A DISSERTATION ON

THE STUDY OF ACINETOBACTER SPECIES AS A NOSOCOMIAL PATHOGEN BY PLASMID PROFILE TYPING

M.D. MICROBIOLOGY – BRANCH - IV

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C E R T I F I C A T E

This is to certify that the dissertation titled **"THE STUDY OF ACINETOBACTER SPECIES AS A NOSOCOMIAL PATHOGEN BY PLASMID PROFILE TYPING"** by **Dr. B. Sophia,** for M.D. Microbiology Examination, September 2006 under the Tamil Nadu Dr. M.G.R. University is a bonafide work carried out under my direct supervision and guidance.

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INTRODUCTION

The genus Acinetobacter originally proposed by Brisou and Prevot (1954) comprised of a heterogeneous collection of non-motile Gram-negative organisms, which could be distinguished from other similar organisms by their lack of pigmentation (Ingram and Shewan, 1960). The history of the oxidase-negative, non-motile. Gram-negative diplobacilli now constituting the genus Acinetobacter has been confused for many years (Henriksen, 1973, 1976). These bacteria were originally classified in to various genera including ***"Bacterium", "Neisseria", "Alcaligenes", "Mima", "Herellea", "Achromobacter"*** and ***"Moraxella"***. For some time this group of bacteria was referred to as the oxidase negative Moraxella. The application of modern methods of taxonomy including carbon source utilisation tests, genetic transformation. DNA hybridisation, and RNA sequence comparison (by hybridisation or direct sequencing) has now resolved the earlier confusion. Progress in Acinetobacter taxonomy has now provided a new insight into Acinetobacter ecology and epidemiology.[10,69]

Acinetobacter species are Gram Negative, strongly aerobic, oxidase negative, catalase positive, non-motile, encapsulated non-fermenting coccobacilli with DNA G+C content of 39-47%. Members of genus Acinetobacter are widely distributed in nature and commonly found as a part of normal flora of humans. They are commonly found on skin and occasionally in the respiratory tract, genitourinary tract, gastrointestinal tract and conjunctiva. They are also found in soil, water and in hospital environment where they can cause serious infections in immunocompromised people and observed

to be highly resistant to commonly used antibiotics and drugs.[68] Members of the genus Acinetobacter are short plump, gram-negative rods that often become more coccoid in the stationary phase. Cells commonly occur in pairs or chains of variable length that are occasionally difficult to destain. No spores are formed.[10,69]

Acinetobacter has become an emerging nosocomial multi drug resistant pathogen all over the world and also in Govt. Rajaji hospital during the recent periods. The Acinetobacter infections increase the morbidity and mortality, increase the cost of treatment, and prolong hospital stay adding to the economical burden on the nation. Despite advancement in medical technology for diagnosis and patient care, a person can still die of an infection caused by a multi-drug resistant Acinetobacter species. Nosocomial infections caused by Acinetobacter species are the main threat in the present scenario.

Yo! ***"Acinetobacter Important!"*** It is difficult to prevent infection because it is ubiquitous in the environment, and it is can be very difficult to treat since it is rapidly evolving towards multi-drug resistance.

SPECIES

By means of genetic transformation DNA hybridisation and RNA sequence comparison, 19 biotypes of Acinetobacter genomic species are identified. The major genomic species comprise ***Acinetobacter baumannii*** (with nine biotypes), ***Acinetobacter calcoaceticus***, ***Acinetobacter haemolyticus, Acinetobacter johnsonii, Acinetobacter junii,*** and ***Acinetobacter lwoffi***.

MICROSCOPIC APPEARANCE

They are Gram-negative rods during log phase, coccobacillary during stationary phase. They are encapsulated. Acinetobacter has a tendency to retain crystal violet and can therefore be incorrectly identified as gram positive.

CULTURAL CHARACTERS

All strains of Acinetobacter are non-fastidious. They grow on Nutrient agar, Macconkey agar, 5% Sheep Blood agar.[68] On Nutrient Agar the colonies are smooth, circular with entire edges convex, semitransparent, white or cream coloured measuring 1.5 – 2mm. They grow well on MacConkey agar medium, and colonies show faint pinkish tint. On 5% Sheep Blood Agar at 37^0C for 24 hours, some of the strains show haemolysis. The colonies are non pigmented and may be mucoid (due to the capsule).

BIOCHEMICAL IDENTIFICATION

Acinetobacter are **Oxidase negative** (The oxidase test provides a rapid test to distinguish them from Neisseria or Moraxella). Acinetobacter can oxidize sugars (respires) but cannot ferment them. **Rapid utilization of 10% glucose** is seen with **OF** medium. Acinetobacter are **unable to reduce nitrate**. Species differentiation is done on basis of glucose oxidation, gelatin liquefaction, hemolysis, growth at 37^0C and 44^0C and susceptibility to penicillin.

VIRULENCE FACTORS

Members of Acinetobacter have a limited number of virulence factors, regulating them to the status of agents of opportunistic infections.[70]

Acinetobacter produces extra cellular enzymes like lipases and esterases,

amylase, collagenase and **β-lactamase**. There are several interesting periplasmic enzymes, including **insulin cleaving metaloprotease**, and membrane bound enzymes in abundant. Acinetobacter genus has a capsule lipopolysaccharide, produces lectins which acts as adhesion factor.

RISK FACTORS FOR ACINETOBACTER INFECTION[39]

Severe illness, Immunocompromised state of the host, an extended stay in the intensive care unit[7,28], previous administration of third generation cephalosporins, procedures such as intubation, ventilator use, indwelling urinary catheter and neurosurgery.[17,32,34]

DISEASES CAUSED BY ACINETOBACTER

Acinetobacter is on opportunist.[22] Acinetobacter can cause suppurative infections in any organ system. They causes ***pneumonia, bacteremia, meningitis, cellulitis, cystitis, pyleonephritis, endophthalmitis, endocarditis.*** It rarely causes disease in healthy individuals with intact host defenses.[19]

RESISTANCE TO ANTIBIOTICS

Acinetobacter has a very high level of resistance to antimicrobials and relatively few antibiotics are active against it.[18,37] A major contributing factor in the emergence of resistant Acinetobacter species is the acquisition and transfer of antibiotic resistance via ***Plasmids and Transposons***. ***Plasmids*** are extra chromosomal pieces of double stranded circular DNA which have the capacity to replicate independently of the host chromosomes. ***Plasmid*** mediated antibiotic resistance can be transferred very easily from one bacterium to another through conjugation, transformation. In addition to the antibiotic resistance, the bacterial ***plasmids*** may confer pathogenicity as well, to

bacterial cells.

A ***plasmid*** type was defined as any plasmid pattern which varied from another pattern with regard to the number and size of the plasmid bands. ***Plasmid*** mediated multiple antibiotic resistance is one of the most demanding problems in the treatment of Acinetobacter infections.[26] The resistance to a number of antibiotics is mediated by ***R-plasmid***. It is established that ***Acinetobacter has the capacity to serve as a potential reservoir of transmissible resistance genes to other microorganisms.***[13,27] The commonest mechanism of resistance to penicillin is inactivation of these agents by β-lactamases which are encoded by ***plasmids*** in Acinetobacter spp.[16]

Plasmid profile typing is shown to be an useful method in diagnosing resistance plasmid band in a strain and is shown to be useful in epidemiological typing of various Acinetobacter species.[21,23,60] ***Plasmid*** DNA may be isolated from small-scale (1–2 ml) bacterial cultures by treatment with alkali and SDS.[29,43]

The following are some of the mechanisms of resistance documented in acinetobacter.

Possession of β–lactamases,[16,47,53] Alteration of penicillin - binding proteins and gyrase. Loss of porins, Possession of an efflux pump. Since the 1970's Acinetobacter has been acquiring increasing drug resistance.[54]

The present study was undertaken to prove Acinetobacter as a nosocomial pathogen by means of plasmid profile analysis, by comparing the isolates from both the clinical specimens and the hospital environmental sources.

AIM AND OBJECTIVES

- To isolate and identify Acinetobacter to species level from various clinical and environmental samples.

- To identify the multidrug resistant Acinetobacter species from various clinical and environmental samples and to isolate plasmids from these resistant isolates.

- To compare the plasmid profile of clinical isolates with the environmental isolates to find the identity between the two and to confirm that Acinetobacter species occur as an important nosocomial pathogen.

- To show that plasmid profile typing is a useful tool for epidemiological investigation of Acinetobacter species.

REVIEW OF LITERATURE

Acinetobacter spp is the second most commonly isolated Non Fermenting Gram Negative Bacilli in hospital environments.

BAUMANN et al studied about a hundred oxidase negative strains of Acinetobacter isolated from soil and water **(1968).**[6]

HUMPHREYS GO, **WILLSHAW GA**, et al studied about a simple method for the preparation of large quantities of pure plasmid DNA. Polyethylene glycol quantitatively precipitates plasmid DNA of molecular weight 6-123-10-6, from cleared lysates of plasmid-carrying bacterial strains, After resuspension and density-gradient centrifugation of the precipitated DNA, it is unchanged in length and in transformation efficiency for Escherichia coli K12. Plasmid DNA can be easily prepared in large quantities by including a polyethylene glycol precipitation step in standard plasmid isolation procedures **(1975)**.[29]

MEYERS. J.A, SANCHEZ. D, et al studied about the simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. Agarose gel electrophoresis may be employed effectively for the detection and preliminary characterization of plasmid deoxyribonucleic acid (DNA) present in clinical isolates and laboratory strains of gram-negative

microorganisms. The method is sensitive and does not require radioisotopes or ultracentrifugation. The estimation of plasmid mass from the extent of DNA migration in gels compares favorably with results obtained by electron microscopy of plasmid DNA purified by equilibrium density centrifugation. The method has proved to be a useful tool for survey work and the epidemiological investigation of plasmid dissemination, as well as an important adjunct to the genetic analysis of plasmids **(1976)**.[46]

SMITH, P.W. and MASSANARI et al studied, room humidifiers as a source of Acinetobacter infections. Twenty four patients contracted systemic infections with Acinetobacter spp during a four month period, unheated room humidifiers at the patients bedsides were implicated as the source of infection. The outbreaks were terminated with the removal of the humidifiers **(1977)**.[66]

BUXTON AE, **ANDERSON RL** et al studied about the Nosocomial respiratory tract infection and colonization with Acinetobacter calcoaceticus, and its epidemiological characteristics. Nosocomial respiratory tract infection with Acinetobacter calcoaceticus occurs frequently in many hospitals. Retrospective studies demonstrated that A. calcoaceticus in sputum was significantly associated with endotracheal intubation ($p = 0.03$) and continuous positive pressure ventilation (p less

than 0.02) After control measures had interrupted the outbreak, a prospective microbiologic investigation demonstrated that one third of the hospital personnel had transient hand colonization with multiple strains of A. calcoaceticus. A pulmonary therapist with chronic dermatitis had persistent hand colonization with the epidemic strain, and he contaminated respiratory therapy equipment. Human skin must be considered an important reservoir of A. calcoaceticus **(1978)**.[11]

