IDENTIFICATION, SPECIATION, ANTIBIOGRAM AND MOLECULAR CHARACTERIZATION OF ACINETOBACTER ISOLATED FROM VARIOUS CLINICAL SAMPLES RECEIVED IN MICROBIOLOGY LABORATORY, THANJAVUR MEDICAL COLLEGE AND HOSPITAL

Dissertation Submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI

In partial fulfillment of the regulations

For the award of the degree of

M.D. (MICROBIOLOGY)

BRANCH – IV

APRIL 2015

THANJAVUR MEDICAL COLLEGE, THANJAVUR

THE TAMIL NADU Dr. MGR MEDICAL UNIVERSITY,

CHENNAI, TAMIL NADU.

CERTIFICATE

This is to certify that the dissertation entitled "**IDENTIFICATION, SPECIATION, ANTIBIOGRAM AND MOLECULAR CHARACTERIZATION OF ACINETOBACTER ISOLATED FROM VARIOUS CLINICAL SAMPLES RECEIVED IN MICROBIOLOGY LABORATORY, THANJAVUR MEDICAL COLLEGE AND HOSPITAL"** submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of regulations required for the award of M.D. Degree in Microbiology is a record of original research work done by Dr. K.MALATHY at the Department of Microbiology, Thanjavur Medical College and Hospital, during the period from June 2013 to May 2014 under my guidance and supervision and the conclusions reached in this study are her own.

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DECLARATION

I, Dr. K,Malathy truly declare that the dissertation entitled "Identification, speciation, antibiogram and molecular characterization of Acinetobacter isolated from various clinical samples received in Microbiology laboratory, Thanjavur Medical College and Hospital" submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of regulations required for the award of M.D. Degree in Microbiology is a record of original research work done by me at the Department of Microbiology, Thanjavur Medical College, Thanjavur during June 2013 to May 2014. I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

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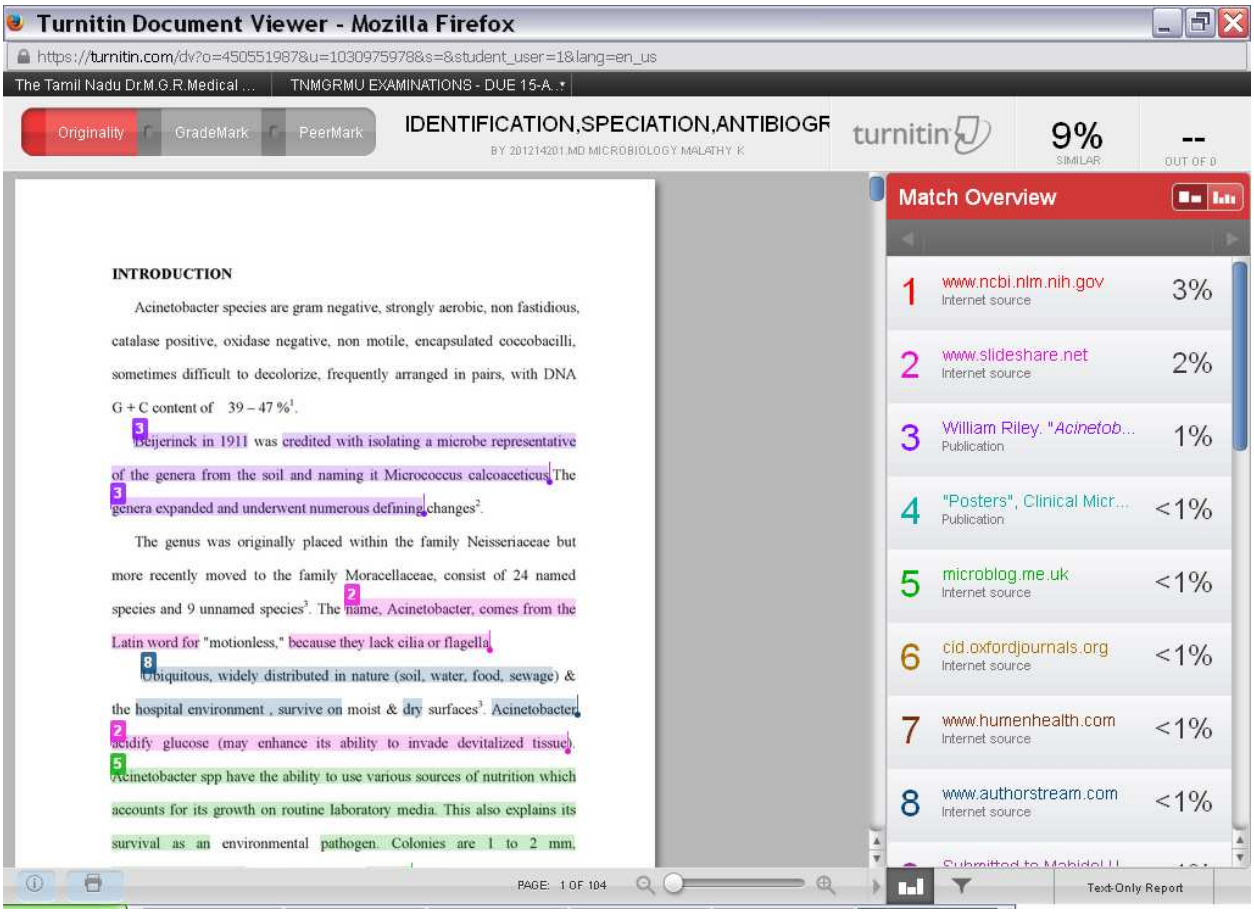

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LIST OF ABBREVIATIONS

1. DNA	-	Deoxy Ribonucleic Acid
2. BLA_{OXA}	-	Beta Lactamase Oxacillinase
3. AFLP	-	Amplified Fragment Length Polymorphism
4. PCR	-	Polymerase Chain Reaction
5. REP PCR	-	Repetitive Sequence Based Polymerase Chain Reaction
6. MLST	-	Multilocus Sequence Typing
7. PCR-ESI-MS	-	PCR followed By Electrospray Ionization Mass Spectrometry
8. MALDI-TOF-MS	-	Matrix Assisted Laser Desorption Ionization-Time of -Flight Mass Spectrometry.
9. ARDRA	-	Amplified Ribosomal DNA Restriction Analysis
10.CAPD	-	Continuous Ambulatory Peritoneal Dialysis
11.MDR	-	Multi Drug Resistant
12.ATCC	-	American Type Culture Collection
13.IAM	-	Collection of Institute of Applied Microbiology
14.CCUG	-	Culture Collection , University of Gothenburg

15.SEIP	-	Collection of Service Enterobacteriaceae Institute of Pasteur
16.NIPH	-	National Institute of Public Health
17.DSM	-	Deutsche Sammlung Von
18.TFP	-	Type Four Pili
19.MIC	-	Minimum Inhibitory Concentration
20.PFGE	-	Pulse Field Gel Electrophoresis
21. ICU	-	Intensive Care Unit
22.LPS	-	Lipopolysaccharide
23.ORF	-	Open Reading Frame
24.MBL	-	Metallo Beta Lactamase
25.IMP	-	Inosine Monophosphate dehydrogenase
26.VIM	-	Verona Integron encoded MBL
27.SIM	-	Single Minded homologue
28.NDM	-	New Delhi MBL
29.ADC	-	Acinetobacter Derived Cephalosporinase
30.IS	-	Insertional
31.OMP	-	Outer Membrane Protein
32.PBP	-	Penicillin Binding Protein
33.ABC	-	ATP Binding Cassette
34.MFS	-	Major Facilitator Superfamily
35.MATE	-	Multidrug And Toxic Compound Extrusion

36. RND	-	Resistance Nodulation Cell Division
37.SMR	-	Small Multidrug Resistance
38.MFP	-	Membrane Fusion Protein
39.AME	-	Aminoglycoside Modifying Enzyme
40.QRDR	-	Quinolone Resistance Determining Region
41.CDC	-	Centre for Disease Control
42.MYSTIC	-	The Meropenem Yearly Susceptibility Test Information Collection
43.CFU	-	Colony Forming Unit
44. MHA	-	Mueller Hinton Agar
45.CLSI	-	Clinical Laboratory Standard Institute
46.dNTP	-	deoxy Nucleotide Triphosphate
47.PBS	-	Phosphate Buffer Saline
48.TAE	-	Tris Acetate EDTA
49.SNN	-	Sepsis Neonatal
50.PICU	-	Paediatric Intensive Care Unit
51.UTI	-	Urinary Tract Infection
52.SICU	-	Surgical Intensive Care Unit
53.IMCU	-	Intensive Medical Care Uni

ABSTRACT

Introduction :

Acinetobacter has emerged as an important nosocomial pathogen, known to cause different kinds of opportunistic infections. Acinetobacter species are gram negative, strongly aerobic, non fastidious, catalase positive, oxidase negative, non motile, encapsulated coccobacilli. Acinetobacter baumanni is now recognized to be the species of great clinical importance being capable of causing life threatening infections including pneumonia, septicemia, wound sepsis, urinary tract infection. One of the striking feature of genus Acinetobacter is the ability to develop antibiotic resistant extremely rapid in response to challenge with new antibiotics.

Aims and objectives :

To know the prevalence of Acinetobacter among various clinical samples. To study different species of Acinetobacter. To assess the antimicrobial susceptibility pattern of Acinetobacter. To study molecular characterization of Acinetobacter.

Materials and Methods :

Clinical samples (urine, blood, pus and sputum) from different wards were inoculated on routine media. Acinetobacter was identified by gram staining ,hanging drop, oxidase, catalase test and other biochemical test. Speciation of Acinetobacter into various genomic species was done

by using a battery of biochemical test. Disc Diffusion susceptibility test was performed on Mueller Hinton agar and Molecular characterization by conventional PCR.

Result :

Out of the 4643 culture positive samples, 80 samples were found to be Acinetobacter. The prevalence of Acinetobacter was 1.7%. Acinetobacter baumannii (77.5%) and Acinetobacter lwoffii (22.5%) were the two species isolated. Acinetobacter strains were resistant to Ampicillin (81%), Gentamicin (36%), Amikacin (29%,), Cephalexin (86%), Ceftriaxone (44%), Ciprofloxacin (49%), Cotrimoxazole (58%), Chloramphenicol (66%). 8% of the total isolates were resistant to Imipenem. The isolates resistant to imipenem were sensitive to Polymyxin and Colistin. $Bla_{OXA-51,}$ an intrinsic gene of Acinetobacter baumannii, was detected in all the 12 Acinetobacter baumannii strains of both Imipenem sensitive and resistant and not detected in the two Acinetobacter lwoffii strains.

Conclusion :

During routine microbiological work, nonfermentative Gram negative bacilli other than Pseudomonas aeruginosa are not taken seriously and are considered as contaminants. But the rate of isolation of Acinetobacter indicates its role in nosocomial pathogen. Acinetobacter baumannii has a remarkable ability to upregulate or acquire resistant

determinants. Acinetobacter baumannii is an emerging pathogen threatening the current antibiotic era.

Key words: Acinetobacter, Speciation, Acinetobacter baumannii, Imipenem resistance, $Bla_{OXA\text{-}51}$ gene.

INTRODUCTION

Acinetobacter species are gram negative, strongly aerobic, non fastidious, catalase positive, oxidase negative, non motile, encapsulated coccobacilli, sometimes difficult to decolorize, frequently arranged in pairs, with DNA G + C content of 39 – 47 %[1].

Beijerinck in 1911 was credited with isolating a microbe representative of the genera from the soil and naming it Micrococcus calcoaceticus.The genera expanded and underwent numerous defining changes[2].

The genus was originally placed within the family Neisseriaceae but more recently moved to the family Moracellaceae, consist of 24 named species and 9 unnamed species[3]. The name, Acinetobacter, comes from the Latin word for "motionless," because they lack cilia or flagella.

It is ubiquitous, widely distributed in soil, water, food, sewage & the hospital environment, survive on moist & dry surfaces[3]. Acinetobacter acidify glucose (may enhance its ability to invade devitalized tissue). Acinetobacter spp have the ability to use various sources of nutrition which accounts for its growth on routine laboratory media. This also explains its survival as an environmental pathogen. On Nutrient agar plate, colonies are 1 to 2 mm, nonpigmented, domed, smooth to slightly mucoid and opaque[2].

However, routine identification in the clinical microbiology laboratory is not (yet) possible, they are divided and grouped into three main complexes[3]:

- Acinetobacter calcoaceticus – baumannii complex : glucose oxidizing, non hemolytic (A. baumannii can be identified by bla_{OXA-51} typing).
- Acinetobacter lwoffii : glucose non saccharolytic, non hemolytic.
- Acinetobacter hemolyticus : hemolytic.

Newer methods of identification of different species[2,4]

Methods include high resolution fingerprinting with AFLP, PCR based typing methods like randomly amplified polymorphic DNA PCR and Repetitive sequence based PCR (REP PCR), Multilocus sequence typing (MLST), Multilocus PCR followed by electrospray ionization mass spectrometry (PCR-ESI-MS), Matrix Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry(MALDI-TOF-MS) and Amplified ribosomal DNA restriction analysis (ARDRA).

Transmission

Acinetobacter can spread to susceptible person by person to person contact or contact with contaminated surfaces.

Major infections due to Acinetobacter[2,5,6]

The respiratory system is the most common site for Acinetobacter infection because of its transient pharyngeal colonization of healthy persons and a high rate of tracheostomy colonization. Acinetobacter has been reported to cause community-acquired bronchiolitis and tracheobronchitis in healthy children. Other manifestation include Ventilator-associated pneumonia, urinary tract & bloodstream infection, Skin/wound infections, conjunctivitis, meningitis, endocarditis, CAPD-associated peritonitis.

Risk factors[6,7]

Prolonged length of hospital stay, exposure to intensive care unit, mechanical ventilation, exposure to antimicrobial agents, recent surgery, invasive procedures and diminished host defenses like alcoholism, tobacco use, diabetes mellitus, renal failure, underlying pulmonary disease.

Acinetobacter baumannii

Most species are not significant sources of infection. However, one opportunistic species, Acinetobacter baumannii , is found primarily in hospitals and poses a risk to people who are immunologically suppressed. Greater than 2/3 of Acinetobacter infections are due to A. baumannii, highly antibiotic resistant organism. A. baumannii has more recently

caused a range of infectious syndromes in military personnel injured in the Iraq and Afghanistan, and has become a **successful pathogen in Afghanistan war**[7] .

Since the 1970s, the spread of multidrug-resistant (MDR) Acinetobacter strains among critically ill, hospitalized patients, and subsequent epidemics, have become an increasing cause of concern. Reports of community-acquired Acinetobacter infections have also increased over the past decade. A recent manifestation of MDR Acinetobacter that has attracted public attention is its association with infections in severely injured soldiers. Acinetobacter baumannii, has emerged as one of the most troublesome pathogens for health care institutions globally. Its clinical significance, especially over the last 15 years, has been propelled by its remarkable ability to up regulate or acquire resistance determinants, making it one of the organisms threatening the current antibiotic era[4].

Mechanisms of resistance in Acinetobacter[4,8]

Enzymatic mechanism includes broad spectrum beta lactamase, carbapenemase, aminoglycoside modifying enzymes. Non enzymatic mechanism includes changes in the outer membrane protein, multidrug efflux pump, and alteration in the penicillin binding protein.

Documented mechanisms for resistance to carbapenems[4,8]

Production of beta lactamases in particular oxacillinase which is a class D beta lactamase in association with promoter gene sequence ISAba1. The most prevalent one is $bla_{OXA-23.}$ Metallo betalactamase (IMP, VIM), altered penicillin binding proteins and changes in the outer membrane protein especially the disruption of 33 kDa carO protein by IS elements also plays a role in carbapenem resistance.

Biofilms enhancing the pathogenicity[8]

A. baumannii forms biofilms with enhanced antibiotic resistance and more recently, that a chaperone-usher secretion system involved in Pilus assembly affects biofilm formation. Acinetobacter commonly colonizes patients in the intensive care setting. Acinetobacter colonization is particularly common in patients who are intubated and in those who have multiple intravenous lines or monitoring devices, surgical drains, or indwelling urinary catheters.

Diagnosis[4]

Infection or colonization with Acinetobacter is usually diagnosed by clinical culture of blood, sputum, urine, pus, body fluid, etc. Microbiologic cultures can be processed by standard methods on routine media. Acinetobacter baumannii isolates were presumptively identified

by using morphology of the colonies, Gram staining, Oxidase and Catalase reactions, growth at 42°C, and the API-20 NE System (Bio-Merieux, Lyon, France).

Current trends in antibiograms[2,5]

Most A. baumannii are now resistant to Ampicillin, Carbenicillin, Cefotaxime and Chloramphenicol. Resistance to Gentamycin, Tobramycin and Amikacin is increasing. Fluoroquinolones, ceftazidime, Trimethoprim- Sulphamethoxazole, Doxycycline, Polymyxin B, Colistin, Imipenem and Meropenem may retain activity against nosocomial Acinetobacter.

Treatment[4,5,9]

First line treatment is with Carbapenem antibiotics such as Imipenem, but carbapenem resistance is increasingly common. Other treatment options include Polymyxin, Tigecycline and Aminoglycosides. Colistin and Polymyxin B have been used to treat highly resistant Acinetobacter infections. The choice of appropriate therapy is further complicated by the toxicity of Colistin which is mainly renal. Acinetobacter isolates resistant to Colistin and Polymyxin B have also been reported.

Preventive measures[10]

Acinetobacter can live on the skin and may survive in the environment for several days. Careful attention to infection control procedures such as hand hygiene, use of alcohol-based hand sanitizers, contact precautions, environmental decontamination, prudent use of antibiotics, healthcare worker education helps in reducing the infection.

As Acinetobacter is an emerging pathogen, this study is undertaken to find out the prevalence and its antimicrobial susceptibility pattern of Acinetobacter in our institution.

AIMS AND OBJECTIVES

1. To know the prevalence of Acinetobacter in various clinical samples received in microbiology laboratory Thanjavur Medical College & Hospital.
2. To study about different species of Acinetobacter prevalent among the various samples by biochemical tests.
3. To assess the in vitro susceptibility and resistance pattern of Acinetobacter.
4. To study the molecular characterization of Acinetobacter by PCR.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Historical review

The history of the genus Acinetobacter dates back to the early 20th century. In 1911, Beijerinck, a Dutch microbiologist, described an organism named Micrococcus calcoaceticus. It was isolated from soil by enrichment in a calcium - acetate - containing minimal medium. In the following decades, similar organisms were described and assigned to atleast 15 different genera and species. It includes Diplococcus mucosus, Micrococcus calcoaceticus, Alcaligenes haemolysans, Mima polymorpha, Herellea vaginicola, Bacterium anitratum, Moraxella lwoffi, Moraxella lwoffi var. glucidolytica, Neisseria winogradskyi, Achromobacter anitratus and Achromobacter mucosus [2,4] .

In 1954 Brisou and Prevot proposed the current genus designation, Acinetobacter, to separate the nonmotile from the motile microorganisms within the genus Achromobacter. This genus was a heterogenous group inclusive of nonmotile, gram negative, catalase positive, and oxidase negative saprophytes distinguishable from other bacteria by their lack of pigmentation. The genus designation was not widely accepted until 1968. Studies of Baumann et al moved the oxidase positive strains to the genus Moraxella[2].

Baumann et al. published a comprehensive survey and concluded that the different species listed above belonged to a single genus, for which the name Acinetobacter was proposed. Their subclassification into different species based on phenotypic characteristics was not possible. In 1971 the subcommittee on the Taxonomy of Moraxella and Allied Bacteria officially acknowledged the genus Acinetobacter. A transformation test was described (Juni 1972; Weyant et al.1996) and is still used for the genus level identification. This research techniques involves the conversion of auxotrophs to prototrophy. Since the organism classed within Acinetobacter were similar, a monotypic genus was proposed, Acinetobacter calcoaceticus, comprising two varieties, var.anitratus (formerly Herellea vaginicola) and var. lwoffi (formerly Mima polymorpha) (Juni 1984) [2,4] .

In the 1974 edition of Bergey's Manual of systematic Bacteriology, the genus Acinetobacter was listed, with the description of a single species, Acinetobacter calcoaceticus. In contrast, in the "Approved List of Bacterial Names," two different species, Acinetobacter calcoaceticus and Acinetobacter lwoffi were included, based on the observation that some Acinetobacter was able to acidify glucose whereas others were not. Based on the same property, the species A.calcoaceticus was subdivided into two subspecies or biovars, A.calcoaceticus bv. anitratus (formerly called Herellea vaginicola) and A.calcoaceticus bv.lwoffi (formerly called Mima

polymorpha). However, these designations were never officially approved by taxonomists[4].

Current taxonomy

As currently defined, the genus Acinetobacter, comprises gram negative, strictly aerobic, nonfermenting, non fastidious, non motile, catalase- positive, oxidase-negative bacteria with a DNA G+C content of 39% to 47% [1,4].Based on recent taxonomic data, it was proposed that members of the genus Acinetobacter should be classified in the new family Moraxellaceae within order Gammaproteobacteria, which includes the genera Moraxella, Acinetobacter, Psychrobacter, Pseudomonas and related organism [4,11].

A major breakthrough in the long and complicated history of the genus was achieved by Bouvet and Grimont in 1986. Based on DNA-DNA hybridization studies, they distinguished 12 DNA groups or genomospecies, some of which were given formal species names, including A. baumaninnii, A. calaoaecticus, A. haemolyticus, A. lwoffi, A. johnsonii, A. junii. Work done by Bouvet and Jeanjean, Tjernberg and Ursing, and Nishimura et al resulted in the description of further Acinetobacter genomic species. It included the named species A.radioresistens, which corresponds to Acinetobacter genomic species 12, as described by Bouvet and Grimont. Some of the independently

described species turned out to be synonyms e.g., A. lwoffii and Acinetobacter genomic species 9 or Acinetobacter genomic species 14, described by Bouvet and Jeanjean and Acinetobacter genomic species 13, described by Tjernberg and Ursing [4,5].

Recently 10 additional Acinetobacter species were described. It included 3 species of human origin, A. parvus, A. ursingi and A. schindleri and 7 species isolated from activated sludge namely A. bayli, A. bouvetii, A. grimontii, A. gerneri, A. tjernbergiae, A. towneri, A. tandoii[12].

Three novel species (Acinetobacter soli, Acinetobacter beijerinckii and Acinetobacter gyllenbergi) were identified in 2008 and 2009 by two research groups. Recently Acinetobacter genomic species 10, Acinetobacter genomic species 11, Acinetobacter genomic species 3, Acinetobacter genomic species 13TU have been named Acinetobacter berezinae, Acinetobacter guilloriae, Acinetobacter pitti, Acinetobacter nosocomialis respectively. They can be phenotypically differentiated from other species within the genus Acinetobacter. At least 33 species within the Acinetobacter genus have so far identified, including 24 named species and 9 unnamed genomic species[4].

Four of the above listed species , namely A. calcoaceticus, A. baumanni, Acinetobacter genomic species 3, and Acinetobacter genomic species 13TU are very closely related and difficult to distinguish from each from each other by phenotypic properties. Therefore it has been

proposed to refer to these species as the A. calcoaceticus - A. baumannii complex[13,14]. This group of organisms, however comprises not only the three most clinically relevant species that have been implicated in the vast majority of both community acquired and nosocomial infections, but also A. calcoaceticus, which has been frequently recovered from soil and water, never been implicated in serious clinical disease. Since environmental species has given its name to the complex, the designation A. calcoaceticus- A. baumannii complex may be misleading and not appropriate if used in a clinical context.

Table 1: Acinetobacter genomic species

Species	Genomic species	Type or reference strain	Reference
A. Calcoaceticus	1	ATCC 23055^{T}	2,4,15
A. Baumannii	2	ATCC 19606^{T}	2,4,15
A. Pitti	3	ATCC 19004	4,15
A. Hemolyticus	4	ATCC 17906^{T}	2,4,15
A. Junii	5	ATCC17908^{T}	4,15
A. gen spp	6	ATCC 17979	4,15
A. Johnsonii	7	ATCC 17909	2,4,15
A. Lwoffii	8/9	ATCC 15309^{T}, ATCC 9957	2,4,15

A. Berezinae	10	ATCC 17924	4,15
A. Guillouiae	11	ATCC 11171	4,15
A. Radioresistens	12	IAM 13186^{T}	2,4,15
A. gen spp	13BJ/14TU	ATCC 17905	4,15
A. gen spp	14 BJ	CCUG14816	4,15
A. gen spp	15BJ	SEIP 23.78	4,15
A. gen spp	16	ATCC 17988	4,15
A. gen spp	17	SEIP Ac87.314	4,15
A. nosocomialis		ATCC 17903	15
A. gen spp	Between 1and 3	10095	4,15
A. gen spp	Close to 13 TU	10090	4,15
A. venetianus		ATCC 3102	4,15
A. ursingii		NIPH137^{T}	4,15
A. parvus		NIPH384^{T}	4,15
A. baylyi		DSM 14961^{T}	4,15
A. bouvetii		DSM 14964^{T}	4,15
A. towneri		DSM 14962^{T}	4,15
A. tandoii		DSM 14970^{T}	4,15
A. tjernbergiae		DSM 14971^{T}	4,15
A. gerneri		DSM 14967^{T}	4,15
A. soli		CCUG 59023^{T}	15

A. beijerinckii		NIPH 838^{T}	15
A. gyllenbergii		NIPH 2150^{T}	15

Species Identification:

Members of the genus Acinetobacter are gram-negative, catalase-positive, and oxidase negative. During periods of rapid growth (exponential phase) the organisms typically appear bacillary to coccobacillary 1- 1.5 by 1.5 – 2.5 microns in size. Notably they become more coccoid or diploccal as the cultures age (stationary phase). The colonies are 1- 2 mm in diameter (smaller than typical Enterobacteriaceae) and are typically domed, smooth to slightly mucoid and opaque. Pigmentation is usually greyish-white, although some strains may appear pale yellow. Hemolytic activity on blood agar is variable, although a diffusible brown pigment has been observed (Pagel and Seyfried 1976) when glucose has been added to the medium (Siau et al. 1998). Acinetobacters are nonlactose fermenters but they may produce a slight pinkish hue that could be mistaken for lactose fermentation[2,16,17,18,19].

The Acinetobacters are nonmotile, but occasionally an odd twitching motility can be demonstrated (Lautrop 1961; Lautrop 1974; Barker and Maxted 1975). Acinetobacter baumannii strain M_2 produces Type IV Pili (TFP) which play a role in natural transformation and twitching motility but not surface associated motility[20].

Older cultures frequently capsulate, occasionally causing problems with destaining the crystal violet. For the most part, the species are not fastidious and capably grow on the standard nutritional medium used within the laboratory. Occasionally strains may be encountered that are fastidious, failing to grow in nutrient broth and forming smaller colonies on blood agar (Weyant et al.1996). Utilisation of carbohydrates varies considerably among the species. Nitrates are not reduced to nitrites. The ability to hydrolyse gelatin and urease is variable. They are strictly aerobic and are capable of growing at a wide range of temperature. However, it is observed that most clinical strains grow optimally at 37◦C, while the environmental isolates prefer lower temperatures. For clinical isolates, growth on MacConkey is variable and presenting as either colorless or light pink colonies[2].

