from clinical

material. Cross relation occurs between many serotypes. A combination of O serotyping, with bacteriophages or pyocin typing is recommended for finer differentiation between strains[29]. Two flagellar antigens (H- antigens) have been distinguished, one complex composed of 5- 7 factors and the other uniform. Totally 6 H factors were detected by Pitt in . H typing is useful for differentiation of strains of the same O serogroup in outbraeaks.

Acinetobacter species: 34 serovars in A.baumanii and 26 serovars in species 3 have been detected by checkerboard agglutination tests and reciprocal cross-absorptions with polyclonal rabbit immune sera. Further work is required to test the utility of serotyping[3].

B.cepacia: The scheme of Werenburg and Monteil (1989) is the most widely used. This differentiates B.cepacia into nine O and five H types.

Pyocin typing:

Pseudomonas aeruginosa: Govan listed 105 types and 25 subtypes using 8 indicator strains, streaked across an area of an agar plate, that had previously supported the growth of the strain being typed. Five additional indicators (A-E) were used for further discrimination.

In 1984 Fyfe and Govan described an improved method by incorporating the indicator strain in a layer over spots of growth of the producer strains.

This method has the following advantages:

- It is rapid.
- It allows differentiation of R and F pyocins from S pyocins, according to the size of zone of inhibition.

- Mucoid strains, usually untypable by the cross streaking method were often typable by the revised method.

B.cepacia: Govan and Harris in 1985 also identified 14 producer and 23 susceptibility types in 44 combinations. There may be variation among outbreak-related systems on this scheme.

Acinetobacter species: The usefulness of bacteriocin typing for clinically important species is being investigated. About 45-65% strains have been found typable[3].

Bacteriophage typing:

Pseudomonas aeruginosa: Both DNA and RNA containing phages active against Pseudomonas have been reported. Phages may attach to LPS, pili, outer membrane proteins or slime polysaccharides as receptors[29]. This diversity of receptors leads to poor reproducibility of phage-typing for Ps. aeruginosa. There is also considerable overlap of patterns, and a 3 major reaction difference rule is required which leads to loss of discrimination[14].Practically phage typing for Ps.aeruginosa has been used as a means of further subdividing strains of the same O serotype.

Acinetobacter species: A substantial proportion of Acinetobacter strains may be non typable. A system using 2 complementary sets of 24 and 14 phages, which allows identification of 125 phage types and 25 phage types respectively has been used. Predominant phage types 17 and 24 have been identified in some outbreaks. Most Acinetobacter phages are lytic and active only against the original strain on which they were isolated. This may be due to the large number of different surface antigens found in this genre. Phage typing for Acinetobacter species is epidemiologically useful when used in conjunction with other methods of typing[3].

Molecular methods:

Ps. aeruginosa: Restriction endonuclease analysis, by pulsed field gel electrophoresis (PFGE) systems such as field inversion gel electrophoresis (FIGE) and contour-clamped homogeneous electric field (CHEF) have been used.

Various DNA probes include those for rRNA, exotoxin – A, pilin protein, alginate and elastase.

Polymerase chain reaction (PCR) such as random amplified polymorphic DNA fingerprinting (RAPD) or arbitrarily primed PCR (AP-PCR), and enterobacterial repetitive intergenic consensus sequence (ERIC-PCR) can be done.

Other methods for Ps.aeruginosa include multilocus enzyme electrophoresis (MLEE) in which electrophoretic mobilities of enzymes from isolates are compared, esterase profiles and pyrolysis mass spectrometry[29].

B.cepacia: Ribotyping and AP-PCR can be used for chromosomal DNA analysis. MLEE is valuable in outbreak investigation, especially in combination with ribotyping. Quantitative differences in fatty acid profiles can also be epidemiologically significant[29].

Acinetbacter species: Both whole cell and cell envelope protein patterns have been done using sodium dodecyl sulphonate (SDS) polyacrylamide gel electrophoresis. MLEE, PFGE and plasmid profiling can also be done. Ribotyping and PCR based methods using either random or specific primers (RAPD or ERIC-PCR) are cumbersome but useful for epidemiological Purpose[3].

MATERIAL AND METHODS

The present study was undertaken at the Department of Microbiology, J.N Medical college, Belgaum during the period from 01/01/2007 to 01/01/2008 and a total of 130 nonfermenters isolated from clinical samples such as pus, sputum, urine, blood, ear swab, catheter tip and pleural fluid were included in the study.

The isolates were characterized by a battery of tests as follows:

1. Gram stain for morphology
2. Hanging drop for motility
3. Study of cultural characters

   On Blood agar at 37^0c

   On MacConkey agar at 37^0c

   On Nutrient agar at 37^0c

4. For production of enzymes

   Oxidase

   Urease

   DNAase

5. TSI Test
6. For substrate utilization

   Citrate test

7. For metabolism of proteins and aminoacids

   Indole production

   Hydrogen sulfide production

8. Oxidative and fermentative tests

   OF Glucose

   OF Lactose

   OF Maltose

   OF Xylose

9. Growth at 42^0c

10. Esculin hydrolysis

11. ONPG test

12. Antibiotic sensitivity testing by Kirby-Bauer's Disk Diffusion method

Gram stain:

A drop of saline taken on the slide, and the colony from the solid medium was picked up and emulsified in the saline, using a sterile loop. The smear was allowed to air dry and was then heat fixed by passing it rapidly through the flame three times[32].

The staining was done using Hucker's modification as follows

- The slide was flooded with aqueous solution of crystal violet and kept for one minute
- The slide was washed in running water.
- The mordant, Gram's iodine was then poured into the slide and left for one minute
- The slide was then washed under running water and decolourised using spirit. One or two drops of spirit were put onto the smear and washed within1-2 seconds to avoid over decolorisation. This was then immediately washed in

running water and the counterstain,1% safranine was added and left for 30 seconds.After washing under running water, it was allowed to air dry.

- The slide was then observed under oil immersion objective of the microscope for the morphology of the organisms.
- Positive control: Staphylococcus aureus ATCC 25923.
- Negative control: Escherichia coli ATCC 25922.

Motility:

It was observed by a hanging drop preparation.

- A drop of peptone water containing a 1-2 hour growth of the organisms[8] was placed on the centre of a coverslip, paraffin wax was applied to the four corners of the coverslip and it was quickly inverted over the concavity of a slide.
- The edge of the drop was then observed under the high power lens and motility of the organisms looked for.
- Interpretation: True motility of the organism was distinguished from Brownian motion, as in the former organisms change their relative position in the field and show a directive movement, while in the latter the relative position of the organisms remain the same[8].

Oxidase test:

- Principle: To determine the presence of an enzyme oxidase which will catalyse the transport of electrons between electron donors in the bacilli and redox dye. The dye is oxidized to indophenol blue producing deep purple colour.

- Procedure: The reagent used was Kovac's reagent, 1% tetramethyl-p-phenylenediamine dihydrochloride. A filter paper was moistened with the Kovac's reagent and by rubbing a speck of the colony on it with a sterile glass rod.
- Interpretation: A deep blue to purple colour appearing within 10 seconds was considered a positive result. A colour appearing between 10-60 seconds was considered a delayed positive result. Any colour appearing after 60 seconds was considered negative[32].
- Positive control: Pseudomonas aeruginosa ATCC 27853.
- Negative control: Escherichia coli ATCC 25922

Urease test:

- Principle: To determine the presence of an enzyme urease which splits urea to ammonia. Ammonia makes the medium alkaline and thus phenol red indicator changes to pink/red in colour.
- Procedure: A well isolated colony is picked from the surface of a primary isolation medium and inoculated on to Christensen's urea agar slope and incubated at 37^0c for 18-24 hours[32].
- Interpretation;

  A. Rapid urea splitters (Proteus)- red colour throughout the medium.

  B. Slow urea splitters (Klebsiella species)- red colour initially in slant only, gradually converting the entire tube.

  C. No urea hydrolysis- medium remains original yellow colour.

DNAase test:

- Principle: To determine the presence of an enzyme DNAase which hydrolyses the nucleic acid in the medium to produce a clear zone around the growth.
- Procedure: By heavily spot inoculating the several colonies of the organism on DNAase test medium and incubated at 37^0c for 24 hours.
- Interpretation: The medium under and around the inoculum will have a clear and uncloudy zone around the DNAase positive culture[32].

Triple sugar iron test:

- Principle: This test detects the capacity of the organism to ferment glucose and/or lactose and sucrose.
- Procedure: With a straight wire, a single colony was stabbed into the butt and streaked on the slant surface and incubated at 37^0c for 18-24 hours. The slant being exposed to atmospheric oxygen has the property to turn alkaline from oxidative decarboxylation of proteins, proteases and aminoacids in the medium and become red. In the butt where oxygen was excluded, protein degradation was minimal and even small quantities of acid can be detected by the appearance of yellow colour. Production of H_2S was indicated by a black colour in the butt, alkaline reactions were indicated by red colour[8].
- Interpretation:
  - * Acid reactions are indicated by yellow colour
  - * Alkaline slant/alkaline butt or no change: no carbohydrate fermented
  - * Alkaline slant/acid butt: glucose fermented, lactose and sucrose not fermented

- * Alkaline slant/acid butt with black discoloration: glucose fermented, lactose snd sucrose not fermented. H_2S gas produced.
- * Acid slant/acid butt: glucose and lactose or sucrose fermented.

Citrate utilization test:

- Principle: It is the ability of an organism to utilize citrate as the sole source of carbon and energy for growth and an ammonium salt as a sole source of nitrogen. Simmon's citrate medium is the most commonly used medium.
- Procedure: A well isolated colony is picked from the surface of a primary isolation medium and incubated at37^{0}c for 24-48 hours.
- Interpretation: A positive test is indicated by the Growth with or without an intense blue colour on the slant.
- Positive control- Enterobacter aerogenes

  Negative control- Escherichia coli ATCC 25922.

Indole production:

- Principle: This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole which accumulates in the medium. Indole is then tested colorimetrically with P- dimethyl amino- benzaldehyde.
- Procedure: The organism to be tested was inoculated on to Tryptophane broth and incubated at 37^{0}c for 18-24 hours. At the end of this time add 15 drops of Kovac's reagent down the innerwall of the test tube.
- Interpretation: The development of a bright fuchsia red colour at the interface of the reagent and the broth within few seconds after adding the reagent indicates positive test.

- Positive control- Escherichia coli ATCC 25922.

  Negative control- Klebsiella pneumoniae

Hydrogen sulfide production:

- Principle: This test demonstrates the ability of certain bacteria to liberate sulfur from sulfur containing aminoacids or other compounds in the form of H_2S.
- Procedure: The organism to be tested was inoculated on to broth rich in sulfur containing aminoacids and lead acetate paper containing 6% of lead acetate was suspended in the tube. Then the tubes were incubated at 37^0c for 18-24 hours.
- Interpretation: The development of black precipitate on the filter paper strip indicates a positive test[32].

Utilization of carbohydrates:

The conventional culture media designed to detect acid production from fermentative bacteria are not suitable for the study of nonfermentative bacilli, most of which grow slowly and produce extremely weak acids. Hugh and Leifson devised OF medium for this purpose.

Procedure and Interpretation:

Two Tubes were used for the OF tests, each inoculated with the unknown organism using a straight wire stabbing the medium. One tube of each pair was covered with a one cm layer of sterile liquid paraffin, leaving the other tube open. Both tubes were incubated at 37^0c for 48 hours or longer.

Acid production is detected in the medium by the appearance of yellow colour. In the case of oxidative organisms, colour production was first noted near surface of the medium. Oxidative metabolism is indicated by the appearance of yellow colour in open tube and green colour in covered tube. Fermentative metabolism was indicated by appearance of yellow colour in both open and covered tube. Green colour in both open and covered tubes indicates non-saccharolytic metabolism[8,32].

Esculin hydrolysis:

Esculin agar was used. An inoculum from an 18-24 hour culture was taken and incubated at 37^0c for 24 to 48 hours[32].

Interpretation:

Positive result – Appearance of a black colour

Negative result – No black colour

Controls:

Positive control – Aeromonas hydrophila

Negative control –Pseudomonas aeruginosa ATCC 27854.

ONPG test:

The -galactosidase(ONPG) test, which determines the presence of the enzyme -galactosidase by utilizing o-nitrophenyl- galactopyranoside, is used to differentiate late lactose fermenting organisms.

Inoculate and incubate for 24 hours at 37^0c . If the test is positive a yellow colour will develop in the fluid. Late lactose fermenters give a positive result more quickly in this medium than in sugar media[32].

Antimicrobial sensitivity testing:

Antimicrobial sensitivity was tested by Kirby-Bauer's disc diffusion method[8,32].

- Growth from an 18-24 hour culture was inoculated into peptone water using a sterile inoculating needle.
- The broth was incubated at 37^0c for approximately one hour
- After this period, the turbidity of the broth was matched with the turbidity of the 0.5 McFarland turbidity standard (i.e 1.5×10^8 CFU/ml).
- A swab was immersed in to the matched broth and then rolled and squeezed against the sides of the tube to remove excess broth.
- This swab was then used to inoculate the plate of Mueller-Hinton agar, in 3 different directions to ensure an even and complete distribution of the inoculum over the entire plate.
- The antibiotic discs were applied within 15 minutes of inoculation of the plate and the plate inverted for incubation.