DEVAUD M, KAYSER FH, et al studied Transposon mediated multiple antibiotic resistance in Acinetobacter strains. A representative isolate of the epidemic strain was found to contain resistance to gentamicin, kanamycin, and streptomycin, respectively. In addition, the strain produced a cephalosporinase and was resistant to penicillins due to the production of a TEM-2 beta-lactamase. The resistant phenotype of this strain was similar to resistance patterns frequently observed in endemic hospital flora, suggesting that the transfer of an R plasmid into Acinetobacter sp, may have occurred. After plasmid RP4 was transferred into an ampicillin and kanamycin susceptible derivative of the epidemic strain, mobilization of resistance to Chloramphenicol, Gentamicin, Streptomycin, Sulfonamides and possibly Tetracycline could be achieved. This mobilization was due to the transposition of a 16-megadalton DNA sequence from the Acinetobacter chromosome into plasmid RP4. Insertion of the transposable sequence occured near the

PSTI and SMAL sites around position 22.5 on the physical map of plasmid RP4 **(1982).**[18]

SAMBROOK. J, MANIATIS et al described a method by which plasmid DNA can be isolated. They isolated plasmids by alkali lysis method the techniques require the use of several chemicals to partition and precipitate the plasmid sample **(1982)**.[43]

ANDREWS HJ, et al described a technique for typing Acinetobacter sp. by bacteriocin production. One hundred and seventy-six cultures from patients in outbreaks, in the community and environmental sources were identified, tested for sensitivity to gentamicin and bacteriocin typed ; 154 were A. anitratus and the remainder were A.lwoffi, only one A.lwoffi strain produced bacteriocin. Ten of 22 were sensitive to bacteriocins and could be used as indicators. A close association was found between bacteriocin production and gentamicin resistance: MIC 4 mg/ml. Using six indicator strains 100/104 (96%) gentamicin-resistant strains were typed with nine distinct patterns of inhibition. Overall typability was 65% but 100/176 (56%) fell into only two groups. The technique may be of value in studying the epidemiology of Acinetobacter **(1986)**.[2]

AVRIL J.L. and MESNARD R. et al studied about the factors influencing the virulence of Acinetobacter. The virulent organisms exhibit

pathogenicity when introduced into the host in very small numbers. This property can be subdivided into invasiveness, multiplication and spread within the host tissues, and including the toxigenicity. The ability of bacteria to assimilate iron appears to be related to invasiveness. The virulence may be due to slime production, lipopolysaccharide (LPS) components of their cell walls that can functions as endotoxin **(1991)**.[70]

LUIS. A. ACTIS et al studied in different clinical isolates of Acinetobacter spp typed by plasmid profile, were able to grow in Iron chelated medium by secreting iron-regulated siderophores. The ability of Acinetobacter spp to colonize and invade the human host suggests that it can obtain essential nutrients, such as iron, which are normally restricted, for bacterial utilization **(1993)**.[41]

SEIFERT.H, et al studied plasmid DNA profiles of Acinetobacter baumanni and clinical application in a complex endemic setting. To study the epidemiological, microbiological and clinical features of infections due Acinetobacter baumannii in a complex endemic situation over an 18-month period and to determine the clinical usefulness of plasmid DNA analysis of A.baumannii in epidemiological investigations **(1994)**.[60]

GARCIA DC, NOCIARI MM, et al studied the use of plasmid profile analysis and ribotyping for typing Acinetobacter baumanni isolates. All multi-drug resistant Acinetobacter baumannii isolates have

multiple resistance plasmid bands. The variable number of plasmids with different molecular weights in each isolate enabled the identification of different multi-drug resistance Acinetobacter baumannii strain. The plasmid typing is a practical method to assist infection control of nosocomial Acinetobacter baumannii **(1996).**[23]

JAWAD. A et al studied about the influence of relative humidity and suspending on menstrual fluid facilitates the survival of Acinetobacter spp **(1996).**[32]

BEIJERNCK M.W, et al in 1911 isolated Acinetobacter from soil and were named micrococcus calcoaceticus **(1997).**[68]

SHAKIBAIE. M.R, CHOPADE, et al studied about the "Plasmid mediated silver and antibiotic resistance" in *Acinetobacter Baumannii* Bl54 Six strains of Acinetobacter baumannii were isolated from clinical samples taken from patients at the Armed Forces Medical College in Pune, India. The genuninity of the isolates was confirmed by chromosomal transformation assay biochemical tests. Minimum inhibitory concentration (MIC) for antibiotics and metals was carried out to determine the sensitivity/resistance of the isolates. Among them, strain BL54 (a blood isolate) was found to be resistant to nine metal ions and multiple antibiotics. The plasmids exhibited different pattern of digestion with EcoRV enzyme. In addition, their data indicates that for resistance to

Ag-sd both plasmids must be expressed in the host **(1998).**[62]

RALPH PANTOPHLET, LORE BRADE, et al studied about the Identification of *Acinetobacter baumannii* Strains with Monoclonal Antibodies against the O Antigens of their Lipopolysaccharides The monoclonal antibodies were characterized by enzyme immunoassay and by Western and dot blot analyses and were investigated for their potential use for the identification of *A. baumannii* strains **(1999).**[56]

KAPPSTEIN. I, et al studied Aerators as a reservoir of Acinetobacter spp causing outbreak of bacteraemia in paediatric oncology patients. Tap water can play role as a source of nosocomial pathogens and faucet aerators have been associated with colonization or infection in hospitalized patients Environment sampling showed the water system to be contaminated with A. junii. Conventional aerators consisting of several wire meshes can serve as a reservoir for low levels of bacteria present in the water system **(2000).**[34]

PEDRO. B. et al showed incidence of Acinetobacter infection is more in patients with central venous lines, vesicle probes, water deposits, dialysis fluid, humidifiers and oxygen systems, hospital mattresses **(2001)**.[50]

CHOPADE. B.A. et al isolated Acinetobacter spp from upper respiratory tract of healthy humans. The colonization of bacteria on human mucosal surface tissue is a pre-requisite for any infection. The attachment of bacteria to human tissue requires adhesion factors like lectins. The lectins are principally carbohydrate binding proteins. They are non-enzyme, non-immunoglobulin proteins that have at least one carbohydrate-binding domain. The lectin carbohydrate interactions could be one of the possible ways for bacterial attachment to mucosal surfaces where the lectins reside on bacteria and the host surfaces present the carbohydrate receptors **(2001).**[14]

HENRIKSEN, PATIL & CHOPADE, et al studied about the inflow of plasmids in Acinetobacter genospecies. *Acinetobacter* contains plasmid mediated resistance to different antibiotics and metals. It also has plasmids which code for antibiotic production and indole acetic acid production **(2001).**[27]

DANES. C studied distribution of beta-lactamases in Acinetobacter baumannii clinical isolates and the effect of syn 2190 (Ampc inhibitor) on the MICS of different (beta) lactam antibiotics **(2002).**[16]

JANN–TAY–WANG et al studied community acquired Acinetobacter baumannii bacteremia in 19 adult patients in Taiwan. They found that malignancy was the most frequent underlying diseases. All 14

isolates obtained from the adult patients were identified as A. baumannii by 16s ribosomal DNA sequencing and were found to be genetically distinct by pulsed-field-gel electrophoresis **(2002).**[31]

LT COL K.K. LAHIRI et al studied that Acinetobacter spp as "emerging nosocomial pathogen". They are usually considered to be opportunistic pathogens, and of recent have been reported to cause a number of outbreaks of nosocomial infections in hospitalized patients like septicemia, pneumonia, wound sepsis, endocarditis and meningitis **(2004).**[40]

PRASHANTH. K. et al studied invitro susceptibility pattern of Acinetobacter species to commonly used Cephalosporins, Guinolones and Aminoglycosides. The combination therapy, using a third generation Cephalosposin and Amikacin could be the best choice for treating Acinetobacter infections **(2004).**[54]

VINODKUMAR. CS. et al highlights Acinetobacter spp as important pathogens in neonatal blood stream infection. Septicaemia due to acinetobacter spp are common in babies with predisposing factors like intravascular catheterization, endotrocheal intubation, parenteral nutrition, broad spectum antibiotis therapy and antificial ventilation **(2004).**[73]

YOHEI DOI, JUN-ICHI WACHINO, et al studied the spread of "Novel Aminoglycoside Resistance Gene aac(6')-Iad among Acinetobacter" from clinical Isolates in Japan A novel aminoglycoside resistance gene, *aac*(*6*')-*Iad*, encoding aminoglycoside 6'-*N*-acetyltransferase, was identified in *Acinetobacter* genospecies 3 strain A-51. The gene encoded a 144-amino-acid protein, which shared modest identity (up to 36.7%) with some of the aminoglycoside 6'-*N*-acetyltransferases. The results of high-pressure liquid chromatography assays confirmed that the protein is a functional aminoglycoside 6'-*N*-acetyltransferase. The enzyme conferred resistance to Amikacin, Tobramycin, Sisomicin and Isepamicin but not to Gentamicin **(2004).**[75]

DENTON M, WILCOX MH et al studied about "Role of environmental cleaning in controlling an outbreak of Acinetobacter baumannii on a neurosurgical intensive care unit". Use of 1000 ppm hypochlorite solution and the introduction of new cleaning protocols reduced the number of environmental isolates. Failure to maintain low levels of environmental contamination with A. baumannii resulted in increase in patient colonization. This study showed that high standards of cleaning play an integral role in controlling outbreaks of A. baumannii in the intensive care unit setting **(2005).**[17]

DIJKSHOORN L, VAN AKEN E, et al studied about the "Prevalence of Acinetobacter baumannii and other Acinetobacter spp. in faecal samples from non-hospitalised individuals". The predominant species were Acinetobactor johnsonii and genomic sp. 11. A. baumannii did not seem to be widespread in the faecal flora of individuals in the community **(2005).**[19]

KEPLER A. DAVIS et al studied multidrug resistant. Acinetobacter spp causing extremity infections in soliders war wound infection and osteomyelitis causing by multidrug resistant (MDR) Acinetobacter species have been prevalent during the 2003 – 2005 miltary operations in Iraq. Twenty three soliders wounded in Iraq were admitted in the hospital and had wound cultures positive for Acinetobacter calcoaceticus – baumannii complex **(2005).**[35]

ZAKEYA BUKHARY et al isolated multidrug resistant Acinetobacter baumannii causing meningitis in female patient who had a pesterior fossa craniotomy with upper cervical laminectomy for excission of a meningioma at the level of foramen magnum. He had mentioned the possibility of occurence of Acinetobacter baumannii in intensive care unit, mechanical ventilation and the use of broad spectrum antimicrobial therapy **(2005).**[77]

MATERIALS AND METHODS

This prospective study was conducted at Government Rajaji Hospital attached to Madurai Medical College. The period of the study was 13 months (December 2004 to January 2006). The study population consisted of 500 patients admitted in different wards – Medicine, Surgery, Orthopaedics and Burns. (125 cases from each ward)

SELECTION OF PATIENTS FOR SAMPLE COLLECTION (500)

- Open injury following accidents – 138
- Wound infections following burns – 119
- Prolonged stay in intensive care unit – 68 (more than 7 days)
- Previous use of antibiotics – 92 (3rd generation cephalosporins)
- History of Malignancies – 21
- Diabetes mellitus patients with ulcers – 71

HOSPITAL ENVIRONMENTAL SAMPLES - (50)

- From pillows in surgical, medical, orthopaedic wards and burns wards – 10.
- From mattresses in surgical, medical, orthopaedic wards and burns wards – 10.
- From trolleys in surgical, medical, orthopaedic wards and burns wards – 10.
- From washbasin in surgical, medical, orthopaedic wards and burns wards – 10.
- From aerators in surgical, medical, orthopaedic wards and burns wards – 10.