Unlike Enterobacteriaceae, some species of Acinetobacter outside the A.calcoaceticus-A.baumannii complex may not grow on Mac Conkey agar. Acinetobacter haemolyticus and several other currently not well defined species, such as Acinetobacter genomic species 6, 13 BJ, 14BJ, 15 BJ, 16 and 17, may show haemolysis on sheep blood agar. This property is never present in isolates belonging to the A.calcoaceticus - A.baumannii complex. No single metabolic test distinguishes Acinetobacter from other similar nonfermenting gram negative bacteria.

A reliable method for identification of Acinetobacter to the genus level is the transformation assay of Juni. It is based on the unique property of mutant Acinetobacter strain BD413 trpE27, a naturally transformable tryptophan auxotroph recently identified as A.baylyi to be transformed by crude DNA of any Acinetobacter species to a wild type phenotype[21].

Enrichment culture at low pH in a vigorously aerated liquid mineral medium supplemented with acetate or another suitable carbon source and nitrate as the nitrogen source has proven useful for the recovery of Acinetobacter from environmental and clinical specimens. Existing media for the isolation of Acinetobacter spp are either non selective, too inhibitory, inhibiting the growth of many Acinetobacter strains, allowing the growth of unwanted bacteria. To facilitate the isolation of Acinetobacter from mixed bacterial population, for rapid isolation and effective control of Acinetobacter infection, Leeds Acinetobacter medium was proposed. According to the results of MIC and viable counts, the concentration of antibiotics and other ingredients in this medium have been determined[22].

Identification of the Acinetobacter is often problematic for all but the reference laboratories. Unfortunately, the databases of most of the commercially available identification systems are variable in their ability to identify all isolates. Gerner - Smidt et al (1991) and Kampfer et al

(1993) have proposed various phenotypic schemes for identification. Review of the schema shows that they are not practical for the typical clinical laboratory owing to their requirement for a large number of biochemicals.

The phenotypic identification scheme was first proposed by Bouvet and Grimont in 1986. It is based on 28 phenotypic tests. This identification scheme was refined in 1987 by the same authors. It includes growth at 37°C, 41°C, 44°C; production of acid from glucose; gelatin liquefication and assimilation carbon sources.

Table 2: Identification scheme of Acinetobacter species[1,6,12,23,24,25]

Organism	Hemo lysis on BAP	Growth		OF test	Arginine	Chloram phenicol sensitivity
		37° C	42°C			
Acb complex	-	+	+	Saccharolytic	+	R
A.lwoffi	-	+	-	NS	-	S
A.hemolyticus	+	+	-	Saccharolytic (75%)	+	R
A.junii	-	+	-	NS	+	R
A.radio resistens	-	+	-	NS	+	R

NS = Non Saccharolytic, R =Resistant, S=Sensitive

Molecular methods

Pulse Field Gel Electrophoresis (PFGE)

PFGE extensively used, gold standard for Acinetobacter baumanni typing in some instance. Still it remains the reference method of choice. Laborious method, that requires several days before generating a typing result. The chromosomal fragments are separated by electrophoresis and digested with ApaI. The fingerprint profiles are visually compared or using specialized computer programs that also allow the storage of profiles in a database. Based on side-by-side comparison of molecular fingerprint patterns of a limited number of strains. Therefore interlaboratory comparison has always been a problematic one and it is not suited for population studies[4,26,27].

PCR based typing methods

Randomly amplified polymorphic DNA PCR – it involves amplification of random fragments of genonomic DNA with single primer with an arbitrary sequence. It has been used successfully to assess the strain relatedness of Acinetobacter isolates.

Repetitive sequence based PCR - REP PCR uses consensus primers for the highly conserved REP sequence located between these DNA motifs.

Both methods do not require specialized equipments. Both are fast, easy and low cost methods that allow grouping of A.baumannii strains with various degrees of genotypic relatedness. However the discriminatory power of these methods are inferior to that of PFGE[4,28].

AFLP analysis:

Amplified Fragment Length polymorphism - AFLP analysis was established in 1990. It is a highly sensitive DNA fingerprinting method. In this method, first DNA is digested with restriction enzymes, followed by selective amplification, electrophoretic separation of fragments and visualization. It is a expensive method, usually performed in a semiautomated procedure, with laser detection of fragments on a sequence platform. The resulting complex profiles are digitalized and analyzed with appropriate software. This high resolution fingerprinting method is useful for the characterization of Acinetobacter strains at the subspecies level and for outbreak investigation. It requires high level of standardization and extensive experience in interpretation of banding patterns. Hence, this method is restricted to reference laboratories and not suited for routine epidemiological studies[4,15]

MLST

Multilocus sequence typing was recently developed for Acinetobacter baumannii by Bartual and coworkers. It is based on the sequence of the conserved regions of the seven house keeping genes (gltA, gyrB, gdhB, recA, cpn60, gpi and rpoD). It is applied to Acinetobacter genomic species 13TU isolates also. The discriminatory power of the MLST system is comparable to that of both PFGE and AFLP. MLST is however expensive and laborious. Therefore not suited for routine outbreak analysis or other limited scale analysis of the epidemiology of Acinetobacter baumannii. It remains to be determined typing system and appropriate for the study of Acinetobacter species. It is suitable for global epidemiological study. It allow the recognition of epidemic, multiresistant and virulent A.baumannii clones and the monitoring of their national and international spread[4,27,28].

PCR – ESI – MS

Multilocus PCR followed by Electrospray ionization mass spectrometry is used for species identification of Acinetobacter baumannii and to determine clonality. The six bacterial house keeping genes – trpE, adk, efp, mutY, fumC and ppa are amplified from each isolate. The amplified products are desalted and purified and then mass spectra are determined. The system was established using 267

Acinetobacter isolates collected from infected and colonized soldiers in an outbreak in the military health care system associated with war in Iraq, outbreaks in European hospitals and culture collections. Correlation with PFGE typing was good. Advantages of PCR-ESI-MS are, it is very fast taking only 4 hours, providing typing results on a time scale which is not achievable with most other system[29].

MALDI-TOF -MS

Matrix-Assited Laser Desorption Ionization-Time of Flight Mass Spectrometry has been developed for the rapid identification of pathogens. Promising results with MALDI-TOF MS obtained for species identification of 552 well characterized Acinetobacter strains representing 15 different species. For MALDI-TOF-MS , bacteria are cultured for 24 hours at 37◦C on MacConkey agar and extracts are prepared. For identification of isolate, the row spectra are compared with those in the Biotyper database and log of greater than 2.3 to represent a secure species identification. Spectra are acquired with a microflex LT mass spectrometer and recorded in the positive linear mode at a laser frequency of 20 HZ, ion source 1 voltage of 20 kv, ion source 2 voltage of 8.5 kv and mass range from 2000 to 20,000 kDa. Reference specra of newly created main spectra is added to the original Biotyper database. To evaluate the spectrum variation within each strain, to estimate the mass

spectral variance of biological replicates, a principal component analysis is performed. It provides similar results in good concordance with rep-PCR. The accuracy and speed of data acquisition can be achieved using MALDI-TOF MS. It can be successfully used in routine clinical microbiology for real time identification of nosocomial outbreaks from A.baumannii isolates before results from DNA based systems are available[30].

ARDRA

Amplified ribosomal DNA restriction analysis is used for the identification of the Acinetobacter genomic species. Restriction analysis with the enzyme AluI, CfoI, MboI, RsaI and MspI of the enzymatically amplified 16 rRNA genes is used to identify all species except genomic species 4, 5,7 17,10,11. It is a rapid and reliable method for the identification of the Acinetobacter genomic species including the closely related DNA groups 1, 2, 3 and 13. ARDRA helps to elucidate the ecology and clinical significance of the different species of this genus. Since ARDRA use universal 16srRNA gene primer, it is applicable to identification of most bacterial species. ARDRA is less prone to contamination problems than PCR, since the use of cultured organism results in a large initial quantity of target DNA[31,32]

Partial rpoB sequence analysis

The degree of polymorphism of house keeping protein gene encoding genes such as recA, gyrB, rpoB genes are higher than that of the non protein encoding 16s rDNA gene. Sequence analysis of this genes provides a method with a better level of resolution for the identification and taxonomic classification of various bacterial species. Sequence analysis of 4 zones of the RNA polymerase beta subunit gene and its flanking space is a useful molecular method for identification of Acinetobacter species. Sequence analysis of the highly discriminative Zone 1, spanning 352 bp between position 2901 and 3250 on the rpoB gene in particular is used to represent a reliable and rapid identification method for Acinetobacter species. rpoB gene sequence analysis has been suited for both collection of Acinetobacter reference strain and a collection of Acinetobacter clinical isolate[15]

Manual and semiautomated commercial identification system

Species identification with manual and semiautomated commercial identification system are currently used in diagnostic microbiology. They are API 20NE, Vitek 2, Phoenix and MicroSan WalkAway Systems. Substrates used for bacterial identification system have not been tailored specifically to identify Acinetobacter. Three clinically relevant members of the A. calcoaceticus-A.baumannii complex

cannot be separated by these systems. A. baumannii, A. genomic species 3, and Acinetobacter genomic species 13TU are uniformly identified as A. baumannii.

Natural Habitats

Acinetobacter are part of the human skin flora. In an epidemiological survey performed to investigate the colonization of Acinetobacter in human skin and mucous membrane, upto 43% of non hospitalized individuals were found to be colonized with Acinetobacter. A.lwoffii (58%),A. johnsonii(20%), A. junii (10%) and Acinetobacter genomic species 3(6%) were most frequently isolated species. In a similar study carrier rate of 44% was found among healthy volunteers, out of which A.lwoffii (61%), Acinetobacter genomic 15BJ (12%), A.radioresistens (8%), and Acinetobacter genomic species 3 (5%) were more prevalent species[33].

Dijkshoorn et al. studied fecal carriage of Acinetobacter, found that carrier rate of 25% among healthy individuals with A.johnsonii and Acinetobacter genomic species 11 predominating. In contrast, A.baumannii which is the most predominant nosocomial species was found rarely on human skin and human feces and Acinetobacter genomic species 13TU was not found at all[34].

Griffith et al.investigated the nares of healthy U.S. soldiers. Since he did not use enrichment culture, he did not find Acinetobacter at all. In the subsequent study, Griffith et al did not detect skin carriage of the A. Calcoaceticus-A. baumannii complex among 102 U.S Army soldiers.The reason was again he performed culture without enrichment and with an extremely long transport time[35].

Chu et al. found 53% of medical students and new nurses to be colonized with Acinetobacter in summer versus 32%in winter. This is due to, seasonal variability in skin colonization contributing to seasonal variation in the prevalence of A.baumannii in clinical samples[36,37].

Although various Acinetobacter species were isolated from animals, A. baumannii was occasionally found as an etiological agent in infected animals. A.baumannii was recovered from 22% of body lice sampled from homeless people. This finding might result from clinically silent bacteremia in these people[38].

Berlau et al. investigated vegetables in the United Kingdom and found that 30 of 177 vegetables (17%) were positive for Acinetobacter. The predominant species were A. baumannii and Acinetobacter genomic species 11 (each at 27%) followed by A.calcoaceticus and Acinetobacter genomic species 3 (each at 13%), while Acinetobacter genomic species 13 was found only once[39].

Houang et al.found Acinetobacter in 22 out of 60 soil samples in Hong Kong. The most frequently isolated species were Acinetobacter genomic species 3(27%) and A.baumannii 23%, with only one A.calcoaceticus.

Recently described Acinetobacter species namely A. baylyi, A. bouvetii, A.grimontii, A.tjernbergiae, A.towneri, and A. tandoii were isolated from activated sludge. Obviously they are environmental species and never been found in humans. A. schindleri and A. ursingii have been recovered from human species only. A. parvus was found in humans and also in dogs[12].

In conclusion, Acinetobacter species seems to be distributed widely in nature. A.calcoaceticus is found in water, soil and on vegetables. Acinetobacter genomic species 3 is found in soil, water, vegetables and human skin. A.johnsonii found in water, soil, human skin and feces. A.lwoffi and A.radioresistens are found in human skin. Acinetobacter genomic species 11 found in water, soil, vegetables and human intestinal tract. In Europe, the carrier rate of A.baumannii in the community is rather low. A.baumannii does not appear to be a typical environmental organism, although it has been found in soil samples in Hong Kong and vegetables in United Kingdom[4].

Epidemiology

Acinetobacters are saprophytic and ubiquitous. They can be found throughout the natural and hospital environment on a wide range of dry and moist inanimate surfaces (Getchell-White et al 1989; Towner et al.1991).

Acinetobacter isolates accounted for 4.25% of the total number of organism isolated[24]. Incidence of Acinetobacter among non fermentor is Acinetobacter baumannii - 24.87 %, Acinetobacter lwoffii – 8.47%. Imipenem resistance of Acinetobacter in Asian countries from 2008-2009 was 67%. The rate was especially high in Malaysia (86.7%), Thailand (81.4 %), India (85.7 %) and China (88.9%)[40].

In large surveillance studies from the United states, about 5 to 10% of ICU acquired pneumonia were due to A.baumannii. Overall crude mortality rate of A.baumannii bloodstream infection was 34 to 43% in ICU and 16.3% outside the ICU. A.baumannii was the 10th most common etiologic agent responsible for 1.3% of all monomicrobial nosocomial bloodstream infections. A.baumannii blood stream infection had the third highest crude mortality rate in the ICU next to Pseudomonas aeruginosa and Candida sp infection. The crude mortality of A.baumannii blood stream infection may be as high as 52%[4].

Outbreak of infections are associated with spread of unique strain and due to contaminated respiratory therapy equipment, bedding materials, intravascular access devices and transmission via the hands of hospital personnel[41]. Recently European investigators suggested that similar to the epidemiology of methicillin resistant staphylococcus aureus, a few epidemic of A.baumannii strains may be involved in outbreaks at various institutions as well as in international spread[41,42].

Wilks et al reported a recent outbreak of multidrug resistant Acinetobacter infection, with environmental contamination found on curtains, laryngoscope blades, door handles, mops, patient lifting equipment and keyboards. This emphasize the need for special attention to disinfection of shared items and extra caution with wound care and respiratory care procedures. One or more epidemic Acinetobacter clones often coexist with endemic strains. This makes it difficult to detect and control transmission[4].

Abbo et al.studied 118 patients with multidrug-resistant Acinetobacter infection in Israel and found 10 different PFGE-typed clones. He also found many clusters of patients with no common source identified, despite molecular testing and extensive investigation[43].

Nemec et al. used ribotyping and amplified fragment-length polymorphisms for demonstrating the genetic relatedness of Acinetobacter isolates in Western Europe. Investigators used PFGE to

demonstrate the inter institutional spread of carbapenem resistant Acinetobacter infection among acute care hospitals in New York, Argentina, the United kingdom, and the Iberian peninsula[4].

Gales et al used PFGE to demonstrate the spread of epidemic Acinetobacter clones between Argentina and Brazil[44].

Multidrug resistant Acinetobacter deep wound infection, osteomyelitis, bacteremia and respiratory infections have been reported among military personnel with traumatic injuries during the conflicts in Iraq and Afghanistan[41].

Brink and colleagues found that 30% of A.baumannii bloodstream isolates in south Africa are carbapenem resistant, 40% are Cefipime And Pipercillin-Tazobactam resistant and 30% are Ciprofloxacin and Levofloxacin resistant[45] .

The first Australian outbreak of hospital acquired A.baumannii was in Western Australia. The isolates were resistant to Gentamicin, Cephalosporins, Ciprofloxacin and Ticarcillin. Molecular epidemiological analysis found that 11% of staff hand samples were positive for the same strain of A.baumannii as that causing patient infection[46].

Aygun et al. 2002 studies have documented that hospital environments, such as intensive care units (ICU), can harbor carbapenem resistant A.baumannii. Only after closing the affected ICU and thorough cleaning of the environment and equipment with hypochlorite and

terminal disinfection, the resistant were eliminated[2].

Brooks et al. 2002 documented that pan resistant acinetobacters could be recovered from the surfaces of dispensers of hand soap containing 2.0 percent chlorhexidine[2].

Taplin et al. 1963 considered Acinetobacters as part of the commensal flora of man and other animals, where they occasionally present as opportunistic pathogens. As commensals, they have been isolated from axillae, groin, digital webs, and other external fossa of about 25 percent of population[2].

Larson et al. 1986 have demonstrated sustained carriage on the hands of health workers. Larson determined that Acinetobacter was the most common gram-negative organisms carried on the skin of hospital personnel. Members of the genus have been isolated from practically every type of patient specimen. This ubiquity often presents problems for the clinician. Therefore, careful evaluation of the role of an isolate to an infectious process is required[2].

Interestingly it appears that the genomic distribution of the various Acinetobacters differs greatly throughout the diverse hospital environments. A.baumannii is the species isolated most frequently. Assimilation test have identified 19 biotypes (Bouvet and Grimont 1987).Traub and Leonhard have described 32 serovars[2].

As with many other genera, many different typing systems have been devised to segregate the various members of this genus. Refer to Bouvet (1991) for a review of early attempts at typing schema. Conventional methods have presented problems that have precluded their adaptation. These methods have included biotyping (Bouvet et al. 1990), phage-typing (Vieu et al.1979), serotyping (Traub and Leonhard 1994) and bacteriocin typing (Andrews 1986)[2].

Currently, several molecular methodologies have been developed and used within the clinical setting for the investigation of nosocomial outbreaks, although it is important to note that no particular system has been accepted as the gold standard. These technique have included plasmid profile analysis (Gerner-smidt and Tjernberg 1993;Seifert et al.1994), restriction endonuclease digestion and pulse field gel electrophoresis of total chrosomal DNA (Gouby et al 1992; Struelens et al.1993; Liu and Wu 1997; Grundmann et al 1997), random amplified polymorphic DNA profiles(Graser et al.1993; Struelens et al1993), ribotyping(Gerner-Smidt 1992; Bernards et al 1997), cell envelope and outer membrane protein profiles(Dijkshoorn et al.1987a,1987b), and multilocus enzyme electrophoretic typing(Thurm and Ritter 1993)[2].

Ackermann et at: strain specificity has been demonstrated for various Acinetobacters against numerous bacteriophages.

Typically, most of the bacteriophages are lytic and are range-restricted to the original strain of Acinetobacter from which they were isolated[2].

Virulence and pathogenic factors

Usually Acinetobacter are opurtunistic pathogens, they have components that are capable of enhancing their virulence in debilitated individuals such as those in burn or intensive care unit and those who have received multiple antimicrobial agents[9.]. Perhaps the most important and similar to other gram negative organisms is the presence of endotoxin. Endotoxin is a lipopolysaccharide (LPS) moiety of the outer membrane and in which the toxic lipid component, lipid A, is embedded. It has been demonstrated that the LPS is responsible for toxicity in mice and pyrogenicity in rabbits. Endotoxin is considered partially responsible for the febrile response during septic episodes. For Acinetobacter pneumonia, Obana demonstrated an enhanced virulence in the presence of mixed infections with other bacteria species in the mouse model. Obana's study could not identify conclusively the definitive nature of this enhancement[2].

The research by Borneleit and Kleber on the matrix of the cell wall and outer membrane demonstrates special properties capable of influencing the permeability properties of the cells.

Fimbriae have been documented that they are potentially capable of facilitating adhesion to human epithelial cells[2].

Anstey et al 1992: A polysaccharide capsule has been described that perhaps limits phagocytosis

Wendt et al 1997: This capsule certainly aids the bacteria to survive under dry condition.

Obana et al 1985; Borneleit and Kleber 1991: The above properties in conjunction with proteins layers and slime also potentially enhance the virulence of the organism.

Interestingly, it has been noted that certain strains have been shown to produce siderophores (e.g.aerobactin) and iron repressible outer-membrane receptor proteins (Smith et al 1990; Echenique et al 1992; Actis et al 1993). These abilities are also potentially important virulence determinants[2].

Clinical significance[2,3,5]

Generally Acinetobacter are not fastidious and are capable of surviving in various environmental niches. Most importantly they are adapt at acquiring resistance to multiple antibiotics. Fortunately, most isolates are simply colonizers in healthy individuals (Bergogne-Berezin and Towner 1996; Cisneros et al 1996). However determing the clinical significance of a particular isolate can be problematic for the clinician,

particularly for intensive care patients and others with extensive surgical or antibiotic intervention. The opportunistic potential of this genus for causing hospital acquired infections is well documented.

Corbella et al (1996) studies have shown that the digestive tracts of patients within ICU often serve as reservoirs for multiresistant A.baumannii strains involved in hospital outbreaks.

The respiratory tract is the prominent site for hospital acquired infections, with endotracheal tubes frequently implicated. Additionally isolates found from the urinary tract, wounds and catheter sites may eventually lead to septicemia (Bergogne-Berezin and towner1996; Bernards et al 1997; Cisneros et al 1996; Seifert et al 1995)

Less frequently, members of the genus have been associated with arthritis, endocarditis, meningitis, osteomyelitis, peritonitis related to continuous ambulatory peritoneal dialysis and ventilator related pneumonias.

A. baumannii is the species most often responsible for hospital acquired infections. A. lwoffii has been more commonly associated with meningitis than other Acinetobacter species. A. ursingii has been shown to cause blood stream infections in hospitalized patients. A. juni is a rare cause of ocular infection and bacteremia, particularly in paediatric patients. A case of community acquired Acinetobacter radioresistens bacteremia in an HIV positive patient has also been reported. A.

schindleri have been recovered from a variety of human specimens (vaginal, cervical, throat, nasal, ear, conjunctiva, and urine) but are mostly regarded as clinically nonsignificant[5].

Risk factor for colonization or infection with multidrug resistant Acinetobacter species include prolonged length of hospital stay, exposure to an intensive care unit, receipt of mechanical ventilation, colonization pressure, exposure to antimicrobial agents, recent surgery, invasive procedures and underlying severity of illness[42].

Genetic resistance[2]

Transformation is probably not a significant event among the Acinetobacters. The process was first demonstrated by Juni and Janik with A. calcoaceticus strain BD4 and its microencapsulated trpE27 mutant derivative BD413. Juni demonstrated that the transformation of strain BD413 trpE27 to the wild phenotype could be used as the basis of the genetic test for identifying members of the genus. The ease with which strain BD413 can be transformed, has been used with a wide range of advanced molecular procedures, allowing the detailed fine structure genetic investigations of the chromosomal organization and metabolic regulation of this strain.

Strain specificity appears to exist with regards to transduction. Using P78, a temperate phage, Herman and Juni demonstrated a low

frequency generalized transduction with its specific host, while failing to lysogenize 389 other strains of Acinetobacter.

Towner and Vivian (1976) and Hinchliffe and Vivian (1980) demonstrated that conjugation could occur with a broad host range of plasmids using Acinetobacter strain EBF 65/65. Although very little is known of the mobilization events , the system has been important for the mapping of mutations on the circular chromosomal linkage group in strain EBF 65/65.

Plasmids and transposons are perhaps the most interesting to researchers and clinicians and probably serve as important role in the biology of Acinetobacter species. It has been observed that more than 80% of Acinetobacter isolates appear to carry multiple indigenous plasmids of variable size. Unfortunately involving plasmids and transposons have been riddled with problems most likely due to difficulties in lysing organism’s cell wall. Transposons, as opposed to plasmids may be more important to the development of antibiotic resistance in clinical isolates. This observation may be due to the lack of a suitable test system for studying plasmid mediated transfer.

Mechanism of Antibiotic Resistance

Treatment for Acinetobacter infections involve the use of beta lactams, aminoglycosides, and quinolones. The increased use of antibiotics, however resulted in a widespread emergence of antibiotic resistant strains. Carbapenem, a class of beta lactam, with a broad spectrum of antibacterial activity, have been widely used as the mainstay of treatment for infections caused by such antibiotic resistant strains. Now, Acinetobacter strains resistant to carbapenems have also emerged worldwide rapidly. Different levels and patterns of antimicrobial susceptibilities have been found among different Acinetobacter species. Several studies reported a higher occurrence of multidrug resistance in A. baumannii compared with the non A. baumannii species[15].

Fournier et al described the broad resistance pattern of Acinetobacter baumannii. He performed whole genome sequencing of a clinical epidemic Acinetobacter baumannii strain found in France (AYE).AbaR1, an 86-kb resistance island, one of the largest to be described so far, was identified. Genomic islands containing resistant markers are referred to as resistance islands. Resistant islands are found mainly in gama proteobacteria like shigella flexneri, vibrio cholaera, salmonella enteric and also in staphylococcus aureus[7].

Among the 88 predicted open reading frames (ORFs) within the genomic region, 82 were predicted to be originated from the other gram negative organisms, such as Pseudomonas species, Salmonella species and Escherichia coli. The G + C content of this region was 52 %, whereas the G + C content of the remaining chromosomes was 38.8%, indicates that the genome was more likely a foreign source. Overall 52 resistance genes were identified , out of which 45 (86.5 %) were localized to the AbaR1 resistance island. The genetic surroundings of these resistance determinants provided more evidence for genetic promiscuity, the presence of genes associated with genomic instability were integrases, transposases and insertion sequence. No plasmid markers were identified in the resistance hot spot. Among the three plasmids found within the AYE strain, none contained any known resistance marker[7].

The resistant AYE strain was compared to the susceptible A. baumannii strain from the same geographic region (SDF) ,a structure AbaG1 was identified in the homologous ATPase like ORF.But it was devoid of resistance determinants.Detailed sequence comparison of the two islands failed to reveal any significant similarity[7].

Beta lactams

Enzymatic mechanism:

In Acinetobacter, the most prevalent mechanism of beta lactam resistance is enzymatic degradation by beta lactamase.

Class B beta lactamases (metallo- beta- lactamases, MBLs) confers higher level of Carbapenem resistance and also resistance to all other beta lactams except aztreonam. MBLs are different from other classes of beta lactamases by being susceptible to EDTA inhibition due to the requirement of Zinc ions in the active site[15].

Several Inosine Monophosphate dehydrogenase (IMP) - (IMP-1, IMP-2, IMP-4, IMP-5, IMP-6, IMP-8, IMP-11) and Verona Integron encoded MBL (VIM) - (VIM-1, VIM-2, VIM-4, VIM-11) variants have been detected among the isolates of A. baumannii-A. calcoaceticus complex[47].

SIM-1 was first described in A. baumannii strain. Studies reported a lower level of carbapenem resistance conferred by SIM-1 compared with that of IMP and VIM variants. So far, $bla_{SIM\text{-}1\text{-}like}$ genes have been found only among Acinetobacter isolates from Korea[48]. All different variants of $bla_{IMP,}$ $bla_{VIM,}$ and bla_{SIM} in A.baumannii isolates have been located on class 1 integrons[47].

NDM-1 has been mainly found in Enterobacteriaceae isolates particularly from the Indian subcontinent, but it has also been detected in

isolates of A.baumannii from India as well as other parts of the world. Resistance to carbapenems are mediated by the coexistence of $bla_{NDM-1,}$ $bla_{OXA23,}$ and bla_{IMP}. It has been detected in pan-drug resistant isolates of A. baumannii from China. Recently NDM-2, a variant of NDM-1 with only one amino acid substitution, has been described in an A.baumannii isolate recovered from a patient transferred from Egypt to Germany[49].