Commercially obtained Himedia discs were used. The strengths of discs used and their zone size interpretative standards were according to guidelines by NCCLS guideline standards. Below table shows disc strengths.

Antibiotic	Strength
Amikacin(AK)	30µg
Cefotaxime(CE)	30µg
Ciprofloxacin(CF)	5µg
Ampicillin(A)	10µg
Doxycycline(Do)	30µg
Co-trimoxazole(Co)	1.25µg/23.75µg

Meropenem(Mr) 10μg

Cefoperazone(Cs) 75μg

Piperacillin(Pc) 100μg

- The plates were incubated at $37^{0}c$ overnight.
- The next day, the plates were checked for presence of confluent growth of the organism between the zones of inhibition and for purity of growth.
- The zone of inhibition for each disc was measured by a scale against a dark background in reflected light. The measurement was made of the diameter of the circle of inhibition, through the centre of the disc, including the diameter of the disc.

Interpretation: The interpretation of sensitivity was done as follows:

Antibiotic	Resistant	Sensitive	Zone Diameter of Control*
Amikacin	14mm	17mm	18-26mm
Cefotaxime	14mm	23mm	18-22mm
Ciprofloxacin	15mm	21mm	25-33mm
Ampicillin	13mm	17mm	0-8mm
Doxycycline	12mm	16mm	0-10mm
Co-trimoxazole	10mm	16mm	0-12mm
Meropenem	13mm	16mm	20-28mm
Cefoperazone	15mm	21mm	23-29mm
Piperacillin	17mm	18mm	25-33mm

* Pseudomonas aeruginosa ATCC 27853

Numbers indicate Zone Diameters in millimeters.

PHOTOGRAPHS

Photo 1: Gram's stain showing gram negative bacilli of P. aeruginosa

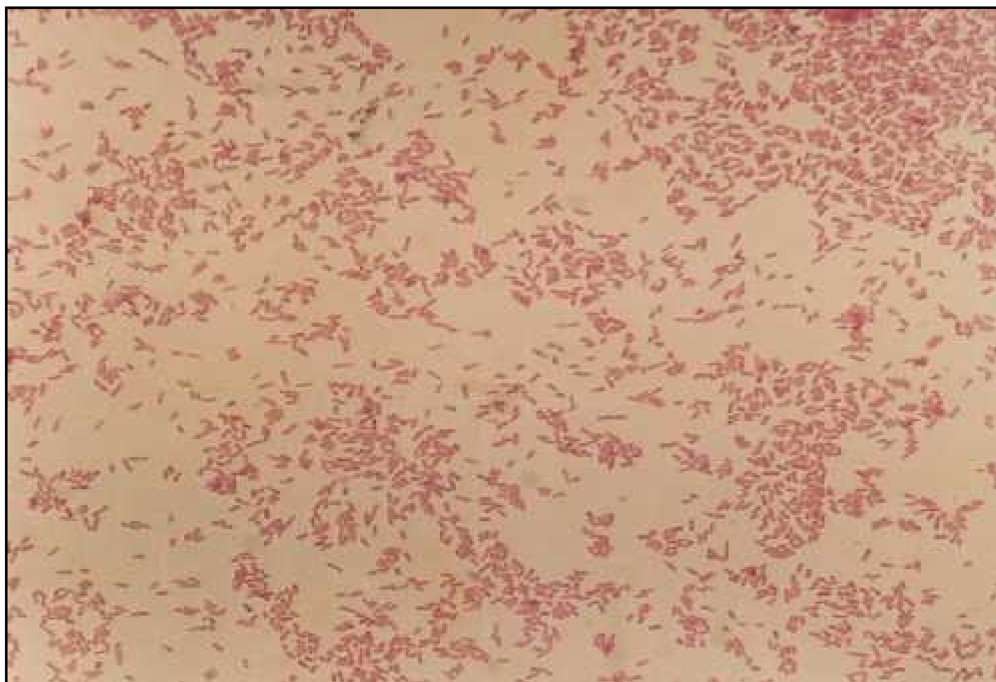


Photo 2: Gram's stain showing gram negative coccobacilli of A. baumanii

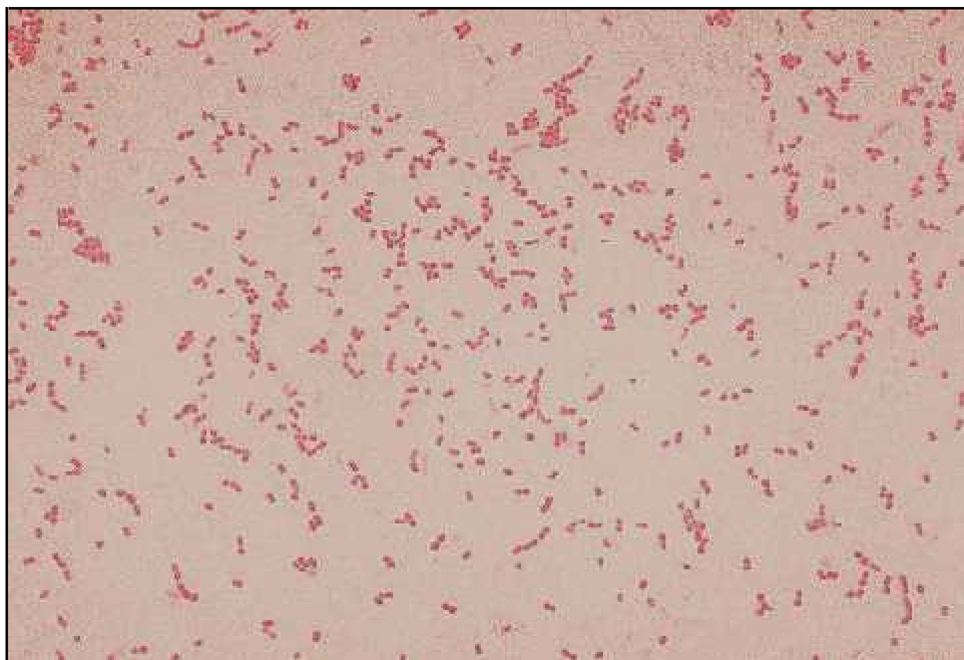


Photo 3: Pigmented colonies of P.aeruginosa on Nutrient agar

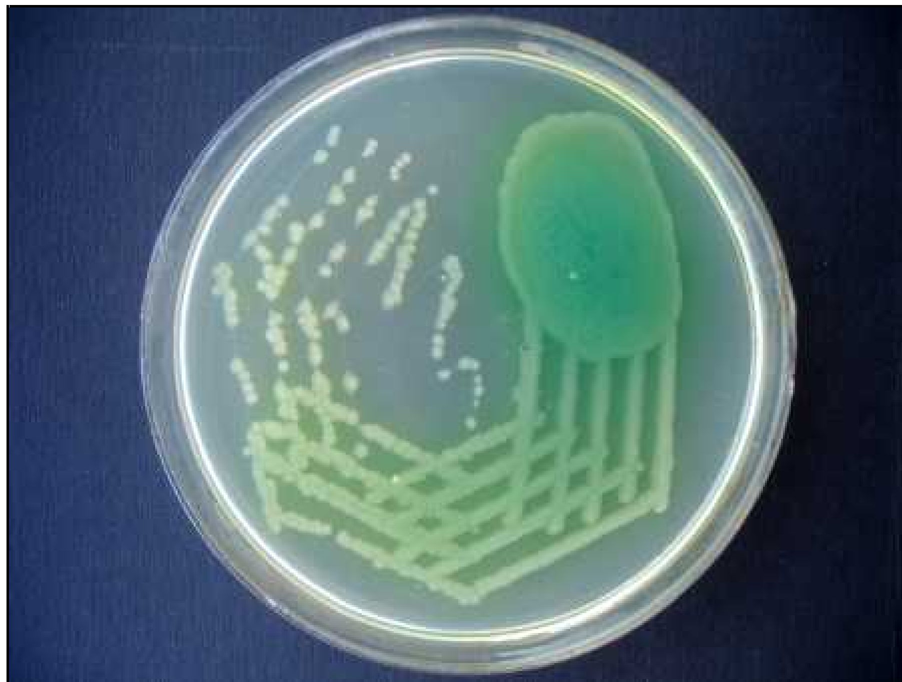


Photo 4: Non kactose fermenting colonies of Burkholderia pseudomallei on Mac-Conkey agar