For the collection of data, standard proforma was used (Annexure I) and details were recorded.

COLLECTION OF SPECIMENS

- Sterilised and moistened cotton swabs were used for the wounds, in open injury following accidents, burns, diabetic ulcer and post operative infections.
- Wounds were wiped with sterile saline, with all aseptic precautions and swabs were taken from the depth of the wound. If the discharge was minimal, edges of the wound were squeezed to expel the contents.
- Using sterile syringes pus was aspirated from the abscesses in open injury following accidents, burns, diabetic ulcer and post operative infections and patients with history of previous use of antibiotics.
- In meningitis cases in intensive care units, cerebro spinal fluid was collected aseptically using sterile syringes through lumbar puncture.
- From patients having pneumonia, respiratory infections and malignancies, coughed out sputum was collected using sterile container.
- Skin was disinfected and pleural fluid was collected from patients having pleural effusion.

- sterile swabs were used for environmental sample collection.
- Sterile, screw capped tubes or screw capped containers were used for the collection of specimen.
- All the clinical samples collected from the patients and the environmental sources were transported to the Microbiology lab in a sterile screw capped container without any delay. In the lab, glucose broth was added to the sample containers and incubated for four hours before processing.

PROCESSING

Day 1

Gram's staining was done for all the samples. Two swabs were taken, one was for Gram staining and the other for culturing.

The plates were selected according to the Gram's reaction. For Gram positive cocci, nutrient agar and 5% sheep blood agar were selected. For Gram negative bacilli, nutrient agar, MacConkey agar and 5% sheep blood agar were selected. The samples were streaked over plates and incubated at 37^0C overnight.

Day 2

Culture plates were examined for colony morphology.

Nutrient agar plate

Colourless, low convex, translucent small colonies were selected and stained for Grams staining.

Gram variable coccobacillary forms in pairs or short chains were considered as Acinetobacter and processed further.

MacConkey agar plate

Small colonies with a pale pinkish tint were selected for Grams staining and motility.

5% Sheep Blood agar plate

Small dull coloured hemolytic or non hemolytic colonies were selected for Grams staining and motility.

Motility

Motility was tested by hanging drop method. The suspected colonies were inoculated into peptone water, incubated at 37^0C for 3-6hrs. Acinetobacter species were non motile.

GRAM VARIABLE COCCOBACILLARY FORMS WHICH WERE NON MOTILE WITH THE ABOVE COLONIAL MORPHOLOGY WERE SUBJECTED FOR THE FOLLOWING BIOCHEMICAL REACTIONS.

Catalase test, Oxidase test, Inoculation into Triple sugar iron agar medium, Indole test, Citrate test, Urease test, Nitrate reduction, Decarboxylation of Arginine, rapid utilisation of O/F Glucose & 10% Lactose, Growth at 44^0C.

CATALASE TEST

Procedure

- Freshly prepared H_2O_2 was placed on colonies on nutrient agar.
- Production of prompt effervescence indicates catalase production. **Acinetobacter species were catalase positive.**

The colonies which were catalase positive were further tested for the following tests.

Oxidase test
Procedure

Freshly prepared 1.0 – 1.5% solution of tetramethyl p-phenylene diamine hydrochloride was poured on a filter paper placed in a pertridish and the colony to be tested was streaked on the filter paper in a line about 5mm long. The smeared line turned dark purple in 10 seconds. This denotes that the organism was oxidase positive.

Oxidase negative organism were considered as Acinetobacter.

Day – 3

FOR CONFIRMATION OF THE ISOLATE OTHER BIOCHEMICAL TESTS AND O/F TEST DONE

Triple sugar iron AGAR medium (TSI)

The triple sugar iron agar medium contains 3 sugars glucose, lactose, sucrose. The medium was distributed in tubes with a butt and

slant. The organism was stabbed to the butt and streaked on the slant. TSI was incubated for 18-24 hours at 37^0C, and then looked for the presence of growth and fermentation. Nonfermenters including Acinetobacter species showed no fermentation in the TSI Agar.

Indole Test

This test demonstrates the production of indole by splitting tryptophane. In peptone water culture after 48 or 72 hours incubation at 37^0C, Kovac's reagent 0.5ml was added gently on the sides.

A red colour was taken as positive. **Acinetobacter species showed indole negative reaction.**

CITRATE UTILIZATION TEST USING SIMMON'S Citrate MEDIUM

The organisms were streaked on the surface of the slant of Simmon's citrate medium and incubated at 37^0C for 18-24 hours. Development of deep blue colour of the medium with growth was taken as positive. **Acinetobacter species were citrate positive.**

UREASE TEST

Christensen's urease medium was used. The organism was streaked on the slop heavily and incubated at 37^0C for 18-24 hours. Urease positive cultures produced a purple pink colour. **Acinetobacter species produced no change in colour due to absence of urease**

enzyme.

NITRATE REDUCTION TEST

This was tested after growing the bacterium for five days at 370C in a broth containing 1% KNO3. The presence of nitrites in the test medium was detected by the addition of α- naphthylamine and sulfanilic acid, with the formation of a red diazonium dye, p-sulfobenzene-azo-α-naphthylamine. A loopful of isolated colonies were inoculated in the nitrate medium and incubated at 350C for 5 days. At the end of incubation, 1ml of each of reagents A and B were mixed just before use and added. A red colour developing within a few minutes signified a positive reaction, while absence of colour indicated a negative reaction. **Acinetobacter species showed negative reaction.**

DECARBOXYLASES
PROCEDURE

Well isolated colonies from the nutrient agar plate were inoculated in two tubes of Moeller decarboxylase medium one containing, amino acids and other devoid of amino acids which was used as a control tube. Sterile mineral oil about 1cm was covered on the surface of the tubes and incubated at 35^0C for 18-24hrs. **Acinetobacter species were arginine decarboxylase positive.**

OXIDATION-FERMENTATION TEST (HUGH AND LEIFSON)
PROCEDURE

Two tubes containing OF medium were inoculated heavily with the isolated colonies using a sterile loop. One tube was covered with a 1-cm layer of sterile mineral oil or melted paraffin, and other tube remained open. Both tubes were incubated at 35^0C and examined daily for 2-3 days. Sugars like 10% Glucose, 1% Lactose, 1% Sucrose, 1% Mannitol and 1% Mannose were tested for oxidative utilization. **Acinetobacter species utilized only 10% glucose and 1% lactose.**

GROWTH AT 44^0C

This was done to identify A. baumanni. Suspected Acinetobacter colonies were inoculated in to the peptone water and incubated at 44^0C in a water bath for 8 hrs. Loopful of colonies from the peptone water which was kept in the water bath were streaked on the nutrient agar plate and incubated overnight. **The presence of growth indicated Acinetobacter baumannii strain.**

Specification of Acinetobacter organisms

The following biochemical tests were done to speciate the acinetobacter

No	Organism	Nitrate reduction	Growth at		Hemolysis Sheep Blood	O/F 10% Glucose	Arginine
			37^0C	44^0C			
1	A. baumanii	-	+	+	-	+	+
2	A. hemolyticus	-	+	-	+	V	+
3	A. calcoaceticus	-	+	-	-	+	-

ANTIBIOTIC SENSITIVITY

Acinetobacter isolated from both the clinical and environmental

samples were subjected to antibiotic sensitivity testing by the "Disc diffusion" method of Kirby Bauer *et al.,* 1960. Hi media discs were used.

DISC SUSCEPTIBILITY TESTING

Acinetobacter colonies from the agar plates were inoculated into peptone water and incubated at 37^0C for 18-24hrs

↓

Spreaded 100µl of incubated peptone water that had McFarland 0.5 standard turbidity of the medium on MHA plate

↓

Antibiotic discs on agar surface were placed aseptically. Discs were evenly distributed on the plate so that they were no closer than 24mm from centre to centre

↓

Incubated at 37^0C / 16 – 18 hrs

↓

Zones of inhibition (in mm) measured using calipers

↓

Results were interpreted as sensitive, intermediate and resistant by comparing the inhibition zone diameters with the ranges recommended by company for standards

All antibiotic resistant Acinetobacter isolates were screened for the presence of plasmids

"Plasmid isolation and detection of plasmid DNA by Agarose Gel Electrophoresis was done at Department of Microbiology, University of Pune".

Method of Plasmid Isolation

For the isolation of DNA plasmid from Acinetobacter, the organism was grown on Luria broth, kept at 37^0C overnight in shaker's incubator

Luria broth contains

Tryptone 10 gms, Yeast extract 5 gms, Nacl 10 gms, pH 7.2, The above components were mixed in 1 lit of sterile distilled water, mixed well, pH adjusted for 7.2 and sterilized by keeping in the autoclave at 121^0C for 15mins. The inoculated Luria broth showing the growth of 10^6 colony forming units was selected for plasmid DNA isolation.

Plasmid isolation by Sambrook & Maniatis Method (1989) (Alkali lysis method)

1.5 ml overnight culture grown in Luria broth (37^0C, 150rpm)

↓

Centrifuged at 8,000 rpm for 5 min at room temperature to get the pellet

↓

Added 100µl of icecold solution I ***(50mM glucose, 25mM Tris HCl, 10mM EDTA, pH8)*** to the microfuge tube.

↓

Kept on ice for 5mins.

↓

The pellet was mixed by vortexing

↓

200µl of freshly prepared solution II ***(1% SDS in 0.2N NaOH)*** added, mixed by inverting the tubes gently and kept on ice for 5mins.

↓

150µl of ice-cold solution III added

(5M potassium acetate 60ml, glacial acetic acid 11.5ml, distilled water 28.5ml, pH5.6) solution III

↓

Kept on ice 10 min

↓

Equal volume of Phenol : chloroform (1:1) added

↓

Mixed by inverting at least for 100-200 times till it became milky

↓

Centrifuged at 10,000 rpm at R.T. for 15 minutes

↓

Avoiding precipitate at interface, transferred aqueous layer to new microfuge tube.

↓

By adding equal volume of Isopropanol, the plasmid DNA precipitated

↓

Kept at R.T. for 1 hr and centrifuged at 10,000 rpm – 15 mins at R.T.

↓

Decant the isopropanol and dry the pellet

↓

The pellet was dissolved in 50µl – T10 E1 buffer (10mM EDTA, 1mM Tris, pH 8)

STEPS IN PLASMID ISOLATION

S. NO	STEPS	OBSERVATION
1	EDTA Buffer	Cell pellet → turbidity
2	Lysis solution	Turbid solution → clear viscous solution string formation
3	Phenol chloroform on centrifugation	Milk white mixture. 3 layers were obtained 1st Layer → aqueous supernatant 2nd Layer → Proteins & Lipid 3rd Layer → Chloroform layers
4	Isopropanol on centrifugation	Cotton ball appeared. Plasmid DNA precipitated
5	$T_{10}E_1$ & Tapping	Plasmid DNA got dissolved

(Tris, EDTA Buffer)

Tris buffer is the recommended buffer for polymerase chain reaction. (10-50mM tris Hcl), pH8.3 - 8.8 at 20^0C.

DETECTION OF PLASMID DNA BY AGAROSE GEL ELECTROPHORESIS

MATERIALS AND METHOD

Isolates of genomic DNA & plasmid DNA, 0.8% agarose solution in TAE buffer (pH 7), Cleaned electrophoresis apparatus, Sterile microtips, Ethidium – Bromide staining solution, Destainer (Distill Water), BPB –

sucrose loading buffer.