Class C beta lactamase: Inherent to all Acinetobacter strains are chromosomally encoded Class C beta lactamases-AmpC cephalosporinase. It is also known as Acinetobacter derived cephalosporinases (ADCs). Unlike AmpC enzymes found in other gram negative organisms, inducible AmpC expression does not occur in A.baumannii.This enzyme when overexpressed hydrolyze most penicillins, cephalothin, cefazolin, cefoxitin, ceftazidime, and beta lactamase inhibitor/ beta lactam combinations, but generally not cefipime or carbapenems. So far, AmpC cephalosporinase have been identified in only a few Acinetobacter species namely A. baumannii, A. pittii, A.baylyi[50].

Phylogenetic analysis demonstrated that AmpC cephalosporinases from A.baumannii and A.pittii are more closely related to each other, comparing to the variants produced by other genera of bacteria. The AmpC cephalosporinase from A.baylyi (designated as ADC-8) was less homologous to ADC-5 of A.pittii and ADC-7 of A.baumannii. But the

phylogenetic proximity of all the ADC cephalosporinases, including ADC-8, evolved from a common ancestor. Normally the bla_{ADC} genes in A.baumannii are normally expressed at basic levels. The key determinant in regulating overexpression of AmpC cephalosporinase in A.baumannii is the presence of an upstream Insertional (IS) element known as ISAba1[50]. The main mechanism responsible for resistance to ceftazidime and other extended spectrum cephalosporins in A. baumannii is ADC enzymes.Till date about 44 bla_{ADC} genes have been identified[10,15].

Class D beta lactamases - OXA type beta lactamases are pencillinases that are able to significantly hydrolyze aminopenicillins (ampicillin and amoxicillin) and carboxypenicillins (carbencillin and ticarcillin). Some of the OXA-type beta lactamase variants also have the ability to hydrolyse extended spectrum cephalosporins. The other variant, described as OXA-type carbapenemases, are able to hydrolyze carbapenems. Most of the extended spectrum OXA type beta lactamases are point mutation derivatives of related narrow spectrum enzymes. So far identified OXA-type carbapenemases are remotely related to the non-carbapenem hydrolyzing OXA-type beta lactamases[51].

OXA-20, OXA-21 and OXA-37 are the only narrow-spectrum OXA type beta lactamases have been so far identified in Acinetobacter isolates. The gene for these enzymes are identified in the form of gene cassettes inserted into the class 1 integrons. Till now, none of the

extended spectrum OXA-type beta lactamases has been found in any Acinetobacter species. In contrast, OXA type carbapenemases, with the exception of $bla_{OXA-48,}$ have been exclusively found among the isolates of the genus Acinetobacter[51].

OXA-58 oxacillinase was the first enzyme identified in Acinetobacter baumannii isolate in France[8]. The first identified OXA type enzyme with carbapenem hydrolyzing activity was from a clinical Acinetobacter baumannii strain isolated in 1985 from Edinburgh. It was initially named as ARI-1.This plasmid encoded reistance determinant was found to be transferable.The gene was later sequenced and named as bla_{OXA-23}[52].

The OXA type carbapenemase genes in Acinetobacter species can be divided into four phylogenetic subgroups - $bla_{OXA-23-like,}$ $bla_{OXA-24-like,}$ $bla_{OXA-51-like}$ and $bla_{OXA-58-like}$ plus five discrete genes - $bla_{OXA-134,}$ $bla_{OXA-104,}$ $bla_{OXA-143,}$ $bla_{OXA-164,}$ and $bla_{OXA-182.}$ The variants within each of the four main subgroups have nucleotide sequence identities of greater than 96%. Whereas the nucleotide sequence identities of the variants from different subgroups, including the discrete genes, range from 46% to 76%. Exception include a 92% nucleotide sequence identity between $bla_{OXA-143}$ and $bla_{OXA-182}$ and 88% nucleotide sequence identity between $bla_{OXA-24-like}$ subgroup with either $bla_{OXA-143}$ or $bla_{OXA-182}$[15].

The OXA type carbapenemase shows a low hydrolytic activity against imipenem and meropenem. It also hydrolyse the narrow spectrum penicillins (benzylpenicillin, ampicillin, piperacillin, ticarcillin) and cephalosporins (cefalotin, cefaloridine) efficiently. The extended spectrum beta lactams (ceftazidime, cefotaxime) and aztreonam are not or very poorly hydrolysed by these enzymes[53].

It has been discovered that $bla_{OXA\text{-}51\ like}$ gene is associated with carbapenem resistance in isolates with an adjacent copy of insertion sequence ISAba1.

Role of ISAba elements

Insertion sequence are the smallest of size less than 2.5 kb. It is the most abundant genetic element capable of independent mobility in microbial genomes. Insertional sequence are responsible for the occurrence of insertional mutations, genome rearrangements, enhanced spread of resistance and virulence determinants within species[54].

Several IS elements are commonly and exclusively detected in the genus Acinetobacter. ISAba1 is a strong promoter, resulting in over expression of the intrinsic bla_{ADC} and $bla_{OXA\text{-}51\text{-}like}$ and the acquired $bla_{OXA\text{-}23\text{-}like}$ genes of Acinetobacter baumannii. ISAba1 acts similarly with other resistant genes such as sulI1 and $bla_{OXA\text{-}58\text{-}like}$. ISAba2, ISAba3, IS18 and ISAba825 also provide strong hybrid promoters for $bla_{OXA\text{-}58\text{-}like}$.

ISAba4 is responsible for an enhanced expression of $bla_{OXA-23-like}$[55]

.Sequence analysis of the genetic environment of bla_{NDM-1} in Acinetobacter baumannii isolate from Germany revealed a chromosomal occurrence of bla_{NDM-1} on a 10.5 kb genetic structure bracketed by two copies of ISAba125. The upstream ISAba125 provides a strong promoter for bla_{NDM-1} expression. The bla_{ADC} gene in the fully sequenced Acinetobacter baumannii strain ACICU is also preceded by an ISAba125 element[56].

Nonenzymatic mechanism

Non enzymatic mechanism is also responsible for beta lactam resistance, including carbapenem resistance . Non enzymatic mechanisms[10] are

1. Changes in the outer membrane proteins (OMPs)
2. Multidrug efflux pumps.
3. Alterations in the affinity or expression of penicillin binding protein

Changes in OMPs & porins

Changes in porins or OMPs reduces the transport of beta lactam into the periplasmic space, this in turn reduces the access to PBPs. Comparing to the susceptible ones, the outer membrane in MDR Acinetobacter baumannii is less permeable to antimicrobial agents.

Alteration in permeability disturbs the beta lactam assimilation into the periplasmic space, this leads to weaker activity of antibiotics[8].

Several porins including the 33-kDa CarO protein constitute a pore channel for influx of carbapenems. This might be involved in the resistance of carbapenem. The distruption of OMP genes by ISAba10 element may sometimes lead to the inactivation of the OMPs like CarO. This reduces the extent of which the antibiotic enters the cell. The chromosomal locus containing the carO gene was cloned from the clinical isolates and it was characterized. It showed, that only a single copy of carO, present in a single transcriptional unit was present in the Acinetobacter baumannii genome.The carO gene encodes a polypeptide of 247 aminoacid residues along with a typical N-terminal signal sequence and predicted transmembrane beta barrel topology[57].

Recent studies revealed that the disruption of the carO gene by the IS elements such as ISAba1, ISAba125 or ISAba825. This results in loss of activity of carO OMP leading to carbapenem resistance in Acinetobacter baumannii[58]. Recent reports of outbreaks caused by carbapenem resistant phenotypes and their characterization revealed that the loss or reduction of porins such as OMPs of 22-29 kDa , 47, 44, and 37 kDa and one of 31 - 36 kDa. This substantiates the findings of previous investigations on OMPs. Gene expression studies along with phenotypic characterization of membrane proteins clarifies the role of

membrane permeability in beta lactam resistance[57,58].

The major protein present in the outer membrane of Acinetobacter baumannii does not belong to the high permeability trimeric porin family. The pore size of the $OmpA_{Ab}$ channel is about 2nm in diameter. $OmpA_{Ab}$ produce very low pore forming activity. It is about 70 fold lower than that of OmpF of E.coli. The outer membrane permeability to cephalothin and cephaloridine is about 100 fold lower than that of E.coli k-12. When the Omp_{Ab} gene is deleted, the permeability of cephalothin and cephaloridine in Acinetobacter baumannii is decreased 2 to 3 fold. The low permeability of this porin together with the presence of beta lactamase and multidrug efflux pumps, such as AdeABC and AdeIJK result in high level of intrinsic resistance to a number of antibiotics[59].

Three porins namely the carbapenem associated OMP carO, Omp33/36 and a 43-kDa porin also called OprD homologue facilitate carbapenem diffusion through the Acinetobacer baumannii outer membrane. These porin channels expression are reduced in several carbapenem resistant strains. Among the three porin channels, the OprD homologue is more responsible for the formation of carbapenem specific channels. It is 49% similar to Pseudomonas aeruginosa[60].

Efflux pumps

Among the well studied mechanisms of resistance in Acinetobacter baumannii, efflux pumps are the one, by which the bacterial cells overcome the action of antibiotics by expelling them out.

According to Fournier et al., 2006 the 3.9 Mb genome of Acinetobacter baumannii AYE is reported to harbor 46 open reading frames (ORFs) encoding putative efflux pumps of different families. It has been reported that, the overexpression of efflux pump in the antibiotic resistant strains provides the evidence for the role of efflux pumps in making the bacteria multi drug resistant[7].

Till date five classes of efflux pumps have been reported to be present in Acinetobacter baumannii. They are[7]

1. ATP binding cassette (ABC)
2. Major facilitator superfamily (MFS)
3. Multidrug and toxic compound extrusion (MATE)
4. Resistance nodulation cell division (RND)
5. Small multidrug resistance (SMR)

The efflux systems in Acinetobacter baumannii that are completely characterized functionally includes[61]

RND type – AdeABC, AdeFGH, AdeIJK

MATE type – AbeM

MFS type – CraA.

According to Iacono et al., and Srinivasan et al : They have only partial knowledge on the function of ABC and SMR efflux pumps.

RND type efflux pump[8]

The RND type efflux pumps that are commonly found in Gram negative bacteria are usually tripartite in nature. It consist of three protein components. They are

1. Cytoplasmic protein
2. Intermembrane or membrane fusion protein (MFP)
3. Periplasmic or outer membrane protein.

These proteins are encoded by three different genes present in single operon. The cytoplasmic protein is otherwise termed as transporter protein. It is involved in the export of substrates such as antibiotics from the cell. MFP and OMP help in export mechanisms. Till date different classes of RND family efflux pumps have been reported in Acinetobacter species. Among them adeABC, adeFGH and adeIJK functions and specificities have been studied extensively. The overexpression of all these efflux pumps is controlled by two component regulatory systems such as sensor and regulator kinase cascade.

According to Magnet et al., adeABC is one of the common types of efflux pumps. It invoved in posing resistance to antibiotics such as Aminoglycosides, Beta Lactams, Chloramphenicol, Tetracycline,

Trimethoprim, Erythromycin and drugs like Ethidium bromide[62].

According to Chau et al., adeABC and adeDE are species specific, whereas adeABC is restricted to Acinetobacter baumannii and adeDE to Acinetobacter genomespecies 3[63]. In AdeABC pump

AdeB – Multidrug transporter

AdeA – Membrane fusion protein

AdeC – Outer membrane protein

AdeB, the efflux transporter, captures the substrate within the phospholipid bilayer or the cytoplasm. The substrate is then transported out via OMP (AdeC). The AdeA acts as intermediate component, which overpass between AdeB and AdeC. AdeR-S two component system controls the expression of AdeB and AdeC components.

According to Marchand et al., point mutation in components of AdeABC and its regulatory proteins leads to overexpression of AdeABC, which in turn leads to multidrug resistance[64].

Penicillin binding protein

Carbapenem resistance in Acinetobacter baumannii is also due to penicillin binding protein (PBP) modifications. The penicillin binding domains of PBPs are transpeptidases or carboxypeptidases, that is involved in peptidoglycan metabolism. The expression of PBP is reduced in multidrug resistant strains in order to resist the activity of antibiotics.

Some of the strategies adopted by Acinetobacter baumannii are acquisition of an additional low affinity PBP, overexpression of an endogenous low affinity PBP and alterations in endogenous PBPs by point mutations or homologous recombination[8].

Resistance to aminoglycosides

Resistance to aminoglycosides by aminoglycoside modifying enzymes (AMEs) is a major threatening problem which leads to resistant phenotypes. All three classes of AMEs are found in Acinetobacter species. The enzymes are[65]

1. O-nucleotidyltransferases – ANT(2”)-Ia, ANT(3”)-Id, ANT(3”)-Id encoded by aadB, aadA1, and aadA4 respectively.
2. O-phosphotransferases – APH(3’)-Ia, APH(3’)-VIa, and APH(3’)-II encoded by aphA1, aphA6, and aphA15 respectively.
3. N-acetyltranferase – AAC(3)-Ia, AAC(3)-IIa, AAC(6’)-Ib, AAC(6’)-Iad, AAC(6’)-Im and AAC(6’)-II encoded by aacC1, aacC2, aacA4, aac(6’)-Iad, aac(6’)-Im and aac(6’)-II respectively.

O-nucleotidyltransferase and O-phosphotransferase catalyse the adenylation and phosphorylation of the hydroxyl groups. The N-acetyltransferases catalyse acetylation of amino groups thereby rendering the antibiotics inactive[8].

It has been reported that the occurrence of a combination of two or more of aminoglycoside modifying resistance genes and association of genes with class 1 integrons[65].

Recently, Acinetobacter baumannii strains producing the 16SrRNA methylase ArmA enzyme have been identified. ArmA is plasmid-encoded. It confers high level of pan aminoglyoside resistance comparing to the moderate level of resistance conferred by aminoglycoside modifying enzymes.This emerging resistance mechanism impairs aminoglycoside binding to target site and confers high level resistance to all clinically useful aminoglycosides like Amikacin, Gentamicin and Tobramycin[66].

AdeABC efflux pump less effectively transports Amikacin and Kanamycin due to their more hydrophilic nature. The MATE family efflux pump AbeM is reported in Acinetobacter which is responsible for exerting resistance to gentamicin and kanamycin[4].

Quinolone resistance

Quinolone resistance is caused by modifications in the structure of DNA gyrase secondary to mutations in the quinolone resistance determining regions (QRDR) of the gyrA and parC genes[67].

DNA gyrase and DNA topoisomerase IV encoded by gyrA and parC genes respectively are the housekeeping genes involved in DNA replication and processing. They are the targets for ciprofloxacin and

other fluoroquinones. A point mutation on the gyrA gene (Ser-83 to leu) was observed in MDR strains of Acinetobacter baumannii. It is responsible for fluoroquinolone resistant phenotype.

According to Deccache et al., sequencing of the parC gene indicates mutations in the parC gene that caused an amino acid change at either Ser-80 or Glu-84[68].

Similar to aminoglycosides, quinolones resistance is also conferred by multidrug efflux pumps including the RND type pump AdeABC and the MATE pump AdeM.

Resistance to Tetracyclin

Resistance to tetracycline and their derivatives are mediated by efflux or ribosomal protection. Tetracyclin efflux pump tet(A) and tet(B) are found in Acinetobacter. tet(A) is found within a transposon, in association with an IS element. Ribosomal protection is mediated by the tet(M) and tet(O)[69]. Apart from tetracycline specific efflux pump, it is also susceptible to efflux by the multidrug efflux systems such as AdeABC pump[62].

Resistance to other antibiotics

Trimethoprim-Sulfamethoxazole resistance in Acinetobacter baumannii is high in many geographical regions. Integrons are very common among strains of Acinetobacter that have a multidrug resistance phenotype. The 3' conserved region of an integron contains a qac gene fused to a sul gene, confers resistance to antiseptics and sulfonamides[70]. Genes coding for Trimethoprim (dhfr) and Chloramphenicol (cat) resistance are also present within integron structures in Acinetobacter baumannii. Efflux pumps may also confers resistance against these agents[71,72].

Management, therapeutic options and susceptibility testing

Guidelines for minimum inhibitory concentration and disk diffusion interpretative criteria for suggested groups of antibiotics have been provided by the National committee for clinical Laboratory standards. Regardless, the clinical management of opportunistic and coinfections with the Acinetobacter is perhaps one of the biggest challenges clinicians facing today, particularly in medical, surgical, rehabilitation, and intensive care units. Resistance among clinical isolates has developed rapidly. This is perhaps the most striking feature of the genus[2].

Clinical isolates are predictably resistant to Penicillin, Ampicillin, first generation Cephalosporins and Chloramphenicol, activity is variable against Carbenicillin, Tetracyclines, Aminoglycosides, second and third generation Cephalosporins, Quinolones, Trimethoprim-sulfamethoxazole and carbapenems[2].

Antimicrobial susceptibility testing for Acinetobacter species is problem prone. Swenson and colleagues at the CDC have shown that results obtained using standardized microbroth dilution do not agree with results obtained with the standardized disk diffusion method for certain antibiotics. Very major errors were frequent with the beta lactam and beta lactam inhibitor combination antibiotics with the microbroth dilution method typically showing greater resistance. At present, there are no data to indicate which method provides more clinically relevant information[5].

Due to the unpredictable multiresistant patterns of nosocomial strains, consideration must be given to the prevalent susceptibility profiles within the institution. Strains of A. calcoaceticus-baumannii complex typically are more antimicrobial resistant than A. lwoffii, probably due to their higher prevalence within the hospital environment and possibly their ability to acquire antibiotic resistance[2].

Treatment

Carbapenems

If isolates retain susceptibility to Carbapenems class of antimicrobial agents, carbapenem remains the treatment of choice. The Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) surveillance program has documented that imipenem as the more potent agent for the treatment of multidrug resistant Acinetobacter infection, on comparing with meropenem. Unfortunately, carbapenem resistant Acinetobacter isolates are increasingly reported worldwide[73,74].

Beta lactamase inhibitors

Beta lactamase inhibitors, particulary sulbactam have intrinsic activity against Acinetobacter strains. For multidrug resistant Acinetobacter infections, several studies have demonstrated clinical efficacy of sulbactam in combination with ampicillin or cefoperazone[75,76].

Tigecycline

Tigecycline, a new glycylcycline agent, has bacteriostatic activity against multidrug resistant Acinetobacter[77]. Studies documented that overexpression of a multidrug efflux pumps in Acinetobacter isolates with decreased susceptibility to tigecyclines.

When adequate peak serum concentration is achieved, tigecycline is best reserved for salvage therapy[78].

Aminoglycosides

Many multidrug resistant Acinetobacter isolates retain intermediate susceptibility to amikacin or tobramycin. Resistance to this class of antimicrobial agents is increasingly associated with aminoglycoside modifying enzymes or efflux pump. These agents are used in combination with another active antimicrobial agent.

Polymyxin

As limited therapeutic options are available, clinicians have returned to the use of polymyxin B or polymyxin E (colistin) for the multidrug resistant Acinetobacter infections[79,80]. Colistin acts by disturbing the bacterial cell membrane, which increases the permeability leading to cell death. Colistin is bactericidal against Acinetobacter. Its effect is concentration dependent. Resistance to polymxin is due to outer cell membrane alterations or efflux pump[81]. Studies have reported that cure or improvement rate for colistin is 57% to 77% among severely ill patients with multidrug resistant Acinetobacter infections[82,83].

Synergy and combination therapy

Combined treatment with an aminoglycoside and Ticarcillin or Piperacillin is synergistic and may be effective in serious infections.

According to Montero et al., the best regimen in a mouse pneumonia model is Colistin and Rifampin, Imipenem and Rifampin, Tobramycin and Rifampin, Imipenem and Tobramycin[84].

According to Yoon et al., synergy with double and triple combinations are obtained with Polymyxin B with Imipenem or Rifampin[85].

According to Tan et al. Colistin and Minocycline combination has synergical effect[86].

According to Kroeger et al., Ceftazidime prevents regrowth and development of Colistin resistance hence Colistin and continuously infused ceftazidime is advisable[87].

According to song et al., Colistin and Rifampin combination has synergy effect compared to Colistin alone[88].

Li et al found heteroresistance (subpopulation with varying level of resistance to Colistin) in Colistin susceptible Acinetobacter isolates studied in vitro[89].

Owen et al suggested that combination therapy is advisable to prevent the emergence of Colistin resistance during monotherapy[90].

Preventive measures[91,92,93]

1. Standard precautions includes hand hygiene, correct and consistent use of gloves, appropriate use of gowns and eye protection.

2. Contact barrier precautions includes dedicate patient care equipment and gowns and gloves for health care personnel on entry to an isolation room.

3. Environmental cleaning and disinfection.

4. Cohorting of health care personnel i.e designating staff to care for only patient colonized or infected with the organism.

5. Clinical unit closure is required in some outbreak settings to interrupt transmission and for thorough environmental disinfection.

6. Antimicrobial stewardship i.e programs to promote judicious antimicrobial use and prevent emergence of resistance.

7. Passive and active surveillance can be done to identify infected or colonized patients, so that interventions can be implemented.

MATERIALS AND METHODS

MATERIALS AND METHODS

Place of study : Thanjavur Medical College and Hospital, Thanjavur.

Period of Study : One year between June 2013 and May 2014

Design of study : Observational study

Collabrating Departments: Medicine, Surgery, Paediatrics, Obstetrics &Gynaecology, Plastic surgery, ENT, Neurosurgery.

Ethical clearance : Prior approval obtained from Ethical Committee

Inclusion Criteria :

1. Patients of all age group
2. Patients of both gender
3. Samples – urine, pus, blood, sputum
4. Patients not taken prior antibiotic therapy.

Exclusion Criteria :

1. Samples – body fluids
2. Patients taken prior antibiotic therapy

Plan of study

Eighty isolates of Acinetobacter were included in this study. These had been isolated from urine, pus, blood and sputum samples received in Microbiology laboratory during the period of June 2013 to May 2014.

Methodology

Presumptive identification of Acinetobacter was made by inoculating the sample on Nutrient agar plate, MacConkey agar plate and Blood agar plate and incubated at 37°C for 24 hours. All non lactose fermentors were subjected to Gram staining, hanging drop and biochemical test like catalase, oxidase, indole, methyl red, voges proskauer, TSI, urease, citrate and nitrate reduction test. Acinetobacter are Gram negative bacilli or coccobacilli, non motile, oxidase negative, catalase positive, indole negative, methyl red negative, voges proskauer negative, non fermentor, citrate positive, urease variable. Acinetobacter was further confirmed by inoculation on selective media, Leeds Acinetobacter media. Speciation was done on the basis of glucose oxidation, hemolysis in blood agar, growth at 37°C and 42°C, Arginine decarboxylation test, 1% and 10% lactose and susceptibility to chloramphenicol. Antimicrobial susceptibility testing was done by Kirby bauer disc diffusion method for Ampicillin, Gentamicin, Amikacin, Cephalexin, Ceftriaxone, Ciprofloxacin, Cotrimoxazole, Chloramphenicol and Imipenem. Molecular characterization of Acinetobacter for bla_{OXA-51} gene was done by conventional PCR.

Culture

Samples received were inoculated on Nutrient agar plate, Macconkey agar plate and Blood agar plate, and incubated at 37°C for 24 hours. Nutrient agar plate – 1-2 mm non pigmented, domed colonies with smooth surfaces.

MacConkey agar plate – Non lactose fermentors, but colonies exihibit a faint pink tint.

Blood agar plate – Non hemolytic, greyish, Smooth, opaque, raised, creamy colonies.

Gram Staining

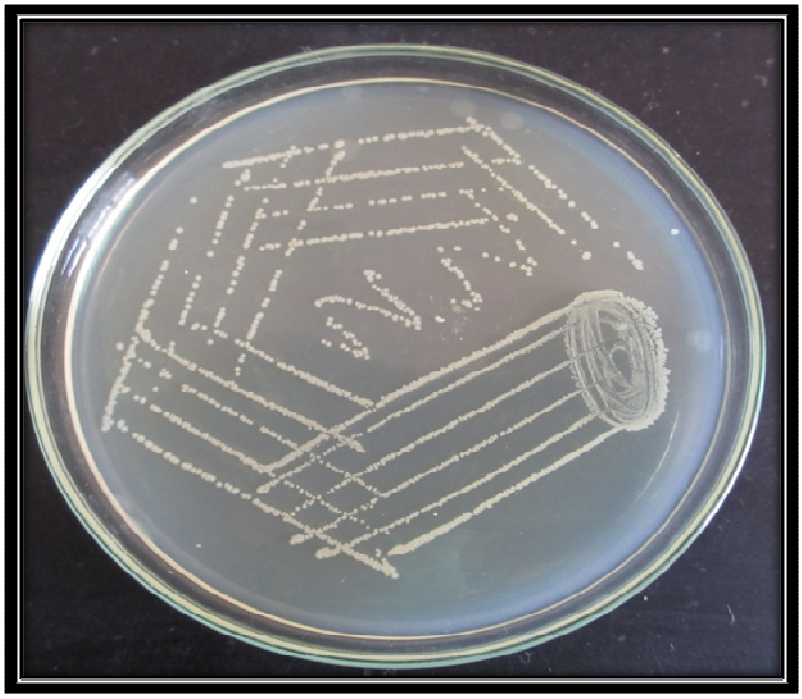
Principle:

In gram positive bacteria, the purple crystal violet/iodine complex is retained within the cell after washing with acetone because the thick peptidoglycan layer does not allow the crystal violet iodine complex to be washed out of the cell. In gram negative bacteria, the crystal violet/iodine complex is leached from the cell because of disruption of the lipid-rich outer membrane by the acetone.

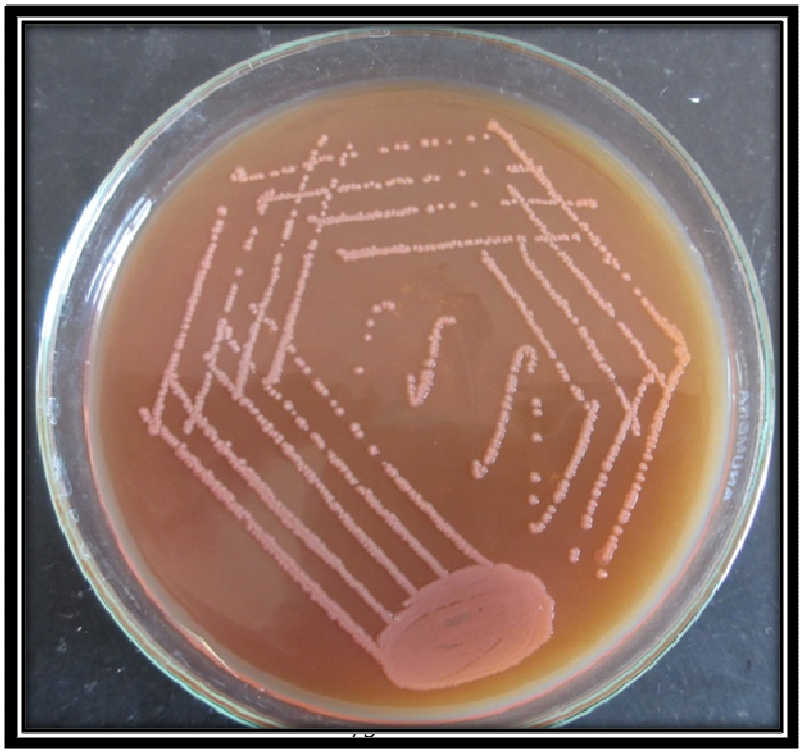
Procedure:

1. Cover the smear with crystal violet for 1 min.
2. Rinse gently under tap water.
3. Cover the smear with Gram’s iodine for 1 min.
4. Rinse gently under tap water.