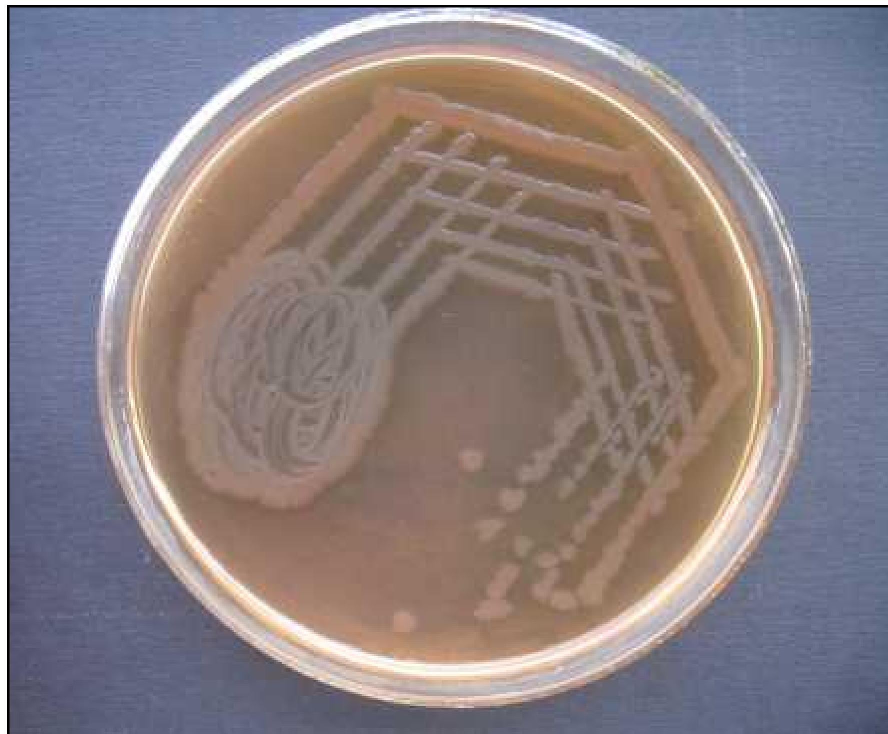


Photo 5: OF reactions of P.aeruginosa

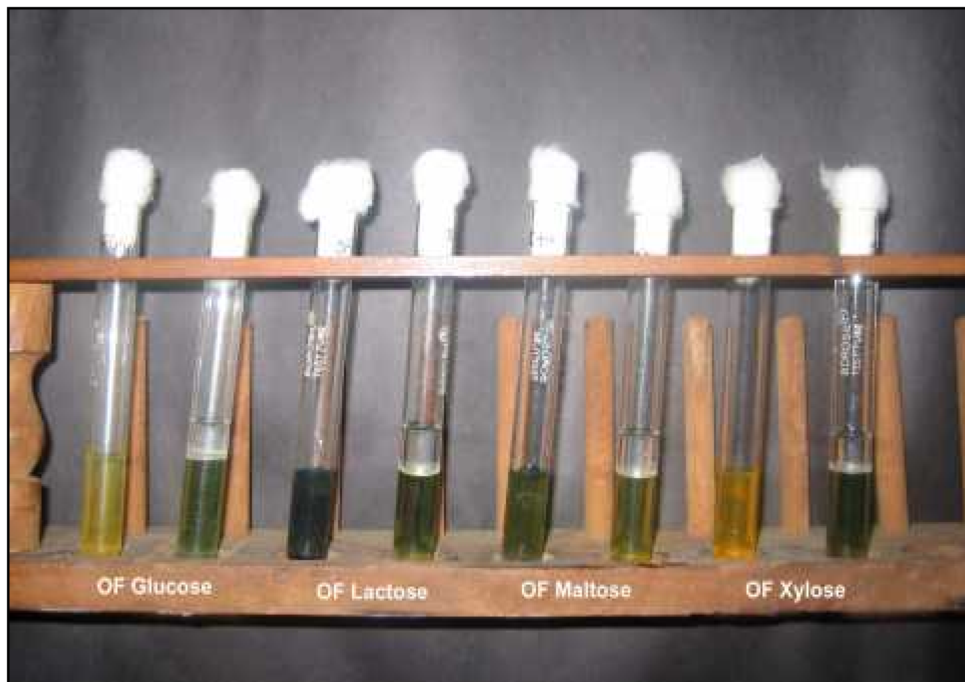


Photo 6: OF reactions of B.pseudomallei

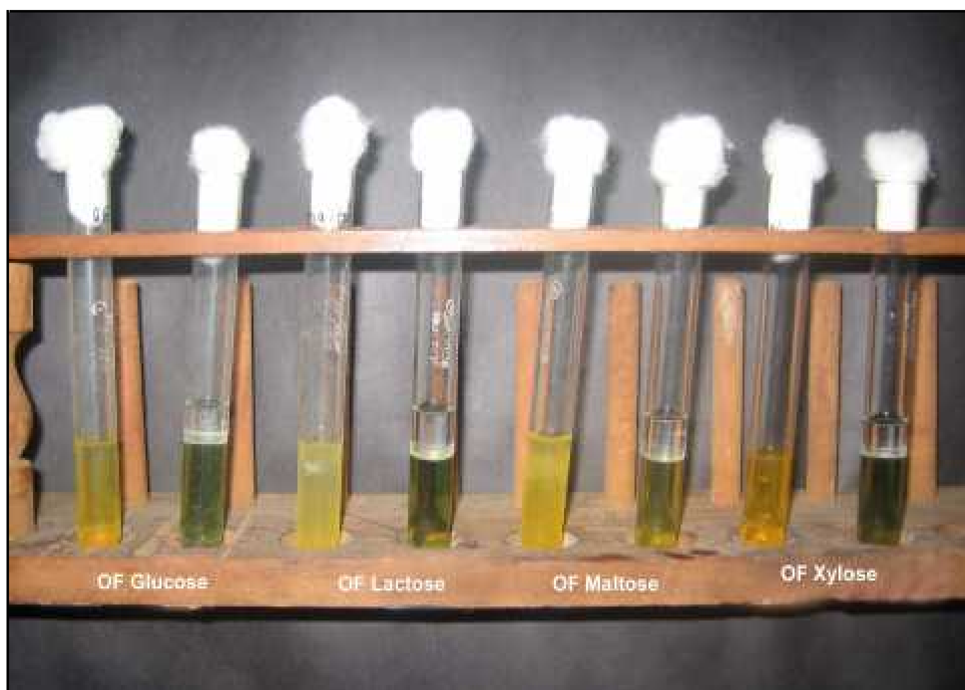


Photo 7: OF reactions of A.baumanii

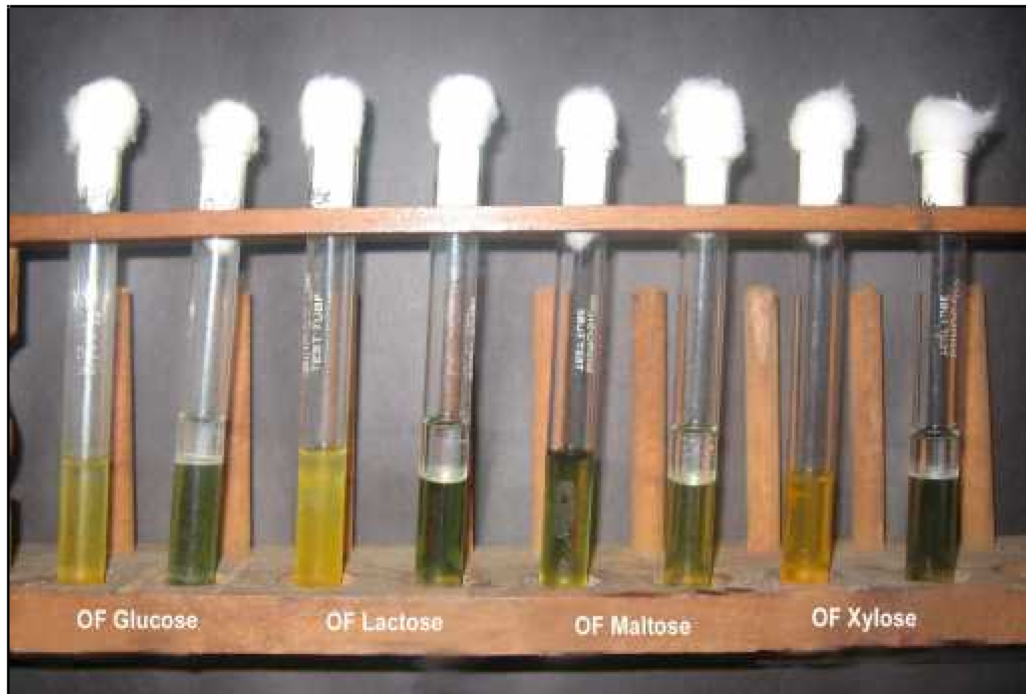


Photo 8: OF reactions of A. lwofii

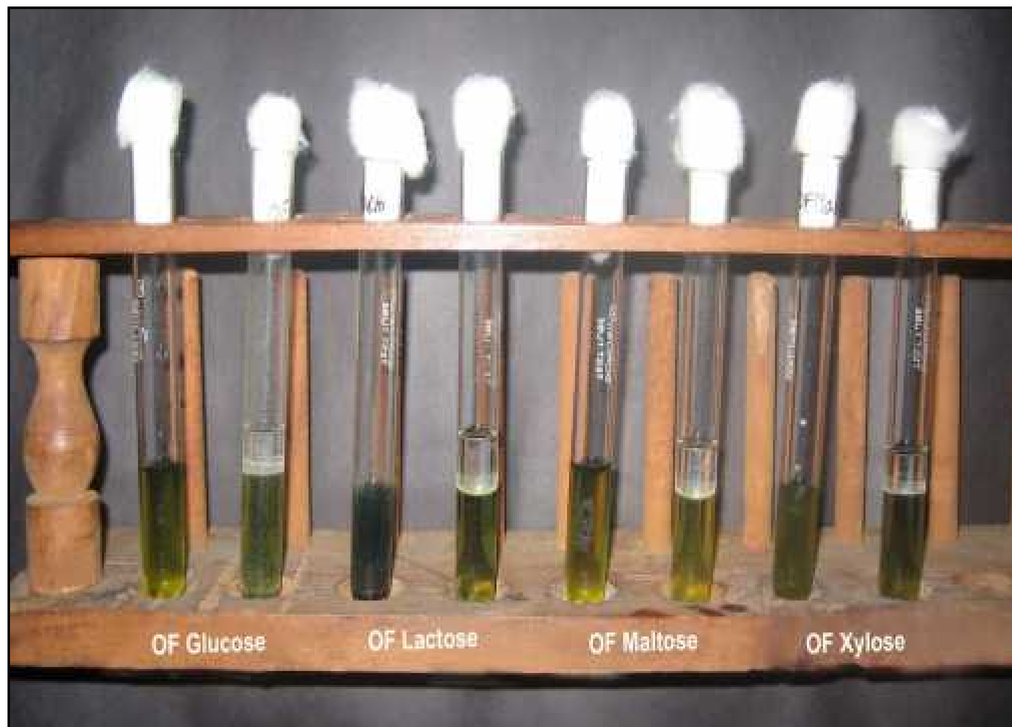


Photo 9: Oxidase Test

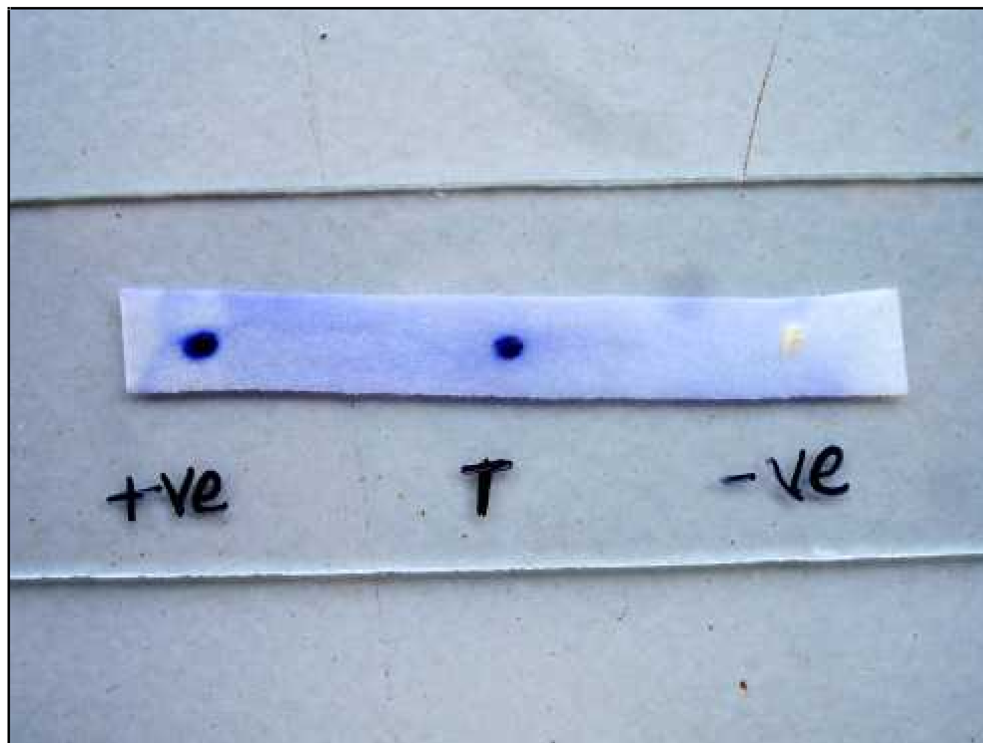


Photo 10: DNAse Test

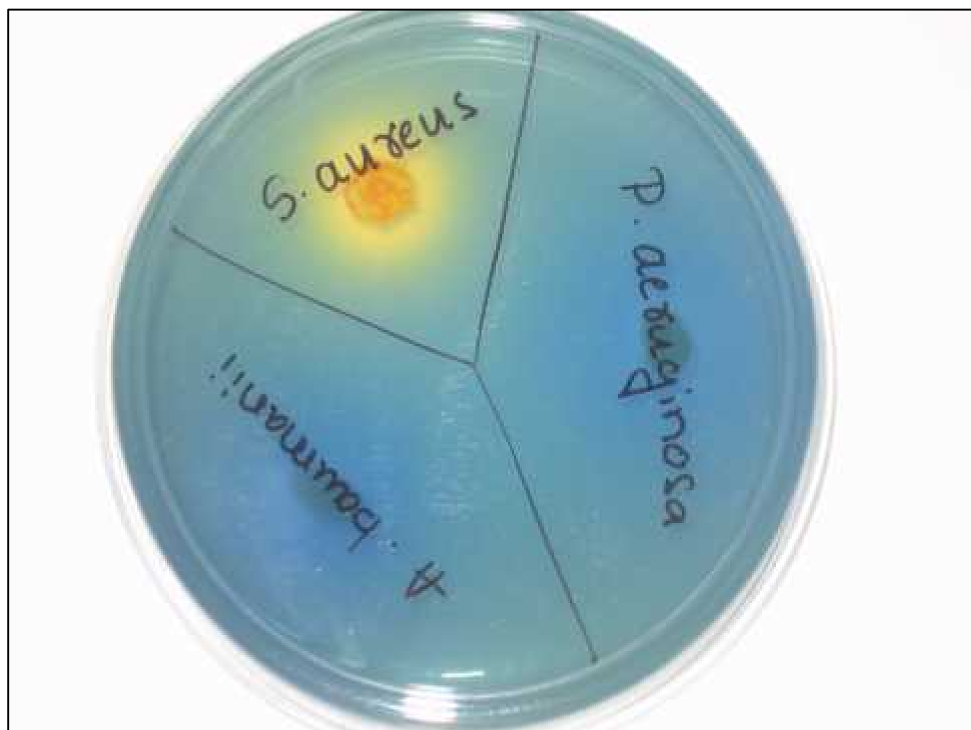
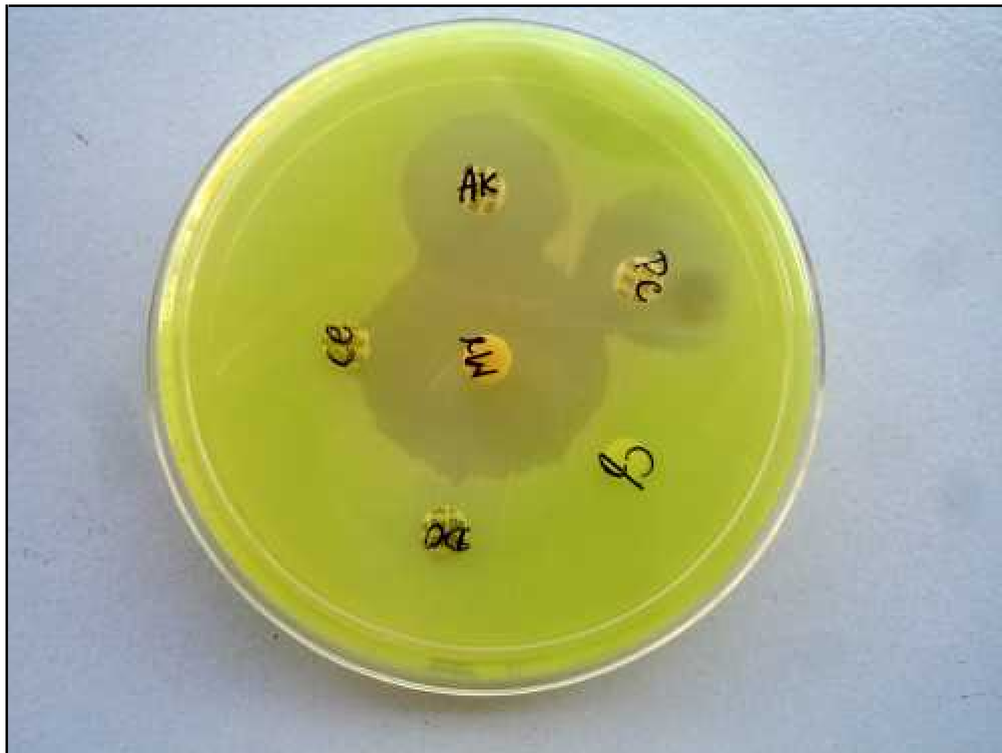


Photo 11: Antibiogram of P.aeruginosa



OBSERVATIONS AND RESULTS

A total of 130 nonfermenters isolated from different clinical samples were identified and characterized.