PROCEDURE

The electrophoresis apparatus was cleaned properly, 0.8% agarose in TAE buffer was prepared and warmed in oven, the molten agarose gel was allowed to cool slightly & poured into the boat in which comb was placed, gel was allowed to solidify & then comb was removed carefully, insulating tape was also removed, the gel was placed in a tank of buffer, 1:1 dilution of DNA isolate & loading buffer was made, 20μl of mixture was applied to cathode end having wells, electrodes were joined to power system & current was allowed to run, current was stopped when tracking dye BPB reached to other end, gel was moved carefully & immerse in 0.5μg/day staining solution of Ethidium - Bromide for 30mints, Gel was destained with distilled water & documented.

RESULTS

The isolated bands of plasmid DNA from clinical isolates along with standard strain plasmids band near dye front were detected in their respective preparation wells.

RESULTS

A total of 500 samples were collected from Surgical, Medical, Orthopaedics and Burns ward of Government Rajaji Hospital, Madurai, 125 samples from each.

In General Surgery, 71 Samples were taken from Diabetic ulcer, 24 from wound infections and 30 from patients with history of use of antibiotics (3rd generation of cephalosporin) within the past 14 days. In Medicine, 68 Samples were taken from patients in Intensive Care Units, 12 from patients with Malignancy and 45 from patients with previous use of antibiotics (3rd generation of cephalosporin) within the past 14 days. In Orthopaedics, 114 samples were collected from patients with wound infection, 11 from patients with previous use of antibiotics (3rd generation of cephalosporin) within the past 14 days. In Burns, 119 samples were collected from patients with burns and 6 from patients with previous use of antibiotics (3rd generation of cephalosporin) within the past 14 days. (Table 1)

Among these 500 samples, 138 samples were from open injury following road traffic accidents; 119 from wound infections following burns; 68 from patients in intensive care unit; 92 from patients who had taken previous use of antibiotics (3rd generation of cephalosporin) within the past 14 days; 71 from patients with diabetic ulcers and 12 from patients with malignancy. (figure 1)

TABLE – 1

SAMPLE COLLECTION BASED ON CLINICAL DIAGNOSIS IN VARIOUS WARDS

Sl.No	**Name of ward**	***Case Selection***	**No. of Cases**	**Total**

1	General Surgery	Diabetic Ulcer	71	125
		Wound Infection	24	
		Patients with previous use of antibiotics with in the past 14 days	30	
2	Medicine	ICU	68	125
		Malignancy	12	
		Patients with previous use of antibiotics with in the past 14 days	45	
3	Orthopaedic surgery	Wound Infection	114	125
		Patients with previous use of antibiotics with in the past 14 days	11	
4	Burns	Burns	119	125
		Patients with previous use of antibiotics with in the past 14 days	6	
			Total	**500**

A total of 50 environmental samples were collected from General Surgery, Medical, Orthopaedics and Burns wards, 10 from each of Pillows, Mattresses, Wash Basins, Trolleys and Aerators. Thus 13 environmental samples from General Surgery, 12 from Medical, 13 from Orthopaedics and 12 from Burns were collected. This is given in Table 2.

TABLE – 2

COLLECTION OF SAMPLES FROM VARIOUS ENVIRONMENTAL SOURCES

Sl. No	Wards	No. of Samples from Environmental Sources					Total
		Trolley	Mattresses	Wash Basin	Aerators	Pillows	
1	General Surgery	3	3	2	2	3	13
2	Medical	2	2	3	3	2	12
3	Orthopaedics	3	3	3	2	2	13
4	Burns	2	2	2	3	3	12
		10	**10**	**10**	**10**	**10**	**50**

All the clinical samples collected showed the growth of either Gram-positive cocci or Gram negative fermenters or Gram negative Non-fermenters. In general surgery wards, out of 125 samples 14 (11.2%) were Gram Positive Cocci, 42 (33.6%) were Gram Negative fermenters and 69 (55.2%) were Gram Negative Non-fermenters. In the medical wards, out of 125 samples, 17 (13.6%) were Gram Positive Cocci, 52 (41.6%) were Gram Negative fermenters and 56 (45%) were Gram Negative Non-fermenters. In orthopaedic wards, out of 125 samples 13 (10.4%) were Gram Positive Cocci, 44 (33.4%) were Gram Negative fermenters and 68 (54.4%) were Gram Negative Non-fermenters. In burns wards, out of 125 samples 15 (12%) were Gram Positive Cocci, 58 (46.4%) were gram-negative fermenters and 52 (42%) were Gram Negative Non-fermenters. In a total of 500 isolates, 59 (11.8%) were Gram Positive Cocci, 196 (39.2%) were Gram Negative fermenters and 245 (49%) were Gram Negative Non-fermenters.

The prevalence of bacteria from the various wards is given in Figure 2.

Out of 245 Gram Negative Non Fermenters isolated from various clinical samples Acinetobacter species were 102 (43%) and other Gram Negative Non Fermenters were 143 (58.4%). In general surgery, out of 69 Gram Negative Non Fermenters, 26 (37.6%) were Acinetobacter species and 43 (62.3%) were other Gram Negative Non Fermenters. In medical wards, out of 56 Gram Negative Non Fermenters, 21 (37.5%) were Acinetobacter species and 35 (62.5%) were other Gram Negative Non Fermenters. In orthopedic wards, out of 68 Gram Negative Non Fermenters, 31 (45.6%) were Acinetobacter species and 37 (54.4%) were other Gram Negative Non Fermenters. In Burns wards, out of 52 Gram Negative Non Fermenters, 24 (46.2%) were Acinetobacter species and 28 (53.9%) were other Gram Negative Non Fermenters.

Acinetobacter species were isolated more in burns ward. The analysis of Acinetobacter according to the clinical presentation showed that above 45% Acinetobacter were isolated only from wound infections and burns. Stay in Intensive Care Units, Malignancy and patients with previous use of antibiotics had less incidence of Acinetobacter isolation.

The incidence of Acinetobacter species and other Non-Fermentive Gram Negative Bacilli isolated in various wards and various clinical presentation are given in Table 3.

Out of 50 samples collected from Environmental sources, 14% were Gram positive cocci, 22% were Gram negative fermenters and 64% were Gram negative Non-fermenters. Gram negative Non-fermenters were isolated more in the burns ward 56.2%. Among the Gram negative Non-fermenters isolated, 22% were Acinetobacter and 42% were other Gram negative Non-fermenters. Acinetobacter species were isolated more in burns ward 36.3%. Prevalence of bacterial isolates from

environmental samples and isolation of Acinetobacter species and other non fermenters in environmental samples are given in figure 3 & Table 4.

TABLE – 4

ISOLATION OF ACINETOBACTER SPECIES AND OTHER NON FERMENTERS IN ENVIRONMENTAL SAMPLES

S. No	**Wards**	**Total Non-fermenters**	**Acinetobacter species**	**Other Gram Negative Non-Fermenters**
1	General Surgery	5 (15.6)	2 (18.1)	3 (14.2)
2	Medical	7 (21.8)	3 (27.2)	4 (19)
3	Ortho	8 (25)	2 (18.1)	6 (28.5)
4	Burns	12 (37.5)	4 (36.3)	8 (38)
Total		**32 (64%)**	**11 (22%)**	**21 (42%)**

(Parenthesis denotes percentage)

Out of 50 samples collected from Environmental sources, 11 (22%) were Acinetobacter species. Out of 11 isolates, 4 (36.3%) were from burns ward, 2 from General surgery (18.1%), 3 from Medical (27.2%) and 2 from orthopedic (18.1%) wards.

Out of 11 Acinetobacter isolates 6 from mattresses, 3 from wash basin, 1 from trolley and 1 from aerator.

Out of 11 Acinetobacter isolates, one was from **trolley** in general surgery ward. Out of 6 Acinetobacter isolates from **mattresses**, 4 were from burns ward, one from orthopaedics ward and one from medical

ward. Three isolates were from **wash basin**, one each from general surgery, medical and orthopaedic wards and one isolate was from **aerator** available in medical ward. Acinetobacter was not isolated from **pillows**. The maximum no. of isolation was from mattresses.

The isolation of Acinetobacter species from environmental sources is given in Table 5.

TABLE – 5

ISOLATION OF ACINETOBACTER SPECIES FROM ENVIRONMENTAL SOURCES

Sl. No	Environmental Sources	No. of Acinetobacter isolates	General Surgery	Medical	Ortho pedics	Burns
1	Trolley	1 (9)	1 (50)	-	-	-
2	Mattresses	6 (54.5)	-	1 (33.3)	1 (50)	4 (100)
3	Wash Basin	3 (27.2)	1 (50)	1 (33.3)	1 (50)	-
4	Aerators	1 (9)	-	1 (33.3)	-	-
5	Pillows	-	-	-	-	-
Total		**11 (22)**	**2 (18.1)**	**3 (27.2)**	**2 (18.1)**	**4 (36.3)**

(Parenthesis denotes percentage)

Acinetobacter were speciated into A. baumannii, A. hemolyticus and A. calcoaceticus according to the biochemical reactions and confirmation by API system. Out of 102 Acinetobacter isolates, 47 (46.1%) were Acinetobacter baumannii, 33 (32.3%) were Acinetobacter hemolyticus and 22 (21.6%) were Acinetobacter calcoaceticus.

In General surgery wards, out of 26 Acinetobacter isolates, 11 (42.2%) were Acinetobacter baumannii, 8 (30.8%) were Acinetobacter hemolyticus and 7

(26.9%) were Acinetobacter calcoaceticus. In Medical wards, out of 21 Acinetobacter, 12 (57.1%) were Acinetobacter baumannii, 6 (28.6%) were Acinetobacter hemolyticus and 3 (14.3%) were Acinetobacter calcoaceticus. In Orthopedics wards, out of 31 Acinetobacter, 18 (58.1%) were Acinetobacter baumannii, 9 (29%) were Acinetobacter hemolyticus and 4 (12.9%) were Acinetobacter calcoaceticus. In Burns wards, out of 24 Acinetobacter, 6 (25%) were Acinetobacter baumannii, 10 (41.7%) were Acinetobacter hemolyticus and 8 (33.3%) were Acinetobacter calcoaceticus. Acinetobacter baumannii was isolated more in orthopaedic wards, Acinetobacter hemolyticus and Acinetobacter calcoaceticus in Burns wards.

The prevalence of Acinetobacter genospecies in various wards is given below in Table No.6.

TABLE – 6

SPECIATION OF ACINETOBACTER ISOLATES FROM CLINICAL SAMPLES IN VARIOUS WARDS

S. No	Wards	Total No Isolates	Acinetobacter baumannii	Acinetobacter hemolyticus	Acinetobacter calcoaceticus
1	General Surgery	26 (37.7)	11 (42.3)	8 (30.8)	7 (26.9)
2	Medical	21 (37.5)	12 (57.1)	6 (28.6)	3 (14.3)
3	Ortho paedic	31 (45.6)	18 (58.1)	9 (29)	4 (12.9)
4	Burns	24 (46.2)	6 (25)	10 (41.7)	8 (33.3)
Total		**102(20.4)**	**47 (46.1)**	**33 (32.3)**	**22 (21.6)**

(Parenthesis denotes percentage)

Out of 11 isolates of Acinetobacter from environmental samples, 5 (45.4%) were Acinetobacter baumannii, 2 (18.1%) were

hemolyticus and 4 (36.3%) were A. calcoaceticus. Among the 5 isolates of Acinetobacter baumannii, 2 were isolated from general surgery, 1 from medical and 2 from orthopedic wards. Among the 2 isolates of Acinetobacter hemolyticus, 1 was from general surgery and 1 from orthopaedic wards. All the 4 Acinetobacter calcoaceticus were only from Burns wards.