Nutrient agar plate

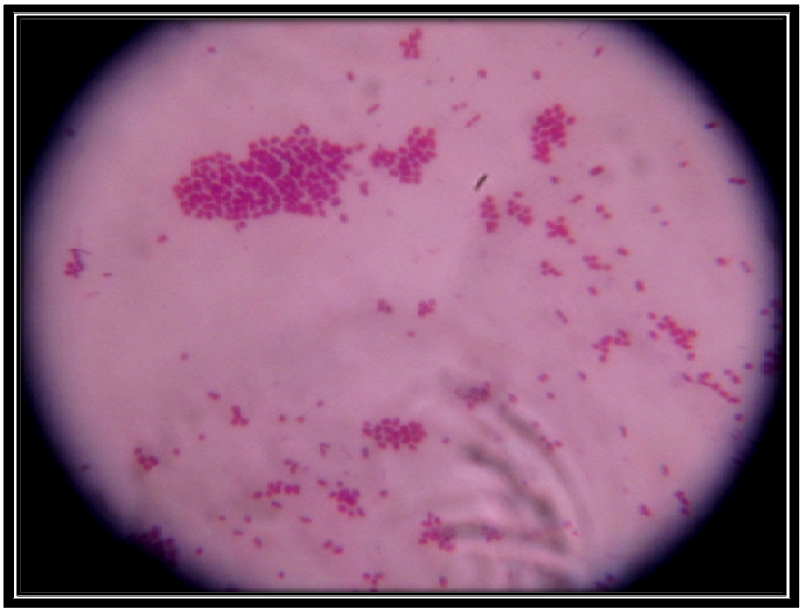


MacConkey agar plate



Blood agar plate

Gram staining – Gram negative coccobacilli



5. Decolourise with acetone.

6. Immediately rinse under tap water.

7. Counterstain with dilute carbol fuchsin for 1 minute.

8. Rinse the smear under tap water and air dry it.

Observe the smear first under low power and then under oil immersion.

Quality control:

Staphylococcus aureus – Purple coloured Gram positive bacteria.

Escherichia coli – Pink coloured Gram negative bacteria

Observation:

Presence of pink coloured coccobacilli

Interpretation:

Gram negative bacilli or coccobacilli

Hanging – drop procedure:

The technique is generally used for studying the motility of the bacteria.

Procedure:

1. Take a clean grease free cavity slide
2. Take a clean coverslip, apply paraffin to four corners of coverslip.
3. Place a drop of colony emulsified in normal saline on the coverslip with the help of inoculating loop.

4. Place the cavity slide over the coverslip so that the drop is placed in the centre.
5. Invert the slide, and observe under microscope
6. First observe under low power, locate the edge of the drop, shift the focus to high power and observe for motility of the organism.

Observation:

The bacteria showed no motility.

Interpretation:

The organism is non motile.

Biochemical Reaction:

Cytochrome Oxidase Test:

Principle:

The cytochromes are iron containing hemoproteins that act as the last link in the chain of aerobic respiration by transferring electrons to oxygen, with the formation of water. The cytochrome system is found in aerobic, microaerophilic, and facultative anaerobic organisms, so the oxidase test is important in identifying organisms that either lack the enzyme or are obligate organisms.

The cytochrome oxidase test uses certain reagent dye, such as Tetra

methyl para phenylenediamine dihydrochloride, that substitutes for oxygen as artificial electron acceptors. In the reduced state, the dye is colourless, however in the presence of cytochrome oxidase and atmospheric oxygen, para phenylenediamine is oxidized , forming indophenols and appears blue.

Procedure:

The indirect paper strip procedure, in which a few drops of the reagent are added to a filter paper strip. The tetramethyl derivative of para phenylenediamine is used because it is more stable in storage and more sensitive to the detection of cytochrome oxidase and is less toxic than the dimethyl derivative. A loopful of colony is smeared into the reagent zone of paper.

Quality Control:

Positive control – Pseudomonas aeruginosa

Negative control – Escherichia coli

Observation:

The colour of the smear in the zone of the filter paper remains unchanged

Interpretation:

The organism is oxidase negative.

Catalase:

Principle:

Catalase is an enzyme that decomposes hydrogen peroxide into water and oxygen. Chemically catalase is a hemoprotein, similar in structure to hemoglobin, except that four iron atoms in the molecule are in the oxidized, rather than the reduced state.

Hydrogen peroxide forms as one of the oxidative end products of aerobic carbohydrate metabolism. If allowed to accumulate, it is lethal to bacterial cells. Catalase converts hydrogen peroxide into oxygen and water.

Procedure:

Take 1 ml of 3% hydrogen peroxide in test tube. Introduce small quantity of bacterial growth into the fluid with the help of a glass rod or plastic loop and touch the side of the tube. Observe for the release of bubbles.

Quality Control:

Positive control – Staphylococcus aureus

Negative control – Streptococcus species.

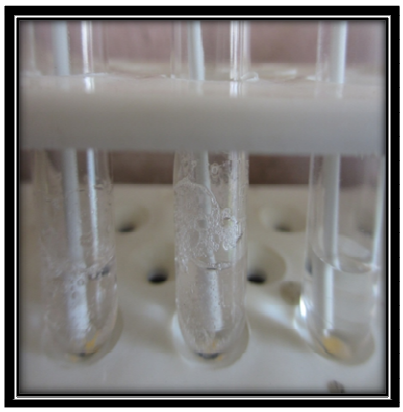
Observation:

The rapid and sustained appearance of bubbles formed.

Interpretation:

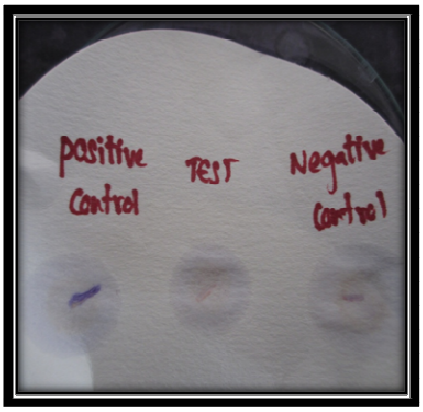
The organism is catalase positive.

Catalase test



PC TEST NC

Oxidase test



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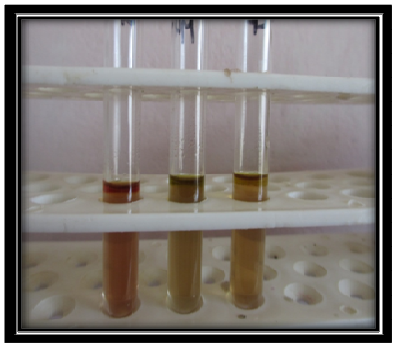
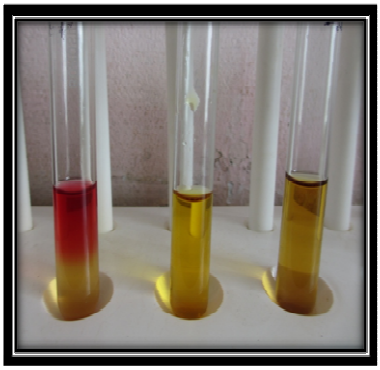
Indole test



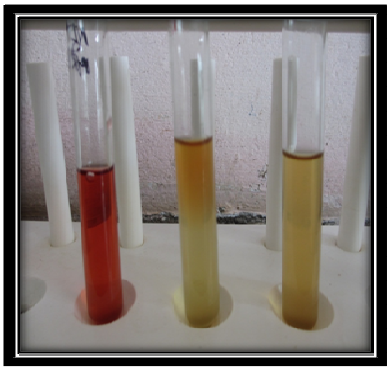
PC TEST NC

MR test



PC TEST NC

VP test



PC TEST NC

Indole:

Principle:

Indole, a benzyl pyrrole, is one of the metabolic degradation products of the amino acid tryptophan. Bacteria that possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan with the production of indole, pyruvic acid, and ammonia.The indole test is based on the formation of a red complex when indole reacts with the aldehyde group of p-dimethylaminobenzaldehyde. This is the active chemical in Kovac and Ehrlich reagent.

Procedure:

Inoculate peptone water with colonies of Acinetobacter and incubate at 35°C for 18 to 24 hours. At the end of this time, add drop of kovac's reagent down the inner wall of the tube.

Quality control:

Positive control – Escherichia coli

Negative control – Klebsiella pneumonia

Observation:

Development of yellowish green colour ring at the interface of the reagent and broth within few seconds after adding kovac's reagent

Interpretation:

Formation of green colour ring – indole is not produced

Methyl Red Test[5]:

Principle:

Methyl red is a pH indicator with a range between 6.0 (yellow) and 4.4 (red). The pH at which methyl red detects acid is considerably lower than the pH for other indicators used in bacteriologic culture media. Hence for producing a color change, the test organism must produce large quantities of acid from the carbohydrate substrate used.

The methyl red test is a quantitative test for acid production, requiring positive organisms to produce strong acids (lactic, acetic, formic) from glucose through the mixed acid fermentation pathway.

Procedure:

1. Inoculate the MR broth with a pure culture of the test organism. Incubate the broth at 35°C for 48 to 72 hours.

2. At the end of this time, add 5 drops of the methyl red reagent directly to the broth.

Quality control:

Positive control – Escherichia coli

Negative control – Enterobacter aerogenes

Observation:

Yellow colour developed

Interpretation:

Yellow color indicates the organism was methyl red negative.

Voges Proskauer Test:

Principle:

Pyruvic acid, the pivotal compound formed in the fermentative degradation of glucose, is further metabolized through various metabolic pathways, depending on the enzyme systems possessed by different bacteria. One such pathway results in the production of acetoin (acetyl methyl carbinol), a neutral reacting end product. In the presence of atmospheric oxygen and 40% potassium hydroxide, acetoin is converted to diacetyl and alpha naphthol serves as a catalyst to bring out a red complex.

Procedure:

Inoculate a tube of VP broth with a pure culture of the test organism. Incubate for 24 hours at 35°C. At the end of this time, 1 ml of broth is taken in a clean test tube. Add 0.6 ml of 5% alpha napthol, followed by 0.2 ml of 40% KOH. Shake the tube gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10 to 15 minutes.

Quality control:

Positive control – Enterobacter aerogenes

Negative control – Escherichia coli

Observation:

Yellow colour formed

Interpretation:

VP test was negative for this organism.

Citrate Utilisation Test:

Principle:

Sodium citrate is a salt of citric acid, a simple organic compound found as one of the metabolites in the tricarboxylic acid cycle (Krebs cycle). Some bacteria obtain energy in a manner other than by the fermentation of carbohydrates by using citrate as the sole source of carbon. The utilization of citrate by the test bacterium is detected in citrate medium by the production of alkaline by products. The medium includes sodium citrate, an anion, as the sole source of carbon, and ammonium phosphate as the sole source of nitrogen. Bacteria that can use citrate can also extract nitrogen from the ammonium salt, with the production of ammonia, leading to alkalinization of the medium from the conversion of the ammonia to ammonium hydroxide. Bromothymol blue-yellow below pH 6.0 and blue above pH 7.6 is the indicator.

Procedure:

A well isolated colony is picked up and inoculated as single streak on the slant surface of the simmon's citrate agar tube. The tube is incubated at 35°C for 24 to 48 hours.

Quality control:

Positive control – Enterobacter aerogenes

Negative control – Escherichia coli

Observation:

Change of medium into deep blue colour.

Interpretation:

Change of medium into deep blue colour indicates the organism has been able to utilize the citrate contained in the medium, with the production of alkaline products.

Urease :

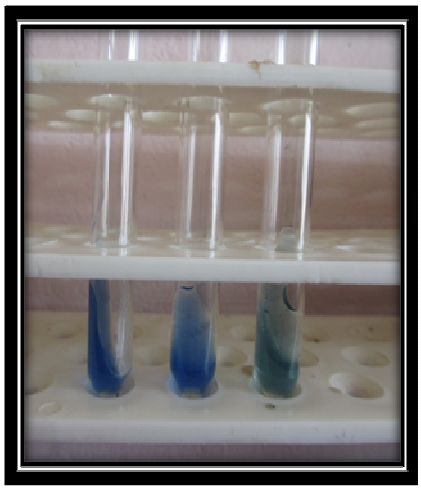
Principle:

Urea is a diamide of carbonic acid. All amides are easily hydrolyzed with the release of ammonia and carbon dioxide. Urease is an enzyme possessed by many species of microorganism that can hydrolyze urea. The ammonia reacts in solution to form ammonium carbonate, resulting in alkalinization and increase in the pH of the medium.

Procedure:

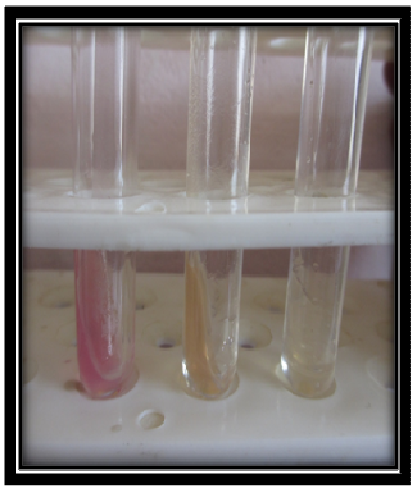
Christensen's urea agar slope is inoculated with colonies of Acinetobacter and incubated at 37°C for 18 hours.

Citrate utilization test



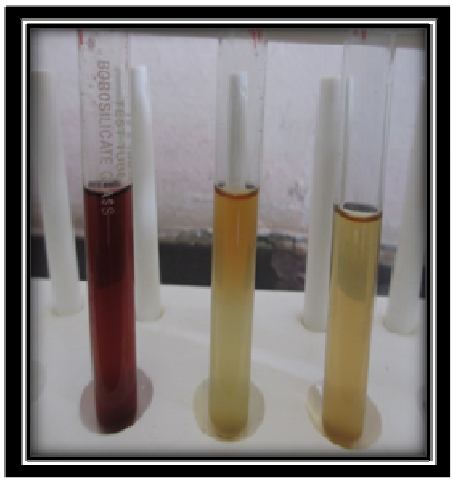
PC TEST NC

Urease test



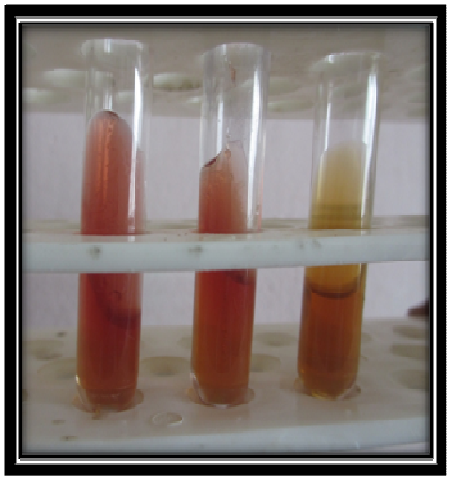
PC TEST NC

TSI test



PC TEST NC

Nitrate reduction test



PC TEST NC

Quality control:

Positive control – Proteus mirabilis

Negative control – Escherichia Coli

Observation:

No colour change in the medium

Interpretation:

No colour change in the medium. So the enzyme urease is not produced by the organism.

Triple Sugar Iron Agar Test (TSI):

Principle:

The TSI agar is distributed in the tube which contains a slant and a butt. TSI medium indicates whether the bacteria ferments glucose only, or lactose and sucrose also with or without production of gas. The medium can detect production of hydrogen sulphide as well as other bacteria which utilizes only glucose. Due to the acid production , the colour of the indicator (phenol red) is changed to yellow and the whole medium appears yellow in colour. After further incubation as the glucose is fully exhausted, the bacteria begin to oxidatively degrade the amino acid present in the medium. Since oxygen is exposed only to the slant portion, oxidative degradation occurs only in the slant portion.This oxidative degradation results in the production of alkali products, which

reverts the colour of the slant to red colour. In the deeper part of the tube, amino acid degradation is insufficient to overcome the acid formed, so medium in the butt part remains yellow in colour.

If the TSI medium is inoculated with lactose fermenting organism, then even after the glucose is completely used up in first 8-12 hours, fermentation continues as the organism is abe to use lactose which is present in concentration 10 times that of glucose. So the acid production continues to occur even after 18-24 hours and both the slant and butt appear yellow.

For the detection of H_2S which is a colourless gas, medium must include an indicator to detect the H_2S. Sodium thiosulphate is the source of sulfur atoms. Ferrous sulfate is the indicator used for the detection of the H_2S which is indicated by the production of insoluble black precipitate.

Procedure:

Inoculate the colony into the tube by means of stab and streak inoculation and incubate for 18-24 hours at 37° C.

Quality Control:

Alkaline slant / alkaline butt – Pseudomonas aeruginosa

Observation:

Alkaline slant / No change (K/ No change) no H_2S production, shows no carbohydrate fermentation.

Interpretation:

The bacteria is non fermentor

Nitrate reduction Test :

Principle:

Organisms demonstrating nitrate reduction have the capability of extracting oxygen from nitrates to form nitrites and other reduction products. The presence of nitrites in the test medium is detected by the addition of alpha naphthylamine and sulfanilic acid, with the formation of a red diazonium dye, p-sulfobenzeneazo- alpha naphthylamine.

Procedure:

Inoculate the nitrate medium with a loopful of test organism and incubate at 35°C for 18 to 24 hours.

At the end of incubation, add 1ml of 0.5% alpha naphthol and sulfanilic acid.

Quality control:

Positive control – Escherichia coli

Negative control – Acinetobacter baumannii

Observation:

No color develops.

Interpretation:

Nitrate was not reduced to nitrite by the test organism.

Culture on selective media:

Leeds Acinetobacter Medium(HI MEDIA)

Recommended for the isolation of Acinetobacter species.

Principle:

Casein acid hydrolysate and soya peptone provides nitrogenous compound and vitamins to the organism. Sucrose, fructose and mannitol provides carbohydrate source. Sodium chloride provides osmotic balance. Phenol red serves as pH indicator. The liberation of ammonia ions by the utilization of nitrogenous material in the medium results in pink colour.

Procedure:

Inoculate the organism on Leeds Acinetobacter medium and incubate at 37° C for 18 to 24 hours.

Quality control:

Appearance

Light yellow to pink coloured homogenous free flowing powder

Gelling

Firm, comparable with 1.2% agar gel

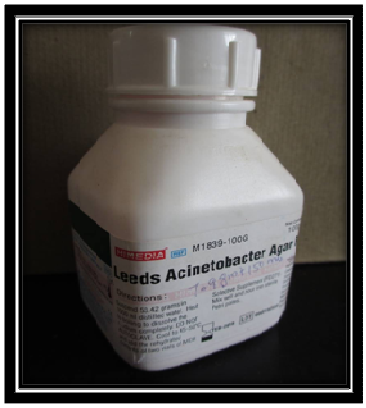
Colour and clarity of prepared medium

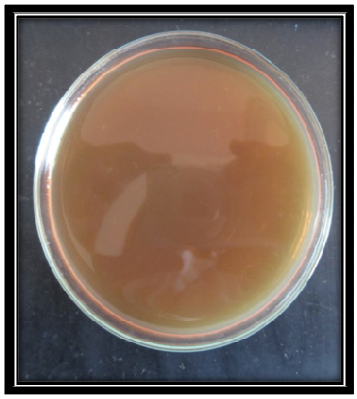
Red colour clears to slightly opalescent gel forms in petri plate

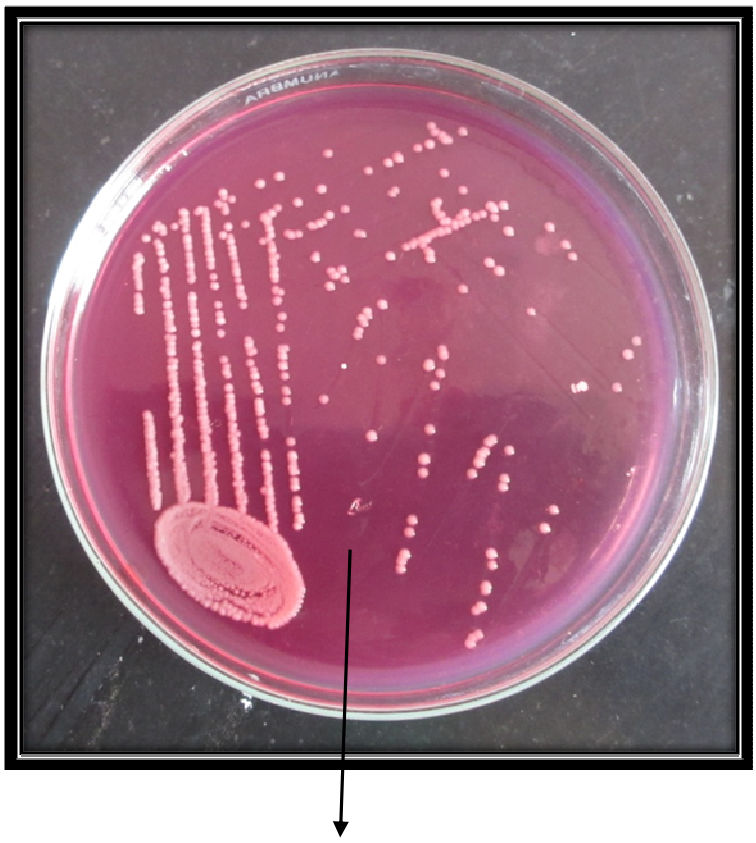
Reaction

Reaction of 5.34% w/v aqeous solution at 25◦C.pH – 7.0

Leeds Acinetobacter medium







Pink mucoid colonies with pink colour diffuses into the medium

Observation:

Pink mucoid colonies with pink colour diffuses into the medium.

Interpretation:

The organism is Acinetobacter.

Oxidative – Fermentative Test (Hugh and Leifson):

Principle:

Saccharolytic microorganisms degrade glucose either fermentatively or oxidatively. The end products of fermentation are relatively strong mixed acids that can be detected in a conventional fermentation test medium. However, the acid formed in oxidative degradation of glucose are extremely weak, and the more sensitive oxidation-fermentation medium of Hugh and Leifson is required for the detection.

The OF medium of Hugh and Leifson differs from carbohydrate fermentation media-

1. Peptone decreased from 1% to 0.2%
2. Carbohydrate is increased from 0.5% to 1.0%
3. Agar is decreased from 1.5% to 0.3%, making it semisolid.

The lower protein/carbohydrate ratio reduces the formation of alkaline amines that can neutralize the small quantities of weak acids that may form from oxidative metabolism. The relatively larger amount of

carbohydrate serves to increase the amount of acid that can potentially be formed. The semisolid consistency of the agar permits acids that forms on the surface of the agar to permeate throughout the medium, making interpretation of the pH shift of the indicator easier to visualize.

Procedure:

Two tubes of OF medium are required. Inoculate the colonies of Acinetobacter into both tubes. One tube is covered with 1 cm layer of melted paraffin, leaving the other tube open to the air. Incubate both tubes at 35°C and examine daily for several days.

Quality control:

Glucose fermenter – Escherichia coli

Glucose oxidizer – Pseudomonas aeruginosa

Nonsaccharolytic – Moraxella species

Observation:

Open tube	Covered tube	Metabolism
Yellow	Green	Oxidative
Green	Green	Nonsaccharolytic

Interpretation:

Oxidative – Acinetobacter baumannii

Nonsaccharolytic – Acinetobacter spp other than Acinetobacter baumannii

Arginine Decarboxylase Test:

Principle:

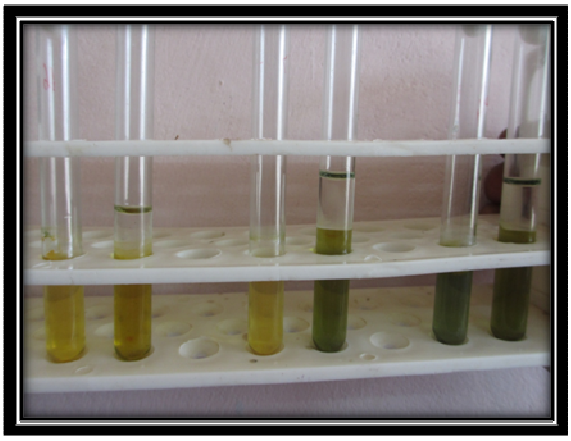
Decarboxylase are a group of substrate specific enzyme that are capable of reacting with the carboxyl portion of amino acids, forming alkaline reacting amines. This reaction, known as decarboxylation, forms carbondioxide as a second product. Decarboxylase enzyme is specific for arginine.

The conversion of arginine to citrulline is a dihydrolase, rather than a decarboxylase reaction, in which an NH_2 group is from arginine as a first step. Citrulline is next converted to ornithine, which then undergoes decarboxylation to form putrescine.

Moeller decarboxylase medium is the base most commonly used for determining the decarboxylase capabilities. The amino acid, arginine is added to the decarboxylase base before inoculation with the organism.The tubes are anaerobically incubated by overlying with mineral oil. During initial stages of incubation the tube turns yellow, owing to the fermentation of the small amount of glucose in the medium. If arginine is decarboxylated, alkaline amines are formed and the medium reverts to its original purple colour.

Acinetobacter baumannii

OF test - Oxidative

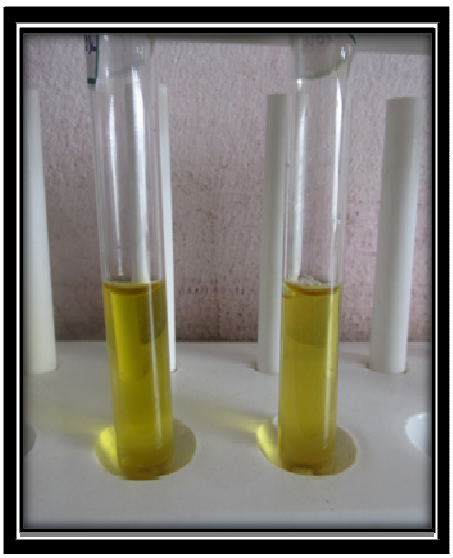


PC TEST NC

Arginine – positive

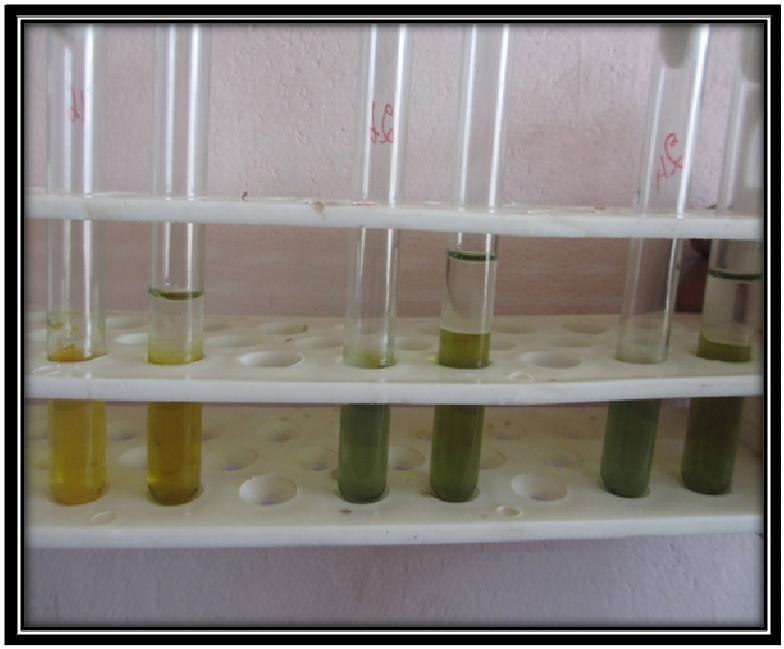
PC TEST NC

1% & 10% lactose-acid production



Acinetobacter lwoffii

OF test – Non saccharolytic

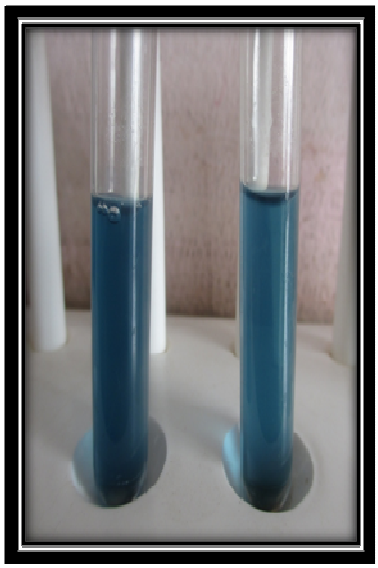


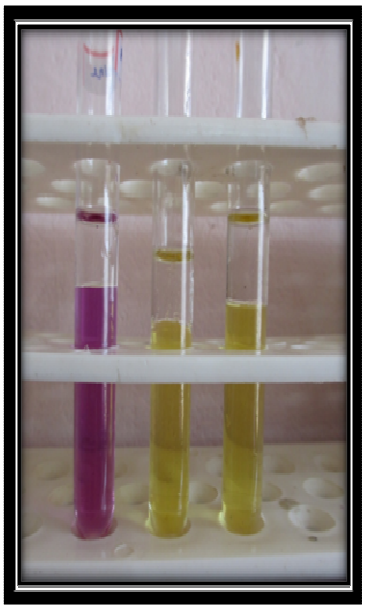
PC TEST NC

Arginine - negative

1% & 10% lactose

No acid production





PC TEST NC

Procedure:

A well isolated colony of Acinetobacter is inoculated into the medium and overlay with sterile mineral oil to cover about 1 cm of the surface and incubate at 35°C for 18 to 24 hours.

Quality control:

Positive control – Enterobacter cloacae

Negative control – Enterobacter aerogenes

Observation:

Conversion of the tube to yellow.

Conversion of the tube to yellow and reversion of the tube to purple colour.

Interpretation:

Yellow – Acinetobacter lwoffii

Yellow to purple – Acinetobacter spp other than Acinetobacter lwoffi

Antimicrobial sensitivity testing

Storage of antimicrobial discs [9,94]

The antimicrobial disc container was stored at 4 - 8° C while the β-lactam antibiotics were stored in the freezer compartment. Some labile agents like Imipenem retained greater stability when stored frozen until the day of use.

Disc container was taken out from refrigerator one or two hours before use and brought to room temperature. Once a cartridge of discs has been removed from its sealed package, after the use it was replaced in a tightly sealed dry container.

Preparation of turbidity standard [9,94]

McFarland standards prepared by adding specific volumes of 1% Suphuric acid and 1.175 % barium chloride to obtain a barium sulphate solution with a specific optical density. The most commonly used is the McFarland 0.5 standard, which contains 99.5ml of 1% sulphuric acid and 0.5 ml of 1.175 % barium chloride. This solution is distributed in tubes and compared to those used for inoculum preparation, which are sealed tightly and stored in the dark at Room temp. The McFarland 0.5 standard provides a turbidity comparable to that of a bacterial suspension containing approximately 1.5 X 10^{8} CFU/ml.

Preparation of Inoculum[9,94]

In order to prepare the inoculum**,** about 3-5 representative colonies were picked up and inoculated in 4-5 ml of peptone water and incubated at 37°C for 2 – 6 hrs to attain 0.5 McFarland's standard which corresponds to 150 million organisms/ml. If it was more turbid, then some more quantity

of peptone water was added and adjusted to 0.5 McFarland's standard by comparing against a card with white background and contrasting black lines.

Inoculation of MHA plates

Within 15 minutes of adjusting the turbidity of the inoculum suspension, dispense the suspension on surface of medium and close the lid. Holding the plate with both the hands, make suspension flow uniformly over entire surface of medium by slowly rotating the plate. Drain out excess fluid and wipe the rim of plate with cotton dipped in Lysol. The plate was closed and left for 3-5 minutes to allow any excess surface moisture to be absorbed before applying drug impregnated discs.

Application of discs to inoculated agar plate:

The predetermined battery of antimicrobial discs of Ampicillin, Gentamicin, Amikacin, Cephalexin, Ceftriaxone, Ciprofloxacin, Cotrimaxazole, Chloramphenicol, Imipenem were tested for all the isolates.

The entire disc were placed on agar plates and pressed down to ensure complete contact with the agar surface. Discs were distributed evenly so that they were not closer than 25 mm from centre to centre of the disc and incubated at 37° C for 16 – 18 hrs.

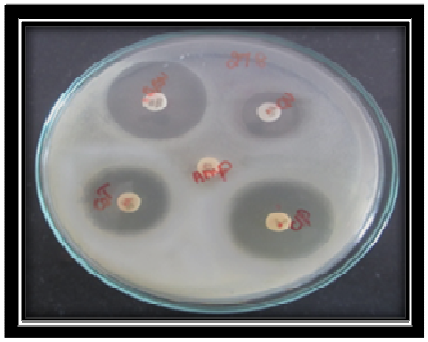
Reading and interpretation of results

After 16-18 hrs of incubation, each plate was examined for satisfactory streaking showing confluent lawn of growth with uniformly circular zones of inhibition around the drug disc.

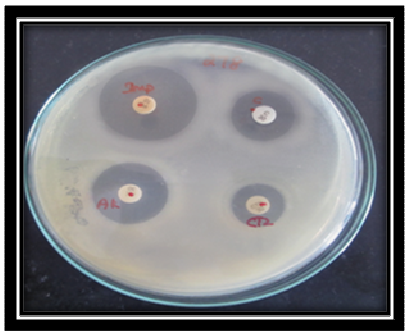
The diameter of the zones of complete inhibition including the diameter of the discs was measured. The zones were measured to the nearest millimetre using a ruler that was held on the back by inverting Petri plate. The Petri plate was held a few inches above a black, non reflecting background and illuminated with reflected light. The zone margin showing no obvious visible growth that could be detected with unaided eyes was considered as a zone of inhibition. The sizes of the zones of inhibition were interpreted by referring to the CLSI standards and reported as ‘susceptible’, ‘intermediate’ or ‘resistant’ to the drugs that were tested.

Antimicrobial susceptibility testing

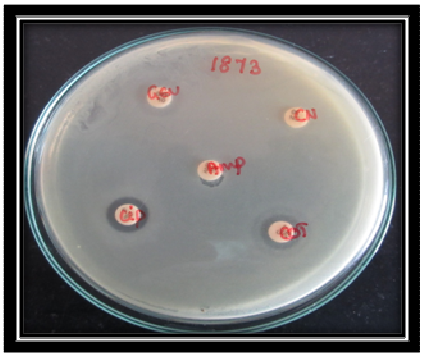
Sensitive pattern



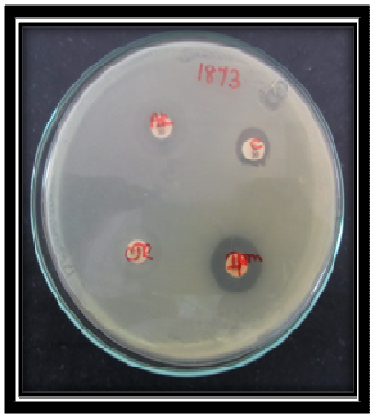
Sensitive pattern



Resistant pattern



Resistant pattern



II line drug

TABLE 3 : ZONE SIZE INTERPRETATIVE CHART ACCORDING TO CLSI

Sl. No.	Antimicrobial agent	Symbol	Drug concentration (µg)	Zone size in mm		
				Resistant	Intermediate	Sensitive
A. Aminoglycosides						
1.	Gentamicin	GEN	10	< 12	13-14	> 15
2.	Amikacin	AK	30	<14	15-16	>17
B. Penicillin						
1.	Ampicillin	AMP	10	<13	14-16	>17
C. Sulphonamides						
1.	Cotrimoxazole	COT	1.25/23.75	<10	11-15	>16
D. Quinolones						
1.	Norfloxacin	NX	10	<12	13-16	>17
2.	Ciprofloxacin	CIP	5	<15	16-20	>21
E. Cephalosporins						
1.	Cephalexin	CN	30	<14	15-17	>18
2.	Ceftriaxone	CTR	30	<13	14-20	>21
F. Carbapenems						
1.	Imipenem	IMP	10	<13	14-15	>16
G. Macrolides						
1.	Chloramphenicol	C	30	<12	13 – 17	18

II line drugs						
1.	Doxycycline	DO	30	12	13 - 15	16
2.	Piperacillin	PI	100	17	18 - 20	21
3.	Piperacillin Tazobactum	PIT	100/10	17	18 - 20	21
4.	Polymyxin-B	PB	300	11		12
5.	Colistin	CO	10	10		11

MOLECULAR CHARACTERIZATION OF ACINETOBACTER

Conventional Polymerase Chain Reaction was done for 14 isolates for the detection of bla_{OXA-51} gene. Isolates were selected randomly based on the following features.

1. PCR was done for twelve Acinetobacter baumannii strains. out of which four isolates were resistant to Imipenem. Remaining eight isolates were sensitive to Imipenem.

2. PCR was also done for two Acinetobacter lwoffi strains.

Gene Identification

Material & Methods:

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

2X Master Mix :

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

Agarose gel electrophoresis:

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

OXA-51 gene primer

Forward Primer: 5'-CTTGCTCGTGCTTCGACCGAGT-3

Reverse Primer: 5'-CGCCTAGGGTCATGTCCTTTTCCC-3'

PCR Product size: 160bp

Procedure

1. Pellet is suspended in 200µl of PBS.
2. Added 50µl of Lysozyme and incubated at 37ºC for 15min.
3. 400µl of Lysis buffer and 40µl of Proteinase K [10mg/ml] is added and gently mixed well.
4. Incubated in water bath at 56°C for 10 min.
5. Add 200µl of Isopropanol and mix well.
6. Transferred whole lysate into PureFast spin column and centrifuged at 10000rpm for 1min.
7. Discard flow through and added 500µl of Wash Buffer and Centrifuge at 12000rpm 1 min.

8. Discard flow through and added 500µl of Wash Buffer-2 and centrifuged at 12000rpm for 1min. Repeated wash one more time.
9. Discarded flow through and Centrifuged column for additional 2 minute to remove any residual ethanol.
10. Eluted DNA by adding 100µl of Elution Buffer and Centrifuged for 1min.
11. Quality and Quantity of extracted DNA is checked by loading in 1% agarose gel and 1µl of extracted DNA is used for PCR amplification

PCR Procedure:

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

1. Reactions set up as follows;

COMPONENTS IN PCR VIAL	QUANTITY
Master Mix	10µl
OXA-51 Primer Mix(2pmoles/µl)	5µl
Genomic DNA	5µl
Total Volume	20µl

2. Mixed gently and spin down briefly.

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

Initial Denaturation : 94ºC for 3 min

Denaturation : 94ºC for 1 min
Annealing : 58ºC for 1min
Extension : 72ºC for 1min
} 35 cycles

Final extension : 72º C for 5 min

Loading:

1. Prepare 2% agarose gel. [2gm of agarose in 100ml of 1x TAE buffer]

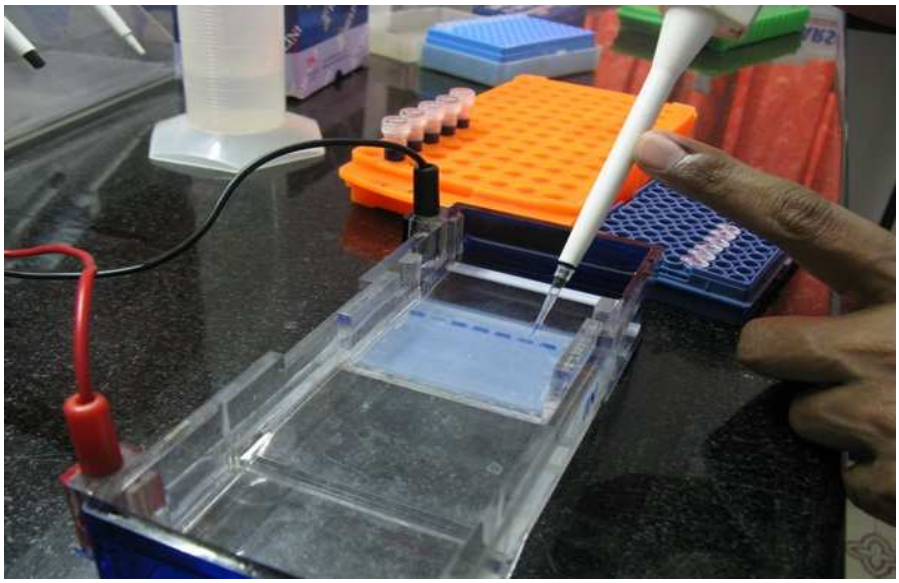
2. Mix 8µl 6X Gel loading dye to each PCR vial and loaded entire PCR product.

3. Run electrophoresis at 50V till the dye reaches three fourth distances and observe the bands in UV Transilluminator.

Agarose gel electrophoresis:

1. Prepared 2% agarose. (2gm agarose in 100ml of 1X TAE buffer and melted using micro oven)
2. When the agarose gel temperature was around 60ºC, added 5µl of Ethidium bromide.
3. Poured warm agarose solution slowly into the gel platform.
4. Kept the gel set undisturbed till the agarose solidifies.

Thermocycler



4. Kept the gel set undisturbed till the agarose solidifies.
5. Poured 1XTAE buffer into submarine gel tank.
6. Carefully placed the gel platform into tank. Maintain the tank buffer level 0.5cm above than the gel.
7. PCR Samples are loaded after mixed with gel loading dye along with 10µl HELINI 100bp DNA Ladder. [100bp, 200bp, 300bp, 400bp, 500bp, 600bp, 700bp, 800bp, 900bp, 1000bp, 1500bp]
8. Run electrophoresis at 50V till the dye reaches three fourth distance of the gel.
9. Gel viewed in UV Transilluminator and observed the bands pattern.

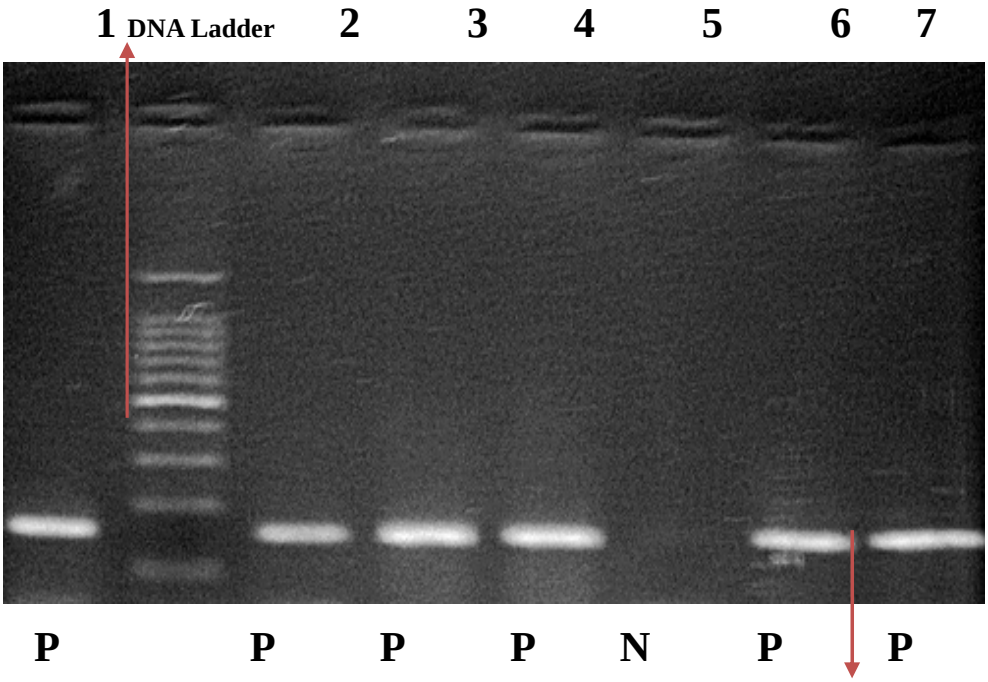
Observation:

Band formation between 100bp and 200bp was formed for all the isolates except for 5^{th} and 12^{th} isolate.

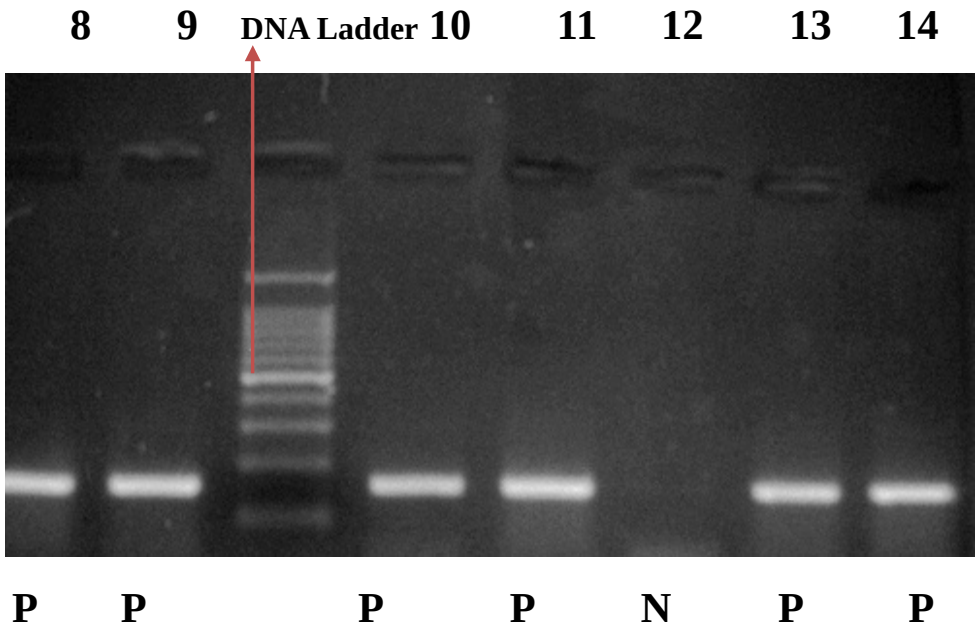
Interpretation:

Bla_{OXA-51} gene was detected in all the 12 Acinetobacter baumannii isolates and not in the two Acinetobacter lwoffii isolates.

Gel Electrophoresis



Band formation between 100 and 200 bp



P – Positive, N - Negative

ACINETOBACTER:

Nutrient agar plate: Circular, 1-2 mm, low convex, regular margin, smooth surface entire edge, colourless colonies, no odour.

Mac Conkey agar plate : Circular, low convex, regular margin, smooth surface, entire edge, non lactose fermenting colonies with a faint pink tint and no odour.

Blood agar plate : Circular, low covex, regular margin, smooth surface, entire edge, non hemolytic grey moist colonies and no odour.

Gram Staining:

Gram negative coccobacilli

Hanging drop:

Non motile

Biochemical Reaction:

Catalase – Positive

Oxidase – Negative

Indole not produced

Methyl red - Negative

Voges proskauer – Negative

Citrate not utilised

Urease not produced

Triple sugar iron agar – Alkaline/ no change (K/NC)

Nitrates not reduced to nitrites

Leeds Acinetobacter Medium:

Pink mucoid colonies with pink colour diffuses into the medium.

TABLE 4 : SPECIATION

Species	Growth		Hemo lysis	OF	Argini ne	Lactose		Chlora mpheni col
	37° C	42° C				1%	10 %	
Acinetobacter baumannii	+	+	-	O	+	A	A	R
Acinetobacter lwoffii	+	-	-	NS	-	-	-	S

O – Oxidative, NS – Non Saccharolytic, A – Acid production, R- Resistant, S – Sensitive.

Antimicrobial Susceptibility test:

The predetermined battery of antimicrobial discs of Ampicillin, Gentamicin, Amikacin, Cephalexin, Ceftriaxone, Ciprofloxacin, Cotrimoxazole, Imipenem and Chloramphenicol were tested for all the eighty isolates. Isolates which were resistant for all the above said drugs, were then tested for Doxycycline, Pipercillin, Piperacillin Tazobactum, Polymyxin and Colistin.

Molecular characterization:

Conventional Polymerase Chain Reaction was done for 14 isolates for the detection of bla_{OXA-51} gene. Isolates were selected randomly based on the following features.

1. PCR was done for twelve Acinetobacter baumannii strains. out of which four isolates were resistant to Imipenem and remaining eight isolates were sensitive to Imipenem.

2. PCR was also done for two Acinetobacter lwoffi strains.

Out of the fourteen isolates, bla_{OXA-51} was detected in twelve Acinetobacter baumannii strains of both Imipenem sensitive and resistant. Bla_{OXA-51} was not detected in the two Acinetobacter lwoffii strains.

RESULT

RESULT

Identification, speciation, antibiogram and molecular characterization of Acinetobacter isolated from the various clinical samples received in 24 hours Microbiology laboratory, Thanjavur Medical College and Hospital during the period of June 2013 to May 2014. Eighty Acinetobacter strains were isolated from the urine, blood, pus and sputum samples.The eighty Acinetobacter strains were analysed for the distribution among sample wise, age wise, sex wise, ward wise, diagnosis wise etc.

TABLE : 5

TOTAL ISOLATES

SPECIMEN	TOTAL NUMBER	CULTURE POSITIVE	ACINETOBACTER
URINE	3444	1170	26
BLOOD	2626	1181	22
PUS	2889	1895	21
SPUTUM	703	397	11
TOTAL	9662	4643	80

Among the 9662 samples received, 4643 were culture positive .Out of the 4643 culture positive samples, 80 samples were found to be Acinetobacter.

TABLE : 6

PREVALENCE OF ACINETOBACTER

	TOTAL NO = 4643	**PERCENTAGE**
ACINETOBACTER	**80**	**1.7%**

In the present study, Acinetobacter isolates accounted for 1.7% of the total number of organisms isolated from urine, blood, pus and sputum during the study period of June 2013 to May 2014.

TABLE : 7

SPECIMEN WISE SCREENING

SPECIMEN	**TOTAL NO = 80**	**PERCENTAGE**
URINE	**26**	**32**
BLOOD	**22**	**28**
PUS	**21**	**26%**
SPUTUM	**11**	**14%**

CHART FOR TABLE - 6

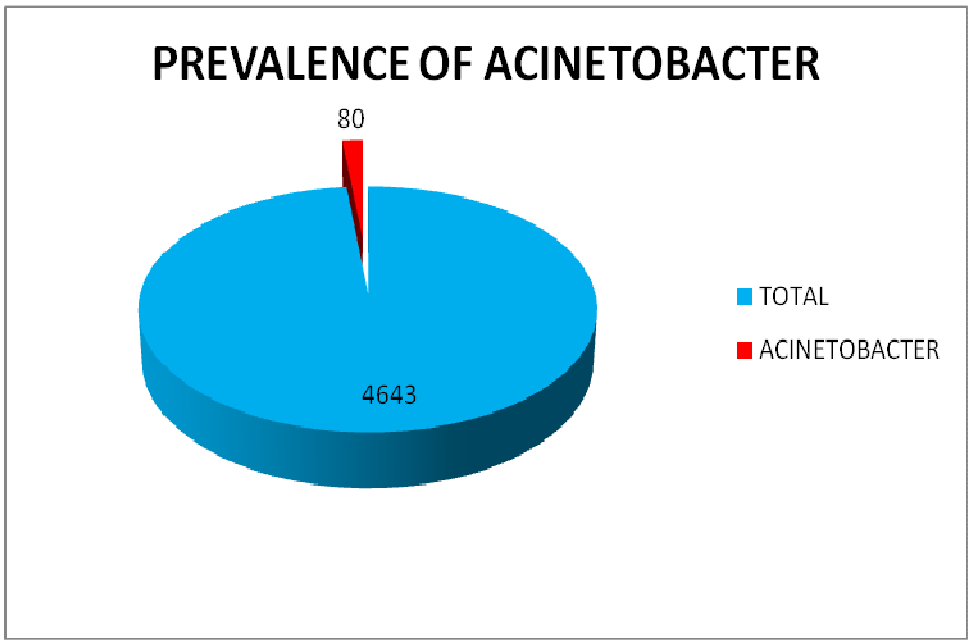
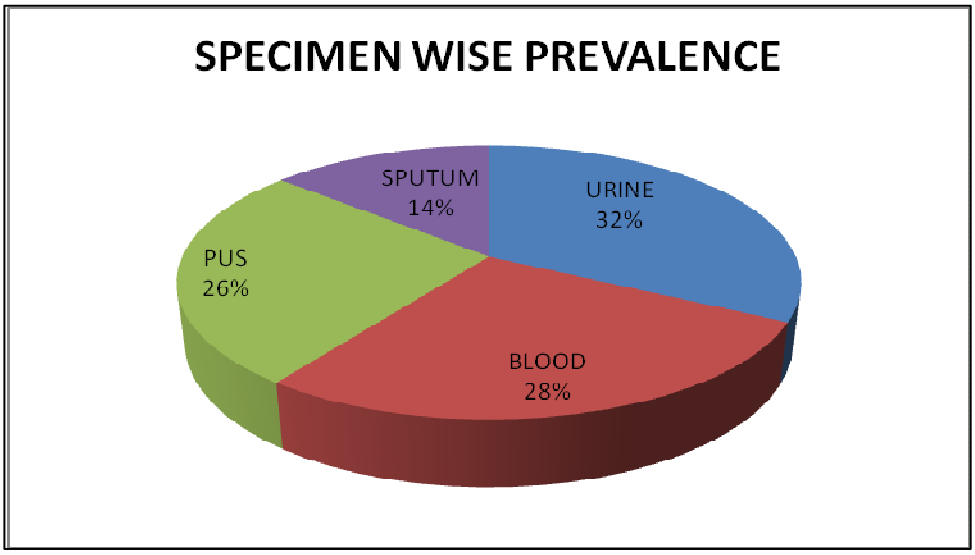


CHART FOR TABLE -7



Among the 80 isolates of Acinetobacter, 32.5% was isolated from urine, 27.5% was isolated from blood, 26% was isolated from pus and 11% was isolated from sputum.

TABLE : 8

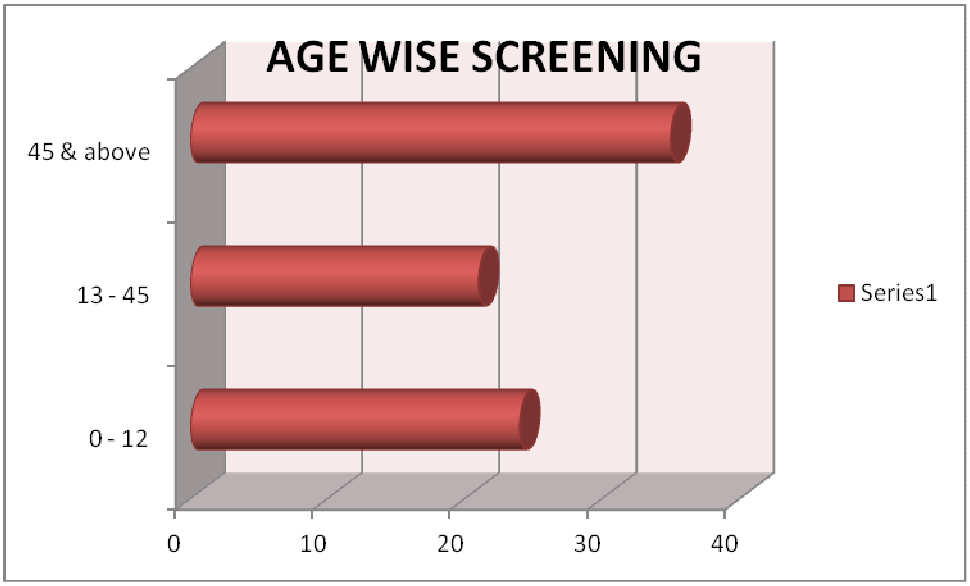
AGE WISE SCREENING

AGE in years	TOTAL NO = 80	PERCENTAGE
0 – 12	24	30%
13 – 45	21	26%
45 and above	35	44%

Age wise screening:

Among the 80 isolates of Acinetobacter, age group of 45 years and above (44%) was most commonly affected, followed by paediatric age group (30%), and then age group of 13-45 years (26%).

CHART FOR TABLE -8



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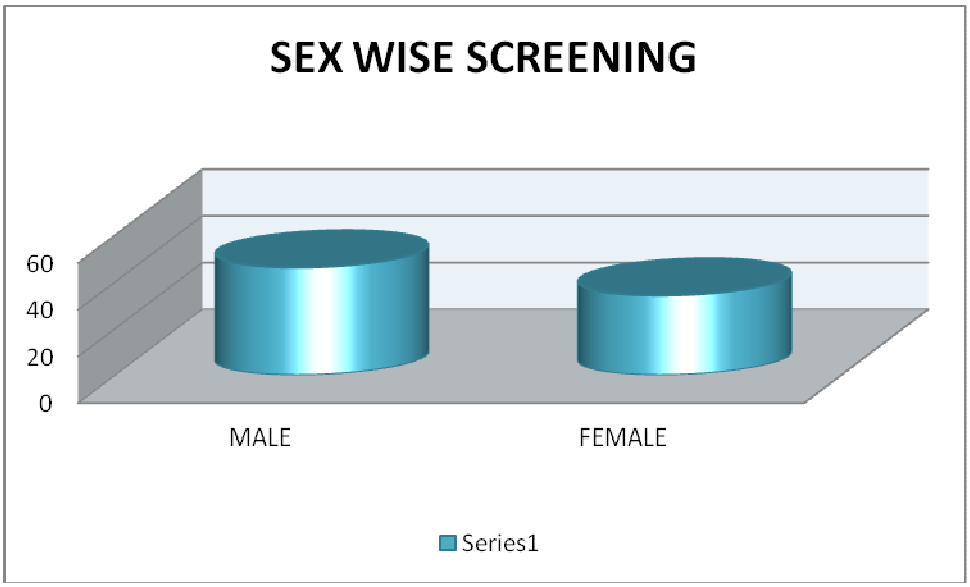

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TABLE - 9

SEX WISE SCREENING

SEX	TOTAL NO =80	PERCENTAGE
MALE	46	57.5%
FEMALE	34	42.5%

Sex wise screening:

On further analysis of sex wise screening among 80 isolates of Acinetobacter, males (57.5%) were more commonly affected than females (42.5%)

TABLE : 10

OUT & IN PATIENT WISE SCREENING

	TOTAL NO =80	PERCENTAGE
IN PATIENT	72	90%
OUT PATIENT	8	10%

In patient & Out patient wise screening:

Among the 80 isolates Acinetobacter, infection was predominant in In-patients (90%) than out patient (10%).

CHART FOR TABLE -10

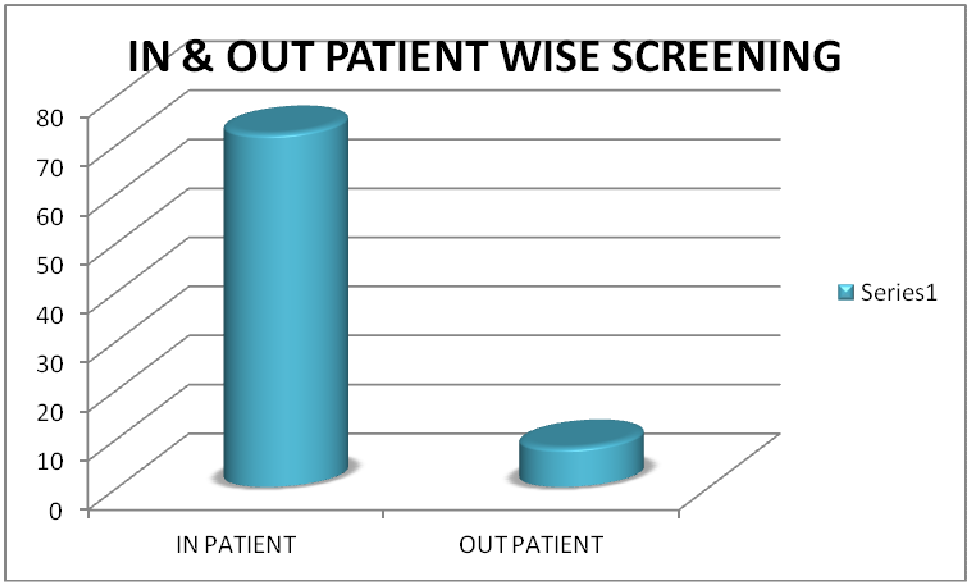


CHART FOR TABLE - 11

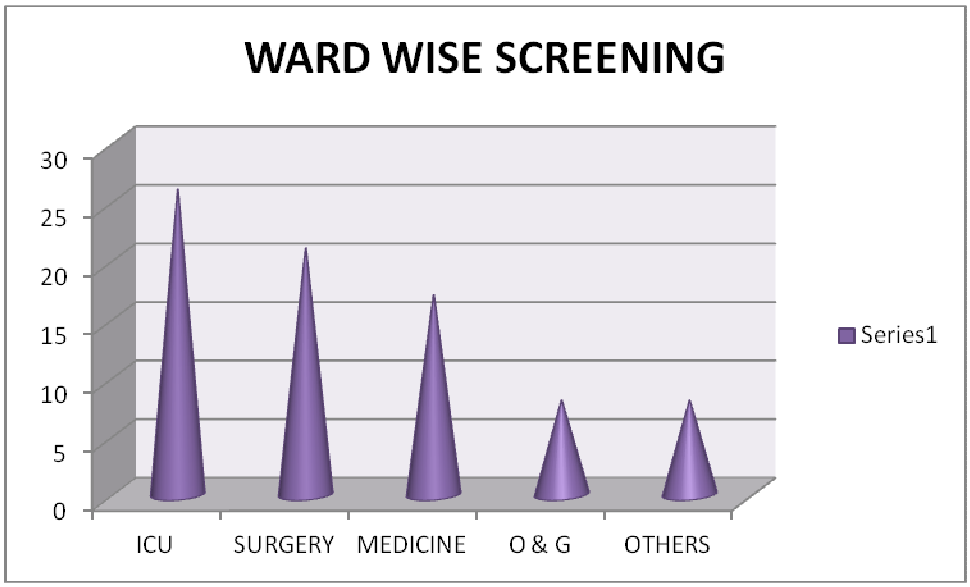


TABLE : 11

WARD WISE SCREENING

WARD	TOTAL NO = 80	PERCENTAGE
ICU	26	32.5%
SURGERY	21	26.5%
MEDICINE	17	21%
OBSTRETIC & GYNAECOLOGY	8	10%
OTHERS	8	10%

Ward wise screening:

In the present study Acinetobacter was isolated more commonly from ICU (32.5%), followed by surgical wards (26.5%), medical wards (21%), obstetrics & gynaecology (10%) and others (10%).All the paediatric cases were from SNN and PICU. So included all the paediatric cases under ICU.

TABLE : 12

DIAGNOSIS WISE SCREENING

DIAGNOSIS	TOTAL NO =80	PERCENTAGE
UTI	25	31.5%
SEPTICEMIA	21	26%
WOUND INFECTION	20	25%
PNEUMONIA	14	17.5%

Diagnosis wise screening:

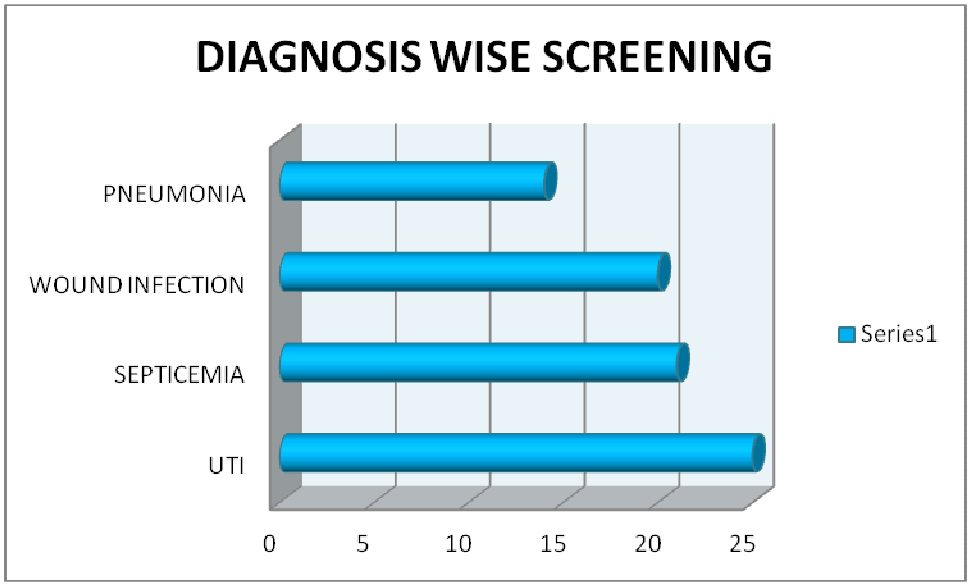
Out of the 80 isolates of Acinetobacter, 31.5% of the patients were affected by UTI, then septicemia (26%), wound infection (25%) and pneumonia (17.5%).

TABLE : 13

DIFFERENT SPECIES OF ACINETOBACTER

SPECIES	TOTAL NO =80	PERCENTAGE
ACINETOBACTER BAUMANNII	62	77.5%
ACINETOBACTER LWOFFII	18	22.5%

CHART FOR TABLE - 12



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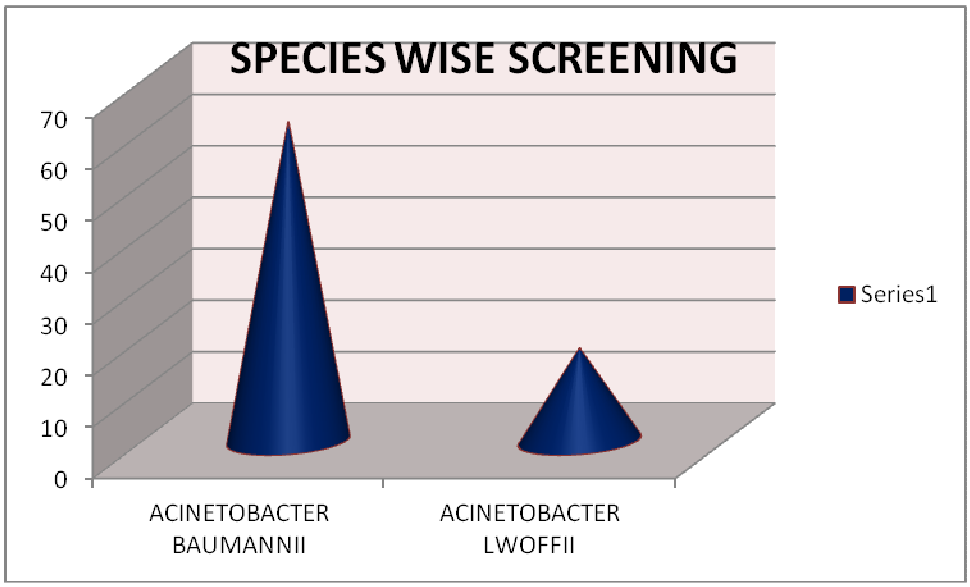

In the present study most of the strains were Acinetobacter baumannii (77.5%) and then Acinetobacter lwoffii (22.5%).

TABLE : 14

SENSITIVITY PATTERN OF ACINETOBACTER TO IMIPENEM

IMIPENEM	TOTAL NO =80	PERCENTAGE
SENSITIVE	73	92%
RESISTANT	7	8%

In the present study strains resistant to imipenem was 8% and sensitive was 92%.

CHART FOR TABLE - 14

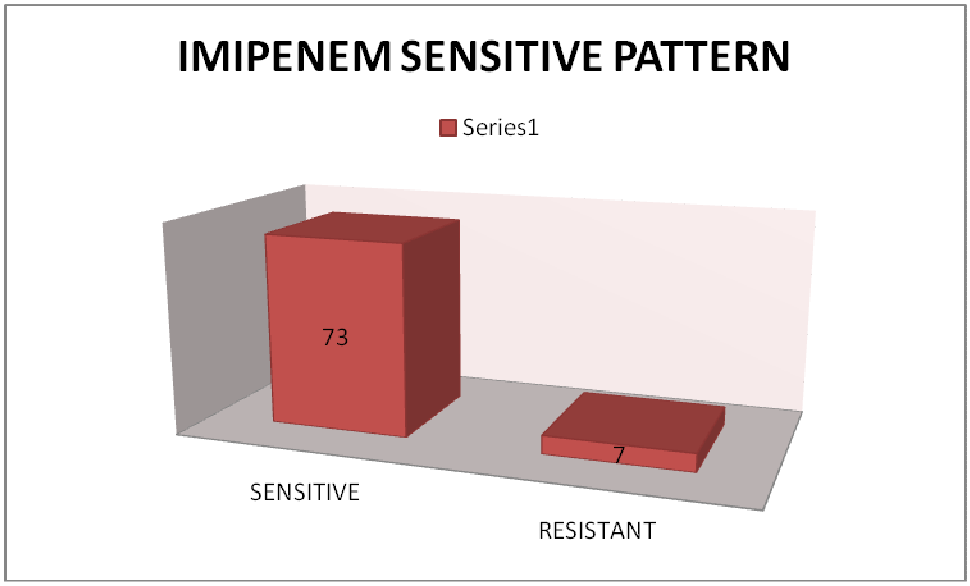

CHART FOR TABLE - 15

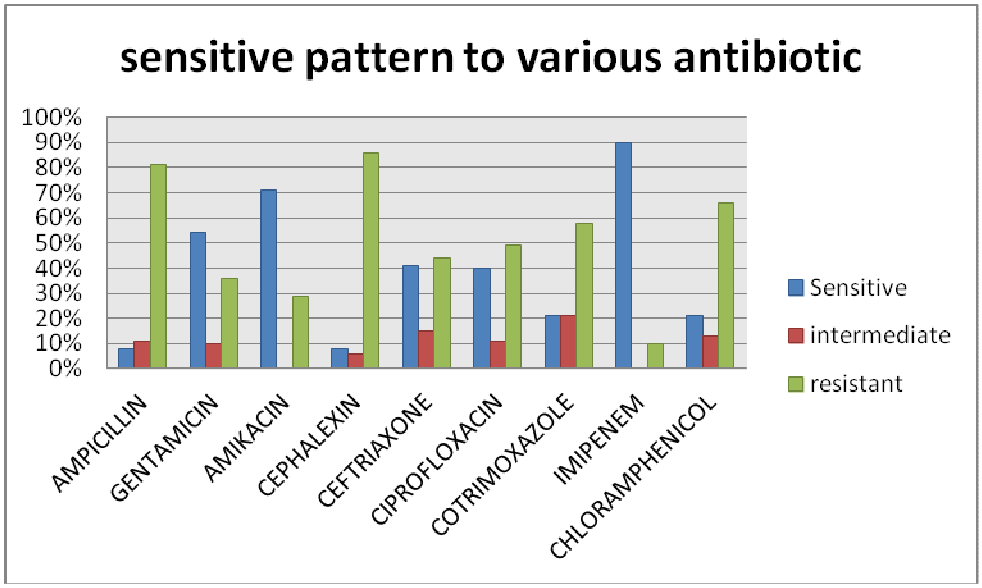

TABLE : 15

SENSITIVITY PATTERN OF ACINETOBACTER ISOLATES TO DIFFERENT ANTIBIOTICS

DRUG	SENSITIVE		INTER MEDIATE		RESISTANT	
	n =80	Per centage	n = 80	Per centage	n =80	Per centage
AMPICILLIN	6	8%	9	11%	65	81%
GENTAMICIN	43	54%	8	10%	29	36%
AMIKACIN	57	71%	Nil	Nil	23	29%
CEPHALEXIN	6	8%	5	6%	69	86%
CEFTRIAXONE	33	41%	12	15%	35	44%
CIPROFLOXACIN	32	40%	9	11%	39	49%
COTRIMOXAZOLE	17	21%	17	21%	46	58%
CHLORAM PHENICOL	17	21%	10	13%	53	66%

Acinetobacter strains were resistant to Ampicillin (81%), Gentamicin (36%), Amikacin (29%), Cephalexin (86%), Ceftriaxone (44%), Ciprofloxacin (49%), Cotrimoxazole (58%), Chloramphenicol (66%)

The isolates which were resistant to all the above drugs were tested for the II line drugs, Doxycycline, Piperacillin, Piperacillin Tazobactum and Polymyxin. Isolates resistant to Imipenem were sensitive to Polymyxin and Colistin.

Molecular characterisation of Acinetobacter

Conventional PCR for detection of bla_{OXA-51} gene was done for 8 Acinetobacter baumannii strains sensitive to Imipenem, 4 Acinetobacter baumannii strains resistant to Imipenem, and 2 Acinetobacter lwoffii strains. Out of which 12 Acinetobacter baumannii strains (both Imipenem sensitive and resistant) were positive for bla_{OXA-51} gene and 2 Acinetobacter lwoffii strains were negative for it.

Result

- Acinetobacter isolates accounted for 1.7% of total number of organisms isolated during the study period.
- Isolation rate was more from urine samples.
- Age group of 45years & above were more commonly affected.
- Males were more commonly affected than females.
- Acinetobacter infection was more common in In-patients than out patients.
- Most of the isolates were from Intensive Care Units (ICU).
- Most of the patients had urinary tract infection.
- Acinetobacter baumannnii was the predominant species isolated among the 80 Acinetobacter strains.
- Imipenem resistance was seen among 8% of the isolates. Isolates resistant to Imipenem were sensitive to Colistin and Polymyxin.
- Acinetobacter strains were resistant to Ampicillin (81%), Gentamicin (36%), Amikacin (29%), Cephalexin (86%), Ceftriaxone (44%), Ciprofloxacin (49%), Cotrimoxazole (58%), Chloramphenicol (66%)
- Conventional PCR was done for 14 isolates of Acinetobacter for the detection of $bla_{OXA-51.}$ It showed positive result in all the 12 Acinetobacter baumannii strains (both Imipenem sensitive and resistant) and negative in 2 Acinetobacter lwoffii strains.

DISCUSSION

DISCUSSION

Eighty isolates of Acinetobacter were included in the present study.They had been isolated from urine, blood, pus and sputum samples. Presumptive identification of Acinetobacter was made by inoculation of the sample on MacConkey agar medium, Nutrient agar medium and Blood agar medium and incubated at 37°C for 24 hours. All non lactose fermentors were subjected to Gram staining, hanging drop and biochemical test like oxidase, catalase, indole, methyl red, voges proskauer, TSI, urease, citrate and nitrate reduction test. Acinetobacter was further confirmed by inoculation on selective media, Leeds Acinetobacter media. Speciation was done on the basis of glucose oxidation, hemolysis on blood agar, growth at 37°C and 42°C, Arginine decarboxylation test, 1% lactose and 10% lactose and susceptibility to chloramphenicol. Antimicrobial susceptibility testing was done by Kirby bauer disc diffusion method. Molecular characterization of Acinetobacter for bla_{OXA-51} gene was done by conventional PCR for fourteen isolates.

All the eighty isolates of Acinetobacter showed growth in the selective media, Leed's Acinetobacter media. On speciation two species of Acinetobacter were detected. The predominant species was Acinetobacter baumannii (77.5%) and next to it is Acinetobacter lwoffi (22.5%). Seven Imipenem resistant Acinetobacter baumannii species were detected. But all the Acinetobacter lwoffii were sensitive to

imipenem. Conventional PCR was done for 14 samples (8 Imipenem sensitive Acinetobacter baumannii, 4 Imipenem resistant Acinetobacter baumannii and 2 Acinetobacter lwoffii) for the detection bla_{OXA-51} gene.Bla_{OXA-51} gene was detected in Acinetobacter baumannii of both imipenem sensitive and resistant strains. The gene was not detected in the Acinetobacter lwoffii strains.

Prevalence of acinetobacter:

In the present study Acinetobacter isolates accounted for 1.7% of the total number of organism isolated from urine, blood, pus and sputum.

Mindolli PB[24] et al stated that Acinetobacter isolates accounted for 4.25% of the total number of organism isolated.

Kalidas Rit[6] et al stated that Acinetobacter isolates accounted for 4.5% of the total number of organism isolated.

Lt Col kk Lahiri et al[95] stated that Acinetobacter isolates accounted for 12.9% of the total number of organism isolated.

Comparing to the above said studies, the isolation of Acinetobacter was less in the present study.

Specimen wise prevalence:

In th present study 32.5% of Acinetobacter were isolated from urine, followed by blood (27.5%), pus (26%) and sputum (14%)

According to Mindolli et al[24] isolation rate was higher in pus,

followed by urine (28%) sputum (25%) and blood (14%).

According to Kalidas Rit et al[6] isolation was higher in pus (48), followed by urine (40), sputum (35) , blood (22).

The two studies are in contrast to the present study as isolation rate was higher in pus.

According to Lt Col KK Lahiri et al[95] isolation was higher in urine (51.9%), blood (9.87%), sputum (12.5%) and pus (11.18%). This study supports the present study as isolation rate was higher in urine. But the isolation of blood in the present study was 27.5% which was high compared to the above study.

TABLE : 16

SPECIMEN WISE PREVALENCE IN VARIOUS COUNTRIES

SPECIMEN	USA	FRANCE	BELGIUM	GERMANY	PRESENT STUDY
URINE	27%	21%	27%	8.2%	32.5%
SPUTUM	28.9%	27%	24.8%	48.8%	14%
PUS	21.5%	27.5%	22.3%	16.4%	26%
BLOOD	9.3%	7.5%	7.6%	26.6%	27.5%
CATHETER TIPS	0	15.5%	0	0	0
OTHERS	13.3%	2%	18.3%	0	0

The isolation rate of blood in the present study was more or less similar to the isolation rate in Germany.

Age wise prevalence

Kalidas Rit et al[6] stated that Acinetobacter infection was more common in patients of age more than 40 years.

Preeti B.Mindoli et al[24] stated that Acinetobacter infection was more common in patients of age more than 45 years.

Vijayan sivaranjani et al[96] stated that Acinetobacter infection was more common in age group of 20-40years.

Hilmar Wisplinghoff et al[41] stated that no significant difference with regard to distribution of age was observed in Acinetobacter infection.

In the present study among the 80 isolates of Acinetobacter, age group of more than 45 was more commonly affected, followed by paediatric age group (0-12) years. Since Acinetobacter infection was common among debilitated infection, in the present study old age were more commonly affected, followed by paediatrics.

The first two studies supports the present study. The third and fourth study are in contrast to the present study.

Sex wise screening:

In the present study among the 80 isolates of Acinetobacter, males (57.5%) were more commonly affected than females (42.5%). The male : female ratio was 1.4 : 1.

According to Kalidas Rit et al[6] the male : female ratio was 1.5 : 1.

According to Mindolli PB et al[24] the male : female ratio was 1.6 : 1.

All the above studies are more or less similar to the present study.

In patient & out patient wise prevalence:

In the present study isolation of Acinetobacter from In- patient was 90% and out patient was 10%.

According to Lt Col KK Lahiri[95] the isolation of Acinetobacter from out patient was 17.1%. This study was more or less similar to the present study. Community acquired Acinetobacter was less compared to the hospital acquired Acinetobacter. Most of the community acquired Acinetobacter species was Acinetobacter lwoffii.

Department wise prevalence:

In the present study the highest rate of isolation was from ICU (32.5%). I included SNN, PICU, SICU, IMCU under ICU. All the isolates from paediatrics were from SNN, PICU. The isolation rate from surgical wards (26.5%), medicine wards (21%), O & G wards (10%).

According to Mindolli PB et al[24], most of the isolates were from surgical wards (30.5%) followed by ICU (27%), paediatric ward (19%), medicine ward (16.5%).

According to Lt Col KK Lahiri et al[95], most of the isolates were from Spinal cord injury centre (28.9%), followed by surgery (16.4%), family ward (11.1%), ICU (7.2%), Burns unit (7.2%), Medicine ward (4.6%) Ortho (3.9%).

In the present study isolates from ICU (32.5%) was higher than the above said studies.

Diagnosis wise prevalence:

In the present study among the 80 isolates of Acinetobacter 31.5% affected by urinary tract infection, 26% affected by septicemia, 25% affected by wound infection and cellulitis and 17.5% affected by pneumonia.

According to Alex studemeister[97] 40% affected by wound infection, 30% affected by urinary tract infection, 20% affected by pneumonia and 10% by septicemia.

In the present study patients were more commonly affected by UTI, but in the above said study, patients were more commonly affected by wound infection.

Different species of Acinetobacter isolated from clinical samples:

In the present study 77.5% of the isolates were Acinetobacter baumannii, and 22.5% were Acinetobacter lwoffii.

According to Mindolli PB et al[24], 78% of the total Acinetobacter isolate were Acinetobacter baumannii, 12.5% Acinetobacter lwoffii, 6% Acinetobacter hemolyticus and 3% Acinetobacter junii.

According to Kalidas Rit et al[6] 74.02% of isolates were Acinetobacter baumannii, 14.2% Acinetobacter lwoffii, 7.79% Acinetobacter hemolyticus, 3.3% Acinetobacter junni.

According to K.Prashant et al[25] 71% of isolates were Acinetobacter baumannii, 20.3% Acinetobacter lwoffi, 3.38%, Acinetobater hemolyticus, 1.6% Acinetobacter johnsonii, 1.6% Acinetobacter junii.

All the above said studies are in correlation with the present study except the number of species, only two species were isolated.

Imipenem resistance pattern of Acinetobacter:

One of the striking features of Acinetobacter is its ability to develop antibiotic resistance extremely rapid in response to challenge with new antibiotic. In the present study Imipenem resistance was seen among 8% of the total isolates. Although Acinetobacter baumannii strains have intrinsic resistance to carbapenems, resistance to carbapenem is seen only, when there is upstream of insertion element ISAba1. Hence only 8% of

the isolates were resistant to Imipenem. All Acinetobacter lwoffii strains were sensitive to Imipenem. Isolates resistant to Imipenem were sensitive to polymyxin and colistin.

Kalidas Rit et al[6] stated that Imipenem resistance was seen among 5.2% of the total isolates.

Vijayan Sivaranjani et al[96] stated that Imipenem resistance was seen among 26.2% of the total isolates.

Comparing to Kalidas Rit et al[6], in the present study imipenem resistance was higher. But comparing to Vijayan Sivaranjani et al, the resistance rate was lower.

Resistance pattern of Acinetobacter to various antibiotics:

In the present study 81% resistant to Ampicillin, 36% resistant to Gentamicin, 29% resistant to Amikacin, 86% resistant to Cephalexin, 44% resistant to Ceftriaxone, 49% resistant to Ciprofloxacin, 55% resistant to Cotrimoxazole, 66% resistant to Chloramphenicol.

Acording to Vijayan Sivaranjani et al[97], 86.8% resistant to Ampicillin, 51.6% resistant to Amikacin, 60.6% resistant to Gentamicin, 59.8% resistant to Cotrimoxazole, 69.6% resistant to Ciprofloxacin.

According to Kalidas Rit et al[6], 14.29% resistant to Amikacin, 70.13% resistant to Gentamicin, 42.21% resistant to Ofloxacin, 92.20% resistant to Chloramphenicol.

According to Mindolli PB et al[24], 37.5% resistant to Amikacin, 47.5% resistant to Gentamicin, 73.5% resistant to Ofloxacin, 88.5% resistant to Chloramphenicol.

The difference in the sensitivity pattern was due to environmental factors and different pattern of antimicrobial usage. In the present study, isolates were more resistant to Ampicillin, Cephalexin and more sensitive to Amikacin and Imipenem.Vijayan Rit et al study is similar to the present study. Mindolli et al and Kalidas Rit et al studies stated that isolates were more resistant to Chloramphenicol, this is in contrast to the present study. But isolates were more sensitive to Amikacin, this is similar to the present study.

Detection of bla_{OXA-51} gene:

Conventional PCR was done for 14 Acinetobacter isolates (8 Imipenem sensitive Acinetobacter baumannii, 4 Imipenem resistant Acinetobacter baumannii and two Acinetobacter lwoffii). Since bla_{OXA-51} gene was intrinsic gene present in Acinetobacter baumannii, all Acinetobacter baumannii of both Imipenem sensitive and resistant were positive for bla_{OXA-51} and the gene was not detected in Acinetobacter lwoffi strains.

According to Jane F turton et al[98], bla_{OXA-51} was detected in all Acinetobacter baumannii strains and it was not detected in other species of Acinetobacter.

This study supports the present study.

Limitation of present study:

- ❖ Restricted use of molecular method due to lack of affordability.
- ❖ Automated systems were not used due to lack of availability.
- ❖ Follow up of cases not done.

Strength of present study:

- ❖ Selective media, Leeds Acinetobacter media was used .
- ❖ Speciation was done by both biochemical test and molecular method.
- ❖ Bla_{OXA} gene, an intrinsic gene of Acinetobacter baumannii was detected by the conventional PCR for confirmation of species.

Suggestions:

1. Avoiding indriscriminate use of antibiotics by ensuring that the indication, the dose and duration of treatment are appropriate.
2. Constantly monitoring the resistance patterns in a hospital or community and change recommended antibiotics used for empirical treatment and limiting the usage of newest group of antimicrobials as long as current drugs are effective.

3. Maintenance of infection control in hospitals, such as isolation and treatment of carriers, hand hygiene practices for ward staff to prevent spread of resistant bacteria.
4. Conducting periodic educational programmes on antimicrobial use for the practitioners in order to limit the use of newer drugs and to minimize the antibiotic resistance.

SUMMARY

SUMMARY

Identification, speciation, antibiogram and molecular characterization of Acinetobacter isolated from various clinical samples received in 24 hours Microbiology diagnostic laboratory, Thanjavur Medical College and Hospital were aimed in finding out the prevalence of Acinetobacter and its resistant pattern during the study period of June 2013 to May 2014. Eighty Acinetobacter were isolated and they were subjected to speciation, antibiogram and molecular characterization. They were analysed on the basis of specimen wise, age wise, sex wise screening etc.

- The prevalence of Acinetobacter was 1.7%. Isolation was more in urine samples. Males and age group of 45 years and above were predominantly affected.
- 90% of Acinetobacter were isolated from In - patients and predominantly from ICU (32.5%).
- Acinetobacter baumannii (77.5%) and Acinetobacter lwoffii (22.5%) were the two species isolated.
- 8% of the total isolates were resistant to Imipenem. The isolates resistant to imipenem were sensitive to Polymyxin and Colistin.
- Molecular characterization by conventional PCR was done for 14 Acinetobacter isolates. $Bla_{OXA\ -51}$ was detected in all the 12 Acinetobacter baumannii strains of both Imipenem sensitive and resistant and not detected in the two Acinetobacter lwoffii strains.

CONCLUSION

CONCLUSION

Identification, speciation, antibiogram, and molecular characterization of Acinetobacter isolated from various clinical samples received in 24 hours Microbiology diagnostic laboratory, Thanjavur Medical College and Hospital reveals that

- The prevalence of Acinetobacter was 1.7%. During routine microbiological work nonfermentative Gram negative bacilli other than Pseudomonas aeruginosa are not taken seriously and are considered as contaminants. But the rate of isolation of Acinetobacter indicates its role in nosocomial pathogen.
- The predominant species isolated was Acinetobacter baumannii more commonly from In patients, that too from ICU. Hence Acinetobacter baumannii more commonly causes hospital acquired serious infections.
- Increasing resistant pattern was seen for the commonly used drugs like Ampicillin, Cephalosporins, Ciprofloxacin, Cotrimoxazole, Chloramphenicol. 8% of the isolates were resistant to Imipenem. All the 8% were Acinetobacter baumannii and none of the Acinetobacter lwoffii strains were resistant to Imipenem.
- Molecular Characterization by conventional PCR revealed that bla_{OXA-51} gene was a intrinsic gene present in Acinetobacter baumannii

Acinetobacter baumannii has beta lactamase oxacillinase 51 gene (bla_{OXA-51} gene) which is an intrinsic gene. But resistance to imipenem is expressed only when insertional element ISAba1 is overexpressed. Acinetobacter baumannii has a remarkable ability to upregulate or acquire resistant determinants. Acinetobacter baumannii is an emerging pathogen threatening the current antibiotic era.

Drug resistance pattern varies from place to place which may be related to the nature of the pathogen and preferred antimicrobial agents. Hence institutional antibiotic policy should be monitored to achieve superior therapeutic outcome and bring about a reduction in healthcare costs.The easiest way to prevent the infection is preventive measures like hand hygiene and health education.

ANNEXURE

PROFORMA

Name: **Ward :**

Age: **Lab No:**

Sex: **OP / IP No:**

Address: **Date of Sample Collection:**

Occupation: **Specimen:**

Income : **Test:**

Chief Complaints:

1.Fever

2.cough

3.Ulcer/Burn

4.Burning micturation

Past History :

1.Tuberculosis

2.Bronchial asthma /COPD

3.Diabetes-

4.Malignancy

5.Trauma

6.Surgery -

Personal History: Smoker- Alcoholic-

CLINICAL DIAGNOSIS

WORK SHEET

Specimen: Urine, Pus, Blood, Sputum

I Culture : 1. MacConkey agar

2. Blood agar

3. Nutrient agar

II Gram Staining

III Hanging drop

IV Biochemical Reaction: 1. Catalase

2. Oxidase

3. Indole

4. Methyl red

5. Voges proskauer

6. Citrate Utilisation test

7. Urease

8.TSI

9.Nitrate reduction test

V Culture on Selective media : Leed's Acinetobacter medium

VI Speciation : 1. Hemolysis

2. Growth at 37◦C and 42◦C

3. OF test

4. Arginine decarboxylase test

5.1% lactose and 10% lactose

6. Susceptibility to Chloramphenical

VII Antimicrobial Susceptibility Testing: Kirby Bauer disc diffusion method

1. Ampicillin
2. Gentamicin
3. Amikacin
4. Cephalexin
5. Ceftriaxone
6. Ciprofloxacin
7. Cotrimoxazole
8. Imipenem
9. Chloramphenicol

II line drugs :

1. Doxycycline
2. Piperacillin
3. Piperacillin Tazobactum
4. Polymyxin
5. Colistin

VII Molecular Characterisation of Acinetobacter by PCR

ANNEXURES

PREPARATION OF MEDIA AND REAGENTS

Gram stain:

Methyl violet:

Methyl violet – 1.5 gm

Distilled water – 100 ml

Grams iodine

Potassium iodide – 2 gm

Iodine - 2 gm

Water – 100 ml

Dilute carbol fuchscin:

Strong carbol fuchscin:

Basic fuchscin – 0.5 gm

Phenol crystal – 2.5 gm

Alcohol – 10 ml

Water - 90 ml

Take 1 ml of strong carbol fuchscin and add 19 ml of distill water.

MacConkey agar plate :

5.5 gm dehydrated MacConkey agar is taken and 100 ml distilled water is added. Then it is heated to dissolve completely. pH adjusted to 7.4. Then autoclaved at 121^{o}C for 15 min. Then plated

Nutrient agar plate:

2.8 gm of dehydrated nutrient agar powder is taken and 100 ml of distill water is added. It was boiled to dissolve it completely. pH is adjusted to 7.4. Autocalved at 121^{o}C for 15 min and plated.

Blood agar plate :

8 ml of human blood is added to the prepared nutrient agar. Mix thoroughly .then autoclaved at 121^{o}C for 15 min and plated

Methyl Red Test:

MR-VP broth :

1.7 gm of glucose phosphate broth is taken and 100 ml distilled water is added and it heated to dissolve completely. pH adjusted to 6.9. Then autoclaved at 121^{o}C for 15 min.Then dispensed in separate tubes.

MR reagent:

Methyl red - 0.1 gm

95 % Ethyl alcohol – 300 ml

VP reagent 1:

α- naphthol – 5 gm

Absolute ethyl alcohol – 100 ml

VP reagent 2:

Potassium hydroxide – 40 gm

Distilled water – 100 ml

Indole Test:

Peptone water:

Peptone - 5gm

Sodium chloride – 2.5 gm

Water – 500 ml

Autoclave at 121^{o}C for 15 minutes

Citrate Utilization test

2.428 gm of dehydrated citrate powder is taken in a conical flask and 100 ml of distilled water is added. It was boiled till it dissolved completely. pH adjusted to 6.8. Then autoclaved at 121^{o}C for 15 min.

Urease Test:

2.401 gm of dehydrated urea agar base is taken and 95 ml of distilled water is added. It was boiled to dissolve it completely. pH adjusted to 6.8. Then autoclaved at 121^{o}C for 15 min and then cooled to 50^{o}C. 5 ml of 40% of urease solution is added.

Triple Sugar Iron agar:

6.452 gm of dehydrated medium is taken and mixed with 100 ml of distill water. Then it is heated to dissolve. pH is adjusted to 7.4.Then autoclaved at 121^{o} C for 15 min. Then it is dispensed in test tubes in such a way to form a slant and butt.

Nitrate broth:

0.9 gm of dehydrated medium is taken and mixed with 100 ml of distilled water. Heat to dissolve completely. pH is adjusted to 7. Then autoclave at 121^{o} C for 15 min. Then it is dispensed in test tubes.

Nitrate reagent:

α- Naphthylamine – 0.5 gm

30% Acetic acid – 100 ml

Leeds Acinetobacter media plate :

5.32 gm of Leeds Acinetobacter media was taken and 100 ml of distilled water is added and kept in water bath at 100^{o}C for 45 min. pH adjusted to 7.Then cooled to 50^{o}C and plated.

Mueller-Hinton agar plate :

3.8 gm of dehydrated media is taken and 100 ml of distilled water is added. Then it is heated to dissolve completely. pH is adjusted to 7.3.Them autoclaved at 121^{o}C for 15 min. Then plated.

1% Lactose :

Take 1 gram of lactose and add 100 ml of distilled water. Add the indicator bromothymol blue to 100 ml of peptone water and adjust the pH to 7.5. Distribute in tubes (add 250 µl of sugar solution to each 5ml of peptone water). Keep it water bath for 15 min.

10% Lactose :

Take 10 gram of lactose and add 100 ml of distilled water. Add the indicator bromothymol blue to 100 ml of peptone water and adjust the pH to 7.5. Distribute in tubes (add 250 µl of sugar solution to each 5ml of peptone water). Keep it water bath for 15 min.

OF Test:

1.935 gm of dehydrated Hugh-Leifson's OF medium is taken and 100 ml of distilled water is added and boiled it to dissolve completely . pH adjusted to 6.8 and then dispenced in separate tubes. Autoclaved at 121^{o}C for 15 min.

Arginine:

1.052 gm of dehydrated decarboxylase base is taken and 100 ml of distilled water is added. 10 gm of arginine is added and boiled to dissolve it completely. pH adjusted to 6. Dispensed in 5 ml amounts in separate tubes and then autoclaved.

MASTER CHART

S.N	NAME	AGE	SEX	IP/OP	MICRONO	DATE	SPECIMEN	HISTORY	DIAGNOSIS	WARD	AMP	GEN	AK	CN	CTR	CIP	COT	IMP	C	II LINE	NOR	GROWTH AT 42	OF	ARG ININE	HEMO LYSIS	1% LACTOSE	10% LACTOSE	SPECIES	bla oxa -51
1	Surya	24	F	264464	1298	16/6/2013	URINE	SURGERY	UTI	OG	S	R	S	R	S	I	R	S	R		R	P	P	P	N	A	A	B	
2	Raju	55	M	OP/41523	1546	15/7/2013	URINE		UTI	MOP	R	R	R	R	R	R	R	S	I		S	N	N	N	N	N	N	L	
3	Perumal	65	M	1462558	1561	17/7/2013	URINE		UTI	M6	S	S	S	R	R	I	I	S	S		S	N	N	N	N	N	N	L	
4	Latha	34	F	290929	1753	7/8/2013	URINE		UTI	OG	R	S	S	R	I	S	S	S	R		I	P	P	P	N	A	A	B	
5	Sujamala D	22	F	277069	2233	24/9/2013	URINE		UTI/Sepsis	ETW	R	R	R	R	R	R	R	R	R	S-PB	R	P	P	P	N	A	A	B	P
6	Manivalan	3 MONTHS	M	OP/70650	2530	23/10/2013	URINE		UTI	PSOP	I	S	S	R	R	S	S	S	I		S	P	P	P	N	A	A	B	
7	Kuppamma	70	F	282081	2651	6/11/2013	URINE		UTI	OG	R	R	R	R	R	R	R	R	R	S-PB,CO	R	P	P	P	N	A	A	B	
8	Alfonsa	50	F	1487094	2902	2/12/2013	URINE	CKD	Obstructive	308/	I	S	S	R	S	S	S	S	R		S	P	P	P	N	A	A	B	
9	Chitradevi	25	F	287167	2928	4/12/2013	URINE	SURGERY	UTI	LW	I	S	S	R	I	S	S	S	R		S	P	P	P	N	A	A	B	
10	Chitra	22	F	1490632	3044	15/12/2013	URINE		FFE	OG	I	S	S	R	S	I	S	I	I		I	P	P	P	N	A	A	B	
11	Seeths	25	F	1492634	3173	26/12/2013	URINE		UTI	M2	R	R	S	R	S	R	R	S	S		R	N	N	N	N	N	N	L	N
12	Harish	7	M	289753	3175	26/12/2013	URINE		FFE	PICU	R	R	R	R	R	R	R	S	R		R	P	P	P	N	A	A	B	
13	Vasantha	45	F	289896	3177	26/12/2013	URINE		UTI	LW	R	S	S	R	R	S	S	S	R		S	P	P	P	N	A	A	B	
14	Saroja	50	F	1492989	3188	27/12/2013	URINE		Acute pyeli	M4	I	S	S	R	R	R	S	S	R		R	P	P	P	N	A	A	B	P
15	Balaji	11	M	291436	108	10/1/2014	URINE		FFE	PICU	R	R	S	R	S	S	S	S	R		R	P	P	P	N	A	A	B	
16	Suresh bat	38	M	OP/9150	405	13/2/2014	URINE		UTI	MOP	I	S	S	R	I	S	R	S	S		S	N	N	N	N	N	N	L	
17	Bhuvanesh	25	F	7848	463	20/2/2014	URINE	DM	DKA	M6	R	I	S	R	R	R	I	S	I		R	P	P	P	N	A	A	B	
18	Gopal	58	M	10651	589	7/3/2014	URINE	SURGERY	DUB	SICU	R	I	S	R	R	R	R	S	R		S	P	P	P	N	A	A	B	
19	Swathy	3 1/2	F	298407	640	12/3/2014	URINE		Fever	PICU	R	I	S	R	R	I	R	S	R		R	P	P	P	N	A	A	B	
20	Deepika	3	F	OP/787	689	15/3/2014	URINE		Neurogenic	PSOP	R	S	S	R	R	R	R	S	S		R	N	N	N	N	N	N	L	
21	Anjali	48	F	300655	848	3/4/2014	URINE		R/O/Urine	OG	R	S	S	I	S	S	S	S	I		S	P	P	P	N	A	A	B	P
22	Thangavel	75	M	16565	885	7/4/2014	URINE	CA prostra	UTI	310	R	R	S	R	S	R	R	S	R		R	P	P	P	N	A	A	B	
23	Palanisamy	39	M	OP/9314	929	14/4/2014	URINE		UTI	STD OP	R	S	S	R	R	S	I	S	S		S	N	N	N	N	N	N	L	
24	Chinnapon	50	F	43215	1234	22/4/2014	URINE		UTI/Abdom	M1	R	S	S	I	I	R	I	S	R		I	P	P	P	N	A	A	B	
25	Denvia	22	F	24354	1258	24/4/2014	URINE		UTI/Abdom	S2	R	R	R	R	R	R	R	S	R		R	P	P	P	N	A	A	B	P
26	Saranish	3	M	OP/91220	1295	29/4/2014	URINE		UTI	POP	I	S	S	R	R	S	I	S	S		S	N	N	N	N	N	N	L	
27	B/O Kauve	NB	M	266030	893	6/7/2013	BLOOD		Sepsis	SNN	I	S	S	R	I	I	I	S	R			P	P	P	N	A	A	B	P
28	Veeramani	48	M	1464574	987	29/7/2013	BLOOD	DM	DM/AK AM	SICU	S	S	S	S	S	S	S	S	R			P	P	P	N	A	A	B	
29	Prakash	28	M	1475802	1393	2/10/2013	BLOOD	ALCOHOL	Acute pan	SICU	R	R	S	R	R	R	S	S	R			P	P	P	N	A	A	B	
30	B/O Sasika	3/365	M	282074	1591	28/10/2013	BLOOD	LBW	sepsis	SNN	R	S	S	R	R	S	R	S	R			P	P	P	N	A	A	B	
31	Sumithra	1 1/2	F	282516	1623	1/11/2013	BLOOD		PUO	PICU	R	S	S	S	S	S	S	S	S			N	N	N	N	N	N	L	
32	Pratheeba	31/365	M	284976	1761	20/11/2013	BLOOD		Bronchopn	PICU	R	R	R	R	R	R	R	S	R			P	P	P	N	A	A	B	
33	Shakila	11/365	F	287163	1873	5/12/2013	BLOOD	LBW	Sepsis	SNN	R	R	R	R	R	R	R	R	R	S-PB,CO		P	P	P	N	A	A	B	P
34	Pradap	1	M	287876	1922	11/12/2013	BLOOD		Fever	PICU	R	S	S	R	S	R	R	S	S			N	N	N	N	N	N	L	
35	Dheena	1 1/2	M	287919	1935	12/12/2013	BLOOD		Pneumonia	PICU	R	R	R	R	R	R	R	R	R	S-PB,CO		P	P	P	N	A	A	B	
36	Karthikeya	10	M	288089	1968	16/12/2013	BLOOD		FFE	PICU	R	R	R	R	I	R	R	S	S			N	N	N	N	N	N	L	N
37	Hariharan	29/365	M	291135	52	7/1/2014	BLOOD	LBW	Sepsis	SNN	S	S	S	R	S	S	S	S	R			P	P	P	N	A	A	B	
38	Anandham	50	M	2769	273	6/2/2014	BLOOD	SURGERY	Sepsis	SICU	R	S	S	R	S	S	I	S	R			P	P	P	N	A	A	B	
39	B/O Rajaki	4/365	M	294345	274	6/2/2014	BLOOD	CHD	Sepsis	SNN	R	S	S	R	I	S	I	S	R			P	P	P	N	A	A	B	P
40	Yasodha	60	F	294274	278	6/2/2014	BLOOD	COPD	Seizure/Fe	PICU	R	S	S	S	S	S	S	S	I			P	P	P	N	A	A	B	
41	B/O Kala	NB	M	294374	290	8/2/2014	BLOOD	LBW	Sepsis	SNN	R	S	S	I	S	S	I	S	R			P	P	P	N	A	A	B	
42	Parthiban	50	M	7173	355	15/2/2014	BLOOD		Fever	M2	R	S	S	R	R	R	R	S	S			N	N	N	N	N	N	L	
43	B/O Anitha	NB	F	296646	429	26/2/2014	BLOOD		Sepsis	SNN	R	R	R	R	S	R	R	S	R			P	P	P	N	A	A	B	
44	B/O/ Seeth	NB	F	296751	466	3/3/2014	BLOOD	LBW	sepsis	SNN	R	R	R	R	R	R	R	S	R			P	P	P	N	A	A	B	
45	B/O Sange	NB	M	298976	514	11/3/2014	BLOOD		Sepsis	SNN	R	R	R	R	R	R	R	S	R			P	P	P	N	A	A	B	
46	Senthil kun	30	M	10602	521	11/3/2014	BLOOD	SURGERY	Sepsis	S3	R	R	R	R	R	R	R	S	R			P	P	P	N	A	A	B	
47	Joyce	30	F	19465	858	17/3/2014	BLOOD	DM	Fever	M2	R	S	S	R	I	I	I	S	I			N	N	N	N	N	N	L	
48	Kokila	1/365	F	306646	902	21/3/2014	BLOOD		Sepsis	SNN	R	R	R	R	R	R	R	S	R			P	P	P	N	A	A	B	
49	Swetha	10	F	263964	1219	13/6/2013	PUS		Burns 60%	PS	R	R	R	R	R	R	R	R	R	S-PB,CO		P	P	P	N	A	A	B	
50	Krishnamo	20	M	1459427	1301	22/6/2013	PUS		BURNS	S2	R	R	R	R	R	R	S	S	R			P	P	P	N	A	A	B	
51	Pachaiyam	45	F	14605287	1499	23/7/2013	PUS		Wound INF	S	R	S	S	R	I	R	R	S	R			P	P	P	N	A	A	B	
52	Mallika	25	F	1459566	1512	24/7/2013	PUS	TB mening	Bed sore	SICU	R	S	S	S	S	S	I	S	I			P	P	P	N	A	A	B	
53	Koliyan	55	M	1463933	1571	30/7/2013	PUS	DM	Diabetic fo	S 27	S	S	S	R	S	R	R	S	R			P	P	P	N	A	A	B	
54	Priya	21	F	1462372	1671	12/8/2013	PUS		Burns 45%	PS	R	R	R	R	S	R	R	S	R			P	P	P	N	A	A	B	
55	Thangama	30	M	1475198	1962	18/9/2013	PUS	SURGERY	Wound INF	ORTHO	R	R	R	R	R	R	R	R	R	S- CO		P	P	P	N	A	A	B	P
56	Singaravel	53	M	1481362	2259	29/10/2013	PUS		Cellulitis	S2	R	R	R	R	R	R	R	S	R			P	P	P	N	A	A	B	
57	Deepan	7	M	1486637	2435	20/11/2013	PUS		Wound INF	305	R	S	S	S	S	S	R	S	R			P	P	P	N	A	A	B	
58	Balayan	55	M	1484618	2570	3/12/2013	PUS	DM	Diabetic fo	S 27	R	R	R	R	R	S	R	S	R			P	P	P	N	A	A	B	
59	Ramu	60	M	1492629	2768	25/12/2013	PUS	DM	Diabetic fo	S 27	S	S	S	R	S	R	S	S	R			P	P	P	N	A	A	B	P
60	Kaliyamoo	48	M	1493103	104	14/1/2014	PUS		Cellulitis	S2 27	R	R	R	R	S	S	R	S	S			N	N	N	N	N	N	L	
61	Raman	24	M	OP 2709	266	3/2/2014	PUS		CSOM	ENT OP	R	S	S	R	S	S	R	S	S			N	N	N	N	N	N	L	

62	Balakrishna	25	M	6344	344	12/2/2014	PUS	SURGERY	Wound INF	NSICU	R	S	S	S	S	S	R	S	R			P	P	P	N	A	A	B		
63	Petchiyaee	65	F	62114	351	12/2/2014	PUS		Filarial leg	PS	R	S	S	I	S	R	R	S	I			P	P	P	N	A	A	B		
64	Govindhara	55	M	8799	512	2/3/2014	PUS		Wound INF	S4	R	S	S	R	R	R	R	S	S			N	N	N	N	N	N	L		
65	Parthasara	86	M	16870	672	21/3/2014	PUS		Abscess	S4	R	S	S	R	S	R	R	S	R			P	P	P	N	A	A	B		
66	Rajkumar	18	M	14288	708	25/3/2014	PUS	SURGERY	VP SHUNT	NSICU	R	S	S	R	S	S	R	S	R			P	P	P	N	A	A	B	P	
67	Marudhapp	60	M	15306	792	7/4/2014	PUS		Pyopneum	M1	R	I	S	R	I	S	I	S	R			P	P	P	N	A	A	B		
68	Nagalaksh	60	F	12784	810	9/4/2014	PUS	CA Breast	Wound INF	S6	R	I	S	R	R	I	R	S	R			P	P	P	N	A	A	B		
69	Muruganar	57	M	17134	865	17/4/2014	PUS		Cellulitis	S4 26	R	I	S	R	S	R	R	S	S			N	N	N	N	N	N	L		
70	Govindhara	65	M	1465116	384	25/7/2013	SPUTUM	TB	PT/Pneum	M3	R	S	S	R	S	S	S	S	R			P	P	P	N	A	A	B		
71	Swetha	7	F	280341	547	15/10/2013	SPUTUM		PE	M	R	S	S	R	I	I	S	S	R			P	P	P	N	A	A	B		
72	Kaliyamoo	47	M	1488604	634	3/12/2013	SPUTUM	TB	PT/Pneum	M16	R	S	S	R	S	I	I	S	R			P	P	P	N	A	A	B		
73	Kalaiselvi	45	F	1488616	643	6/12/2013	SPUTUM		Pneumonia	M6	R	I	S	R	S	S	I	S	I			P	P	P	N	A	A	B		
74	Natesan	55	M	1490500	676	21/12/2013	SPUTUM		Pneumonia	M3	S	R	S	S	S	S	S	S	R			P	P	P	N	A	A	B	P	
75	Revathi	16	F	3588	72	26/1/2014	SPUTUM	BA	Pneumonia	306	I	S	S	I	S	S	I	S	S			N	N	N	N	N	N	L		
76	Manimegal	45	F	3417	74	28/1/2014	SPUTUM	COPD	Pneumonia	306	R	S	S	R	S	S	I	S	R			P	P	P	N	A	A	B		
77	Kandasam	63	M	6161	95	7/2/2014	SPUTUM	TB	PT /Pneme	306	R	R	R	R	R	R	R	R	R	S-PB,CO		P	P	P	N	A	A	B	P	
78	Tamilselvi	43	F	294666	124	15/2/2014	SPUTUM		Pneumonia	LW	R	S	S	R	R	S	R	S	S			N	N	N	N	N	N	L		
79	Perumal	55	M	7796	127	15/2/2014	SPUTUM	COPD	Pneumonia	M5	R	S	S	R	S	R	R	S	R			P	P	P	N	A	A	B		
80	Arunachala	46	M	OP/168860	256	3/4/2014	SPUTUM	TB	Pneumonia	TMOP	R	I	S	R	I	I	R	S	R			P	P	P	N	A	A	B		

ABBREVIATION

P - POSITIVE
N - NEGATIVE
A - ACID PRODUCTION
B - ACINETOBACTER BAUMANNII
L - ACINETOBACTER LWOFFII
S - SENSITIVE
I - INTERMEDIATE
R - RESISTANT

BIBLIOGRAPHY

BIBLIOGRAPHY

1.Jyoti R Patil, Neelakshi R Jog, *Balu A Chopde . Isolation and characterization of Acinetobacter spp.from upper respiratory tract of healthy humans and demonstration of lectin activity.Indian Journal of Medical Microbiology (2001). 19 (1):30-35.

2.Topley and Wilson's Microbiology and Microbial Infections. 10th edition.volume2 - edited by S.Peter Borriello, Patrick R.Murray and Guido, chapter 49, Acinetobacter and Moraxella, ASM Press Holder Arnold – 2005 - page.no - 1181-1203.

3.Connie R.Mahon Text book of Diagnostic Microbiology, Donald C.Lehman, George Manuselis -4th edition- chapter 21- Nonfermenting and Miscellaneous Gram – Negative Bacilli, W.B Saunder's company Elsevier 2011- page no 489-490.

4. Anton Y. Peleg,[1]* Harald Seifert,[2] and David L. Paterson[3,4,5]. Acinetobacter baumannii: Emergence of a Successful Pathogen. Clinical Microbiology Reviews July 2008. page no 538-582.

5.Koneman's color Atlas and Textbook of Diagnostic Microbiology – 6th edition, chapter 7, The Nonfermentative Gram Negative Bacilli, Lippincott Williams and Wilkins 2006, page no 353-355.

6. Patrick R.Murray, Ellen Jo Baron, James H Jorgensen, marie Louise Landry, Mitchael A.Pfaller, Manual of Clinical Microbioligy,Volume 1,6th edition, chapter 24- Identification of

Aerobic Gram Negative Bacteria, ASM Press-Washington, page no-412-425.

7.Pierre-Edouard Fournier[1], David Vallenet[2], Valerie Barbe[2], Stephane Audic[1], Hiroyuki Ogata[1], Laurent Poirel[3], Herve richet[4], et al.Comparative Genomics of Multidrug Resistance in Acinetobacter baumannii, PLOS Genetics (2006) 2(1): e7.

8.K. Prashanth, T. Vasanth, R. Saranathan, Abhijith R. Makki and Sudhakar Pagal. Antibiotic Resistance, Biofilms and Quorum Sensing in Acinetobacter Species.

9.Bailey and Scott's Diagnostic Microbiology, 13th edition, chapter 21,Acinetobacter, Stenotrophomonas and similar organism, Mosby , Inc , an affliliate of Elsevier, 2014, page no 329-334.

10.Lisa L. Maragakis[1,2] and Tris M. Perl[1,2]. Acinetobacter baumannii : Epidemiology, Antimicrobial Resistance and Treatment options. Clinical Infectious Disease 2008; 46:1254-63.

11.Prescott, Harley and Kleins Microbiology, 8th edition, chapter–20, Bacteria: The Proteobacteria, MC Graw Hill – 2011, page-no 828-829.

12.Emma L. Carr[1], Peter Kampfer[2], Bharat K. C. Patel[3], Volker Gurtler[4] and Robert J. Seviour. Seven novel species of Acinetobacter isolated from activated sludge. International Journal of Sytematic and Evolutionary Microbiology (2003). 53, 953-963.

13. Peter Gerner-Smidt. Ribotyping of the Acinetobacter calcoaceticus-Acinetobacter baumannii Complex. Journal of Clinical Microbiology 1992.page no 2680-2685.

14.Peter Gerner-Smidt[1],* Ingela Tjernberg[2], And Jan Ursing[2]. Reliability of Phenotypic Tests for Identification of Acinetobacter Species 1991. page no 277-282.

15. Nabil Karah. Identification, molecular epidemiology, and antibiotic resistance characterization of Acinetobacter spp. clinical isolates. University Hospital of North Norway 2011.

16.Surinder kumar, Text Book of Microbiology, 1st edition, chapter 50, Miscellaneous Bacteria, Jaypee Brother Medical Publisher 2012, page no 477-478.

17.P. Chakraborty, Text Book of Microbiology, 3rd edition, chapter 36, Pseudomonas and Related Nonfermenters, New Central Book Agency 2013, page no 373-374.

18.D. R. Arora, B. Arora, Text Book of Microbiology, 3rd edition, chapter 24, Neisseria, Moraxella and Acinetobacter, CBS publishers, page no 254-255.

19. R. Vasanthakumari, Text Book of Microbiology, 2nd edition, chapter 43, Miscellaneous Bacteria, BI publication, page no 335-336.

20. Christian M. Harding, Erin N. Tracy, Michael D. Carruthers, et al. Acinetobacter baumannii strain M2 produces Type IV pili which

play a role in natural transformation and twitching motility but not surface associated motility 2014. mBio 4 (4).

21.Juni. E. Interspecies transformation of Acinetobacter: genetic evidence for a ubiquitous genus. J. Bacteriol,1972. 112:917–931.

22.A. Jaward, P.M. Hawkey, J.Heritage and A.M Snelling. Description of Leed's Acinetobacter Medium, a new selective and differential medium for isolation of clinically Acinetobacter species and comparison with Herellea agar and Holtan's agar. Journal of Clinical Microbiology 1994. p-2353-2358.

23. Sofia Constantiniu, Angela Romaniuc, Luminița Smaranda Iancu, Raluca Filimon, Iuliana Taraşi.Cultural and biochemical characteristics of Acinetobacter spp. strains isolated from hospital units. The Journal of Preventive Medicine 2004. 12 (3- 4):35-42.

24.Preeti B. Mindolli[1]*, Manjunath P. Salmani[2], Vishwanath G[3] and Hanumanthappa A.R[3]. Identification And Speciation Of Acinetobacter And Their Antimicrobial Susceptibility Testing. Al Ameen J Med Sci (2010). 3 (4): 345-349.

25.K. Prasanth and S. Badrinath. Simplified phenotypic tests for identification of Acinetobacter Spp. and their antimicrobial susceptibility status. J.Med.Microbiol vol. 49 (2000). 773-778.

26.Yaun Hu, Boqing Li, Dazhi Jin, Zhigang Cui, Xiaoxia Tao,Binghua Zhang and Jianzhong Zhang. Comparison of Multiple-Locus

Variable-Number Tandem-Repeat Analysis with Pulsed-Field Gel Elecrophoresis Typing of Acinetobacter baumannii in China. J. Clini. Microbiol.2013,51(4): 1263.

27.Mei Deng, Man-Hua Zhu, Jun-Jie Li, Sheng Bi, Zi-Ke Sheng, Fei-Shu Hu, Jia-Jie Zhang, Wei Chen, Xiao-Wei Xue, Ji-Fang Sheng and Lan-Juan Li. Molecular Epidemiology and Mechanisms of Tigecycline Resistance in Clinical Isolates of Acinetobacter baumannii from a Chinese University Hospital. Antimicrobial Agents and Chemotherapy 2014. 58(1):297.

28. Mette V. Larsen, Salvatore Cosentino, Simon Rasmussen, Carsten Friis, Henrik Hasman, Rasmus Lykke Marvig, Lars Jelsbak, Thomas Sicheritz-Pontén David W. Ussery, Frank M. Aarestrup and Ole Lund. Multilocus Sequence Typing of Total-Genome-Sequnced Bacteria. J. Clin. Microbiol 2012, 50(4):1355.

29.Ecker, J. A., C. Massire, T. A. Hall, R. Ranken, T. T. Pennella, C. Agasino Ivy, L. B. Blyn, S. A. Hofstadler, T. P. Endy, P. T. Scott, L. Lindler, T. Hamilton, C. Gaddy, K. Snow, M. Pe, J. Fishbain, D. Craft, G. Deye, S. Riddell, E. Milstrey, B. Petruccelli, S. Brisse, V. Harpin, A. Schink, D. J. Ecker, R. Sampath, and M. W. Eshoo. Identification of Acinetobacter species and genotyping of Acinetobacter baumannii by multilocus PCR and mass spectrometry. J. Clin. Microbiol.2006, 44:2921–2932.

30. Seifert H, Dijkshoorn L, Gielen J, Nemec A, Osterhage K, Erhard M, Krut O: Evaluation of MALDI-TOF MS for identification of Acinetobacter species. In: 107th General Meeting of the American Society for Microbiology. vol. abstract C- 172; 2007.

31. Ni Tien, Bang-Jau You, Hui-Lan Chang, Hsiu-Shen Lin, Chin-Yi Lee, Tung-Ching Chung, Jang-Jih Lu and Chao-Chin Chang, Comparison of Genospecies and Antimicrobial Resistance Profiles of Isolates in the Acinetobacter calcoaceticus-Acinetobacter baumannii Complex from Various Clinical Specimens , Antimicrob. Agents Chemother. 2012, 56(12):6267.

32. Mario Vaneechoutte[1],* Lenie Dijkshoorn[2], Ingela Tjernberg[3], Abdeslam Elaichouni[1], Paul De Vos[4], Geert Claeys[1], And Gerda Verschraegen[1], Identification of Acinetobacter Genomic Species by Amplified Ribosomal DNA Restriction Analysis, Journal of Clinical Microbiology, 1995, p-11-15.

33.Seifert, H., L. Dijkshoorn, P. Gerner-Smidt, N. Pelzer, I. Tjernberg, and M. Vaneechoutte. . Distribution of Acinetobacter species on human skin: comparison of phenotypic and genotypic identification methods. J. Clin. Microbiol. 1997 35:2819–2825.

34. Dijkshoorn, L., E. van Aken, L. Shunburne, T. J. van der Reijden, A. T. Bernards, A. Nemec, and K. J. Towner. Prevalence of Acinetobacter baumannii and other Acinetobacter spp. in faecal samples from

non-hospi- talised individuals. Clin. Microbiol. Infect.2005, 11:329–332.

35. Griffith, M. E., D. R. Lazarus, P. B. Mann, J. A. Boger, D. R. Hospenthal, and C. K. Murray. Acinetobacter skin carriage among US army soldiers deployed in Iraq. Infect. Control Hosp. Epidemiol.2007, 28:720–722.

36.Chu, Y. W., C. M. Leung, E. T. Houang, K. C. Ng, C. B. Leung, H. Y. Leung, and A. F. Cheng. Skin carriage of acinetobacters in Hong Kong. J. Clin. Microbiol. 1999,37:2962-2967.

37.McDonald, L. C., S. N. Banerjee, W. R. Jarvis, et al. Seasonal variation of Acinetobacter infections: 1987–1996. Clin. Infect. Dis. 1997 ,29:1133– 1137.

38. La Scola, B., and D. Raoult. Acinetobacter baumannii in human body louse. Emerg. Infect. Dis.2004,10:1671–1673.

39.Berlau, J., H. M. Aucken, E. Houang, and T. L. Pitt. Isolation of Acinetobacter spp. including A. baumannii from vegetables: implications for hospital-acquired infections. J. Hosp. Infect.1999, 42:201–204.

40.Cheol-In Kang and Jae Hoon Song. Antimicrobial resistance in Asia: Current epidemiology and clinical implications. Infection and Chemotherapy 2013; 45(1):22-31.

41.Cisneros JM[1], Reyes MJ, Pachón J, Becerril B, Caballero FJ, García-Garmendía JL, Ortiz C, Cobacho AR. Bacteremia due to Acinetobacter baumannii: epidemiology, clinical findings, and prognostic features.Clin Infect Dis. 1996 Jun;22(6):1026-32.

42. Hilmar Wisplinghoff[1], Michael B. Edmond[2], Michael A. Pfaller[3], Ronald N. Jones,[3] Richard P. Wenzel[2], and Harald Seifert[1]. Nosocomial Bloodstream Infections Caused by Acinetobacter Species in United States Hospitals: Clinical Features, Molecular Epidemiology, and Antimicrobial Susceptibility. Clinical Infectious Diseases 2000; 31:690-7.

43. Abbo A, Navon-Venezia S, Hammer-Muntz O,Krichali T, Seigman-Igra Y,CarmeliY. Multidrug resistant Acinetobacter baumannii. Emerg Infec Dis 2005;11:22-9.

44. Gales AC, Pfaller MA, Sader HS, Hollis RJ, Jones RN. Genotypic characterization of carbapenem-nonsusceptible Acinetobacter spp.isolated in Latin America. Microb Drug Resist 2004;10:286–91.

45. Brink, A., J. Moolman, M. C. da Silva, and M. Botha. Antimicrobial susceptibility profile of selected bacteraemic pathogens from private institutions in South Africa. S. Afr. Med. J. 2007, 97:273–279.

46. Riley, T. V., S. A. Webb, H. Cadwallader, B. D. Briggs, L. Christiansen, and R. A. Bowman. Outbreak of Gentamicin-resistant Acinetobacter baumannii in an intensive care unit: clinical,

epidemiological and microbiological features. Pathology 1996 28:359–363.

47. Poirel L, Nordmann P: Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect 2006, 12(9):826-836.

48. Lee K, Yum JH, Yong D, Lee HM, Kim HD, Docquier JD, Rossolini GM, Chong Y: Novel acquired metallo-ß-lactamase gene, blaSIM-1, in a class 1 integron from Acinetobacter baumannii clinical isolates from Korea. Antimicrob Agents Chemother 2005, 49(11):4485-4491.

49. Chen Z, Qlu S, Wang Y, Liu S, Wang Z, Du X, Wang L, Guo J, Liu N, Yuan J et al: Coexistence of blaNDM-1 with the prevalent blaOXA23 and blaIMP in pan-drug resistant Acinetobacter baumannii isolates in China. Clin Infect Dis 2011, 52(5):692-693.

50. Jacoby GA: AmpC ß-lactamases. Clin Microbiol Rev 2009, 22(1):161-182.

51. Poirel L, Naas T, Nordmann P: Diversity, epidemiology, and genetics of class D ß-lactamases. Antimicrob Agents Chemother 2010, 54(1):24-38.

52. Paton, R., R. S. Miles, J. Hood, and S. G. B. Amyes. Beta-lactamase mediated imipenem resistance in Acinetobacter baumannii. Int. J. Antimicrob. Agents 1993 2:81–88.

53. Marque S, Poirel L, Heritier C, Brisse S, Blasco MD, Filip R, Coman G, Naas T, Nordmann P: Regional occurrence of plasmid-mediated carbapenem- hydrolyzing oxacillinase OXA-58 in Acinetobacter spp. in Europe. J Clin Microbiol 2005, 43(9):4885-4888.

54. Segal H, Garny S, Elisha BG: Is IS_{ABA-1} customized for Acinetobacter? FEMS Microbiol Lett 2005, 243(2):425-429.

55. Heritier C, Poirel L, Nordmann P: Cephalosporinase over-expression resulting from insertion of ISAba1 in Acinetobacter baumannii. Clin Microbiol Infect 2006, 12(2):123-130.

56. Pfeifer Y, Zander E, Göttig S, Hunfeld KP, Seifert H, Higgins PG: Complete DNA sequence of blaNDM-1 gene cassette integrated into the chromosome of Acinetobacter baumannii. 21st European Congress of Clinical Microbiology and Infectious Diseases/27th International Congress of Chemotherapy 2011, Abstract O 215.

57. Siroy, A. Molle, V. Guillier, C.L. Vallenet, D. Caron, M.P. Cozzone, A.J. Jouenne, T. & De, E. (2006). Channel Formation by CarO, the Carbapenem Resistance-Associated Outer Membrane Protein of Acinetobacter baumannii, Antimicrobial Agents and Chemotherapy Vol.49, No.12, (December 2005) pp. 4876-4883.

58. Mussi, M.A. Limansky, A.S. & Viale, A.M. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of Acinetobacter baumannii: natural insertional inactivation of a gene

encoding a member of a novel family of b-barrel outer membrane proteins. Antimicrob Agents Chemother, Vol.49 (April 2005), pp.1432–1440.

59. Etsuko Sugawara and Hiroshi Nikaido . OmpA Is the Principal Nonspecific Slow Porin of Acinetobacter baumannii. J. Bacteriol. 2012, 194(15): 4089-4096

60. Manuella Catel-Ferreira, Rony Nehmé, Virginie Molle, Jesús Aranda, Emeline Bouffartigues, Sylvie Chevalier, Germán Bou, Thierry Jouenne and Emmanuelle Dé.Deciphering the Function of the Outer Membrane Protein OprD Homologue of Acinetobacter baumannii. Antimicrob. Agents Chemother 2012, 56(7):3826-3832.

61. Roca, I., Espinal, P. Marti, S. & Vila, J. First Identification and Characterization of an AdeABC-Like Efflux Pump in Acinetobacter Genomospecies 13TU, Antimicrob. Agents and Chemother, Vol.55, No.3, (March 2011) pp. 1285-1286.

62. Magnet, S. Courvalin, P. & Lambert, T. Resistance-Nodulation-Cell Division-Type Efflux Pump Involved in Aminoglycoside Resistance in Acinetobacter baumanStrain BM4454, Antimicrob. Agents and Chemother, Vol.45, No.12, (December 2011) pp.3375-3380.

63. Chau, S.L. Chu, Y.W. Houang, E.T. Novel resistance-nodulation-cell division efflux system AdeDE in Acinetobacter genomic DNA group 3. Antimicrob Agents and Chemotherapy Vol. 48, No. 10, (October

2004) pp. 4054-4055.

64. Marchand, I. Piolle, L.D. & Courvalin, P. Expression of the RND-type efflux pump AdeABC in Acinetobacter baumannii is regulated by the AdeRS two-component system. Antimicrob Agents Chemother, Vol.48 (September 2004) pp. 3298– 30.

65. Nemec A, Dolzani L, Brisse S, van den Broek P, Dijkshoorn L: Diversity of aminoglycoside-resistance genes and their association with class 1 integrons among strains of pan-European Acinetobacter baumannii clones. J Med Microbiol 2004, 53(Pt 12):1233-1240.

66. Cho YJ, Moon DC, Jin JS, Choi CH, Lee YC, Lee JC: Genetic basis of resistance to aminoglycosides in Acinetobacter spp. and spread of armA in Acinetobacter baumannii sequence group 1 in Korean hospitals. Diagn Microbiol Infect Dis 2009, 64(2):185-190.

67. Jain R, Danziger LH: Multidrug-resistant Acinetobacter infections: an emerging challenge to clinicians. Ann Pharmacother 2004, 38(9):1449-1459.

68. Deccache, Y. Irenge, L.M. Savov, E. Ariciuc, M. Macovei, A. Trifonova, A. Gergova, I. Ambroise, J. Vanhoof, R. & Gala, J.L. Development of a pyrosequencing assay for rapid assessment of quinolone resistance in Acinetobacter baumannii isolates. Journal of Microbiol Methods Vol.86, No. 1, (July 2011), pp. 115-118.

69. Ribera, A., J. Ruiz, and J. Vila. Presence of the Tet M determinant in a clinical isolate of Acinetobacter baumannii. Antimicrob. Agents Chemother. 2003,47:2310–2312.

70. Gu, B., M. Tong, W. Zhao, G. Liu, M. Ning, S. Pan, and W. Zhao. Prevalence and characterization of class I integrons among Pseudomonas aeruginosa and Acinetobacter baumannii isolates from patients in Nanjing, China. J. Clin. Microbiol. 2007,45:241–243.

71. Houang, E. T., Y. W. Chu, W. S. Lo, K. Y. Chu, and A. F. Cheng. Epidemiology of rifampin ADP-ribosyltransferase (arr-2) and metallo-beta- lactamase (blaIMP-4) gene cassettes in class 1 integrons in Acinetobacter strains isolated from blood cultures in 1997 to 2000. Antimicrob. Agents Chemother.2003, 47:1382–1390.

72. Lee, .K., J. H. Yum, D. Yong, H. M. Lee, H. D. Kim, J. D. Docquier, G. M. Rossolini, and Y. Chong. Novel acquired metallo-beta-lactamase gene, bla(SIM-1), in a class 1 integron from Acinetobacter baumannii clinical isolates from Korea. Antimicrob. Agents Chemother. 2005,49:4485–449.

73. Jones RN, Sader HS, Fritsche TR, Rhomberg PR. Carbapenem susceptibility discords among Acinetobacter isolates. Clin Infect Dis 2006;42:158.

74. Jones RN, Deshpande L, Fritsche TR, Sader HS. Determination of epidemic clonality among multidrug-resistant strains of

Acinetobacter spp. and Pseudomonas aeruginosa in the MYSTIC Programme (USA, 1999–2003). Diagn Microbiol Infect Dis 2004;49:211–6.

75. Ikonomidis A, Pournaras S, Maniatis AN, Legakis NJ, Tsakris A. Discordance of meropenem versus imipenem activity against Acinetobacter baumannii. Int J Antimicrob Agents 2006;28:376–7.

76. Brauers J, Frank U, Kresken M, Rodloff AC, Seifert H. Activities of various beta-lactams and beta-lactam/beta-lactamase inhibitor combinations against Acinetobacter baumannii and Acinetobacter DNA group 3 strains. Clin Microbiol Infect 2005;11:24–30.

77. Pachon-Ibanez ME, Jimenez-Mejias ME, Pichardo C, Llanos AC, Pachon J. Activity of tigecycline (GAR-936) against Acinetobacter bau- mannii strains, including those resistant to imipenem. Antimicrob Agents Chemother 2004;48:4479–81.

78. Peleg AY, Potoski BA, Rea R, et al. Acinetobacter baumannii bloodstream infection while receiving tigecycline: a cautionary report. J Antimicrob Chemother 2007;59:128–31.

79. Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis 2005;40:1333–41.

80. Li J, Nation RL, Turnidge JD, et al. Colistin: the re-emergingantibiotic for multidrug-resistant gram-negative bacterial

infections. Lancet In- fect Dis 2006;6:589–601.

81. Gales AC, Jones RN, Sader HS. Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of gram-neg- ative bacilli: report from the SENTRY antimicrobial surveillance pro- gramme (2001–2004). Clin Microbiol Infect 2006;12:315–21.

82. Holloway KP, Rouphael NG, Wells JB, King MD, Blumberg HM. Polymyxin B and doxycycline use in patients with multidrug-resistant Acinetobacter baumannii infections in the intensive care unit. Ann Pharmacother 2006;40:1939–45.

83. Kallel H, Bahloul M, Hergafi L, et al. Colistin as a salvage therapy for nosocomial infections caused by multidrug-resistant bacteria in the ICU. Int J Antimicrob Agents 2006;28:366–9.

84. Montero, A., J. Ariza, X. Corbella, A. Domenech, C. Cabellos, J. Ayats, F. Tubau, C. Borraz, and F. Gudiol. Antibiotic combinations for serious infections caused by carbapenem-resistant Acinetobacter baumannii in a mouse pneumonia model. J. Antimicrob. Chemother.2004, 54:1085–1091

85. Yoon, J., C. Urban, C. Terzian, N. Mariano, and J. J. Rahal. In vitro double and triple synergistic activities of polymyxin B, imipenem, and ri- fampin against multidrug-resistant Acinetobacter baumannii. Antimicrob. Agents Chemother.2004, 48:753–757.

86. Tan, T. Y., L. S. Ng, E. Tan, and G. Huang. In vitro effect of minocycline and colistin combinations on imipenem-resistant Acinetobacter baumannii clinical isolates. J. Antimicrob. Chemother.2007, 60:421–423.

87. Kroeger, L. A., L. B. Hovde, I. F. Mitropoulos, J. Schafer, and J. C. Rotschafer. Colistin methanesulfonate against multidrug-resistant Acinetobacter baumannii in an in vitro pharmacodynamic model. Antimi- crob. Agents Chemother. 2007,51:3431–3433

88. Song, J. Y., S. Y. Kee, I. S. Hwang, Y. B. Seo, H. W. Jeong, W. J. Kim, and H. J. Cheong. In vitro activities of carbapenem/sulbactam combination, colistin, colistin/rifampicin combination and tigecycline against carbapenem resistant Acinetobacter baumannii. J. Antimicrob. Chemother.2007, 60: 317–322.

89. Li J, Rayner CR, Nation RL, et al. Heteroresistance to colistin in multidrug-resistant Acinetobacterbaumannii. Antimicrob Agents Chemother 2006;50:2946–50.

90. Owen RJ, Li J, Nation RL, Spelman D. In vitro pharmacodynamics of colistin against Acinetobacter baumannii clinical isolates. J Antimicrob Chemother 2007;59:473–7.

91. Wilks M, Wilson A, Warwick S, et al. Control of an outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus colonization and infection in an intensive care unit (ICU) without 10

closing the ICU or placing patients in isolation. Infect Control Hosp Epi- demiol 2006;27:654–8.

92. Zarrilli R, Casillo R, Di PA, et al. Molecular epidemiology of a clonal outbreak of multidrug-resistant Acinetobacter baumannii in a university hospital in Italy. Clin Microbiol Infect 2007;13:481–9.

93. Kraniotaki E, Manganelli R, Platsouka E, Grossato A, Paniara O, Palu G. Molecular investigation of an outbreak of multidrug-resistant Acinetobacter baumannii, with characterisation of class 1 integrons. Int J Antimicrob Agents 2006;28:193–9.

94. Mackie & McCartney Practical Medical Microbiology. 14th edition, chapter 8,Laboratory control of antimicrobial therapy, Churchill Livingstone 2012, page no 151-177.

95. Lt Col KK Lahiri*, Lt Col NS Mani+, Lt Col SS Pur.Acinetobacter spp as Nosocomial Pathogen : Clinical Significance and Antimicrobial Sensitivity.MJAFI 2004;60:7-10.

96. Vijayan Sivaranjani, Sivaraman Umadevi, [...], and KS Seetha. Multi-drug resistant *Acinetobacter* species from various clinical samples in a tertiary care hospital from South India.Australas Med J.2013; 6(12):697-700.

97. Alex Studemeister* D. Community-Acquired Acinetobacter baumannii Infections in Northern California.Clin.Microbial 2013, 2-6.

98. Jane F. Turton,1* Neil Woodford,2 Judith Glover,1 Susannah Yarde,1 Mary E. Kaufmann,1 and Tyrone L. Pitt1. Identification of Acinetobacter baumannii by Detection of the blaOXA-51-like Carbapenemase Gene Intrinsic to This Species. Journal of Clinical Microbiology 2006.2974-2976.