TABLE-1: NONFERMENTER ISOLATES FROM DIFFERENT CLINICAL SPECIMENS

SPECIMEN	NO.	%
PUS / WOUND DISCHARGE	76	58.4
BLOOD	30	23.0
URINE	11	8.2
SPUTUM	6	4.5
PLEURAL SWAB	3	2.3
CATHETER TIP	2	1.5
EAR SWAB	2	2.3

Nonfermenters were most commonly isolated from Pus/Wound discharge (58.4%), followed by Blood (23%).

GRAPH-1: NONFERMENTER ISOLATES FROM DIFFERENT CLINICAL SPECIMENS

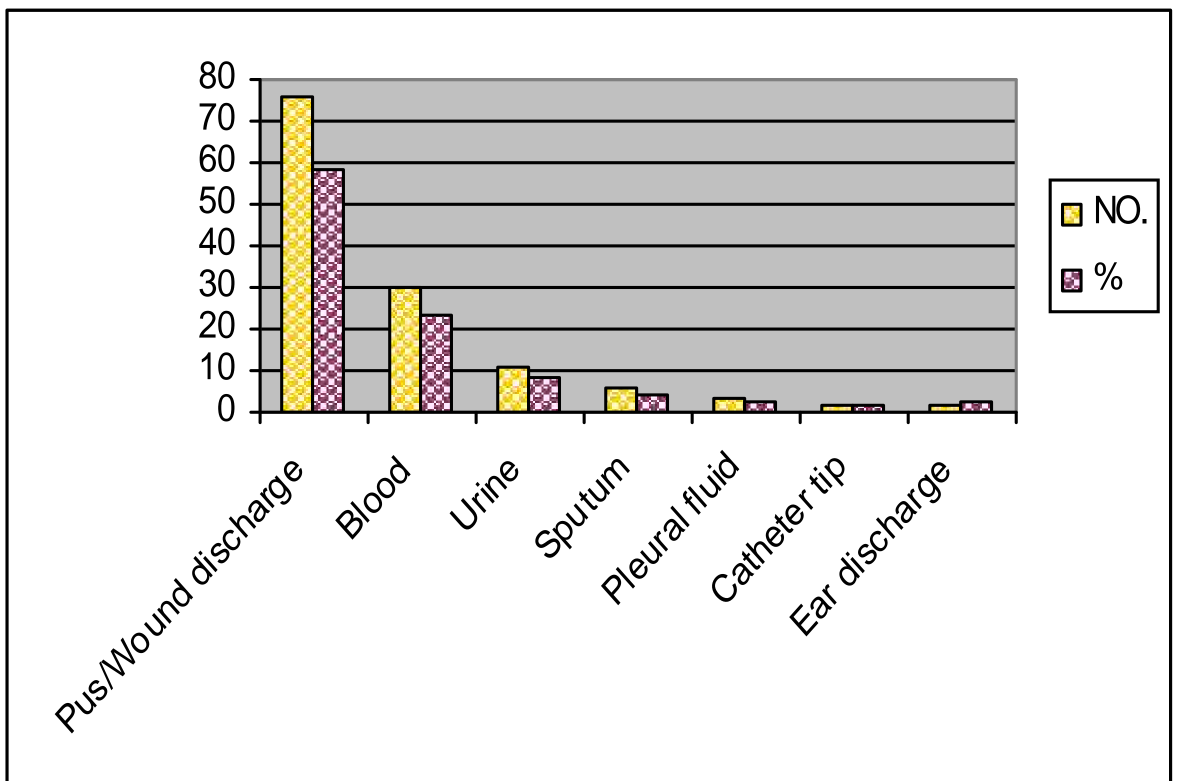


TABLE-2: DISTRIBUTION OF NONFERMENTERS IN DIFFERENT AGE AND SEX GROUPS

Sex	0-10yrs	11-20yrs	21-30yrs	31-40yrs	41-50yrs	51-60yrs	60yrs	Total
Male	22	06	11	09	13	06	09	76
Female	13	07	13	06	05	03	07	54
Total	35	13	24	15	18	09	16	130

GRAPH-2: : DISTRIBUTION OF NONFERMENTERS IN DIFFERENT AGE AND SEX GROUPS

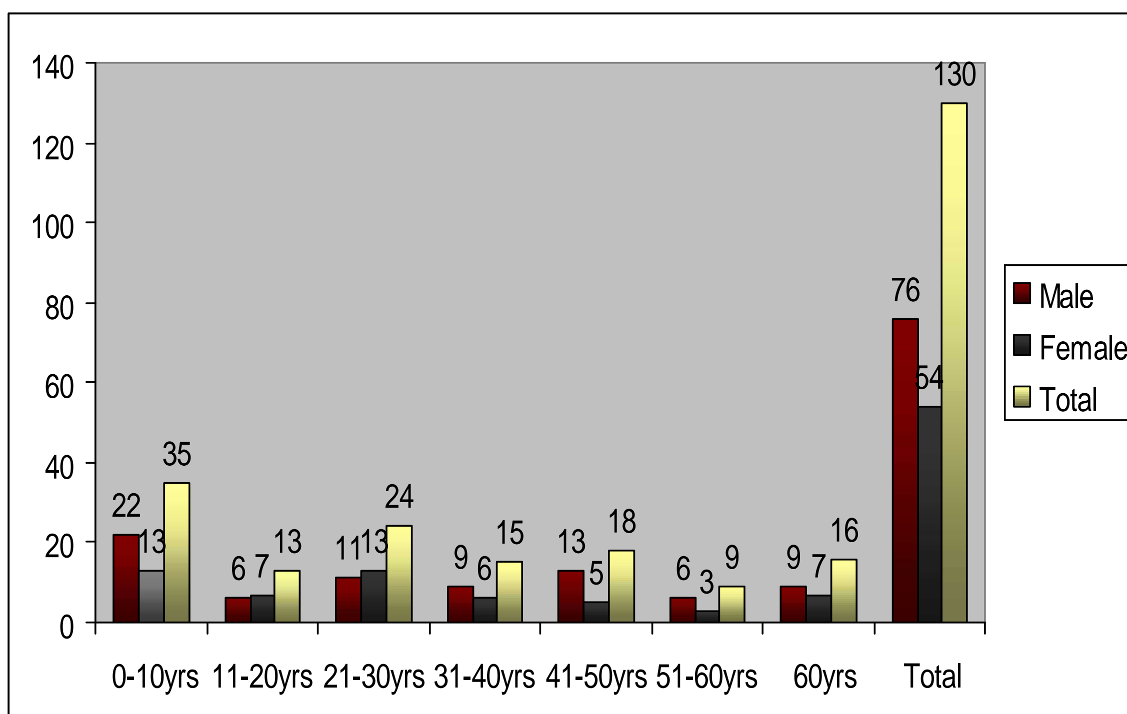


TABLE-3: NONFERMENTATIVE BACILLI IN CLINICAL SPECIMENS n =130

Isolate	No.	%
P.aeruginosa	107	82.3
A.baumanii	20	15.4
A.lwofii	01	0.76
B.pseudomaleii	01	0.76
Moraxella	01	0.76

GRAPH-3: NONFERMENTATIVE BACILLI IN CLINICAL SPECIMENS n=130

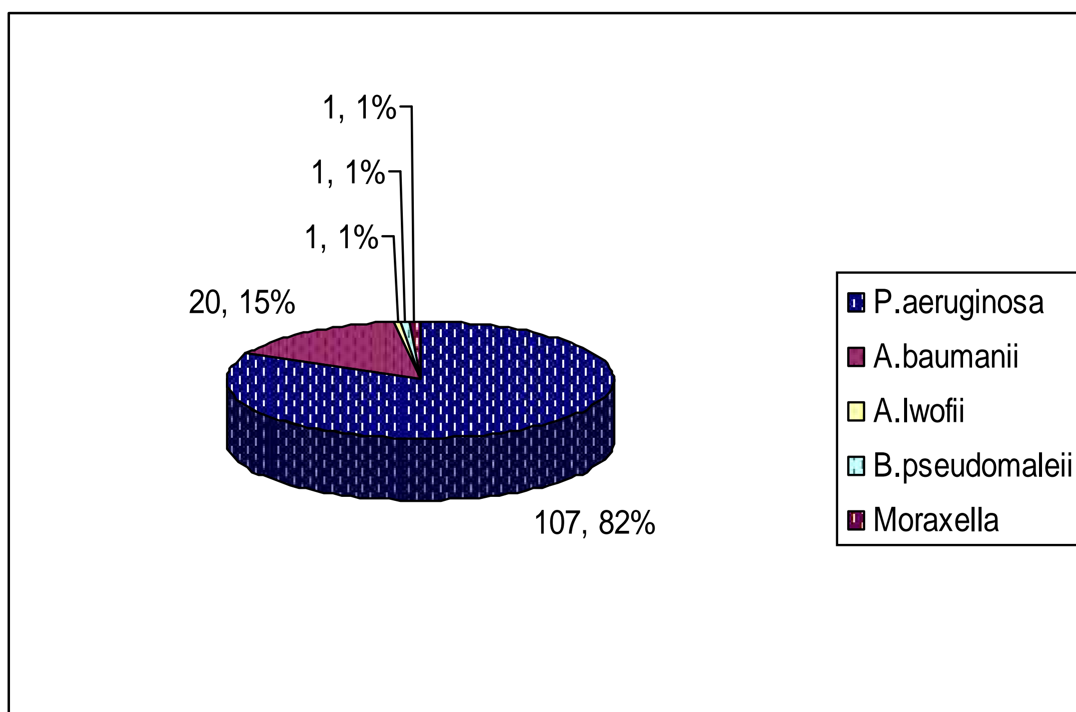


TABLE-4: BIOCHEMICAL CHARACTERISTICS OF NONFERMENTERS

Test	P.aeruginosa	A.baumanii	A.lwofii	B.pseudomallei	Moraxella
Oxidase	+ve	-ve	-ve	+ve	+ve
Pigment	+ve(93%)	-ve	-ve	-ve	-ve
Indole	-ve	-ve	-ve	-ve	-ve
H_2S	-ve	-ve	-ve	-ve	-ve
Urease	+ve(19%)	+ve(25%)	-ve	-ve	-ve
TSI	K/NC	K/NC	K/NC	K/NC	K/NC
MMM	NF&M	NF&NM	NF&NM	NF&M	NF&NM
OF Glu	A	A	-ve	A	-ve
OF Xylo	A	A	-ve	A	-ve
OF Lactose	-ve	A	-ve	A	-ve
OF Maltose	-ve	-ve	-ve	A	-ve
Growth at 42^0c	+ve	+ve	+ve	+ve	+ve
Esculin	-ve	-ve	-ve	-ve	-ve
ONPG	-ve	-ve	-ve	-ve	-ve
DNAse	-ve	-ve	-ve	-ve	-ve

TABLE-5: ANTIBIOTIC SENSITIVITY PATTERN OF NONFERMENTERS

n=130

Antibiotic	No.sensitive	% sensitive
Ampicillin(A)	3	2.3
Co-trimoxazole(Co)	12	9.2
Cefoperazone(Cs)	15	11.5
Cefotaxime(Ce)	19	14.6
Piperacillin(Pr)	21	16.1
Doxycycline(Do)	24	18.4
Amikacin(Ak)	60	46.1
Ciprofloxacin(Cf)	64	49.2
Meropenem(Mr)	122	93.8

GRAPH-4: ANTIBIOTIC SENSITIVISTY PATTERN OF NONFERMENTERS
n=130

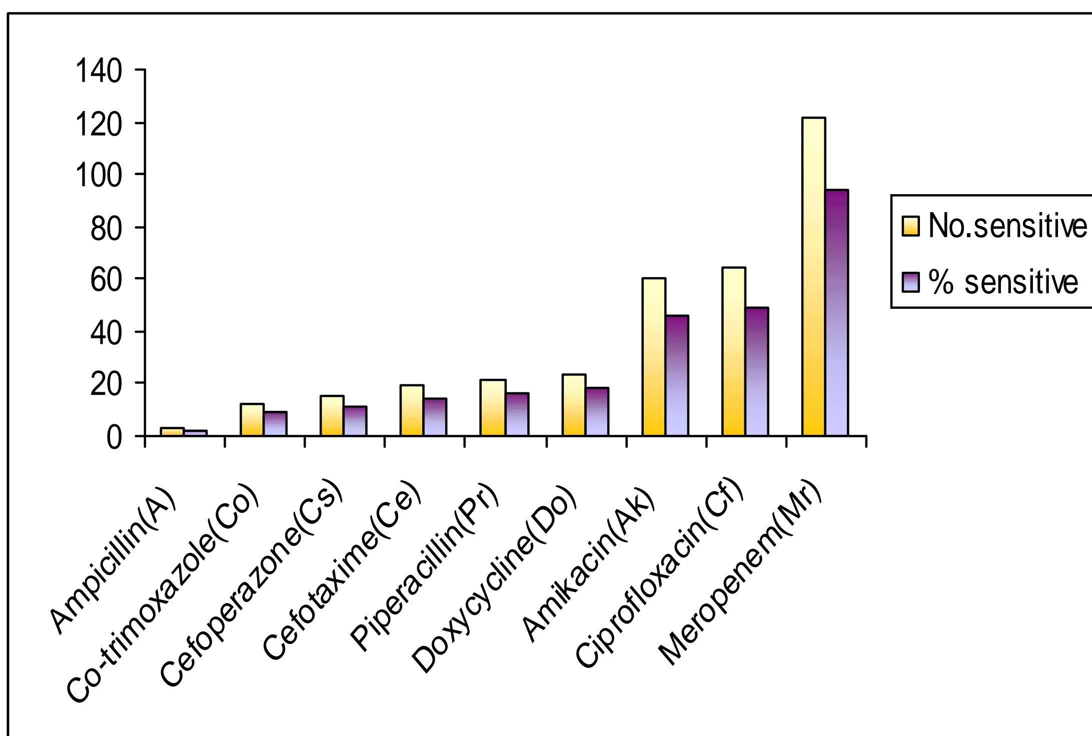


TABLE-6: ANTIBIOTIC SENSITIVITY PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATES n=107

Antibiotic	No.sensitive	% Sensitive
Ampicillin(A)	1	0.9
Co-trimoxazole(Co)	5	4.6
Cefoperazone(Cs)	11	10.2
Doxycycline(Do)	14	13
Cefotaxime(Ce)	15	14
Piperacillin(Pr)	17	15.8
Amikacin(Ak)	53	49.5
Ciprofloxacin(Cf)	54	50.4
Meropenem(Mr)	103	96.2

GRAPH-5: ANTIBIOTIC SENSITIVITY PATTERN OF PSEUDOMONAS AERUGINOSA ISOLATES n=107

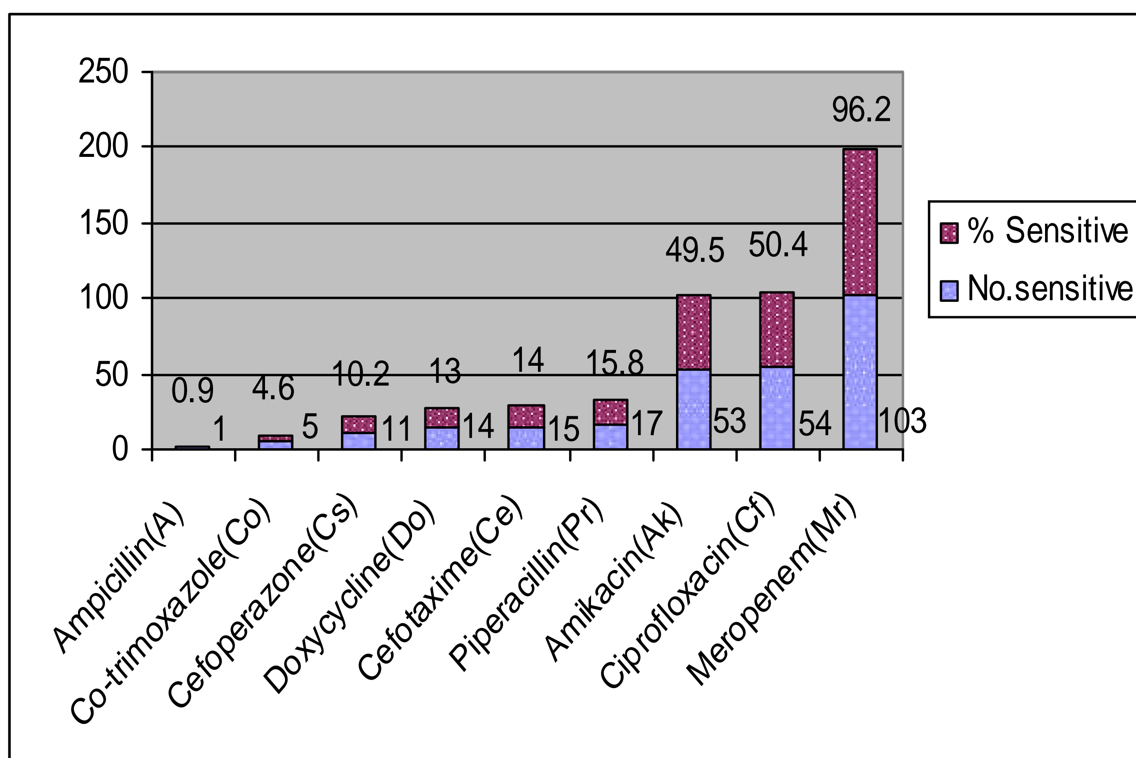
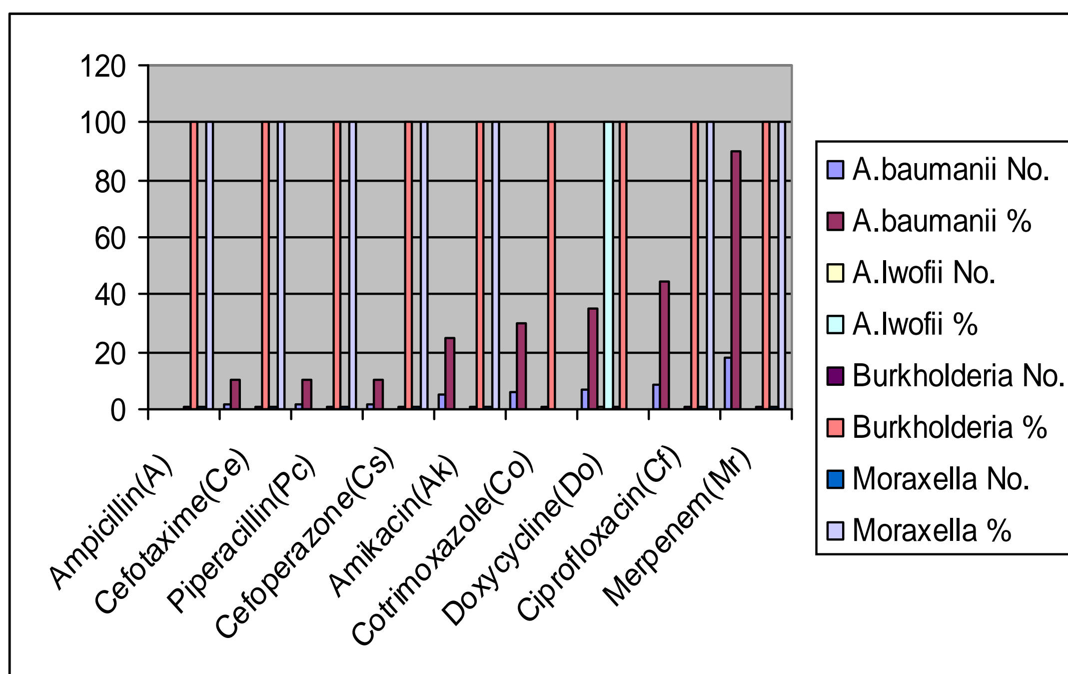


TABLE-7: ANTIBIOTIC SENSITIVITY OF ACINETOBACTER SPECIES AND OTHER NONFERMENTERS

Antibiotic	A.baumanii n=20		A.lwofii n=1		Burkholderia n=1		Moraxella N=1	
	No.	%	No.	%	No.	%	No.	%
Ampicillin(A)	0	0	0	0	1	100	1	100
Cefotaxime(Ce)	2	10	0	0	1	100	1	100
Piperacillin(Pc)	2	10	0	0	1	100	1	100
Cefoperazone(Cs)	2	10	0	0	1	100	1	100
Amikacin(Ak)	5	25	0	0	1	100	1	100
Cotrimoxazole(Co)	6	30	0	0	1	100	0	0
Doxycycline(Do)	7	35	1	100	1	100	0	0
Ciprofloxacin(Cf)	9	45	0	0	1	100	1	100
Merpenem(Mr)	18	90	0	0	1	100	1	100

GRAPH-6: ANTIBIOTIC SENSITIVITY OF ACINETOBACTER SPECIES AND OTHERNONFERMENTERS



DISCUSSION

Gram negative nonfermenting aerobic bacteria are being increasingly implicated in human disease55.The complex physicochemical properties of these organisms necessitate a battery of tests for their precise identification. In addition there is still much confusion regarding the taxonomic status of many of these nonfermenters. Identification of these nonfermenters has often being neglected. Therefore we intended to identify commonly encountered, clinically significant gram negative nonfermenting bacteria from clinical specimen along with their antimicrobial susceptibility pattern.

During a one year period (Jan 2007 to Jan 2008), we collected 130 isolates from clinical samples which gave an alkaline/alkaline (K/K) or alkaline/neutral (K/N) reaction on Triple sugar iron agar. All these were identified by a battery of tests as per standard laboratory techniques[16,18].

In our study most of the isolates were from pus samples (76) followed by blood (30), urine (11), sputum(6), pleural fluid (3), catheter tip and ear swab 2 each.

Pseudomonas aeruginosa was the most common isolate. Majority of the isolates were from pus/wound discharge(62) with 57.9% followed by blood (24) with 22.4%, urine(9), sputum(6), pleural fluid(3), earswab(2) and catheter tip(1) . This has correlated with study conducted by Veenu et al were in 53% of the P.aeruginosa isolates were from pus. Lolitha et al reported 60% of P.aeruginosa isolates to be isolated from pus samples followed by blood (25%) and urine(10%). In our study out of 6 Pseudomonas isolates from sputum samples 2 were from cystic fibrosis patients and the isolates were mucoid in nature.

Second common isolate was Acinetobacter.baumanii (15.3%). Most of the isolates were from pus samples accounting for 65% followed by blood samples (25%),catheter tip (5%) and urine (5%). Veenu et al reported that 59% of the isolates were from pus/wound discharge followed by blood samples(30%), which correlates well with our study.

A.lwofii was isolated from urine sample, Burkholderia was isolated from wound discharge and Moraxella was isolated from blood.

In our study, the commonest isolate was P.aeruginosa with 82.4%. This has correlated well with the study conducted by Akalin HE et al who reported P.aeruginosa to be isolated from 95.28% of cases. Vijaya et al reported the same isolate from 78.94%, Arora et al reported from 72.83% of cases and veenu et al reported from 72.6% of cases.

Some studies had a much lower rate of isolation of P.aeruginosa. Shenoy et al reported the same isolate from only 31.5% of cases. Merino LA et al reported from 48.8% of the cases. Elthaway et al isolated the same organism from 56% of the cases.

Suzuki Y et al reported P.aeruginosa from 68.4% of cases, Grover et al isolated from 67.2% and Frota et al reported it from 69.9% of cases.

In the present study the second commonest isolate was Acinetobacter baumanii with 15.4%. This result has correlated well with the study conducted by Merino LA et al who reported the same organism in 16.8% of cases. Lolitha LA et al reported the same in 21.4% of cases and Arora et al reported it to be 8.4%. In all these studies A.baumanii was the second commonest isolate after P.aeruginosa.

However Elthaway et al had reported a much higher rate of isolation of A.baumanii (34%).

Grover et al reported A.baumanii from 6.2% of cases, Vijaya et al reported 6.1% of isolates to be A.baumanii, Suzuki et al reported the same organism from 5.6% of cases. Frota et al had isolated from 5.48% and Veenu et al had reported it to be 3.66%. Whereas in a study conducted by Shenoy S et al did not have any A.baumanii isolate.

In this study the other nonfermenters isolated were A.lwofii(0.76%), Moraxella species(0.76%) and Burkholderia pseudomallei(0.76%). Suzuki et al isolated A.lwofii from 2.6%. Frota et al isolated from 4.4%. However Grover et al had reported a higher rate of isolation from 7.8% of cases. Lolitha et al had reported Burkholderia from 3.1% of cases. Veenu et al has reported a single isolate of B.pseudomallei from urine sample.

In the present study nonfermenters were commonly isolated from 0-10yrs age group(35 isolates). Most of the isolates were from Blood samples of neonates with h/o birth complications and features of septicaemia. In this study 24 isolates were isolated from 21-30yrs age group followed by 60yrs age group with 16 isolates.

In our study, we found that, 76 nonfermenters were isolated from the male patients where as 54 were from females. The male:female to ratio was 0.7:1. This is in accordance with the study conducted by Lolitha et al, who reported 70% of the isolates from male patients. However in a study conducted by Gardener et al where in nonfermenters were divided equally among male and female patients.

In general nonfermenters appear inert in the typical tests used for fermentative gram negative bacilli. Conventional sugar media used for fermentative bacteria do not support the growth of many nonfermenters and the acids produced are often too weak

to convert the pH indicator. Hugh and Leifson's OF medium that accommodated the metabolic properties of nonfermenters is used for the identification and speciation.

Most common isolate in our study, P.aeruginosa oxidized Glucose and Xylose to produce acid, A.baumanii oxidized Glucose, Lactose and Xylose sugars in OF sugar media. A.lwofii and Moraxella were nonsacharolytic, have not oxidized any of the sugars. B.pseudomallei oxidized all the sugars.

Different types of pigments are produced by nonfermenters, which are helpful in making a species identification. P.aeruginosa produces pyocyanin pigment in 92-97% of strains. In our study 94% of P.aeruginosa isolates have produced pyocyanin pigment.

Commonly urea hydrolysis is not seen in P.aeruginosa isolates. In our study urea hydrolysis was seen in 19% of P.aeruginosa isolates which is a variable and 25% of A.baumanii strains hydrolysed urea.

Nonfermenters are resistant to a large number of commonly used antibiotics. In our study most of the strains showed sensitivity to Meropenem(93.8%) followed by Ciprofloxacin(49.2%) and Amikacin(46.1%). Doxycycline, Cefotaxime, Piperacillin and Cefoperazone sensitivity was 18.4%, 14.6% 16.1% and 11.5% respectively. The isolatesshowed least sensitivity to Ampicillin(2.3%) and Co-trimoxazole(9.2%).

Meropenem sensitivity as reported by various workers varies from 90-99%. 93% of the strains were sensitive to Meropenem in our study is in accordance with the study conducted by Karlowsky JA et al who reported 96% sensitivity and Elting et al reported it to be 92%.

Amikacin sensitivity varies from 55-82% as reported in various studies. Amikacin sensitivity of 46.1% in our study is less as compared to the study conducted by Chaudhary et al with 71.8% and Veenu et al with 68%, which is statistically not significant. Ciprofloxacin sensitivity varies from 48-90%. Ciprofloxacin sensitivity of 49.2% correlates well with the study conducted by Mahapatra et al.

In our study out of 107 Pseudomonas aeruginosa isolates 103 (96.2%) were sensitive to Meropenem which correlates well with studies conducted by Fadda G et al and Elting et al. ciprofloxacin sensitivity in this study was 50.4% which correlates well with study conducted by Mahapatra et al. amikacin sensitivity of 49.5% in our study is less as compared to the studies conducted by the Karlwosky et al and Mahapatra et al. Piperacillin sensitivity of 15.8% in our study is very less as compared to the study by Veenu et al and Grover et al Doxycycline, Cefotaxime, Cefoperazone sensitivity was 13%, 14% and 10.2% respectively.

Mehta et al had reported Amikacin, Ciprofloxacin and Piperacillin as the most effective anti-psuedomonadal agents- sensitivity being 89%, 88% and 86.5% respectively. However in the study by Nagoba et al, though Amikacin sensitivity was high(90%), Piperacillin and Ciprofloxacin sensitivity was less than 60%. In our study Amikacin and Ciprofloxacin sensitivity was 49.5% and 50.4% respectively. Thus sensitivity of these antibiotics is on the decline.

A total of 20 A.baumanii isolates were tested for the antibiotic sensitivity testing. Meropenem sensitivity of 90% has been reported in our study, correlates well with study conducted by Karlwosky et al(90%) and Taneja et al(88%).

Ciprofloxacin, Doxycycline, Co-trimoxazole and Amikacin sensitivity was 45%, 35%, 30% and 25% respectively. The sensitivity of Cefotaxime, Piperacillin and Cefoperazone was 10%.

Though multidrug resistance is common among A.baumanii, Meropenem has shown high in-vitro activity against the same. Amikacin and Ciprofloxacin are moderately active.

Fleischer et al have shown that Acinetobacter species are highly resistant to first and second generation Cephalosporins and 70% sensitive to third generation cephalosporins.

A.lwofii was isolated from an adult female patient with post operative wound infection. It showed resistance to multiple antibiotics. It was resistant to Amikacin, Ciprofloxacin, Cefotaxime, Piperacillin, Cefoperazone and Ampicillin. It was sensitive only to Doxycycline.

B.pseudomallei strain was a sensitive strain and sensitive to all antibiotics, similar to the study by Anuradha et al.

Moraxella isolate was a sensitive strain, resistant only to Doxycycline and Co-trimoxazole.

Thus this study shows that nonfermenters are isolated frequently from various clinical specimens and they are resistant to the routinely used antibiotics. This emphasize that these organisms need to be taken more seriously and not discarded as contaminants or nonpathogens. Identification of these organisms must be done and isolation of organisms can throw more light on their prevalence and pathogenic role.

CONCLUSION

1. In the present study nonfermenters were isolated from different clinical samples. P.aeruginosa was the commonest isolate. Out of 130 nonfermenters 107(82%) were P.aeruginosa followed by A.baumanii with 20(15%).
2. The majority of the isolates were isolated from pus/wound discharge(78) followed by blood (30) and urine(11)
3. Maximum number of isolates were from patients of age group 0-10yrs (35) most of them were neonates with history of LBW and septicemia. The least number of isolates were from age group 51-60yrs(9).
4. Males were commonly affected than females.
5. Meropenem was the most effective drug with 93.8% sensitivity followed by Ciprofloxacin of 49.2% and Amikacin of 46.9% sensitivity.
6. Out of 107 isolates of P.aeruginosa 96.2% were sensitive to Meropenem, 50% to Ciprofloxacin, 49.5% to Amikacin.
7. A.baumanii isolates were more sensitive to Meropenem(96.2%) followed by Ciprofloxacin (45%), Doxycycline (35%) and Amikacin(25%).

SUMMARY

The present study was conducted between Jan 2007-Jan 2008 in the department of Microbiology, J.N.Medical College, Belgaum.

A total of 130 isolates from various clinical samples were taken and identified to the species level by using conventional tests[16,18].

Out of 130 isolates, 107(82.3%) were Pseudomonas aeruginosa, 20(15.4%) were Acinetobacter baumanii, 1(0.76%) each of A.lwofii, B.pseudomallei and Moraxella were isolated.

The majority of the isolates were isolated from pus/wound discharge(78) followed by blood (30), urine(11), sputum(6) and 2 each from catheter tip and ear swab.

Maximum number of isolates were from patients of age group 0-10yrs (35) most of them were neonates with history of LBW and septicemia and 21-30yrs (24). The least number of isolates were from age group 51-60yrs(9).

Nonfermenters were more commonly isolated from males(76) as compared to females(54).

All the isolated strains were tested for antimicrobial susceptibility by using Kirby Bauer disc diffusion method. Out of 130 isolates, 122 (93.8%) isolates were sensitive to Meropenem , 64(49.2%) to Ciprofloxacin, 60(46.1%) to Amikacin, 24(18.4%) to Doxycycline and 19(14.6%) to Cefotaxime.

Out of 107 isolates of P.aeruginosa 96.2% were sensitive to Meropenem, 50% to Ciprofloxacin, 49.5% to Amikacin. Isolates were least sensitive to Ampicillin, doxycycline, Cefotaxime and Piperacillin.

A.baumanii isolates were more sensitive to Meropenem(96.2%) followed by Ciprofloxacin (45%), Doxycycline (35%) and Amikacin(25%).

A.lwofii isolate was resistant to multiple antibiotics. It was sensitive only to Doxycycline.

B,psuedomallei isolate was a sensitive strain and was sensitive to all the antibiotics tested.

Moraxella isolate was also a sensitive strain, resistant only to Doxycycline and Co-trimoxazole.

BIBLIOGRAPHY

1. Mishra B, Bhujwala RA, Shrinivas. Non fermenters in human infections. Indian J Med Res 1986 Jun; 83: 561-6.
2. Gulati S, Kapil A, Goel V, Das B, Dwivedi SN, Mahapatra AK. Biotyping of Acinetobacter species isolated from clinical samples. Indian J Med Res 1999 Nov; 110: 160-3.
3. Bergogne-Berezin E, Towner KJ. Acinetobacter spp. as Nosocomial Pathogens: Microbiological, Clinical and Epidemiological Features. Clin Microbiol Rev 1996 Apr; 9(2): 148-65.
4. Vijaya D, Kamala, Bavani S, Veena M. Prevalence of nonfermenters in clinical specimens. Indian J Med Sci 2000 Mar; 54(3):87-91.
5. Gardner, B Griffin, Swartz, Kunz. Nonfermentative gram negative bacilli of nosocomial interest. American J Med 1970 Jun ; 48: 735-749.
6. Allan J, Morrison, Jr and Richard P.Wenzel. Epidemiology of infections due to Psuedomonas aeruginosa. Rev Infect Dis 1984 Sept-Oct; 6 Suppl. 3: S627-42.
7. Kreig R, Holt J. Bergey's Manual of Systemic Bacteriology. 9th Edition, Volume 1, The William's and Wilkin's, Baltimore 1984.
8. Koneman E, Allen S, Janda W, Schreckenberger P, Winn W. Colour Atlas and textbook of Diagnostic Microbiology. 5th Edition, Lippincott –Raven Publishers, Philadelphia, 1997.
9. Pitt TL. Psuedomonas, Burkholderia and related genera in Topley and wilson's Microbiology and Microbial infections 9th edition, Collier L, Balows A, Sussman M Arnold Publishers 1998, Vol 2; 1109-1129.

10. 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.
11. Mehta M, Punia JN, Joshi RM. Antibiotic resistance in Pseudomonas aeruginosa strains isolated from various clinical specimens- a retrospective study. Indian J Med Microbiol 2001; 19(4):232.
12. Rao PS, Shivananda PG. Bacteremia due to nonfermenting gram negative bacilli in immuno-compromised patients. Indian J Med Microbiol 1993; 11: 95-99.
13. Christopher A, Ohl, Mathew Pollack. Infections due to Pseudomonas species and related organisms in Harrison's principles of Internal Medicine. Brunwald, Fauci, Kasper, Hauser, Longo and Jameson. McGraw Hill Companies Inc. 2001: 963-970.
14. Aliaga L, Mediavilla JD, Cobo F. A clinical index predicting mortality with Pseudomonas aeruginosa bacteremia. J Med Microbiol. 2002 Jul; 51(7): 615-9.
15. Kiska DL, Kerr A, Jones MC, Carraciolo JA, Eskridge B, Jordan M, Miller S, Hughes D, King N, Gilligan PH. Accuracy of four commercial systems for identification of Burkholderia cepacia and other gram negative nonfermenting bacilli recovered from patients with cystic fibrosis. J Cli Microbiol 1996 34: 886-891.
16. Forbes B, Sahm D, Weissfeld A. Baily and Scott's Diagnostic Microbiology. 10th Edition,Mosby Inc, 1998.
17. Veenu RS, Arora DR. Isolation and susceptibility pattern of nonfermenting gram-negative bacilli from clinical samples. Indian J Med Microbiol 1998 17: 14-17.

18. Morrison AJ, Hoffman KK, Wenzel RP. Associated mortality and clinical characteristics of nosocomial Pseudomonas maltophilia in a university hospital. J Clin Microbiol 1986; 24: 52-55.

19. Muder RR, Haris AP, Muller S M, Edmond M, Chow JW, Padadakis K, Wagener MW, Bodey GP, Steckelberg JM. Bacteremia due to Stenotrophomonas maltophilia- a prospective, multicenter study of 91 episodes. Clin Infect Dis 1996; 22: 508-512.

20. Berger SA, Siegma-Igra Y, Stadler J. Group VE-1 septicemia. J Clin Microbiol 1983 ; 17 : 926-27.

21. Verhasselelt B, Claeys G, Elaichouni A. Case of recurrent Flavimonas oryzihabitans bacteremia associated with an implanted central venous catheter (Port A- Cath) assessment of clonality by arbitrarily primed PCR. J Clin Microbiol 1995; 33: 3047-48.

22. Morrison AJ, Sxhulman JA. Community acquired bloodstream infection caused by Ps.paucimobilis: A case report and review of literature. J Clin Microbiol 1986; 29: 853-55.

23. Marne C, Pallares R, Sitges-Serra A. Isolation of Pseudomoans putrefaciens in intraabdominal sepsis. J Clin Microbiol 1983; 17: 1173-1174.

24. Hollis DG, Weaver RE. Chemical and cultural characterization of CDC group WO-1, a weakly oxidative gram negative group of organisms isolated from clinical sources. J Clin Microbiol 1992 30: 2474-2475.

25. Morrison AJ, Boyce K. Peritonitis caused by Alcaligenes dentrificans subsp. xylosoxidans- case report and review of the literature. J Clin Microbiol 1986; 24:879-881.

26. Bauwens JE, Spach DH, Schacker TW, Mustafa MM, Bowden RA. Bordetella bronchiseptica pneumonia and bacteremia following bone-marrow transplantation. J Clin Micrbiol 1992; 30:2474-2475.

27. Dunne WM Jr, Tillman J, Murray JC. Recovery of a strain of Agrobacterium radiobacter with a mucoid phenotype from an immunocompromised child with bacteremia. J Clin Microbiol 1993; 31: 2541-2543.

28. Freney J, Gruer LD, Bornstein N et al. Septicemia caused by Agrobacterium species. J Clin Microbiol 1985; 22: 683-685.

29. Towner KJ. Acinetobacter in Topley and Wilson's Microbiology and Microbial infections 9th edition, Collier L, Ballows A, Sussman M. Arnold Publishers 1988, Vol II; 1229-1236.

30. G. Wauters, A.Boel, G.P.Voorn, Verhagen, F.Meunier, M.Janssens et al. Evaluation of a new identification system, crystal enteric/nonfermenter, for gram negative bacilli. J Clin Microbiol 1995;33:845-849.

31. Helio S, Sader, Thomas R, Fritsche and Ronald jones. Accuracy of three automated systems (Microscan walkaway, VITEK and VITEK 2) for susceptibility testing of Pseudomonas aeruginosa against five broad spectrum beta lactam agents. J Clin Microbiol 2006;44:1101-1104.

32. Collee JG, Doguid JP, Fraser AG and Marmion BP. Mackie and Mc Cartney practical medical microbiology: 14th ed. USA: Churchill Livingstone; 1989.

33. MacFaddin J, Biochemical tests for identification of medical bacteria. The Williams and Wilkins company, Baltimore, 1976.

34. Veenu, Sikka R and Arora DR. Isolation and Susceptibility pattern of nonfermenting gram negative bacilli from clical samples. Ind J Med Microbiol 1999; 17(1): 14-17.

35. Lolitha Madhta and K. Gopala Krishna Bhat. Nonfermenting gram negative bacilli infections in hospitalized children. Ind Assoc Biomedical Scientists 1999; 19(2): 99-102.

36. Akalin HE and Bakayal M. Nonfermentative gram negative bacilli: their distribution to clinical materials and antibiotic susceptibility. Mkrobiyol Bul 1980 Jan; 14(1): 21-26.

37. Vijaya D, Kamala, Bhavani S and Veena M. Prevalence of nonfermenters in clinical specimens. Indian J Med Microbiol 2000; 54(3): 87-91.

38. Arora U, Aggarwal A and Sofat S. Nonfermenters in human infections. Indian J Pathol Microbiol 2003 Apr; 46(2): 256-57.

39. Merino LA, Ronconi MC, Marin M, Hrenuk GE and Pato AM. Nonfermenting gram negative bacilli: distribution in clinical specimens and antimicrobial susceptibility. Rev Lationam Microbiol 1999 Oct-Dec; 41(4): 279-84.

40. Elthaway AT and Khalaf RM. Antibiotic resistance among gram negative nonfermentative bacteria at a teaching hospital in Saudi Arabia. J Chemother 2001 Jun; 13(3): 260-64.

41. Suzuki Y, Koguchi M, Tanaka S, Fulkayama S, Ishihara R, Deguchi K et al. Frequency of clinical isolation of glucose nonfermentative gram negative rods and their susceptibilities to antibacterial agents. Jpn J Antibiot 1995 sept; 48(9): 1264-73.

42. Kaushal ML, Grover PS and Gupta ML. Nonfermenters in urinary tract infections. J Assoc Physician India 1998 sept; 46(9): 798-800.

43. Frota, Cristiane Cunha, Moreira Jose and Luciano Bezerra. Frequency of nonfermentative gram negative bacilli isolated from clinical materials of patients at universdade Federal do Ceara Hospital Complex, Brazil. Rev Microbiol 1998 sept; 29(3): ISSN 0001-3714.

44. Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Surveillance for antimicrobial susceptibility among clinical isolates of Pseudomonas aeruginosa and Acinetobacter baumanii from hospitalized patients in the United States, 1998 to 2001. Antimicrob Agents Chemother 2003 May; 47(5): 1681-1708.

45. Elting LS, Bodey GP. Septicemia due to Xanthomoans species and non aeruginosa Ps. species: increasing incidence of catheter related infections. Medicine 1990; 69: 296-306.

46. Mahapatra A, Samal B, Pattnaik D, Mishra S and Ghosh SK. Antimicrobial susceptibility pattern of clinical isolates of nonfermentative bacteria. Indian J Pathol Microbiol 2003 Jul; 46(3): 526-27.

47. Fadda G, Sapnu T, Ardito F, Taddei C, Santangelo R, Siddu A et al. Antimicrobial resistance among nonfermentative gram negative bacilli isolated from the respiratory tracts of Italian inpatients: a 3 year surveillance study by the Italian epidemiological survey. Int J Antimicrob Agents 2004 Mar; 23(3): 254-61.

48. Mehata M, Punja JN, Joshi RM. Antibiotic resistance in Pseudomonas aeruginosa strains isoalated from various clinical specimens – a retrospective study. Indian J Med Microbiol 2001; 19(4): 232.

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

50. Fleischer M, Przondo-Mordarska A, Ruzkowska J. Sensitivity of bacteria of the genus Acinetobacter to antibiotics and ofloxacin. Med Dosw Mikrobiol. 1991; 43(3-4): 103-10.

51. Anuradha K, Meena AK and Lakshmi V. Isolation of Burkholderia pseudomallei from a case of septicemia : A case report. Indian J Med Microbiol 2003; 21: 129-132

ANNEXURE - I : MEDIA

I) Blood agar

Nutrient agar, commercially available from Himedia, was prepared.

Ingredients, per 1000ml:

Peptic digest of animal tissie	-	5gms
Sodium chloride	-	5gms
Beef extract	-	1.5gms
Yeast extract	-	1.5gms
Agar	-	15gms

Final pH (at 25^0 c) 7.4±0.2

28 grams were dissolved in 1000ml of distilled water. This was boiled to dissolve, and sterilized at 15 lbs pressure(121^0c) for 15 minutes.

The nutrient agar was cooled to 50^0c and sterile human blood was added aseptically in a concentration of 5%.this was then poured in to sterile plates of 9 cms diameter, to a thickness of about 4mm.

II) MacConkey agar(Commercially available from Himedia)

Ingredients, per 1000ml:

Pancreatic digest of gelatin	-	17gms
Peptic digest of animal tissue	-	1.5gms
Casein enzymatic hydrolysate	-	1.5gms
Lactose	-	10gms

Bile salts	-	1.5gms
Sodium chloride	-	5gms
Neutral red	-	0.03gms
Crystal violet	-	0.001gms
Agar	-	15gms

Final pH (at 25^0 c) 7.1±0.2

51.5gms were dispensed in one litre distilled water and boiled to dissolve. This was autoclaved at 15lbs pressure (121^0c) for 15 minutes and then poured in to plates.

III) Mueller-Hinton agar: (Commercially available from HiMedia)

Beef extract	-	300gms
Peptone	-	17.5gms
Starch	-	1.5gms
Agar	-	17gms
Distilled water	-	1000ml

Final pH (at 25^0 c) 7.1±0.2

Suspend the medium in distilled water mix thoroughly and heat with frequent agitation. Boil for about 1 min, dispense, sterilize by autoclaving at 116^0c-121^0c for not more than 15 min and cool by placing immediately in a 50^0c water bath before pouring.

IV) Triple sugar iron agar (Commercially available from Himedia)

Ingredients per 1000 ml

Peptic digest of animal tissue	-	10gms
Casein enzymatic hydrolysate	-	10gms
Yeast extract	-	3gms
Beef extract	-	3gms
Lactose	-	3gms
Sucrose	-	10gms
Dextrose	-	10gms
Sodium chloride	-	1gm
Ferrous sulphate	-	5gms
Sodium thiosulphate	-	0.20gms
Phenol red	-	0.024gms
Agar	-	12gms

Final pH (at 25^0 c) 7.4±0.2

65 grams of medium was dispensed in 1000ml of distilled water and heated to boiling to dissolve. The medium was dispensed in test tubes and autoclaved at 15lbs pressure (121^0c) for 15 minutes. The tubes were set in sloped form with a 1 inch butt.

V) Christensen's urease medium

Peptone	-	1gm
Sodium chloride	-	5gms
Monopotassium phosphate	-	2gms
Glucose	-	1gm
Phenol red	-	0.012gm
Agar	-	15gms
Distilled water	-	1000ml

Final pH – 6.8

Ingredients were dissolved in water and dispensed into test tubes. This was autoclaved at 15lbs pressure (121^0c) for 15 minutes.

20% urea solution, sterilized by filteration was added aseptically to the tubes to a concentration of 10% and the medium was set to form slants.

VI) Simmon's citrate medium

Sodium chloride	-	5gms
Magnesium sulphate	-	0.2gm
Ammonium dihydrogen phosphate	-	1gm
Potassium dihydrogen phosphate	-	1gm
Sodium citrate	-	5gms
Distilled water	-	1000ml

Final pH - 6.8

The ingredients were dissolved in water by boiling and the medium was dispensed in test tubes and sterilized by autoclaving at 15 lbs pressure (121^0c) for 15 minutes.

VII) Hugh and Leifson's Oxidation-Fermentation medium

Peptone	-	2gms
Sodium chloride	-	5gms
Dipotassium phosphate	-	0.3gm
Agar	-	3gms
Bromothymol blue	-	0.03gm
Distilled water	-	1000ml

Final pH – 6.8

The ingredients were dissolved in water by boiling and 1% of sugars were added and sterilized by autoclaving at 15 lbs pressure (121^0c) for 15 minutes. The medium was dispensed in test tubes.

VIII) DNAse agar: (Commercially available from HiMedia)

Ingredients per 1000ml

Tryptose	-	20gms
Deoxyribonucleic acid	-	2gms
Sodium chloride	-	5gms
Bromothymol blue	-	0.03gm

IX) ONPG:

o-nitrophenyl- -galactopyranoside - 0.6gm

Phosphate buffer 0.01mol/litre, pH 7.5 - 100ml

Dissolve without heat and sterilize by filtration

Add aseptically 1 part of the above to the 3 parts of 1% peptone in water at pH - 7.5. Distribute in 2ml amounts in test tubes.

ANNEXURE - II

Proforma

Name: **Age:** **Sex:**

Hospital no:

Ref by:

D.O.A:

Presenting complaints:

History of presenting illness:

Past history:

History of antibiotic intake:

Diagnosis:

Specimen:

Microbiological investigations:

Growth on culture media:

1) Nutrient agar
2) Mac Conkey agar
3) Blood agar

Gram's stain:

Biochemical tests:

1) Oxidase
2) Motility
3) Indole
4) H_2S
5) Urease
6) TSI

7) Mannitol motility medium

8) OF Glucose

9) OF Xylose

10) OF Lactose

11) OF Maltose

12) Growth at 42^0c

13) Esculin hydrolysis

14) ONPG

15) DNAse

Species:

Antibiotic susceptibility test:

Ak -

Co -

Ce -

A -

Do -

Cf -

Mr -

Pc -

Cs

ANNEXURE - III
CONSENT

Mr./Mrs. ______________________________ we are requesting you to enroll yourself in study titled conducted by Dr. Shilpa K. Gokale, Postgraduate student in Microbiology under the guidance of Dr. (Mrs). S. C. Metgud at J.N.M.C., Belgaum under KLE Academy of Higher Education and Research Centre, Belgaum.

You have been requested to participate in research because you are into the study group. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge.

Your participation in research in voluntary. Your decision whether or not to participate in the study will not affect your relationship with J.N.M.C. If you decide to participate you are free to withdraw at any time.

The purpose of research is to isolate and identify the aerobic and anaerobic bacteria from clinically diagnosed cases of chronic sinusitis and to carry out antibiotic susceptibility testing for aerobic isolates.

PROCEDURE INVOLVED

Microbiological study will be done on the various clinical samples to detect the nonfermenters.

RISK AND BENEFITS

There are no extra risks involved and benefits are to be evaluated.

PRIVACY AND CONFIDENTIALITY

The only people to know that you are a research subject are members of the research team. No information about you or provided by you during research will be disclosed to others without your written permission, except:

1. In emergency to protect your rights and welfare.
2. If required by law.

AUTHORIZATION TO PUBLISH RESULTS

When the results of the research are published or discussed, in a conference, no information will be displayed that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

FINANCIAL INCENTIVES FOR PARTICIPATION:

You will not be paid/offered any free gifts for participating in the research. You will not be reimbursed for expenses.

CONSENT STATEMENT

I undersigned ________________________ have been explained in my vernacular lanuage about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

In case you have any questions related to the study, you can contact Dr. Shilpa K. Gokale (Phone No. 9945410438).

In case you have any questions about your rights as a study participant, you can contact Dr. V. D. Patil (0831-2471350).

Signature or left hand thumb print of participant or legally authorized representative.

Participant's Name ________________________ Signature ___________________

Witness Name ____________________________ Signature ___________________

Experimenter's Name ______________________ Signature ___________________

Date: ____________

Place: ___________

ANNEXURE – IV - MASTER CHART

Sl.No.	OP/IP No.	Age	Sex	Specimen	Species	Antibiotic susceptibility pattern								
						Ak	Co	Ce	A	Do	Cf	Mr	Pc	Cs
1	214496	20y	F	Wound discharge	P.aeruginosa	S	R	R	R	S	R	S	R	R
2	217882	60y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
3	198524	60y	F	Pus	P.aeruginosa	S	R	S	R	S	S	S	R	S
4	201242	48y	M	Pus	P.aeruginosa	S	R	R	R	R	R	S	R	R
5	201242	45y	M	Pus	P.aeruginosa	S	R	R	R	R	R	S	R	R
6	147246	45y	F	Pus	P.aeruginosa	S	R	R	R	R	S	S	R	R
7	202483	30y	F	Ear discharge	P.aeruginosa	S	R	R	R	R	S	S	R	R
8	203683	35y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
9	203290	70y	F	Pus	P.aeruginosa	S	R	R	R	R	S	S	R	R
10	207169	65y	F	Sputum	P.aeruginosa	S	R	R	R	R	S	S	R	R
11	206422	64y	F	Blood	Moraxella	S	R	S	S	S	S	S	S	S
12	204342	14y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
13	222372	32y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
14	224770	38y	F	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
15	424720	45y	F	Wound discharge	A.baumanii	R	R	R	R	R	R	S	R	R
16	204343	14y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
17	206422	64y	M	Pus	P.aeruginosa	S	R	R	R	S	S	S	R	R
18	218206	28y	F	Wound discharge	A.baumanii	R	R	R	R	S	R	S	R	R
19	215049	28y	M	Pus	P.aeruginosa	R	R	R	R	R	R	S	R	R
20	217979	7y	M	Pus	P.aeruginosa	S	R	R	R	R	R	S	S	R
21	219920	8y	F	Blood	P.aeruginosa	R	R	R	R	R	R	S	R	R
22	205113	60y	M	Pleural fluid	P.aeruginosa	R	R	R	R	R	R	S	R	R
23	220474	1d	F	Blood	A.baumanii	R	S	R	R	R	S	S	R	S
24	215594	7d	M	Blood	P.aeruginosa	S	R	R	R	R	S	S	R	R
25	220335	2y	F	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R

Sl.No.	OP/IP No.	Age	Sex	Specimen	Species	Antibiotic susceptibility pattern								
						Ak	**Co**	**Ce**	**A**	**Do**	**Cf**	**Mr**	**Pc**	**Cs**
26	200473	50y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	S	S	R	R
27	200473	60y	M	Blood	P.aeruginosa	S	R	S	R	R	S	S	R	R
28	240221	48y	M	Wound discharge	P.aeruginosa	S	R	R	R	S	S	S	R	R
29	220335	2y	F	Wound discharge	P.aeruginosa	R	R	R	R	R	S	S	R	R
30	220306	80y	M	Wound discharge	A.baumanii	R	R	R	R	S	R	S	R	R
31	118107	25y	F	Wound discharge	P.aeruginosa	R	R	R	R	S	S	S	R	R
32	223864	65y	M	Pleural fluid	P.aeruginosa	S	R	R	R	R	S	S	R	R
33	240412	18y	F	Wound discharge	P.aeruginosa	S	R	R	R	R	S	S	R	R
34	241487	60y	M	Wound discharge	P.aeruginosa	S	R	R	R	R	S	S	R	R
35	241579	30y	M	Pus	P.aeruginosa	R	R	R	R	R	R	S	R	R
36	235031	45y	M	Wound discharge	P.aeruginosa	S	R	R	R	R	R	S	R	R
37	246072	10m	M	Wound discharge	A.baumanii	R	R	R	R	R	R	S	R	R
38	227124	5d	F	Blood	P.aeruginosa	R	S	S	R	R	S	S	R	R
39	227939	74y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	S	S	R	R
40	242132	38y	M	Pus	P.aeruginosa	R	R	R	R	R	R	S	R	R
41	244515	1d	M	Blood	P.aeruginosa	R	R	R	R	R	R	S	R	R
42	244513	1d	M	Blood	P.aeruginosa	R	R	R	R	R	R	S	R	R
43	242284	42y	M	Wound discharge	P.aeruginosa	S	R	R	R	R	S	S	R	R
44	242710	1d	M	Blood	P.aeruginosa	R	R	S	R	S	S	S	R	R
45	242136	26y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
46	241934	65y	F	Wound discharge	P.aeruginosa	S	R	R	R	R	R	S	R	R
47	241899	14d	M	Blood	P.aeruginosa	R	R	S	R	R	S	S	R	R
48	242696	26y	F	Wound discharge	P.aeruginosa	S	S	S	R	S	S	S	R	R

Sl.No.	OP/IP No.	Age	Sex	Specimen	Species	Antibiotic susceptibility pattern								
						Ak	**Co**	**Ce**	**A**	**Do**	**Cf**	**Mr**	**Pc**	**Cs**
49	249154	8m	F	Blood	P.aeruginosa	R	S	S	R	R	R	S	R	S
50	250260	1d	F	Blood	P.aeruginosa	S	S	R	R	S	S	S	R	R
51	248511	18y	F	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
52	248817	34y	F	Urine	P.aeruginosa	R	R	R	R	S	R	S	R	S
53	248977	9y	F	Sputum	P.aeruginosa	S	R	S	R	R	S	S	R	R
54	249904	24y	F	Wound discharge	A.baumanii	R	R	R	R	R	R	S	R	R
55	244514	1d	M	Blood	P.aeruginosa	R	R	R	R	R	R	R	R	R
56	243333	35y	F	Sputum	P.aeruginosa	S	R	R	R	S	R	S	R	R
57	245221	15y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
58	242294	45y	F	Wound discharge	A.baumanii	S	R	R	R	R	R	S	R	R
59	250667	5y	M	Wound discharge	A.baumanii	R	R	R	R	R	R	R	R	R
60	244869	79y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	R	R	R
61	221262	8y	M	Sputum	P.aeruginosa	S	R	R	R	R	S	S	R	R
62	219449	50y	M	Wound discharge	A.baumanii	R	S	S	R	R	S	S	R	R
63	222372	32y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
64	224770	30y	F	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
65	249954	65y	F	Wound discharge	A.baumanii	R	R	R	R	R	R	S	R	R
66	250917	20y	F	Urine	A.lwofii	R	R	R	R	S	R	R	R	R
67	250290	35y	F	Wound discharge	P.aeruginosa	R	R	R	R	R	S	S	R	R
68	253574	20y	F	Urine	P.aeruginosa	R	R	R	R	R	S	S	R	R
69	228999	35y	F	Wound discharge	P.aeruginosa	R	R	R	R	R	S	S	R	R
70	253518	30y	F	Urine	A.baumanii	R	R	R	R	S	S	S	R	R

Sl.No.	OP/IP No.	Age	Sex	Specimen	Species	Antibiotic susceptibility pattern								
						Ak	Co	Ce	A	Do	Cf	Mr	Pc	Cs
71	252279	22y	F	Pus	A.baumanii	S	R	R	R	R	S	S	R	R
72	252630	42y	M	Pus	P.aeruginosa	R	R	R	R	R	R	S	R	R
73	253040	26y	F	Urine	P.aeruginosa	S	R	R	R	R	S	S	R	R
74	252711	68y	F	Wound discharge	P.aeruginosa	S	R	R	R	R	S	S	R	R
75	253102	22y	F	Pleural fluid	P.aeruginosa	R	R	R	R	R	S	S	R	R
76	243845	62y	F	Wound discharge	P.aeruginosa	S	R	R	R	R	R	S	R	R
77	247605	1d	M	Blood	P.aeruginosa	S	R	R	R	R	S	S	S	S
78	247754	45y	F	Wound discharge	A.baumanii	R	R	R	R	S	S	S	R	R
79	246290	1d	M	Blood	P.aeruginosa	S	R	R	R	R	S	S	S	S
80	246420	1d	M	Blood	P.aeruginosa	R	S	R	R	R	S	S	S	R
81	253870	20y	M	Blood	P.aeruginosa	R	S	R	R	R	S	S	S	R
82	253442	30y	M	Urine	P.aeruginosa	R	R	R	R	R	S	R	R	R
83	253648	6y	M	Blood	A.baumanii	R	S	R	R	R	S	S	R	R
84	253442	30y	M	Pus	P.aeruginosa	R	R	R	R	R	R	R	R	R
85	253449	49y	M	Sputum	P.aeruginosa	S	R	S	R	R	S	S	S	S
86	254369	1m	F	Blood	P.aeruginosa	R	S	R	R	R	S	S	R	R
87	254428	24y	M	Pus	B.Pseudomallei	S	S	S	S	S	S	S	S	S
88	253632	10y	M	Urine	P.aeruginosa	S	R	R	R	R	S	S	S	R
89	251017	51y	M	Pus	P.aeruginosa	R	R	R	R	R	R	S	R	R
90	254624	49y	M	Pus	P.aeruginosa	R	R	R	R	R	R	S	R	R
91	229413	24y	M	Wound discharge	P.aeruginosa	S	R	R	R	R	S	S	R	R
92	221522	20y	F	Earswab	P.aeruginosa	R	R	R	R	R	R	S	R	R

Sl.No.	OP/IP No.	Age	Sex	Specimen	Species	Antibiotic susceptibility pattern								
						Ak	**Co**	**Ce**	**A**	**Do**	**Cf**	**Mr**	**Pc**	**Cs**
93	235312	24y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
94	236746	60y	F	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
95	238107	25y	F	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
96	238767	17y	M	Blood	P.aeruginosa	R	R	R	R	R	R	S	R	R
97	237721	24y	M	Wound discharge	P.aeruginosa	S	R	S	R	R	S	S	S	R
98	237783	40y	M	Wound discharge	A.baumanii	R	R	R	R	S	R	R	R	R
99	238998	65y	M	Wound discharge	P.aeruginosa	S	R	R	R	R	S	S	R	R
100	238008	35y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
101	222965	35y	M	Wound discharge	P.aeruginosa	S	R	R	R	R	S	S	S	S
102	224441	25y	F	Wound discharge	P.aeruginosa	S	R	R	R	R	R	S	R	R
103	225117	46y	F	Urine	P.aeruginosa	S	R	R	R	R	R	S	R	R
104	224076	68y	M	Wound discharge	P.aeruginosa	S	R	R	S	R	S	S	S	R
105	227329	75y	M	Urine	P.aeruginosa	S	R	R	R	R	R	S	S	S
106	228010	2d	F	Blood	P.aeruginosa	R	R	R	R	R	R	S	S	R
107	228389	14y	M	Sputum	P.aeruginosa	S	R	R	R	R	S	S	S	R
108	228562	9m	M	Blood	P.aeruginosa	R	R	R	R	R	R	S	S	R
109	238403	3d	F	Blood	A.baumanii	R	R	R	R	R	S	S	S	R
110	238557	32y	F	Blood	A.baumanii	R	S	R	R	S	R	R	R	R
111	257853	1y	M	Wound discharge	A.baumanii	S	S	R	R	S	S	S	R	S
112	296514	1d	M	Blood	P.aeruginosa	S	R	R	R	S	S	S	R	R
113	268667	45y	M	Wound discharge	P.aeruginosa	S	R	R	R	S	S	S	R	R
114	247604	20y	F	Wound discharge	P.aeruginosa	S	R	R	R	S	S	S	R	R
115	254369	1m	F	Blood	P.aeruginosa	S	R	R	R	S	S	S	R	R

Sl.No.	OP/IP No.	Age	Sex	Specimen	Species	Antibiotic susceptibility pattern								
						Ak	Co	Ce	A	Do	Cf	Mr	Pc	Cs
116	254428	24y	M	Pus	P.aeruginosa	S	R	S	R	R	S	S	R	R
117	254624	49y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
118	242072	40y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	R	R	R
119	242284	42y	M	Wound discharge	P.aeruginosa	S	R	R	R	R	R	S	R	R
120	242710	1d	M	Blood	P.aeruginosa	S	R	R	R	R	S	S	R	R
121	258738	2y	M	Wound discharge	A.baumanii	R	R	R	R	R	S	S	S	R
122	256738	4y	M	Catheter tip	P.aeruginosa	S	R	R	R	S	S	S	R	R
123	259051	22y	M	Urine	P.aeruginosa	S	R	S	R	R	S	S	R	R
124	260851	1m	M	Central linetip	A.baumanii	S	S	S	R	R	S	S	R	R
125	259055	55y	M	Wound discharge	P.aeruginosa	S	R	S	R	R	S	S	R	R
126	259250	70y	F	Blood	P.aeruginosa	R	R	R	R	R	R	S	R	R
127	262527	1d	F	Blood	P.aeruginosa	R	R	R	R	R	S	S	R	R
128	262184	52y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
129	261257	30y	F	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R
130	263457	64y	M	Wound discharge	P.aeruginosa	R	R	R	R	R	R	S	R	R

KEY TO MASTER CHART

A	:	Ampicillin
A.baumanii	:	Acinateobacter baumanii
Ak	:	Amikacin
A.lwofii	:	Acinetobacter lwofii
B.pseudomallei	:	Burkholderia pseudomallei
Co	:	Cotrimoxazole
Ce	:	Cefotaxime
Cf	:	Ciprofloxacin
Cs	:	Cefoperazone
d	:	Days
Do	:	Doxycycline
F	:	Female
Ip	:	Inpatient
m	:	Months
M	:	Male
Mr	:	Meropenem
P.aeruginosa	:	Pseudomoans aeruginosa
Pc	:	Piperacillin
R	:	Resistant
S	:	Sensitive
y	:	Years