The speciation of Acinetobacter isolates from environmental sources is given in Table No.7.

TABLE – 7

SPECIATION OF ACINETOBACTER ISOLATES FROM ENVIRONMENTAL SOURCES IN VARIOUS WARDS

S. No	Wards	Total No Isolates	Acinetobacter baumannii	Acinetobacter hemolyticus	Acinetobacter calcoaceticus
1	General Surgery	3 (27.2)	2 (40)	1 (50)	-
2	Medical	1 (9)	1 (20)	-	-
3	Ortho paedic	3 (27.2)	2 (40)	1 (50)	-
4	Burns	4 (36.3)	-	-	4 (100)
Total		**11 (22)**	**5 (45.4)**	**2 (18.1)**	**4 (36.3)**

(Parenthesis denotes percentage)

Clinical Isolates of Acinetobacter species were further analysed according to the various specimens collected. Out of 47

Acinetobacter baumannii isolated, 23 (48.9%) were from Pus, 9 (19.1%) from wound swab, 10 (21.2%) from sputum and 5 (10.6%) from pleural fluid. **Out of 33 Acinetobacter hemolyticus isolated,** 23 (69.6%) from pus, 3 (9%) from wound swab, 5 (15.1%) from sputum and 2 (6%) from cerebro spinal fluid. **Out of 22 Acinetobacter calcoaceticus isolated,** 14 (63.6%) from Pus, 5 (22.7%) from wound swab, 2 (9%) from sputum and 1(4.5%) from CSF. Among the culture positive samples, 59% were from pus, 16.6% from the wound swab, 16.6%, from the sputum, 4.9% from the pleural fluid and 2.9% from the CSF.

Acinetobacter baumannii was isolated more from pus (48.9%) and not isolated from CSF, Acinetobacter hemolyticus was isolated more from pus (69.6%), and not isolated from pleural effusion. Acinetobacter calcoaceticus was isolated more from pus (63.6%) and not isolated from pleural effusion. Acinetobacter baumannii was not isolated from CSF and Acinetobacter hemolyticus and Acinetobacter calcoaceticus were not isolated in pleural fluid. Pus was the common specimen for isolation of all the three species.

The Isolation of Acinetobacter species from various samples is given in Figure-4.

Environmental isolates of Acinetobacter were further analysed according to the various sources collected. Out of 5 (45.5%) Acinetobacter baumannii isolated, 1 (20%) was from trolley, 1 (20%) from mattress, 2 (40%) from washbasin and 1 (20%) from aerators. Out of 2 (18.1%) Acinetobacter hemolyticus 1 (50%) was from mattress and 1 (50%) from wash basin. All the 4 (36.3%) Acinetobacter calcoaceticus were from mattresses (100%). Thus it was found that 6 isolates were from mattresses 1 A. baumanni, 1 A. hemolyticus and 4 A. calcoaceticus. No isolates of Acinetobacter hemolyticus were from trolley and aerators. No isolates of Acinetobacter calcoaceticus were from trolley, washbasin and aerators.

The Isolation of Acinetobacter species from various environmental sources specimens is given in Figure-5.

The drugs sensitivity patterns for the various Acinetobacter species showing more than 30% sensitivity of clinical isolates were analysed. **Acinetobacter baumannii** were sensitive to Carbenicillin (36.2%), Ceftriaxone (30%) and Imipenem (34.1%). **Acinetobacter hemolyticus** were sensitive to Imipenem (39.3%) and Ceftriaxone (39.3%) **Acinetobacter calcoaceticus** were sensitive to Imipenem (31.8%). Thus Imipenem was the only antibiotic for which all the three species of Acinetobacter showed more than 30% sensitivity.

Antibiotic microbial sensitivity pattern of Acinetobacter species for common antibiotics is given in Table 8.

The drug resistant patterns for the various Acinetobacter species showing more 60% resistance of clinical samples were analysed. **Acinetobacter baumannii** were resistant to Carbenicillin (63.8%), Ceftriaxone (70.2%), Imipenem (65.9%) Cotrimoxazole (78.7%), Ampicillin (87.2%), Gentamicin (82.9%), Ciprofloxacin (78.7%) Cefotaxime (89.4%), Doxycycline (87.2%), Amikacin (83%) and Piperacillin (89.4%). **Acinetobacter hemolyticus** were resistant to Imipenem (60.6%), Carbenicillin (75.8%), Amikacin (75.8%) Cotrimoxazole (81.1%), Ampicillin (87.8%), Gentamicin (93.9%), Ciprofloxacin (75.7%), Cefotaxime (72.7%), Doxycycline (87.9%), Ceftrioxazone (60.6%) and Piperacillin (90.9%). **Acinetobacter calcoaceticus** were resistant to Imipenem (68.2%) Carbenicillin (77.3%), Ceftriaxone (77.3%), Cefotaxime (90.9%), Amikacin (86.4%), Ampicillin (95.4%), Gentamicin (95.4%), Ciprofloxacin (81.8%), Cotrimoxazole (86.4%), Doxycycline (90.9%) and Piperacillin (95.5%). It was found that Ampicillin, Gentamicin and Piperacillin showed more than 95% resistance in Acinetobacter calcoaceticus only.

Antibiotic resistant pattern of Acinetobacter species for common antibiotics is given in Table 9.

The drug sensitivity patterns for the various Acinetobacter species showing more than 30% sensitivity in the environmental samples were analysed. Acinetobacter baumannii were sensitive to Carbenicillin (40%), Imipenem (40%), Ampicillin (60%), Gentamicin (40%), Ciprofloxacin (40%), Piperacillin (60%). Cotrimoxazole (40%) Cefotaxime (80%), Doxycycline (60%), Ceftrioxazone (60%) and Amikacin (40%) **Acinetobacter hemolyticus** were sensitive to Ampicillin (50%), Gentamicin (50%), Cotrimoxazole (50%), Ciprofloxacin (100%), Doxycycline (50%), Ceftrioxazone (50%), Amikacin (50%), Piperacilin (50%), Carbenicillin (50%) and Imipenem (50%), **Acinetobacter calcoaceticus** were sensitive to Imipenem (50%), Carbenicillin (50%) and Cefotaxime (75%).

Thus imipenem and carbenicillin were the only antibiotics for which all the three species were sensitive.

Antibiotic sensitivity pattern of Acinetobacter species for common antibiotics is given in Table 10.

The drug resistant patterns for the various Acinetobacter species showing more than 60% resistance of environmental samples were analysed. Acinetobacter baumannii were resistant to Carbenicillin (60%), Imipenem (60%), Gentamicin (60%), Cotrimoxazole (60%), Cefotaxime (60%), Amikacin (60%) and Ciprofloxacin (60%). **Acinetobacter hemolyticus** were resistant to Cefotaxime (100%). **Acinetobacter calcoaceticus** were resistant to Ceftriaxone (75%), Ampicillin (100%), Gentamicin (100%), Cotrimoxazole (75%), Doxycyline (75%), Amikacin (75%), Piperacillin (100%), Carbenicillin (100), Imipenem (100%) and Ciprofloxacin (75%). It was found that in Acinetobacter calcoaceticus all the antibiotics except Cefotoxime, Imipenem and Cabenicillin showed more than 60% resistance and the antibiotics Ampicillin, Gentamicin and Piperacillin showed more than 95% resistance.

Antibiotic resistant pattern of Acinetobacter species for common antibiotics of environmental samples is given in Table 11.

The antibiotic sensitivity and resistance for more than 4 common antibiotics in isolates from clinical and environmental samples were analysed. The antibiotics used were Ampicillin, Gentamicin, Cotrimoxazole, Cefotaxime, Doxycycline, Ceftriaxone, Ciprofloxacin, Amikacin, Piperacillin, Carbenicillin, Imipenem. **Out of 47 Acinetobacter baumannii isolates from clinical samples**, 25 (53.2%) showed sensitivity and 22 (46.8%) were resistant to more than 4 antibiotics. **Out of 33 Acinetobacter hemolyticus isolated from clinical samples**, 13 (39.4%) showed sensitivity to common antibiotics and 20 (60.4%) were resistant to antibiotics. **Out of 22 Acinetobacter calcoaceticus from clinical samples**, 8 (36.4%) showed sensitivity to common antibiotics and 14 (63.6%) were resistant to antibiotics. Among the isolates, Acinetobacter calcoaceticus showed maximum resistance (63.6%), followed by Acinetobacter hemolyticus showed moderate resistance (60.4%) and then Acinetobacter baumannii showed least resistance (46.8%).

Out of 5 Acinetobacter baumannii isolates from Environmental samples 3 (60%) were sensitive for more than 4 antibiotics and 2 (40%) were resistant for more than 4 antibiotics. **Out of 2 Acinetobacter hemolyticus isolates from Environmental samples** 1 (50%) was sensitive and 1 (50%) was resistant and **Out` of 4 Acinetobacter calcoaceticus isolates from Environmental samples** 1 (25%) was sensitive and 3 (75%) were resistant.

Thus Acinetobacter calcoaceticus in environmental samples were more resistant when compared to Acinetobacter baumannii and Acinetobacter hemolyticus.

Clinical and environmental isolates of Acinetobacter species showing sensitivity and resistance to common antibiotics to more than 4 drugs is given in Table 12 & 13.

TABLE – 12

CLINICAL ISOLATES OF ACINETOBACTER SHOWING SENSITIVITY AND RESISTANCE TO COMMON ANTIBIOTICS

S.No	SPECIES	TOTAL NO OF ISOLATES	SENSITIVITY FOR > 4 ANTIBIOTICS	RESISTANCE FOR > 4 ANTIBIOTICS
1	Acinetobacter baummannii	47 (46)	25 (53.2)	22 (46.8)

2	Acinetobacter hemolyticus	33 (32)	13 (39.4)	20 (60.4)
3	Acinetobacter calcoaceticus	22 (21)	8 (36.4)	14 (63.6)

(Parenthesis denotes percentage)

TABLE – 13

ENVIRONMENTAL ISOLATES OF ACINETOBACTER SHOWING SENSITIVITY AND RESISTANCE TO COMMON ANTIBIOTICS

S.No	SPECIES	TOTAL NO OF ISOLATES	SENSITIVITY FOR > 4 ANTIBIOTICS	RESISTANCE FOR > 4 ANTIBIOTICS
1	Acinetobacter baummannii	5 (45.4)	3 (60)	2 (40)
2	Acinetobacter hemolyticus	2 (18.1)	1 (50)	1 (50)
3	Acinetobacter calcoaceticus	4 (36.3)	1 (25)	3 (75)

(Parenthesis denotes percentage)

All the clinical isolates showing resistance to all common antibiotics were further studied for plasmid profile and the following results were obtained.

General Surgery Ward

Out of 5 antibiotic resistant ***Acinetobacter baummannii***

isolated, 1 isolate showed 1 band, 2 showed 2 bands, 1 showed 4 bands and ***1 isolate showed 8 bands.*** *Out of 6 isolates of antibiotic resistant Acinetobacter hemolyticus, 4 isolates showed 1 band, 1 isolate 5 bands, 1 isolate showed 6 bands. Out of 3 isolates of antibiotic resistant Acinetobacter calcoaceticus, 2 isolates showed 1 band, 1 isolate showed 2 bands.* ***Thus Acinetobacter baumannii showed more number of plasmid bands depicting that, it was the most common isolate causing multi drug resistance in general surgery wards.***

Medical Ward

Out of 6 isolates of antibiotic resistant ***Acinetobacter baummannii*** *isolated,* ***1 isolate showed 7 bands****, 1 showed 4 bands, 1 showed 3 bands, 2 isolates showed 2 bands, 1 isolate showed 1 band. Out of 5 isolates of antibiotic resistant Acinetobacter hemolyticus, 4 isolates showed one band, 1 isolate showed 2 bands. Out of 3 isolates of antibiotic resistant Acinetobacter calcoaceticus, 2 isolates showed one band, 1 isolate showed 1 band.* ***Thus Acinetobacter baummannii showed more number of plasmid bands depicting, it was the most common***

isolate causing multi drug resistance in medical wards.

Orthopaedic ward

Out of 8 isolates of antibiotic resistant ***Acinetobacter baummannii*** *isolated, 3 isolates showed 3 bands, 2 showed 4 bands, 1 showed 2 bands, 1 showed 1 bands* ***1 isolate showed 8 bands****. Out of 3 isolates of antibiotic resistant Acinetobacter hemolyticus, 1 isolate showed 4 bands, 1 isolate showed 2 bands, 1 isolate showed 1 band. Out of 3 isolates of antibiotic resistant Acinetobacter calcoaceticus, 1 isolate showed 7 bands, 1 isolate showed 4 bands, 1 isolate showed 3 bands. Thus* ***Acinetobacter baummannii showed more number of plasmid bands depicting, it was the most common isolate causing multi drug resistance in Orthopaedic wards.***

Burns

Out of 3 isolates of antibiotic resistant Acinetobacter baummannii, 1 showed 2 bands, 1 showed 3 bands and 1 showed 4 bands. Out of 6 isolates of antibiotic resistant Acinetobacter hemolyticus , 2 showed 2 bands, 1 showed 3 bands, 1 isolate showed 4 bands, 1 isolate showed 5 bands, 1 isolate showed 8 bands. Out of 5 isolates of antibiotic resistant

Acinetobacter calcoaceticus, 1 isolate showed 2 bands, 2 isolates showed 3 bands, 1 isolate showed 4 bands, **1 isolate showed 9 bands. Thus Acinetobacter calcoaceticus showed more number of plasmid bands indicating that, it is the most common isolate causing multi drug resistance in Burns wards.**

The plasmid profiles of Acinetobacter species isolated from various wards are given in the Table 14, 15, 16 & 17.

TABLE – 14

PLASMID PROFILES OF ACINETOBACTER SPECIES ISOLATED FROM CLINICAL SAMPLES SHOWING RESISTANCE TO COMMON ANTIBIOTICS IN GENERAL SURGERY WARD

Acinetobacter species	No. of Plasmid bands									No of isolates
	1	2	3	4	5	6	7	8	9	
Acinetobacter baumannii	1	2	-	1	-	-	-	1	-	5
Acinetobacter hemolyticus	4	-	-	-	1	1	-	-	-	6
Acinetobacter calcoaceticus	2	1	-	-	-	-	-	-	-	3

TABLE – 15

PLASMID PROFILES OF ACINETOBACTER SPECIES ISOLATED FROM CLINICAL SAMPLES SHOWING RESISTANCE TO COMMON ANTIBIOTICS IN MEDICAL WARD

Acinetobacter species	No. of Plasmid bands									No of Isolates
	1	2	3	4	5	6	7	8	9	
Acinetobacter baumannii	1	2	1	1	-	-	1	-	-	6
Acinetobacter hemolyticus	4	1	-	-	-	-	-	-	-	5
Acinetobacter calcoaceticus	2	1	-	-	-	-	-	-	-	3

TABLE – 16

PLASMID PROFILES OF ACINETOBACTER SPECIES ISOLATED FROM CLINICAL SAMPLES SHOWING RESISTANCE TO COMMON ANTIBIOTICS IN ORTHOPAEDIC WARDS

Acinetobacter species	No. of Plasmid bands									No of Isolates
	1	2	3	4	5	6	7	8	9	
Acinetobacter baumannii	1	1	3	2	-	-	-	1	-	8
Acinetobacter hemolyticus	1	1	-	1	-	-	-	-	-	3
Acinetobacter calcoaceticus	-	-	1	1	-	-	1	-	-	3

TABLE – 17

PLASMID PROFILES OF ACINETOBACTER SPECIES ISOLATED FROM CLINICAL SAMPLES SHOWING RESISTANCE TO COMMON ANTIBIOTICS IN BURNS WARD

Acinetobacter species	No. of Plasmid bands									No of Isolates
	1	2	3	4	5	6	7	8	9	
Acinetobacter baumannii	-	1	1	1	-	-	-	-	-	3
Acinetobacter hemolyticus	-	2	1	1	1	-	-	1	-	6
Acinetobacter calcoaceticus	-	1	2	1	-	-	-	-	1	5

The plasmid profile typing of Environmental samples in **surgery wards** showed that among the two isolates of Acinetobacter baumannii, first isolate showed 6 bands and second showed 2 bands and one isolate of Acinetobacter hemolyticus showed 4 bands. In **medical wards** one isolate of Acinetobacter baumannii showed two bands. In **orthopaedic wards** among the two isolates of Acinetobacter bauamannii first showed 2 bands and second showed 1 band. One isolate of Acinetobacter hemolyticus showed 3 bands. In **Burns wards** among the four isolates of Acinetobacter calcoaceticus, first showed 9 bands,

second showed 3 bands, third showed 2 bands and fourth isolate showed 1 band. Thus the plasmid bands shown in surgery, medical, and ortho wards of environmental isolates do not correlate with the plasmid bands shown in the clinical isolates of these wards whereas the plasmid bands shown in Acinetobacter calcoaceticus of environmental samples correlated with Acinetobacter calcoaceticus of clinical samples in burns ward.

Distribution of plasmids in different species of Acinetobacter isolated from Environmental sources is given in Table 18, 19, 20 & 21.

TABLE – 18

PLASMID PROFILE TYPING OF ACINETOBACTER SPECIES FROM ENVIRONMENTAL ISOLATES IN SURGERY WARD

Acinetobacter species	**No of Isolates**	**Various Isolates showing No. of Plasmid bands**								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Acinetobacter baumannii	2	-	1	-	-	-	1	-	-	-
Acinetobacter hemolyticus	1	-	-	-	1	-	-	-	-	-
Acinetobacter calcoaceticus	-	-	-	-	-	-	-	-	-	-

TABLE – 19

PLASMID PROFILE TYPING OF ACINETOBACTER SPECIES ENVIRONMENTAL ISOLATES IN MEDICAL WARDS

Acinetobacter species	No of Isolates	Various Isolates showing No. of Plasmid bands								
		1	2	3	4	5	6	7	8	9
Acinetobacter baumannii	1	-	1	-	-	-	-	-	-	-
Acinetobacter hemolyticus	-	-	-	-	-	-	-	-	-	-
Acinetobacter calcoaceticus	-	-	-	-	-	-	-	-	-	-

TABLE – 20

PLASMID PROFILE TYPING ACINETOBACTER SPECIES ENVIRONMENTAL ISOLATES IN ORTHOPAEDIC WARD

Acinetobacter species	No of Isolates	Various Isolates showing No. of Plasmid bands								
		1	2	3	4	5	6	7	8	9
Acinetobacter baumannii	2	1	1	-	-	-	-	-	-	-
Acinetobacter hemolyticus	1	-	-	1	-	-	-	-	-	-

Acinetobacter calcoaceticus	-	-	-		-	-	-	-	-	-

TABLE - 21

PLASMID PROFILE TYPING ACINETOBACTER SPECIES ENVIRONMENTAL ISOLATES IN BURNS WARD

Acinetobacter species	**No of Isolates**	**Various Isolates showing No. of Plasmid bands**								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Acinetobacter baumannii	-	-	-	-	-	-	-	-	-	-
Acinetobacter hemolyticus	-	-	-	-	-	-	-	-	-	-
Acinetobacter calcoaceticus	4	1	1	1	-	-	-	-	-	1

Among the 22 Acinetobacter baumannii clinical isolates, 20 bands were found in molecular weight ranging between (9-7 kb), 8 bands ranging in molecular weight between (6-4 kb) and 4 bands ranging in molecular weight between (3-1). **Among the 20 Acinetobacter hemolyticus clinical isolates**, 19 bands were found in molecular weight ranging between (9-7 kb), 6 bands ranging in molecular weight between (6-4 kb) and 9 bands ranging in molecular weight between (3-1). **Among the 14 Acinetobacter calcoaceticus clinical isolates**, 14 bands were found in molecular weight ranging between (9-7 kb), 7 bands ranging in

molecular weight between (6-4 kb) and 3 bands ranging in molecular weight between (3-1).

Distribution of plasmids in different species of Acinetobacter isolated from clinical specimens is given in Table 22.

TABLE – 22

DISTRIBUTION OF PLASMIDS IN DIFFERENT SPECIES OF ACINETOBACTER ISOLATED FROM CLINICAL SPECIMENS

Acinetobacter species	***No. of Isolates***	**Plasmid bands in various Molecular weight (Kb)**		
		9 – 7	**6 - 4**	**3 - 1**
Acinetobacter baumannii	22	20	8	4
Acinetobacter hemolyticus	20	19	6	9
Acinetobacter calcoaceticus	14	14	7	3

Distribution of plasmids in different species of acinetobacter isolated from environmental samples were analysed. Out of the plasmid bands in **Acinetobacter baumannii environmental isolates**, 6 bands were found in molecular weight ranging between (9–7 kb) and 4 bands ranging in molecular weight between (6-4 kb). Out of the plasmid bands in **Acinetobacter hemolyticus environmental isolates**, 2 bands were found in molecular weight ranging between (9-7 kb) and 2 bands ranging in molecular weight between (6-4 kb). Out of the plasmid bands in **Acinetobacter calcoaceticus from environmental source**, 4 bands were found in molecular weight ranging between (9-7 kb), 3 bands

ranging in molecular weight between (6-4 kb) and 2 bands ranging in molecular weight in between (3-1).

The distribution of plasmid bands in various molecular weight in the clinical isolates of Acinetobacter baumannii, Acinetobacter hemolyticus and Acinetobacter calcoaceticus were in the range of 9-7, 6-4, 3-1kbs whereas in environmental samples of Acinetobacter baumannii and Acinetobacter hemolyticus had not shown any bands in molecular weight 3-1kbs. But one isolate of Acinetobacter calcoaceticus showed the band in molecular weight 3-1kbs. Thus Acinetobacter calcoaceticus was the only environmental isolate that correlated with the clinical isolate.

Distribution of plasmids in different species of Acinetobacter isolated from Environmental samples is given in Table 23.

TABLE – 23

DISTRIBUTION OF PLASMIDS IN DIFFERENT SPECIES OF ACINETOBACTER ISOLATED FROM ENVIRONMENTAL SAMPLES

Acinetobacter species	***No. of Isolates***	**Plasmid bands in various Molecular weight (Kb)**		
		9-7	**6-4**	**3-1**
Acinetobacter baumannii	5	6	4	-
Acinetobacter hemolyticus	2	2	2	-
Acinetobacter calcoaceticus	4	4	3	2

SUGGESTIONS

Hospitals have always acted as a source of infection to patients admitted. Suppuration is the most common postoperative consequences in hospitals.

The incidence of hospital infections has been reported to be 2-12 per cent in the advanced countries, it is much higher in the crowded hospitals in the developing countries.

In this present study Acinetobacter is isolated in 22% of environmental sources and 20% of hospital occurred infections in patients. The present study reveals the multidrug resistance Acinetobacter infections (20%) as hospital acquired infections.

This incidence may be reduced by taking preventive and control measures at three links in the chain of infections, namely by elimination of the reservoirs of agents by interrupting transmission of infections and by protecting host against infections and disease.

Modification of environmental resources

- Can be achieved by provision of safe air, water, milk and food supply.
- Appropriate handing of sewage and waste products.

- Destruction of the agent in the environmental niche by chemical or physical means.
- Safe medical care environment can be achieved by proper sterilization of surgical instruments, the use of disinfectants and antiseptics and appropriate aseptic techniques.

Interrupting of transmission can be achieved by insisting proper hand washing between tasks in the preparation for the food, caring for the children, caring for the sick and before and after surgery.

By proper use of gloves, gowns, and eye protection should be emphasized to prevent the transmission of blood borne disease between the patients and health care workers.

Use of high filtration masks for protection from respiratory transmission.

Protecting the host can be achieved

- Through immunization
- Administering antibiotics to assure the presence of anti-infective agent at the site of a potential infection is a more recent addition to the control programs protecting the host.

- The use of single dose or short course of preoperative antibiotics reduce the probability of infections with agents in the hospital environments.

DISCUSSION

A total of 500 clinical samples and 50 environmental samples were collected from various wards of Govt. Rajaji Hospital, Madurai during a period of 13 months to find out the prevalence of Acinetobacter species. The antibiotic resistant pattern was studied, the plasmids were isolated from the resistant organisms and the plasmid profile of clinical and environmental isolates were compared and confirmed that Acinetobacter calcoaceticus occurred as a common nosocomial pathogen in the burns ward.

STUDY OF CLINICAL SAMPLES

In the present study, among the 500 samples collected, Gram negative Non-Fermenters were predominately isolated (49%), followed by Gram negative Fermenters (39.2%) and Gram positive cocci (11.8%) **(Figure 2)**. Among the Gram-negative Non-Fermenters, Acinetobacter species were isolated from orthopaedic wards (41.3%), burns wards (46%), from medical (37.5%), and general surgery wards (37.5%). The maximum isolation of Acinetobacter species was found in wound infections of orthopeadic (41.3%) and burns wards (46%) **(Table 3).** Pavlica R, et al[49] had discussed that Acinetobacter was commonly isolated from wounds and burns and Pedro et al[50] demonstrated multidrug resistant Acinetobacter in burns cases, which is in supportive of

our study. The common occurrence of Acinetobacter species in wound infections of orthopaedic wards and burns ward may be due to the ability of this organism to attach to the human tissues by the linkage of adhesion factor lectin and carbohydrate in exposed tissue or due to the ability of the organism to invade tissues for obtaining essential nutrients like iron or due to the virulent factors in these organisms like slime production and lipopolysaccharide components of the cell wall that can function as endototoxins in exposed tissues.[14]

The speciation of the Acinetobacter showed that Acinetobacter baumannii (58.1%) was commonly isolated in the orthopaedic wards **(Table 6)** in contrast to the previous reports by Zakeya, Bukhary et al[77] in which they showed that Acinetobacter baumannii were associated commonly with the patients in Intensive care units, mechanical ventilation and patients with previous use of antibiotics. But Kepler et al[35] had explained Acinetobacter baumannii in extremities infection in soldiers and isolated Acinetobacter baumannii in the soil in Iraq and proved that it was the cause for dirty battle field wounds with serious infections. As, many of our samples from wound infections in the orthopaedic wards were secondary to road traffic accidents the possibility of Acinetobacter baumannii isolation in open injury in orthopaedic ward is justified.

It was also shown in our study that Acinetobacter hemolyticus (41.7%) and Acinetobacter calcoaceticus (33.3%) were common in burns

wards. Stererts R.J. et al[63] had shown that Acinetobacter calcoaceticus was the most common organism causing infections in “university burns centre” and found that wet mattresses served as an environment reservoir of Acinetobacter. It was also discussed by Buxton AE et al and Towner et al[11, 69] that Acinetobacter hemolyticus and Acinetobacter calcoaceticus can occur as common nosocomial pathogens in the hospital area where there is an accumulation of moisture. Hence occurrence of these organisms in the skin of these burns patients which is always moist is justified. Moreover the burns site is ideal for the growth of microorganism, serum and debris provide nutrients, and the burn injury compromises blood flow, blocking effective inflammatory responses. Further more, cellular and humoral defenses against infections are compromised and both lymphocytes and phagocytes functions are impaired. Also there is a development of a hypermetabolic state with excess heat loss and increased need for nutritional supports. The immune suppressive state of the burns patient and the moisty surface of the skin might have given way for the Acinetobacter hemolyticus and Acinetobacter calcoaceticus to colonize on the exposed surface area of the burns skin. All these studies and factors are in supportive of my study report.

In our study, it was observed that Acinetobacter baumannii was commonly isolated in PUS (48.9%) but not in CSF and the same was

supported by the study of LT. Col KK Lahiri et al[40] who had also shown that Acinetobacter baumannii was more commonly isolated from PUS (11.8%). Also it was shown in our study that Acinetobacter calcoaceticus and Acinetobacter hemolyticus were not isolated from pleural fluid. Similar study by Veenu et al[71] also showed that Acinetobacter calcoaceticus were not demonstrated in CSF and pleural fluid.

The present study on the antibiotic susceptibility pattern of Acinetobacter species showed that they were sensitive to common antibiotics like Ampicillin (9.8%), Piperacillin (8.8%), Gentamicin (12.7%) and Ciprofloxacin (21.5%) **(Table 8)**. This is in accordance with the study conducted by Prashanth et al[54] who had shown sensitivity to Ampicillin (9.4%), Piperacillin (4.7%), Gentamicin (34.4%) and Ciprofloxacin (37.5%).

The resistance pattern of these antibiotics in this study showed that 90% Acinetobacter species resistant to Ampicillin, 91.1% to Pipercillin, 89.2% to Gentamicin and 78.4% to Ciprofloxacin **(Table 9)**. This is in accordance with the study by Prasanth et al[54] who had shown that 84.4% Acinetobacter species were resistant for Ampicillin, 87.5% for Pipercillin, 62.5% for Gentamicin, and 48.4% for Ciprofloxacin.

It was also observed in our study that 53.2% of Acinetobacter baumannii, 39.4% of Acinetobacter hemolyticus and 36.4% Acinetobacter calcoaceticus showed sensitivity for more than 4

antibiotics. Thus Acinetobacter baumannii was more sensitive to antibiotics in contrast to other studies where more resistant pattern was shown for Acinetobacter baumannii[16,20,23].

It was also shown in our Study that 46.8% of Acinetobacter baumannii, 60.4% of Acinetobacter hemolyticus and 63.6% of Acinetobacter calcoaceticus showed resistance for more than 4 antibiotics from the clinical isolates **(Table 12)**. This is in accordance with the study of Devaud et al[18] that Acinetobacter calcoaceticus is unusually resistant to multiple antibiotics which may be due to the aminoglycoside modifying enzymes 3-N-acetyl transferase, 3'-phospho transferase & 3"-adenyl transferase or cephalosporinase or TEM2 betalactamase. Henriksen et al[27] had explained the possibility of plasmid mediated multiple drug resistance in Acinetobacter calcoaceticus. Devaud et al[18] also explained transposon mediated multiple antibiotic resistance in Acinetobacter calcoaceticus.

Plasmid profile typing of Acinetobacter species from clinical isolates showed that Antibiotic resistance in these organism in General Surgery ward revealed that 7 bands were seen in 1 isolate of Acinetobacter baumannii, 8 bands were seen in 1 isolate of Acinetobacter hemolyticus and 2 bands were seen in 1 isolate of Acinetobacter calcoaceticus. Thus **Acinetobacter hemolyticus showed more bands indicating more resistance to antibiotics in**

surgical wards (Table 14).

Similarly in **medical wards**, one isolate of Acinetobacter baumannii showed a maximum of 7 bands and one isolate of Acinetobacter hemolyticus showed 2 bands and one isolate of Acinetobacter calcoaceticus showed 2 bands. Thus **Acinetobacter baumannii showed more resistance to antibiotics in medical wards (Table 15)**. In **orthopaedic wards**, one isolate of Acinetobacter baumannii showed a maximum of 8 bands, one isolate of Acinetobacter hemolyticus showed 4 bands and one isolate of Acinetobacter calcoaceticus showed 7 bands. Thus **Acinetobacter baumannii showed more bands indicating more resistance to antibiotics in orthopaedic wards (Table 16)**. In **burns ward** one isolate of Acinetobacter calcoaceticus showed a maximum of 9 bands, one isolate of Acinetobacter hemolyticus showed 8 bands and one isolate of Acinetobacter baumannii showed 4 bands. Thus **Acinetobacter calcoaceticus showed more bands depicting more resistance to antibiotics in burns wards (Table 17)**. This study reports are in accordance with the study of Seifert H et al[60] who had shown more antibiotic resistance by plasmid profile typing in Acinetobacter bauamannii species in medical and orthopaedic wards. But in surgery ward he had demonstrated more antibiotic resistance in Acinetobacter baumannii, in contrast to our study where we had demonstrated

Acinetobacter hemolyticus as more antibiotic resistant. Devaud et al[18] had demonstrated antibiotic resistance in Acinetobacter calcoaceticus to be more common in burns wards by plasmid typing[18,26] which is in supportive of our study.

The distribution of plasmid bands in various molecular weight in clinical isolates showed that Acinetobacter baumannii, Acinetobacter hemolyticus and Acinetobacter calcoaceticus showed plasmid bands of molecular weights 9-7, 6-4, 3-1kbs. This is in accordance with the study by Lucis Actis et al[41], Davaud et al[18] who had demonstrated similar molecular weight bands in all the three species of Acinetobacter.

STUDY OF ENVIRONMENTAL SAMPLES

In our study, among the 50 environmental samples collected, Gram negative Non-Fermenters were predominant (64%), followed by Gram negative Fermenters (22%) and Gram positive cocci (14%) **(Figure 3)**. Among the Gram negative Non-Fermenters, Acinetobacter species were more commonly isolated in burns ward (36.3%), less common in general surgery (27.2%) and in orthopaedic wards (27.2%). The maximum isolated Acinetobacter species were from mattresses (54.5%) in burns ward. This is in accordance with the study by Sherertz et al[63] who had demonstrated Acinetobacter colonization in mattresses of burns ward which may be due to the moisty exposed skin surface which is a good nidus for Acinetobacter which comes into direct contact with the

mattresses.

The speciation of Acinetobacter in environmental samples showed that Acinetobacter baumannii (27.2%) was isolated in general surgery wards and Acinetobacter hemolyticus (27.2%) in orthopaedic wards whereas Acinetobacter calcoaceticus was isolated only (36.3%) in burns ward especially in mattress. This is in accordance with the study by Zaer et al[76] in which he had demonstrated maximum isolates of Acinetobacter calcoaceticus (38.8%) from mattress, lockers and linen in burns ward.

The study on antibiotic resistant patterns of Acinetobacter species in environmental samples showed 40% Acinetobacter bowmanni, 50% Acinetobacter hemolyticus and 75% Acinetobacter calcoaceticus were resistant for more than 4 antibiotics. Thus Acinetobacter calcoaceticus was more resistant for common antibiotics which in accordance with the study by Zaer et al[76] in which he had also demonstrated antibiotic resistance for common antibiotics in Acinetobacter calcoaceticus found in the environmental samples.

The plasmid profile typing of Environmental samples in surgery ward showed that **among the two isolates of Acinetobacter baumannii, one isolate showed 6 bands**, one isolate showed 2 bands and one isolate of Acinetobacter hemolyticus showed 4 bands **(Table 18)**. In medical wards one isolate of Acinetobacter baumannii showed two bands **(Table 19)**. In orthopaedic wards among the two isolates of

Acinetobacter bauamannii, one showed 2 bands and another one showed 1 band and one isolate of Acinetobacter hemolyticus showed 3 bands **(Table 20)**. In Burns wards, **among the four isolates of Acinetobacter calcoaceticus, one isolate showed 9 bands**, one isolate showed 3 bands, one isolate showed 2 bands and one isolate showed 1 band **(Table 21)**. The plasmid profile typing of environmental samples in various wards showed that Acinetobacter calcoaceticus is the only isolate showing more number of bands, and Acinetobacter baumannii, Acinetobacter hemolyticus showed less number of bands suggestive of more antibiotic resistance for Acinetobacter calcoaceticus in burns ward. This was already proved by Devaud et al[18] who had demonstrated more than 7 bands in Acinetobacter calcoaceticus in environmental source of burns wards.

The distribution of plasmid bands in various molecular weight in environmental samples showed that there were bands only in molecular weight 9-7, 6-4 kbs and no bands in molecular weight 3-1 kbs in Acinetobacter baumannii and Acinetobacter hemolyticus whereas Acinetobacter calcoaceticus showed the bands in molecular weight 9-7, 6-7, 3-1 kbs . This is also in accordance with the study conducted by Devaud et al[18] in which he had demonstrated plasmid bands in molecular weight 3-1kbs in Acinetobacter calcoaceticus.

COMPARISON OF CLINICAL AND ENVIRONMENTAL ISOLATES

The above study demonstrated that Acinetobacter calcoaceticus in the clinical samples (63.6%) showed resistance for common antibiotics like Ampicillin, Piperacillin, Gentamicin, Ciprofloxacin and in environmental sources showed 75% resistance for the above antibiotics. But the other species did not show any correlation in the antibiotic resistant pattern in clinical and environmental samples. Zaer et al[76] in his study demonstrated Acinetobacter calcoaceticus as the commonest organism among the three species which showed more antibiotic resistance.

The plasmid profile study of both clinical and environmental isolates showed that Acinetobacter calcoaceticus isolated from the burns ward showed 9 bands in clinical isolate and 9 bands in environmental isolate confirming the possibility of A. calcoaceticus as the environmental pathogen. This confirmation is further supported by the study of plasmid profile according to the molecular weight in which it was shown that Acinetobacter calcoaceticus from both clinical and environmental samples in burns ward alone showed the molecular weight 3-1 kbs whereas the other species didnot show any correlation in the molecular weight. The study by Devaud et al[18] who had similar correlation in the plasmid profile like the number of bands and the molecular weight in the clinical and environmental isolates is in support of our study.

Isolation and identification of plasmids according to various

molecular weights in both clinical and environmental Acinetobacter isolates was done by simple Agarose Gel Electrophoretic Method in our study and showed that it was an easy, sensitive and useful method for epidemiological investigation. Similar study of plasmid profile by Agarose gel electrophoresis by Jane Aldrich Meyers et al[46] also proved that it was an effective method for the detection and preliminary characterization of plasmid DNA present in clinical isolates and laboratory strains of gram negative organisms, thus supporting our study.

SUMMARY

This study which aimed at demonstrating the Acinetobacter species of organisms as a nosocomial pathogen was conducted on both clinical and environmental samples isolated from various wards like medical, surgical, orthopaedic and burns in Govt. Rajaji Hospital, Madurai during a period of 13 months. (December 2004 to January 2006).

The study of Acinetobacter species in the clinical samples from various wards of Govt. Rajaji Hospital, Madurai. showed the following results:

- Among the 500 heterogenous samples tested, 49% showed Gram negative Non-Fermenters and among the Gram negative Non-Fermenters, Acinetobacter species were isolated in 20.4% samples.
- The speciation of Acinetobacter showed that Acinetobacter baumannii was the commonest isolate which was from 46.1% of samples, Acinetobacter hemolyticus from 32.2% of samples and Acinetobacter calcoaceticus from 21.6% of samples. Acinetobacter baumannii was predominantly isolated from orthopaedic ward (58.1%), Acinetobacter hemolyticus was from burns (41.7%) and Acinetobacter calcoaceticus was isolated from burns (33.3%).

- The Pus sample was proved to be the most suitable specimen for the isolation of Acinetobacter baumannii (48.9%). The CSF sample did not show Acinetobacter baumannii and the pleural fluid did not show positivity for Acinetobacter hemolyticus and Acinetobacter calcoaceticus.

- The antibiotic susceptibility of varied species of Acinetobacter showed that 53.2%. Acinetobacter baumannii showed sensitivity and 46.8% showed resistance to more than 4 antibiotics. Similarly 39.4%. Acinetobacter hemolyticus showed sensitivity and 60.4% of showed resistance to more than 4 antibiotics and. Acinetobacter calcoaceticus 36.4% showed sensitivity and 63.6% showed resistance for more than 4 antibiotics. Among the three species Acinetobacter calcoaceticus was the one showing higher resistance to antibiotics.

- The plasmid typing of the varied species of Acinetobacter showed that in surgical ward, one isolate of Acinetobacter hemolyticus showed 8 bands suggesting higher antibiotic resistance caused by Acinetobacer hemolyticus in this ward. Similarly Acinetobacter baumannii showed 7 bands in medical ward, 8 bands in orthopaedic wards and Acinetobacter calcoaceticus showed 9 bands in burns wards. Thus Acinetobacter baumannii showed higher antibiotic resistance in medical and orthopaedic wards and Acinetobacter calcoaceticus in burns wards.

- The distribution of plasmid bands in various molecular weights showed that all the species showed molecular weights of 9-7, 6-4 and 3-1kbs.

THE STUDY OF ENVIRONMENTAL SAMPLES;

- Out of 50 environmental samples 64% were gram negative non fermenters and among gram negative non fermenters 22% were Acinetobacter species.
- Speciation of Acinetobacter showed 45.4% Acinetobacter baumannii, 18% Acinetobacter hemolyticus and 36.3% Acinetobacter calcoaceticus. Acinetobacter baumannii was commonly found in surgery and orthopaedic wards, Acinetobacter hemolyticus was in surgery and orthopaedic wards, Acinetobacter calcoaceticus in burns ward.
- Antibiotic susceptibility pattern showed that 60% **Acinetobacter baumannii** were sensitive for more than 4 antibiotics 40%, resistant for more than 4 antibiotics. **Acinetobacter hemolyticus** showed 50% sensitive for more than 4 antibiotics and 50% resistant for more than 4 antibiotics. **Acinetobacter calcoaceticus** showed 25% sensitive for more than 4 antibiotics and 75% resistant for more than 4 antibiotics. **Thus Acinetobacter calcoaceticus showed more resistance for antibiotics and it was the only species isolated in burns**

ward.

- Plasmid typing showed 9 bands in Acinetobacter calcoaceticus isolated from burns and the molecular weight showed 9-7, 6-4 and 3-1 kbs in one isolate whereas Acinetobacter baumannii and Acinetobacter hemolyticus showed no bands with molecular weight 3-1kbs.
- Comparison of clinical and environmental samples showed that Acinetobacter calcoaceticus isolated from burns ward was the only one which showed similarlity in the antibiotic resistance pattern, number of molecular bands and the molecular weight in both clinical and environmental samples. Thus Acinetobacter calcoaceticus occured as a nosocomial pathogen in burns ward.
- Simple Agarose Gel Electrophoretic method was employed for the detection and preliminary characterization of plasmid (DNA) present in clinical and environmental Acinetobacter isolates. The estimation of plasmid mass from the extend of DNA migration in gels compared favourably with the results obtain from both environmental and clinical isolates. This method had proved to be useful tool for survey work and the epidemoiolgical investigation of plasmid dissemination, as well as an important adjunct to the genetic analysis of plasmids.

CONCLUSION

Gram-negative Non-Fermenters were commonly isolated from the medical wards, surgery wards, orthopedic wards and burns ward. Among the Non-Fermenters, Acinetobacter was shown to be the commonest organism isolated in these wards. The Acinetobacter was speciated by the biochemical reactions and it was found that Acinetobacter baumannii was commonly isolated from orthopaedic wards and Acinetobacter calcoaceticus and Acinetobacter hemolyticus were commonly isolated from burns wards.

The antimicrobial susceptibility pattern of the three species of Acinetobacter showed that 63.6% Acinetobacter calcoaceticus were resistant to common antibiotics like ampicillin, piperacillin, gentamicin and ciprofloxacin. One of the antibiotic resistant isolate of Acinetobacter calcoaceticus showed 9 bands in plasmid profile typing showing that Acinetobacter calcoaceticus was showed higher resistance to antibiotics and this sample was isolated from the pus sample from a burns patient.

Similar study on the environmental samples also showed that 75% antibiotic resistance for Acinetobacter calcoaceticus in burns ward. One isolate from the mattress of burns ward showed 9 bands, similar to the one in the pus sample of burns patient confirming that the infection in the burns patient may be from the environment. This is further supported by

the same molecular weight of bands identified from both clinical and environmental samples in the burns ward. Thus Acinetobacter calcoaceticus occurs as a nosocomial pathogen in burns wards.

Simple Agarose Gel Electrophoretic Method was employed for the detection and preliminary characterization of plasmid DNA present in clinical and environmental isolates and proved to be useful tool for survey work and the epidemoiolgical investigation of nosocomical infection.

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PROFORMA

CASE HISTORY

Name : Address:

Age :

Sex :

Occupation :

Education :

Income :

IP No. :

Ward No. :

Diagnosis :

Date of admission :

Date of discharge :

Complaints of :

Fever : Continuous, intermittent, low grade, high grade, associated with chills / sweating.

Cough : Productive / non productive, diurnal variation

Sputum : Colour, purulent, non purulent, foul smelling, blood stained.

Pus : Colour, discharge – watery, purulent, blood stained.

Present history :

Past history	:	H/O DM, HT, Anaemia, Jaundice, Convulsions.
General Examination	:	H/O ear discharge, conjunctivitis, trauma, anaemia, Generalized Lymphadenopathy, cyanosis, clubbing, icterus, skin hair nail changes.
Systemic examination	:	
CVS	:	
RS	:	
Per abdomen	:	
CNS	:	
Treatment given previously	:	

Treatment given as per antibiogram:

Outcome :

Cured / Severity reduced / Worsened / death.

Lab investigations	:
Specimen	:
Lab number	:
Date	:
Time	: