Species Prevalence, Strain Characterisation, Antibiotic / Non-Antibiotic Susceptibility And Epidemiology of Nosocomial Pathogens in Cardiac Surgery Patients

SINDHU .C.P.

Ph.D THESIS-2000

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SPECIES PREVALENCE, STRAIN CHARACTERISATION, ANTIBIOTIC/ NON-ANTIBIOTIC SUSCEPTIBILITY AND EPIDEMIOLOGY OF NOSOCOMIAL PATHOGENS IN CARDIAC SURGERY PATIENTS

A Thesis Presented
by

SINDHU.C.P.

to

The Division of **Microbiology**
in partial fulfilment of the requirements
for the degree of
Doctor of Philosophy

SREE CHITRA TIRUNAL INSTITUTE
FOR
MEDICAL SCIENCES AND TECHNOLOGY
TRIVANDRUM

March 2000



CERTIFICATE

I **SINDHU .C.P**, hereby certify that I had personally carried out the work depicted in the thesis entitled **"SPECIES PREVALENCE, STRAIN CHARACTERISATION, ANTIBIOTIC/NON-ANTIBIOTIC SUSCEPTIBILITY AND EPIDEMIOLOGY OF NOSOCOMIAL PATHOGENS IN CARDIAC SURGERY PATIENTS"** except where external help sought and acknowledged.

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Submitted

by

SINDHU C.P.

for

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of

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This work is dedicated to my parents

Shri. D. HARIHARAN NAIR

and

Smt. C.P. SASIKALA

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SYNOPSIS

Nosocomial infections (hospital-acquired infections) constitute a major public health problem in terms of frequency, severity, management and cost of treatment. They result in substantial morbidity, mortality and prolongation of hospitalization. Nosocomial infections due to multiple drug resistant microorganisms are emerging as difficult problems.

The source of infecting organisms can be either exogenous (from another patient or a member of the hospital staff or from inanimate objects or environment in the hospital) or endogenous (from the patient's own body flora which can be from nose, throat, gut, skin or genitourinary tract).

NORMAL FLORA OF HUMAN BODY

Humans harbour a wide array of microorganisms either on or in their bodies. Microbial flora of humans can be broadly divided in to (a) Helpful symbionts (b) Harmless commensals and (c) Harmful opportunists. But no hard-and-fast rule divides the human microbia into clear-cut categories of harmless commensal organisms and pathogenic species. Inspite of all the advances in modern science including genotyping, it is sometimes difficult for a Microbiologist to differentiate whether the bacterial culture obtained is a contaminant or the actual infective agent responsible for the disease.

The normal commensals of mouth, nose, throat, gut and skin include many Gram positive and Gram negative organisms. These microorganisms usually found in healthy persons can become opportunistic pathogens when the host resistance decreases or when they move from their original habitat to the other areas of the body. Also, when sensitive commensals are removed by some antimicrobial therapy, some residual resistant strains can turn virulent to cause disease.

The common types of nosocomial infections include : (a) Urinary Tract Infection (UTI), (b) Respiratory Tract Infection (RTI), (c) Bacteremia and Septicaemia and (d) Surgical Wound Infections (WI) (which may range from minor infections by normal resident skin flora to severe spreading infections due to exogenous pathogens).

WOUND INFECTIONS IN CARDIAC SURGERY PATIENTS (WITH PARTICULAR REFERENCE TO MEDIASTINITIS AND STERNAL WOUND INFECTION)

The incidence of post-operative infections by various pathogenic bacteria among cardiac surgery patients ranges from 0.2 to 5% in developed countries and from 3 to 15% in developing countries.

Surgical wound infection and mediastinitis can range from sepsis with few localizing symptoms or signs of obvious wound infection to purulent drainage and wound dehiscence. The mortality rate due to sternal infections range from 0 to 52%.

The important risk factors that are likely to lead to wound infection in cardiac surgical population includes (a) CABG, (b) Uremia, (c) General health, (d) Re-operation, (e) Chronic pulmonary obstructive diseases, (f) Corticosteroids, (g) Obesity, (h) Diabetes mellitus, (i) Old age (j) Carriage rate of *S.aureus* and other pathogenic bacteria in nose or other sites.

The important Gram positve microorganisms associated with nosocomial infections in Cardiac Surgery Patients include *Staphylococcus aureus, Coagulase - Negative Staphylococci* (CNS), *β-hemolytic Streptococci, St. pneumoniae, Diphtheroids* and other anaerobic organisms. The Gram negative organisms responsible for such infections include *Eschericia Coli, Klebsiella* sp., *Acinetobacter* sp., *Enterobacter* sp., *Proteus* sp., *Pseudomonas* sp., *Serratia marcescens* and *Morganella morgaani.*

NASAL CARRIAGE OF S.AUREUS AND ITS CORRELATION WITH POST-OPERATIVE WOUND INFECTION

An important factor leading to sternal wound infection is the nasal carriage of *S.aureus* and other pathogenic bacteria. About 10-15% of normal population harbour *S.aureus* in their anterior nares. Studies have shown that patients who are carriers of pathogenic bacteria are more prone to develop post-operative wound infection than the patients who are non-colonizers.

AIM AND OBJECTIVE OF THE STUDY

The proposed study is aimed at :

(a) understanding the rate of carrier state of potential pathogenic bacteria in patients undergoing open heart surgery

(b) to correlate the incidence of post-operative wound infections with the carrier state and

(c) to suggest effective control measures in order to reduce such endogenous infections.

METHODS

The study involved isolation, identification and characterisation of different strains of nosocomial pathogens in patients undergoing cardiac surgery at SCTIMST hospital.

Four hundred patients admitted for cardiac surgery were investigated to identify the carrier state of potential pathogenic bacteria in their throat, nose and sternal skin area on three different occassions namely ,

(a) on the day of admission to the hospital,

(b) the day of shifting from ICU to ward and

(c) on the day of discharge from the hospital.

Simultaneously one hundred health-care personnel (HCP) working in the hospital, were also screened for the carrier state of pathogenic bacteria in their nose, throat and skin of hands and forehead. All specimens were cultured within two hours after collection. On the next day, the suspected colonies were subcultured and the bacterial strains were identified as per standard techniques.

The antibiotic susceptibility tests of CNS strains, *S.aureus* strains and *Klebsiella* strains, isolated from patients and health-care personnel were carried out by Kirby Bauer method on Mueller Hinton Agar plates. The tests were used to determine the susceptibility pattern of these strains against various antibiotics. Discs of twenty -five different antibiotic drugs were used to obtain the antibiogram pattern of the bacterial strains isolated from both groups.

In order to control the spread of nosocomial infections, it is essential that the source of infection be identified using discriminatory typing methods. The different typing methods

used in the present study to find out the correlation between the strains isolated from the carrier state and the pathogenic strains isolated from infectious specimens included Antibiogram, Bacteriophage typing, Biotyping, Bacteriocin typing and analysis of cell envelope protein patterns using Poly Acrylamide Gel Electrophoresis (PAGE). A similarity observed in the results of three or four typing systems is taken to infer that the isolates belong to the same strain.

Identification up to species level of bacterial strains were done to find out the significance of the strains causing infection, in the case of CNS in view of the emergence of multiple drug resistance among them. 750 strains of CNS and 113 strains of *Klebsiella* sp. isolated from patients and 111 strains of CNS and 14 strains of *Klebsiella* sp. isolated from health-care personnel were subjected to speciation using different biochemical tests.

Tests were conducted for the detection of Beta lactamases among 644 strains of CNS and 134 strains of *S.aureus* belonging to patient isolates and 111 strains of CNS and 27 strains of *S.aureus* isolated from health-care personnel using Iodometric techniques, (test tube, filter paper, microplate and agar plate methods) and Acidimetric techniques (filter paper and microplate methods) each with two indicators - Bromocresol purple and phenol red. The phenol red filter paper method was the most sensitive followed by test tube and filter paper iodometric methods, the least sensitive being Bromocresol purple microplate method.

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an agent that inhibits the visible growth of an organism in a culture medium. The MIC of antibiotics such as Ciprofloxacin, Cefuroxime, Tobramycin and Amikacin were tested using Agar dilution method. 100 strains of CNS - 50 isolates each from patients and health-care personnel (HCP); 75 strains of *S.aureus* - 50 from patients and 25 from HCP, and 64 strains of *Klebsiella* - 50 from patients and 14 from HCP were tested. In addition, MIC of Penicillin and Cloxacillin were tested against the strains of CNS and *S.aureus*, while MIC of Gentamicin and Augmentin were checked against *Klebsiella* strains.

The elaboration of slime has been suggested as an indicator of clinical significance and a marker of virulence. The slime producing variants usually show a significantly higher colonization capacity than do the non-slime producing variants of the same strain. Hence, tests to detect slime production by *Staphylococcus* sp. were performed routinely inorder to predict the virulence of these organisms. Two methods were compared to study the slime production among the CNS and *S.aureus* strains (a) Test tube method of Christensen using 0.1% safranine

and (b) Congo Red Agar plate method of Freeman. The Agar plate method was found to be more sensitive.

A preliminary screening of some of the indigenous medicinal plants and spices were done to find out their antibacterial activity using two types of extracts (filter sterile aqueous extract and autoclaved aqueous extract) by Agar well diffusion method.

A total of 15 indigenous medicinal plants and 10 indigenous spices were screened against 50 CNS strains (25 from Cardiac Surgery Patients (CSP) and 25 from Health-Care Personnel HCP); 50 *S.aureus* strains (25 from CSP and 25 from (HCP)); 25 *Pseudomonas* strains from cardiac surgery patients and 39 *Klebsiella* strains (25 from CSP and 14 from HCP) to find out their antibacterial activity.

RESULTS

A total of 1160 bacterial strains belonging to seven species were isolated from 3600 swab specimens - 1200 each from throat, nose and sternal skin area of patients. From health-care staff, a total of 175 bacterial strains belonging to five species were isolated from 300 swab specimens-100 each from throat, nose and skin.

The leading bacterial species identified from patients as well as from health-care personnel were the CNS species followed by *S.aureus* and *Klebsiella* sp - 66.03%, 12.67%, 10.08% in the former group and 63.4%, 15.4% and 8.0% in the latter group. Most of the CNS and *S.aureus* strains were isolated from the nasal swab followed by skin - 59.92%, 31.85% from patients and 54.95% and 35.14% respectively from health-care personnel.

The maximum number of bacteria were isolated from the nose followed by skin. *Beta-hemolytic Streptococci* were mainly isolated from the throat. The maximum isolation of *Klebsiella* was from the throat in both groups. *Streptococcal* strains and *Ps.aeruginosa* were not found in the health-care personnel. The study have found a marked reduction of bacteria in the skin carriage while with nasal carriage a slight tendency to get colonized at the end of hospitalization was noticed.

Multiple antibiotic resistance was exhibited by pathogenic bacteria like *Klebsiella sp* and *S.aureus* isolated from patients rather than those from health-care personnel. But with CNS, most of the strains tested showed a higher rate of sensitivity.

In the speciation of CNS using different biochemical tests, *S.epidermidis* was the leading isolate among the CNS strains-40.80% and 44.14% from patients and health-care

personnel respectively. Among the *Klebsiella* sp., the leading isolate was *K.Pneumoniae* (50.44%) among patients and *K.aerogenes* (64.3%) among health-care personnel. *S.warneri, S.xylosus* and *K.ozanae* were not isolated from the health-care personnel, but could be isolated from patients - 1.33%, 1.06% and 13.27%.

The results obtained from the MIC tests suggest that patient-isolates demonstrate more drug resistance than those isolated from HCP in most cases. Amikacin and Tobramycin were found to be very effective against CNS, *S.aureus* and *Klebsiella* sp, but cannot be administered as prophylactic agents, due to their nephrotoxicity. Cefuroxime seems an ideal drug for prophylaxis with fairly low MIC values. Cloxacillin continues to hold out promise against the Gram positive organisms.

The findings obtained in the present study for the antibacterial screening of few medicinal plants and spices indicated that the autoclaved aqueous extract of *Tamarindus indicus* and filter sterile aqueous extract of *Allium sativum* are most effective antistaphylococcal agents followed by *Eucalyptus globulus, Ocimum sanctum, Phyllanthes niruri, Lawsonia inermis, Eupatorium urticifolium, Adhatoda vasica, Moringa oleifera* and *Eugenia Caryophyllata*.

Against Gram negative organisms, *Tamarindus indicus* was very effective against *Klebsiella* sp. followed by *Ocimum Sanctum*, while against *Pseudomonas* sp. *Tamarindus indicus* was the most effective one-all *Pseudomonas* strains were sensitive even at 2.5% concentration indicating the potential uses of these native pant extracts in treating multiresistant *Pseudomonas* sp. and *Staphylococcal* sp. which often causes treatment problem during the post operative periods.

ANALYSIS OF THE ISOLATES FROM INFECTED PATIENTS

Urine, sputum and blood samples were collected from patients suffering from UTI, RTI, Bacteremia or Post-Operative Wound Infections (W.I). The specimens collected were processed for isolation and identification of bacterial strains were carried out to find out the correlation between these pathogens and the carrier state.

Our patient population of 400 included 196 females and 204 males (49% and 51%). 73 (18.25%) patients developed post-operative infection including UTI, RTI, Bacteremia and W.I.

Among these 400 patients, 31 patients (7.75%) developed W.I. of which 21 were males and 10 were females. The most common bacteria encounterd in wound infections was

CNS (38.70%) followed by *S.aureus* (32.25%) and *Non- Fermentors* (19.35%). Other Gram negative bacteria isolated included *Ps.aeruginosa, C.freundii, K.pneumoniae and Enterobacter* sp.

Of the 31 positive cultures obtained from the wound swabs, correlation with carrier state was found in 21 patients where carrier isolates as well as the isolates from specimens showed similarity by more than three typing methods. But we could not find any correlation in 10 of the 31 patients who developed post-operative wound infection.

Out of the 21 cultures which showed correlation, seven had correlation with carrier state in the post operative samples only, which suggests that the infection might have been acquired exogenously or endogenously. In 14 cases, we got correlation with the pre-operative samples confirming endogenous infection.

Ten patients (2.50%) developed UTI with equal incidence in both sexes. Among the patients suffering from UTI, the most common organisms isolated were the *Candida* sp. (30.00%) followed by CNS (20.00%). But no correlation was found in the bacterial strains isolated from patients suffering from UTI.

Among the 27 patients (6.75%) who developed lower respiratory tract infections (LRI), 17 were males and 10 were females. The leading isolates were *K.Pneumoniae* (44.44%) and *Non-Fermentors* (29.62%). Among the 27 positive cultures, correlation was found with the carrier state in 10 patients and no correlation was observed with 17 patients. Of the 10 cultures which showed correlation with the carrier state, three had correlation with carrier state only in the post-operative samples which suggests that the infection might have been acquired exogenously or endogenously. In seven cases we got correlation with the pre-operative samples attributing endogenous infection as the cause.

Bacteremia developed in 18 patients (4.50%). *Non-Fermentors* were the predominant organisms in the blood cultures (55.55%). Among the patients who developed bacteremia 12 were males and six females. Of the 18 positive blood cultures, correlation was found with carrier state in seven patients and no correlation observed in 11. Of the seven cultures which showed correlation; three had correlation with carrier state only with the post-operative samples which reveals that infection might have been acquired exogenously or endogenously. In four cultures we got correlation with the pre-operative samples showing endogenous infection.

CONCLUSION

The leading bacterial strains identified from Cardiac Surgery Patients were CNS sp. followed by *S.aureus* and *Klebsiella* sp., of which CNS and *S.aureus* were mainly isolated from the nose and *Klebsiella* from throat.

During hospitalization a marked reduction of bacteria in the skin carriage was observed.

Beta hemolytic *Streptococci* and *Ps.aeruginosa* were not isolated from health-care personnel.

S.epidermidis was the leading isolate among the CNS strains isolated from both groups.

80.12% of CNS and 96.27% of *S.aureus* strains isolated from patients and 87.38% of CNS and 96.30% of *S.aureus* strains isolated from health care personnel were Beta lactamase producers.

Acidimetric phenol red filter paper method and Iodometric test tube method are the most sensitive methods for Beta lactamase detection.

63.47% of CNS and 92.14% of *S.aureus* isolated from patients and 69.36% of CNS and 77.77% of *S.aureus* isolated from health-care personnel were slime producers.

Of the six antibiotics tested for MIC; Cefuroxime and Cloxacillin proved to be drug of choice for prophylaxis.

A correlation of the incidence of post-operative wound infection with carrier state clearly revealed that over half the incidence of post operative infection is endogenous. Thus screening the patients pre-operatively and subsequently prophylactic administration of proper antibiotic is of great importance to reduce the chances of post operative infection. Thus it can be suggested that the antibiotic susceptibility screening of the potential pathogenic bacteria pre-operatively is preferable in deciding the specific antibiotic in its proper dose. This will help in ensuring the reduction in hospital stay and expenses with lower morbidity and mortality which is the ultimate aim of medical science.

In the wake of increasing multiple drug resistance among the nosocomial pathogens, screening for new antibacterial agents is of utmost relevance. In this context the very high antibacterial activity of *Allium sativum* and *Tamarindus indicus* offers promise.

CHAPTER - I

INTRODUCTION

Nosocomial infections (hospital acquired infections), especially those due to multiple drug resistant microorganisms, have now become a major public health concern in terms of frequency, severity and cost of treatment (Platt et al 1992), notwithstanding the many surveillance programmes being devised for their control.

The source of infecting organism can be either exogenous (from another patient, a member of the hospital staff, from inanimate objects or environment in the hospital) or endogenous (from the patients own normal body flora from nose, throat, gut, skin or genitourinary tract).

A carrier is a person who harbours the pathogenic bacteria without suffering any ill effect from it throughout the life time. A carrier can be (a) healthy carrier (b) convalescent carrier (c) temporary carrier (d) chronic carrier (e) intermittent carrier (f) contact carrier or (g) paradoxical carrier.

NORMAL FLORA OF HUMAN BODY

A human harbours a wide array of microorganisms either on or in his body. The normal microbial flora for each species is more or less constant. There are two types (a) Resident flora and (b) Transient flora.

The resident flora are those that constitute a constant population and cannot be removed permanently; the latter vary from time to time and are not permanent. But there are no set rules to divide the human microbes into definite categories of harmless commensal organisms or pathogenic species.

Microbial flora of humans can be broadly divided in to.

(a) Helpful symbionts - Eg: *Escherichia coli*

(b) Harmless commensals - Eg: Coagulase Negative Staphylococci (CNS).

(c) Harmful Opportunists - Eg: *Staphylococcus aureus*.

The normal commensals of throat and oral cavity, skin, nasal canal and gut include many gram positive and gram negative organisms as shown in (Table - 1.1)

TABLE 1.1

THE NORMAL COMMENSAL BACTERIA OF THROAT, ORAL CAVITY, NASAL CANAL, GUT AND SKIN

Throat & Oral Cavity	Nasal Canal	Gut	Skin
Viridans *Streptococci* (+)	*S.epidermidis* (+)	*Clostridium perfringens* (+)	Diphtheroids (+)
Non-hemolytic *Streptococci* (+)	Enterococci (+)	*Cl.difficile* (+)	*St.viridans* (+)
Streptococcus pneumoniae (+)	*St. pneumoniae* (+)	*Bacillus cereus* (+)	*St.faecalis* (+)
Staphylococcus epidermidis (+)	*St.viridans* (+)	*S.aureus* (+)	*S.aureus* (+)
Micrococci (+)	Micrococci (+)	*E.coli* (-)	*St.Pyogenes* (+)
Neisseria pharyngitidis (-)	Diphtheroids (+)	*Campylobacter* spp. (-)	Enterococci (+)
Coliforms (-)	*S.aureus* (+)	*Yersinia enterocolitica* (-)	*E.coli* (-)
Haemophilus influenzae (-)	*St.pyogenes* (+)		*Proteus* spp. (-)
	Klebsiella spp.(-)		*Pseudomonas aeruginosa* (-)

(+) Gram positive, (-) Gram negative.

The commensal microorganisms can become opportunistic pathogens when the host resistance decreases or when they move to other areas of the body from their original sites.

NOSOCOMIAL INFECTIONS

Urinary Tract Infection (U.T.I) is responsible for about 35% to 45% of hospital acquired infections (Walter 1986). Even with adequate precautions, catheterisation in hospitals leads to urinary infections in about 2% which with indwelling catheters may mount to 20%. The microorganisms causing infections enter the catheterized urinary tract either through the intraluminal route or transurethral route or extraluminal route. Only 25% to 35% of patients with catheter associated bacteruria experience symptoms attributable to infection and most cases are asymptomatic bacteruria.

Respiratory Tract Infection (RTI) constitute 8% to 33% of nosocomial infections. The rate of nosocomial pneumoniae varies from 0.5% to 5% (Jay 1986). Microorganisms may invade the alveoli and produce pneumoniae by one of three routes (a) aspiration from the oropharynx (b) suspension in inhaled gas or (c) lymphohematogenous spread.

Bacteremia and Septicaemia are consequences of infection at any site but are commonly caused by infected intravenous canulae. A nosocomial bacteremic episode is defined as the occurrence of any bacteremia beginning at least 72 hours after admission according to Garner et al (1988) elicited by isolation of one or more organisms from the same patient on one or more occassions when clinical evidence suggested a common source. Nosocomial blood stream infections carry a high fatality rate and the crude mortality associated with blood stream infections ranges from 35% to 60% especially in patients in the ICUs (Gatell et al 1988; Weinstein et al 1983).

A wide variety of bacterial, mycobacterial, fungal and rickettsial species have been identified from infections associated with cardiovascular prostheses. The incubation period of post-operative infections related to prosthetic materials is often uncertain and varies within broad limits depending on factors like virulence of organisms, size of inoculum, prophylactic use of antibiotics and host resistance. But generally only those infections that become apparent within 60 days after placement of cardiovascular prostheses is considered as nosocomial.

In the case of infections of skeletal prostheses associated with total hip replacement or total knee replacement or any other skeletal prostheses, the early infections occur within the first month of the post operative period. Late infections

are noted months or even years after conduct of the surgery. Infection rates range from approximately 6% in prosthetic knee replacement (Kettle Kamp, and Leach 1973) to less than 1% in prosthetic hip replacement.

Newborns are readily susceptible to infection as their immune system is not well developed. Cutaneous and conjunctival infections are the most common neonatal infections in both term and premature infants.

Following major burns, nosocomial infections are more prevalent and it is sometimes difficult to determine the presence and degree of infection in burn wounds, since they are invariably contaminated with microbes.

Nosocomial infections in Intensive Care Unit (ICU) patients comprise approximately 20% of all nosocomial infections. The incidence of nosocomial infections in ICU's are usually much higher than in wards according to studies of Maki (1989), Donowitz et al (1982), Lennox Archibald et al (1997). Severity of underlying disease, invasive diagnostic and therapeutic procedures, contaminated life support equipment and the prevalence of resistant microorganisms are critical factors in the high rate of infection in ICUs (Massanari and Heirholzer 1986).

Surgical Wound Infection (W.I) are most common after UTI and they are responsible for increasing the cost and hospital stay as well as the mortality related to surgery. The severity of infection may vary from stitch abscess by normal resident skin flora to severe spreading infections due to exogenous pathogens (Peter Cruse 1986).

Major Pathogens In Nosocomial Infections

The major Gram positive microorganisms associated with nosocomial infections include *S.aureus*, CNS, Group D *Streptococci*, Beta hemolytic *Streptococci, St. Pneumoniae*, Diphtheroids and some anaerobic organisms. The major Gram negative organisms responsible for such infections include *E.Coli, Klebsiella* spp., *Acinetobacter* spp., *Proteus* spp., *Enterobacter* spp., *Pseudomonas aeruginosa, Serratia marcescens* and *Morganella Morganii. Mycobacterial* spp., *Rickettsial* spp., Viral, Candidial and Other fungal infections also contribute to nosocomial infections.

WOUND INFECTION IN CARDIAC SURGERY PATIENTS (WITH PARTICULAR REFERENCE TO MEDIASTINITIS AND STERNAL WOUND INFECTION)

The post-operative infections leads to severe complications and even mortality among the cardiac surgery patients. They lead to increased duration of hospitalization and additional cost for treatment. Green and Wenzel (1977), and Nelson and Dries (1986) studies have shown an increased hospitalization of 5 to 25 days and an extra cost of 2 to 8 times. Wound infections range from 0.2% to 5% in the developed countries and from 3 to 15% among developing countries. (Belcher et al 1990, Kouchoukos et al 1990, Kohman et al 1990).

The impact of sternal wound infection on the health care system is significant. The mortality rate due to sternal infection ranges from 0 to 52%. (Loop et al 1990, Weintraub et al 1989, Rutledge et al 1985, Belcher et al 1990, Demmy et al 1990, Kouchoukos et al 1990, Ulicny et al 1990).

A post-sternotomy suppurative mediastinitis is defined as the detection of purulent material behind the sternum at surgical reexploration or at autopsy. Surgical Wound Infection and Mediastinitis can range from sepsis with few localizing symptoms to signs of obvious wound infection with dehiscence and purulent drainage.

The important risk factors that are likely to lead to wound infection in cardiac surgery patients include: (a) CABG (b) Uremia (c) Patients general health (d) Re-operation (e) Chronic pulmonary obstructive diseases (f) Breast size (g) Corticosteroids (h) Obesity (i) Diabetes mellitus (j) carriage rate of *S.aureus* or any other pathogenic bacteria in nose or in any other body sites.

Wound infections may occur by direct contamination of the operative field or hematogenous spread that can occur intra-operatively or post-operatively. Intraoperative contamination occurs from operating room personnel, Glove puncture, instruments and patients own flora as well as from extra corporeal circuit, intravenous infusions, blood transfusions and invasive monitoring lines (Kluge et al 1973). Post-operative contamination may occur from infusion lines, drains, urinary catheters, pulmonary infections and post-operative inotropic support (Firmin and Wood 1987, Engelman et al 1973).

Intra-operative contamination is found to be mainly caused by Gram positive organisms and post-operative by Gram negative organisms (Stiver et al 1979, Weinstein et al 1976, Sarr et al 1984).

Organisms that are commonly isolated in infected median sternotomy wounds include *S.aureus*, CNS, *Ps.aeruginosa, Klebsiella* spp., *Serratia marcescens, Enterobacter* spp., *Acinetobacter* spp., anaerobic *Pepto Streptococcus, B.fragilis, Wuchereria bancroftii, Mycobacterium fortuitium, M.hominis, Corynebacterium Xerosis*, Fungus *Cuevalaria* and *Aspergillus* spp.

CARRIAGE OF PATHOGENIC BACTERIA AND ITS CORRELATION WITH POST-OPERATIVE WOUND INFECTION

S.aureus is one of the most important causes of nosocomial and community acquired infections. An important factor leading to sternal wound infection is the carriage of *S.aureus* and other pathogenic bacteria in the nasal canal or on other body sites.

About 10-15% of normal population harbour *S.aureus* in their anterior nares. It has been shown that patients who are carriers of pathogenic bacteria are more prone to develop post-operative wound infections, than the patients who are noncolonisers. While colonisers had incidence of WI at around 8%, the noncolonisers had WI incidence of only 1% in a study by Klutyman et al (1995).

IDENTIFICATION AND DRUG RESISTANCE IN NOSOCOMIAL PATHOGENS

It is important to identify the exact source and specific organism responsible for infection and to select and optimise the dose of antimicrobial. Various methods have been devised to achieve these aims. They include different typing methods, methods for speciation, methods to fix the dose, methods to assess virulence and drug-resistance.

TYPING OF BACTERIAL STRAINS

The various typing methods include Antibiogram (Tenover et al 1994); Phage typing (Mulligan et al 1988, 1991); Biotyping (Tenover et al 1997); Bacteriocin typing (Fyfe et al 1984); Analysis of cell envelope protein pattern using PAGE

(Mulligan et al 1988) slime production (Hedin 1996) and Genotyping (prevost et al 1992). In many cases it is often very difficult to establish whether the bacterial strains isolated are from same source or belonging to same strain. But control of nosocomial infections requires the ability to distinguish different strains.

SPECIATION OF BACTERIAL STRAINS

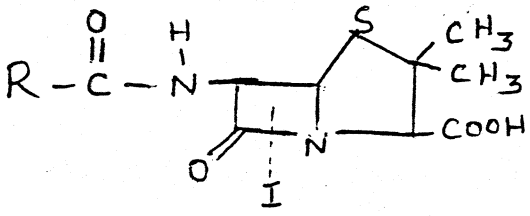
Identification upto species level of bacterial strains are rarely indicated, but are important to find out the significance of the strains causing infection. This is because organisms once considered to be the constituent of normal flora in human beings are gaining importance nowadays as opportunistic pathogens, especially in case if they possess multiple antibiotic resistance. They form an important source of nosocomial infections occuring among hospitalized patients and are often multidrug resistant. Hence speciation of such bacterial strains has to be done by standard procedures (Cowan and Steel 1993, Kloos and Bannerman 1995).

PRODUCTION OF BETA-LACTAMASE

Hospital infections occuring due to Staphylococcus species derives special attention because of their resistance to antibiotics including penicillins and cephalosporins.

Penicillins (Fig a) and Cephalosporins (Fig b) are widely used antibiotics having a beta-lactam ring as part of their basic structure. They can be used against a wide variety of pathogens. However the induction of drug resistance in pathogens has become a great problem, especially in a hospital environment.

Fig (a)



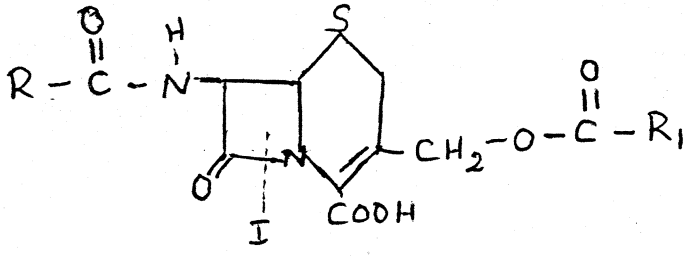


Fig (b)

Resistance to Beta-lactam antibiotics are due to the innate ability of the pathogen to produce the Beta-lactamase enzyme which hydrolyses the cyclic amide bond in the Beta-lactam ring of penicillins, cephalosporins and other related compounds, producing acidic derivatives having no antibacterial properties.

In this context, it is interesting to note that the presence of small amount of drug in the immediate environment may lead to substantial increase in the potency of the enzyme produced (Horton et al 1982, Dyke 1979). Thus the gravity of this problem is much higher in hospital environment where chances of drug induced drug resistance is more.

The Beta-lactamase activity can be detected by Acidimetric method, Iodometric method (Sykes and Matthews 1979), chromogenic cephalosporin substrate method (Callaghan et al 1976) or Microbiological method (McGhie et al 1977).

MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC is the lowest concentration of an agent that inhibits the visible growth of an organism. The lower the MIC, the more susceptible the organism tested. Agar dilution and tube dilution methods are commonly employed methods to find out the MIC (Pamela 1978). It helps to select an ideal bactericidal drug with low toxicity and to use it at a low enough concentration to achieve therapeutic effect.

PRODUCTION OF SLIME

Slime is a capsular material composed of glycerol phosphate, Alanine, Glucose and Glucosamine (Endl et al 1983). Production of extracellular slime by bacteria offers distinct pathogenic advantages in adherence (Carsenti-Etesse et al 1992), protection from host's immune response as well as protection from antimicrobial agents (Gray et al 1984; Johnson et al 1986). The slime producing variants showed a significant higher colonization capacity than did the non-slime producing variants of the same strain used (Baselga et al 1993). This can be used as a typing method for Staphylococcal species to rule out whether two or more isolates from the patient are similar or not (Hedin 1996).

DIAGNOSIS

The use of proper diagnostic method is of great importance in the prevention of any infection. An apt correlation of the clinical symptoms with the microbiological findings helps in arriving at correct diagnosis. Serological, biochemical, culture and microscopic methods are of common use as diagnostic tools.

CONTROL MEASURES

Hospital infection may occur sporadically or as outbreaks. The explosiveness of spread of classical epidemic diseases lead to the concept of quarantine, from which evolved modern techniques of isolation. The universal threat of wound infection following surgery stimulated the stringent aseptic tradition in the operating room including meticulous aseptic surgical techniques, environment cleanliness, disinfection and sterilization processess. The unique observation and management abilities of Florence Nightingale established the modern basis for the design of hospitals and the strategies for patient care that minimize the risk of infection.

The introduction of antibiotics has helped in reducing the risk of infection to a great extent; but the unfortunate emergence of multiple antibiotic-resistance among infectious pathogens had a dampening effect. It has been shown that even with employing the most effective infection surveillance and control programmes, the percentage of nosocomial infection prevented is only at the range of 13% to 38% (Haley and Garner 1986).

The increasing prevalence of antimicrobial drug resistance among hospital acquired pathogens is a major concern world wide. So immediate attention is the need of the hour to fight against these multiple drug resistant pathogens so as to save the life and time through careful precautions, better antimicrobial agents and awareness. Thus the proper adaptation of scientific screening techniques in time, use of appropriate antimicrobial agent and the search for new and better therapeutic agents are indispensable.

In this context some attempts for screening of indigenous medicinal plants and spices for their antimicrobial activity (Sharma 1977, Vijaya and Ananthan 1994, Valsaraj et al 1997, Naqvi et al 1991, Vanden Berghe and Vlietinck 1991) is interesting. An attempt was also made to study the *in vivo* anti-Staphylococcal effect

of Garlic (*Allium Sativum)* and Tamarind *(Tamarindus indicus*) in comparison with the antibiotic cefuroxime in experimental mice.

AIM AND OBJECTIVE OF THE STUDY

The proposed study is mainly aimed at

(a) understanding the rate of carrier state of potential pathogenic bacteria in patients undergoing open heart surgery.

(b) to correlate the incidence of post-operative wound infections with the carrier state and

(c) to suggest effective control measures in order to reduce such endogenous infections.

The study includes isolation, identification and characterization of different strains of pathogens from cardiac surgery patients and to study their antibiotic / non antibiotic susceptibility pattern as well as to understand the epidemiology of nosocomial infections.

CHAPTER - II

REVIEW OF RELATED LITERATURE

Nosocomial infections are one of the most feared complications among patients in hospitals. The consequences of hospital infections are manifold and of major public health concern. Since ancient times, hospitals have always acted as sources of infection for patients admitted to them. In the middle of the nineteenth century, James Simpson reported that the mortality rate following amputations was four times higher in hospitalized patients, compared to those who remained at home and also that it was directly proportional to the size of the hospital (Maxwell and Donald 1986).

In 1843, Oliver Wendell Holmes in his paper, "On the Contagiousness of child bed fever, postulated that puerperal infections were spread physically to parturient women by physicians from infected materials in autopsies they had performed or from infected women they had attended. (Maxwell and Donald 1986).

Joseph Lister, in his historic essay, "On the Antiseptic Principle in the practice of Surgery", introduced the principles of antisepsis (Maxwell and Donald 1986). The concept of asepsis and its applications in hospital practice reduced the incidence of infections, but still caused substantial morbidity, mortality and prolongation of hospitalization in addition to increased cost of patient care.

According to Haley et al (1985), approximately 80% of nosocomial infections fall into four categories: Urinary Tract Infections (UTI); Surgical Wound Infections (W.I); Pneumoniae (RTI) or Bacteremia. Wenzel (1990) reported that nosocomial infection occurs in 5-10% of patients admitted to hospitals in U.S. Daschner (1979) reported that the incidence of hospital acquired infections in surgery fluctuated between 1.5% and 29%, the average being 5%. According to him, the most frequent infections included UTI, W.I, RTI, Sepsis and infections of skin and the most commonly isolated bacteria included *E.coli* and *S.aureus*.

Gastmeier et al (1998) had demonstrated an overall prevalence rate of 3.5% of nosocomial infections among patients in German hospitals with a variation of 0 to 8.9%. In his study, the commonest nosocomial infections reported included UTI (42.1%), Lower RTI (20.6%), S.W.I (15.8%) and primary sepsis (8.3%).

23% of bacteremia was reported in a study of Munoz et al (1997). Jordivalles et al (1997) reported 3.6% of bacterial infections in their study and confirmed the intravascular catheter as the most frequent source of nosocomial bacteremia in I.C.U patients. However, the incidence of catheter related blood stream infection in a study by Herruzo et al (1998) was reported as 5.5%.

Shell (1980) suggested some proposals for reducing the Respiratory Tract Infections. According to him, proper nursing intervention, maintaining the integrity of patient's defence mechanisms and augmenting those that are impaired will help to reduce nosocomial RTI.

Of late, the rate of nosocomial infections are increasing due to multiple drug resistant microorganisms as evident from the reports of Friedland and McCracken (1994), Threlfall et al (1992), Gold and Moellering (1996), Cisneros et al (1996) and Vickery and Pegler (1991).

Gould (1994) suggested that hospital is an ecological niche, where the use of antimicrobial agents tends to create a selective pressure that promotes the emergence of resistant organisms and predisposes patients to colonization with such organisms.

Nobel laureate Sir Burnet (1962) wrote that "One can think of the middle of the twentieth century as the end of one of the most important social revolutions in history, the virtual elimination of the infectious disease as a significant factor in social life".

Nobel laureate Dr. Lederberg (1988) wrote, "The ravaging epidemic of AIDS has shocked the world. We will face similar catastrophes again ... we have too many illusions that we can ... govern the remaining vital Kingdoms, the microbes that remain our competitors of last resort for dominion of the planet".

Studies of Towner (1997), De Gheldre et al (1997) and Cheng et al (1996) stated that hospital outbreaks of multiresistant Gram negative bacteria can result from the interplay of several mechanisms, including the selection of resistance during therapy and subsequent cross-colonization between patients.

Schaberg et al (1991) had observed a disturbing move towards more resistance among many Gram negative isolates causing nosocomial infections. Legakis and Tsakris (1992) have observed that *Ent. cloacae, Acinetobacter* species and *S.marcescens* which were considered as harmless normal flora are increasingly becoming important causes of nosocomial infection.

49.8% of the bacteria isolated from blood cultures in the study of Jordivalles et al (1997) belonged to aerobic Gram positive cocci and 32.6% belonged to Gram negative bacilli. A similar incidence of aerobic Gram positive cocci were reported in the studies of Rello et al (1994) and Pittet et al (1994). Comparably studies of Jordivalles et al (1997), Banerjee et al (1991), Martin et al (1989) and Weinstein et al (1997) have shown CNS as the most prevalent cause of Gram positive bacteremia.

Depending on the source of acquisition of the pathogen, nosocomial infection can be exogenous in origin as reported by the studies of Bosi et al (1996), Sanders et al (1970), Richards and Levitsky (1970), Reyes et al (1973), Kalervo et al (1998) or endogenous in origin as per studies of Klutyman et al (1995), Egebo et al (1996), Alkhoja et al (1979), Seifert et al (1993), Wenzel and Perl (1995), Taplin et al (1963).

Dusan et al (1981) stated that correlation between nonspecific immunity and contamination allows endogenous colonization. Crane et al (1980) reported that endogenous or autoinfection is amplified by the use of canulae, catheters and other hospital devises. According to him, meticulous nursing care is important in reducing the incidence of endogenous spread of microbes. He added that exo and endogenous infections in neutropenic patients are reduced using laminar airflow rooms combined with prophylactic antibiotics as per his studies in an oncology unit.

However, in a study conducted by Waller et al (1963) post operative wound infection were found to have resulted from the airborne spread of Staphylococci from a staff member who remained at the periphery of the operating room throughout the surgical procedure, the only route for transmission of organism being the air and no other opportunity for contact or common vehicle spread.

WOUND INFECTION IN CARDIAC SURGERY PATIENTS WITH SPECIAL REFERENCE TO SURGICAL WOUND INFECTION AND MEDIASTINITIS

The impact of surgical wound infections on the health care system is significant. Post-operative infection by various pathogenic bacteria among the cardiac surgery patients range from 0.2 to 5% in developed countries and from 3 to 15% among developing countries. The incidence of mediastinal and sternal wound infection in various studies is shown in Table 2.1.

TABLE - 2.1

THE INCIDENCE OF MEDIASTINAL AND STERNAL INFECTION AFTER CARDIAC SURGICAL PROCEDURES

Sl. No.	Author	Journal / Year	% of post-operative Surgical complications	Place of study
1	El Oakley	J. Cardio Vasc. Surg. (1997)	0.4%	London
2	Ochsner	J. Cardio Vasc. Surg. (1972)	0.4%	-
3	Grmoljez	Am. J. Surg. (1975)	0.7%	-
4	Sarr	Ann. Thorac. Surg. (1984)	0.7%	Baltimore
5	Breyer	Ann. Thorac. Surg. (1984)	0.8%	Springfield
6	Farinas	Cli. Inf. Dis. (1995)	0.9%	Spain
7	Loop	Ann. Thorac. Surg. (1990)	1.1%	Ohio
8	Rutledge	Surgery (1985)	1.4%	-
9	Jurkiewicz	Ann. Surgery (1980)	1.5%	-
10	Engelman	Arch. Surg. (1973)	1.6%	-
11	Leslie	Ann. Thorac. Surg. (1990)	1.9%	New York
12	Klutyman	J. Inf. Dis. (1995)	2.0%	Netherland
13	Thurer	J. Thorac. Cardio Vasc. Surg. (1974)	2.2%	-
14	Munoz	Cli. Inf. Dis. (1997)	2.2%	Spain
15	Sutherland	J. Thorac. Cardio Vasc. Surg. (1977)	2.86%	-
16	Russel	Ann. Thorac. Surg. (1986)	3.46%	Saltlake City
17	Jimenez	Surgery (1970)	5.0%	_
18	Culliford	J. Thorac. Cardio Vasc. Surg. (1976)	8.0%	New York
19	Marroni	Eur. J. Cli. Microbiol. Inf. Dis. (1999)	5.9%	Italy

Green and Wenzel (1977), Nelson and Dries (1986) have shown an increased hospitalization of 5 to 25 days and an extra cost of 2.8 times in their study due to post-operative wound infections. Ulicny et al (1990) too found a significant lengthening of mean post-operative hospitalization by nearly 13.5 days with development of sternal wound infection. The mortality rate due to sternal wound infection ranged from 0 to 52% as per studies conducted by Loop et al (1990), Weintraub et al (1989), Rutledge et al (1985), Belcher et al (1990), Demmy et al (1990), Kouchoukos et al (1990), and Ulicny et al (1990).

The important risk factors for the development of wound infection among cardiac surgical population have been well defined: Firmin et al (1987) showed concurrent medical problems, nutritional status and immune state as pre-disposing factors. Ulicny et al (1990) have observed a noted significant relationship between age and bodyweight and risk of sternal infection. Reexploration for post-operative bleeding has been implicated as a major risk factor by Rutledge et al (1985), Sarr et al (1984) and Ulicny et al (1990).

Sternal disruption caused by external cardiac massage used in early post operative cardiac arrests has been associated with a two fold to four fold risk of wound infection as per the studies conducted by Rutledge et al (1985), Grossi et al (1985) and Sarr et al (1984). A 50% sternal infection rate in patients undergoing closed chest massage was reported by Ulicny et al (1990).

Sternal wound infections are caused by contamination of the operative field or by hematogenous spread which occur intraoperatively or postoperatively (Kluge et al 1973). According to Firmin et al (1987), Sarr et al (1984) and Miholic et al (1986), the contaminating organisms were derived from operating room personnel, equipment, contamination of extra corporeal circuit, I.V. infusion, blood transfusion, invasive monitoring lines, urinary catheters and pulmonary infections.

The incidence of sternotomy and sepsis when both mammary arteries were used were found to go as high as up to 8% according to Rutledge et al (1985), Grossi et al (1985), Sarr et al (1984) and Nkongho et al (1984). Ulicny et al (1990) concluded that particular attention has to be given to hemostasis, maintenance of hemodynamic stability, pre-operative weight reduction and avoidance of internal mammary grafting in elderly.

In the study of Loop et al (1990), Staphylococcus species dominated among the bacterial strains isolated (67.2%) followed by Enterobacteriaceae (27.86%). El Oakley (1997) reported that 57% of patients in his study developed infection due to *S.aureus* including Methicillin Resistant *Staphylococcus aureus*. Munoz et al (1997) reported *S.aureus* as the main organism isolated in his study which was followed by Gram negative rods; while Vickery and Pegler (1991) have reported that 25% of all the Staphylococcus isolates in their study belonged to Methicillin resistant *Staphylococcus aureus*.

10 to 15% of healthy adults carry *S.aureus* in the nares, and in hospital personnel this may even rise to 20 to 35% as per studies of Williams (1990). Luzar et al (1990) were of the opinion that contributing factor to the problem of nosocomial infections is the nasal carriage of *S.aureus* among hospital personnel. According to him, the reservoir of potential pathogens has to be recognized as a significant source for new carriers among patients and staff.

The carriage rate of *S.aureus* among hospital personnel is associated with an increased risk of infection. The carriage rate of all *S.aureus* among hospital personnel has been studied in various countries and a british study reported 35% rate in 1955 (Blowers et al 1955). US study in 1969 reported 39% (Maxwell et al 1969). Nigerian study in 1991 reported 39% (Ako-Nai et al 1991), but Rountoee and Beard (1958) states that high carriage rate of non MRSA have been reported during epidemics. However nasal carriage rate of MRSA in hospital personnel have been found to be lower according to Boyce (1991) and Casewell (1983) study; whereas higher rates has been reported in situations of outbreak of MRSA infections by Cafferkey et al (1984). Shagren (1984) is of the opinion that role of asymptomatic carriers pertains more to epidemics, but 15% of the healthy population are chronic asymptomatic nasal carriers of *Staphylococcus aureus*.

Kingdom et al (1983) have shown that a study of medical undergraduates at Trinity College Dublin, with differing periods of hospital exposure showed no change in carrier rates, but however he noticed a tendency towards more antibiotic resistance in the group with longer exposure. Rafalowski et al (1991) in his study in Polish medical undergraduates, showed a carrier rate of 33% with low antibiotic resistance. Lamikanra et al (1985) reports among non medical undergraduates in Nigeria, having carriage rate of 56.4% with a considerable proportion of the strains resistant to

commonly used antibiotics. Ward (1992) proposed that MRSA and MSSA have an equal likelihood of nasal carriage.

Weinke et al (1992) reported that many patient groups have high rates of *S.aureus* nasal carriage in comparison to the general population and health care workers. Nasopharyngeal cultures have been correlated with organisms from the infected mediastinum as per studies of Engelman et al (1973).

Weinstein (1959) study described that, of the 125 patients undergoing major surgery, patients with a positive pre-operative *S.aureus* nasal culture demonstrated a post-operative wound infection rate of 37%. On the contrary, those patients with negative pre-operative nasal culture demonstrated a 11% infection rate. Several other studies which support the view that patients who are carriers of *S.aureus* are more prone to develop post operative wound infection are shown in Table 2.2.

Tullock et al (1954) reported that Staphylococci isolated from skin lesions in patients with chronic Staphylococcal skin infections in the study were of the same phage type as Staphylococci isolated from anterior nares of patients in 88% of instances where Staphylococci were typable.However the reverse has also been shown by Basset et al (1963) who found 5% of postoperative wound infection in colonized patients and 8% of post operative wound infection in noncolonized patients.

Wenzel et al (1992) suggested that eradication of the carrier state in patients pre-operatively may reduce the rate of post-operative wound infection with *S.aureus* which was supported by Klutyman et al (1995).

A ten year prospective study of Cruse and Foord (1980) concluded that short pre-operative hospitalization, showering with antiseptic soap before operation, shaving of patient kept to a minimum, contamination eschewed, punctilous surgical techniques, scrupulous care in operation performed on elderly, obese, malnourished and diabetic patients, no drains brought out through the operative wound, meticulous electro-surgical coagulation techniques and informing each surgeon of his/her clean wound infection rate and average rates among peers-all will help to reduce the rates of post-operative wound infection to a certain extent.

TABLE 2.2

COMPARISON OF POST-OPERATIVE WOUND INFECTION AMONG NASAL CARRIERS / NONCARRIERS

Author	Journal	% Infected / Colonized	% Infected / Non colonized
White	Antimicrob. Age. Chemother.	19%	8%
Calia	J. Hygiene	17%	9%
Mc. Neill	BMJ	16%	10%
P.H.L.	Lancet	9%	7%
Henderson	BMJ	8%	3%
Williams	BMJ	7%	2%
Klutyman	J. Inf. Dis.	8%	1.1%

Clark et al (1976) is of the opinion that careful attention to possible endogenous sources of infection from the patient and a multifaceted program directed to exogenous sources of infection can lower infection rates in cardiac surgical patients. In his study, he found a reduction of deep wound infection rate from 2.9% to 0.6% and a concomitant total wound infection decrease from 6.6% to 3.3% by taking appropriate measures like identification of shedders and carriers, satisfactory practice of hair removal, skin preparation of patient and use of high flow HEPA filtered vertical ventilator system.

Platt et al (1992) and Wenzel and Streed (1989) are of the opinion that although National Nosocomial Infection Surveillance programs have been implemented in many countries, they rarely include information on risk factors in relation to specific epidemiology of various bacterial species. Such studies need detailed prospective surveillance of specific procedures or services. According to them, such surveillance studies can provide a more comprehensive means of analyzing the epidemiology of hospital acquired infections, defining risk factors and determining trends of resistance and pattern of virulence.

Carriage of any pathogenic bacteria in any body site can lead to endogenous type of hospital infection. This has been proved by the studies of Vatopoulos et al (1996) and Ekdahl et al (1997) who reported that the carriage of any pathogenic bacteria like *Enterobacter cloacae, Acinetobacter* spp., *Streptococcus pneumoniae, Serratia marcescens* - all of which have been considered as non pathogenic normal flora in the past, have emerged as the leading causes of endogenous hospital acquired infection.

Investigation of an outbreak requires the use of discriminating typing systems to understand the dynamics of the problem, to find out the source and similarity of the strain as well as to guide infection control measures (Struelens 1993). Typing systems are based on the principle that clonally related isolates share characteristics by which they can be differentiated from unrelated isolates (Arbeit 1995).

In clinical practice, the use of antibiogram for epidemiological typing is a natural first step (Hedin 1996). Works showing that antibiogram pattern has been used as an important typing method in the epidemiological studies to verify strain similarity includes the studies of Ten Over et al (1994), Ayliffe (1990), Jacques et al (1994), Hedin (1996) and Hedin and Hambraeus (1993).

Biotyping has been used as an important typing method in the epidemiological studies to verify the strain similarity as per the works of Aber and Mickael (1981), Christensen et al (1982), Maslow et al (1993) and Philippe et al (1990). Biotyping involves comparison of biochemical or substrate utilization pattern and qualitative or quantitative measurement of metabolic end products (Balows and Isenberg 1978).

According to Ivannovics (1962), Gagliano and Hinsdill (1970), Heczko (1971), Fyfe (1984), Pitt (1988) and Pfaller (1995), Bacteriocin - typing increases the sensitivity of epidemiologic typing when used in combination with other typing systems such as antibiogram or biotyping.

In the study of epidemics or outbreaks, especially with *S.aureus* strains, Bacteriophage - typing has been employed as an important typing method. (Mulligan et al 1988,1991, Blair et al 1961, Tenover et al 1994, Cookson et al 1986, Aubry Damon et al 1997, Mathew et al 1983, Klutyman et al 1995, Rossney et al 1994). Production of slime should also be considered as a typing method according to Hedin (1996) and Etesse - Carsenti et al (1990). Typing the strains using PAGE has also helped to draw a conclusion to find out whether the strains belonged to same type or not. Works that have been done to find out the strain similarity using PAGE include the studies of Mulligan (1988) and Costas (1989).

Microbial typing has provided a powerful set that facilitate both epidemiologic surveys and patient management. According to Arbeit (1995) typing data are most appropriately evaluated in the context of hypotheses and questions fully developed by the clinician or epidemiologist and thus are used to augment rather than to replace those analysis.

VIRULENCE AND DRUG RESISTANCE

It is important to identify the exact source and specific organism responsible for infection. Timely detection and control of epidemic strains are important to prevent spread of nosocomial infections (Goldmann et al 1996). The Clinical Microbiology Laboratory plays an important role providing accurate and rapid diagnostic method by identifying the organism.

Species of bacteria (CNS) which were once considered as harmless commensals of normal bacterial flora of man are nowadays considered as important

opportunistic pathogens causing various diseases including infections associated with Prosthetic heart valves, Cerebrospinal shunts, Arthritis associated with artificial joints, UTI, Endocarditis and other Post-operative wound infections according to studies of Patrick (1990), Boyce et al (1990), Patricia et al (1985), Speller and Mitchell (1973), Weinstein et al (1976), Nicolle et al (1983), Gruer et al (1984) and Schebaum et al (1975).

Several systems of classification have been proposed for the identification of CNS species (Wood et al 1987, Cowan and Steel 1993, Baird and Parker 1963, Kloos and Bannerman 1995). Of the various species of CNS, *S.epidermidis* is considered as the most important pathogenic species due to the emergence of multiple antibiotic resistant strains among them (Lyyttikainen et al 1996, Sindhu et al 1997).

Narayani et al (1988) who worked on the speciation of CNS have found that 49.1% of healthy carriers and 39% of patient isolates belonged to *S.epidermidis* whereas Shanmugam et al (1994) have found it as 49% and 32.1% respectively. According to Nystrom (1992), the increased rate of prevalence of this strain among patients gives an idea on the continuous persistence of them in the hospital environment.

Naseema et al (1988) reported that among CNS, the leading species in their study for carrier state of pathogens among hospital personnel were found to be 49.1% of *S.epidermidis*. Thurn et al (1992) reported 89.1% of strains of CNS in their study belonged to *S.epidermidis*.

Nonfermentors like *Acinetobacter* Spp. which were thought to be nonpathogenic and part of normal flora of skin are causing increased infections of late. Hence with Gram negative bacteria also, identification upto species level is important as there are increasing reports on hospital infections caused due to these organisms (Cisneros et al 1996, Forster and Daschner 1998, Consuelo et al 1990, Hol et al 1996, Struelens et al 1993, Mishra et al 1986).

Antibiotic resistance is now generally accepted as a major public health issue. The rate of bacterial resistance to antibiotics is significant epidemiologically and ecologically. Antimicrobial resistant pathogens are challenges to progress in controlling emerging infections, especially in hospital acquired infections as reported by Bryan et al (1994). According to Hughes and Tenover (1997) antimicrobial resistance has a number of very important implications for clinicians caring for

patients, infection control practitioners monitoring health care institutions, public health professionals responsible for infectious disease surveillance, clinical microbiologists responsible for detection of resistant strains, persons and organizations providing continuous medical education for physicians, health education specialist responsible for communicating public health messages to the public and individuals in government and industry responsible for setting drug and vaccine development priorities.

Studies of Detsky and Naglie (1990) and Freeman et al (1987) have proved that in hospital infections, the resistant microorganism and antibiotic therapy increased the risk of colonization, infection and spread of resistance.

Brumfitt and Hamilton (1989) report that MRSA is one of the major causes of hospital acquired infections, challenging clinicians and infection control teams through out the world. Voss et al (1994) found 12.8% of *S.aureus* strains in his study were resistant to the antibiotic methicillin. He also found that the rates of resistance to the nonglycopeptide antibiotics were lowest for rifampin and highest for ciprofloxacin.

MRSA have also been found to be efficient colonizers of patients causing outbreak of serious infections that are difficult to control as per studies of Boyce (1990), Cookson and Phillips (1988) and Wenzel et al (1991). MRSA strains have been found to develop resistance to many antibiotics including erythromycin, tetracycline, gentamicin and other fluoroquinolones in the studies of Boyce et al (1990), Cookson and Phillips (1988) and Mulligan and Arbeit (1991) and Aubry Damon et al (1997).

Acar and Goldstein (1997) have reported high resistance to fluoroquinolones among *S.aureus, K.pneumoniae, Serratia marcescens* and *Ps.aeruginosa*. Similar reports on resistance to fluoroquinolones among *E.coli* have been reported by Casellas et al (1994), Goldstein and Acar (1995) and Turnidge (1995). Increasing resistance to oxacillin and fluoroquinolones have been reported in the studies of Pegues et al (1998).

Emori and Gaynes (1993) and Swartz (1994) report that the frequency of infections caused by Staphylococci both CNS and *S.aureus*, Enterococci and *C.albicans* is increasing. Bergogne-Berezin and Towner (1996) and Spencer (1995) report that in addition to the enteric bacilli, *Ps.aeruginosa, Acinetobacter* species,

S.maltophilia and *B.cepacia* have become important nosocomial pathogens worldwide.

Hospital infections occuring especially due to Staphylococcus species derives special attention because of their resistance to antibiotics including penicillins and cephalosporins. This extracellular enzyme having high activity against penicillins and cephalosporins are generally called Staphylococcal penicillinase or beta-lactamase. Beta-lactamases inactivate benzyl penicillin by destroying the beta-lactam ring (Bauern feind 1986, Abraham and chain 1940 and Lyon and Skurray 1987).

According to Antone (1997), the number and variety of new beta-lactamases detected have risen at an alarming rate, in response to the clinical use of new classes of beta-lactam antibiotics. Beta-lactamases produced by Gram positive organisms are inducible enzymes which appear in increased quantity only in the presence of an indicator. These are extracellular (Dyke 1979, Peter and Ian 1993), while beta-lactamases produced by Gram negative organisms produce both inducible and constitutive enzymes and are mostly cell bound. (Normark et al 1990). Bennet and Chopra (1993) studied the molecular basis of beta-lactamase induction in bacteria.

The most widely used methods for detecting beta-lactamases are mainly of four categories : Acidimetric methods, Iodometric methods, Microbiological methods and Chromogenic substrate methods (Sykes and Matthew 1979).

Sindhu and Shanmugam (1998) reported the beta-lactamase production among CNS and *S.aureus* strains isolated from patients admitted for cardiac surgery and from healthy hospital personnel by both iodometric and acidimetric techniques and have reported about the alarming rate of beta-lactamase positive Staphylococcal species prevalent among the patients and hospital staff in their study, where they got 80% and 96% of positivity for CNS and *S.aureus* strains indicating the problem of treatment by beta-lactamase susceptible antibiotics.

Bhatia et al (1982) have found 96% of strains tested by them as beta-lactamase producers; whereas Horton et al (1982) have found all penicillin resistant strains in their study as beta-lactamase producers. Shanmugam and Beena Panicker (1996) have reported 95.5% of CNS strains as beta-lactamase producers in their study.

The production of extracellular slime offers distinct pathogenic advantages in adherence, protection from host's immune response as well as protection from

antimicrobial agents according to Gray et al (1984), Johnson et al (1986) and Peters (1988).

Slime production has been considered as a typing method for Staphylococcus species to identify whether two or more isolates from the patient are similar or not in the study of Hedin (1996). The elaboration of slime in conjunction with other characteristics has been suggested as a predictor of significance in clinical aspects as well as a marker of virulence by Makhija et al (1995), Ishak et al (1985), Martin et al (1989) and Davenport et al (1986).

The detection of slime production can be done by two methods. Christensen et al (1982) described the test tube method using 0.1% safranine while Freeman et al (1989) described a new method of detecting slime by means of Congo red agar plate method.

Slime production of *S.epidermidis* has been found by several investigators to be an efficient discriminator between infectious and noninfectious strains (Christensen et al 1982 and Diaz Mitoma et al 1987). However other investigators are of the opinion that slime production is not a universal determinant of strains causing infection (Kristinsson et al 1986, and Needham and Stempsey 1984).

Alexander and Rimland (1987) suggested that slime production was not associated with specific antibiotic susceptibility pattern or speciation. In their prospective study, slime production was not associated with pathogenicity in CAPD peritonitis caused by CNS.

Etesse Carsenti et al (1990) in their study among the *S.aureus* have shown that (64.1%) are not slime producers and are not adherent isolates, thus giving the least chance to find any correlation between these properties and pathogenesis. However according to them though biotyping, phagetyping, serotyping, antibiotic susceptibility profiles and plasmid pattern analysis have been used in an attempt to identify the strains of Staphylococci, there is still a need for a simple rapid and cost effective method of distinguishing true pathogens from simple contaminants. They are of the opinion that testing isolates for slime positivity and for adherence property may fullfill the task.

Baselga et al (1993) reported that in their experiments, the slime producing variants showed a significantly higher colonization capacity than the non-slime producing variant of the same strain used.

On studying the adhesive activity of clinical Staphylococcal strains on a model of human erythrocytes by Arutcheva and Petrakov (1991) no relationship was noted between the adhesion indices and Staphylococcal antibiotic resistance. It is important to consider the adherence property and bactericidal activity, when selecting agents for antibiotic prophylaxis.

Post-operative infections are serious and often life threatening complications. They are often associated with substantial morbidity, prolonged hospital stay and increased hospitalization costs. For this reason antibiotic prophylaxis has been used in cardiac surgery for many years in order to reduce patient morbidity,hospital stay and hospitalization cost.

In the wake of the observation that hospital pathogens are becoming resistant to a number of available drugs, the MIC is gaining importance. The intepretation of antibiotic susceptibility test result as "Sensitive and Resistant" may not be always clear cut (Pamela 1978). In such situations the only way to find out the apt bactericidal concentration is to determine the Minimum Inhibitory Concentration (MIC) of the particular drug for the organism concerned.

Despite the fact that patients undergoing open heart surgery are at risk for serious post operative infections, there is no consensus about the kind of antibiotic prophylaxis and the duration of its administration (Kriaras et al 1997, Lainez et al 1994, Orita et al 1992, Slaughter 1993). There is evidence from clinical trials that a single dose of antibiotic prophylaxis has the same efficacy as multiple doses given perioperatively and that longer antibiotic administration increases risk of super infections, resistance and toxicity in cardiac surgery patients (Nichols 1994, Sisto 1994, Balbrath 1993).

The efficacy of single dose prophylaxis with Ceftriaxone and Cefuroxime in cardiac surgery has been demonstrated in clinical studies of Hall et al (1993), Nooyen et al (1994), Vusorisalo et al (1997). Kriaras et al (1997) study compare the effectiveness of single dose Cefuroxime with a four day combination of Amoxycillin - Netilmicin as antibiotic prophylaxis in patients undergoing open heart surgery and found that a single shot of Cefuroxime prophylaxis is equally effective and safe as a four day regimen with Amoxycillin and Netilmicin.

World wide, Cephalosproins are the most widely used antibiotics for surgical prophylaxis (Gorbach 1982). A second generation Cephalosporin is usually selected

because of its low toxicity, increased stability to beta-lactamase enzymes, broad antibacterial spectrum and good tissue penetration (Maki et al 1992, Conklin et al 1988, Gentry et al 1988).

So far evidences are insufficient to recommend antimicrobial prophylaxis to intraoperative period alone (Ariano and Zhand 1991, Page et al 1993). Though amikacin and tobramycin have been advocated as effective drugs for prophylaxis (Muscato et al 1991), their potential for nephrotoxicity and auditory or vestibular toxicity limits their use as prophylactic agents (Joseph and Robert 1995).

The different systems of Indian medicine like Ayurveda, Siddha, Unani and other local traditions utilize a large number of plants for the treatment of human diseases. The Vedhas, particularly the Atharvavedha, contain many medical references ranging from mere witchcraft to alkaline surgery. Ayurvedic literature such as "Charaka Samhitha and Sushrutha Samhitha" have mentioned about 2000 drugs (Kannabiran 1978), most of which have plant origin.

The medicinal plants are vital resources having important bearing on human health and the regions economy. WHO has estimated that 80% of the world's population rely on traditional medicine, a major part of which involves the use of plant extracts or their active constituents (Vijaya and Ananthan 1994).

A large number of Indian medicinal plants are bestowed with antimicrobial actions (Chopra et al 1956). Research on natural resources has been encouraged by WHO since 1978. In recent years, attention has been given to plant products for the following reasons.

(a) Drugs from plants are effective, easily available, less expensive and rarely have side effects.

(b) Synthetic drugs not only have side effects, but also are very expensive to develop. For the successful introduction of a new product, approximately 3000 to 4000 compounds are to be synthesized, tested and screened. The cost of development ranges from 0.5 million $ to 5 million $ (Ikram 1984).

(c) Plants synthesize complex molecules from simpler ones by highly specific reactions, that would either be too difficult or too costly to duplicate by artificial methods.

(d) Intensive use of antibiotics has often resulted in the development of resistant strains (Sydney et al 1980).

Researchers are increasingly turning their attention to folk medicine inorder to develop better drugs against cancer as well as viral and microbial infections (Galal et al 1991, Hoffman et al 1993). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not yet been adequately evaluated (Balandrin et al 1985).

Antibacterial activity of ether extract of medicinal plants have been reported by Carlson et al (1984). Beloy et al (1976) reported the antimicrobial activity of 106 plant species. Chhabra and Viso (1991) studied antibacterial activity of some Tanzanian plants used in traditional medicine while Hernandez et al (1980) reported the antibacterial and antifungal activities of nine Cuban higher plants.In the screening of 21 UAE folk medicinal plants by Tanira et al (1994) only ten showed antimicrobial activity. Muanza et al (1994) report that on screening nine Zaire medicinal plants for invitro antibacterial and antifungal activity, six plants demonstrated marked bactericidal activity.

The preliminary chemical investigation of *M.oleifera* bark revealed the presence of sterols and terpenes and absence of alkaloids, saponins, tannins and mucilage (Bhattacharjee and Das 1969, Atal et al 1978). The seed oil contained palmitic, stearic, behenic and oleic acids. The juice from the leaves and stem bark of *M.oleifera* inhibited *S.aureus* but not *E.coli* in a study by Bhawasar et al (1965).

The antimicrobial activities of *Moringa oleifera* leaves, roots, bark and seeds were investigated *in vitro* against bacteria, yeast, dermatophytes and helminths that are pathogenic to man in a study by Cacerer et al (1991). In his study, he demonstrated that the fresh leaf juice and aqueous extracts from seeds inhibit the growth of *Ps.aeruginosa* and *S.aureus* and that extraction temperatures of above 56°C inhibited the activity.

In the study of Valsaraj et al (1997), they observed antibacterial activity of ethanolic extract of *Olerodendron infortunatum* against *S.aureus, E.coli* and *Ps.aeruginosa* at a concentration of 25 mg/ml. Similar studies were reported by Pushpangadan and Atal (1984).

In the study using ethanol extract of leaf of *Murraya Koenigii*, Valsaraj et al (1997) reported activity against *E.coli, Ps.aeruginosa* and *S.aureus*. With *Leucas aspera* no activity was observed against *E.coli* and *Ps.aeruginosa,* (Ambasta et al 1992).

Ethanolic extract of *Adhatoda vasica* did not gave any activity against *E.coli* while antibacterial activity was observed against *E.coli, S.aureus* and *Ps.aeruginosa* in the study of Valsaraj et al (1997).

Aqueous extracts of *Camellia sinensis* of different types inhibited a wide range of pathogenic bacteria including MRSA in the study by Yam et al (1977). Tea extracts were bactericidal to Staphylococci at well below "Cup of tea" concentration according to them. Testing of pure tea compounds and closely related chemicals suggested that the antibacterial activity of extracts of green tea can be explained by its content of epigallocatechin, epigallocatechin gallate and epicatechin gallate according to this study group. In black tea extracts, theaflavin and its gallates are additional active components. Bactericidal effect of tea has also been reported by Masakotoda (1989).

However no antibacterial activity in the aqueous extracts of tea was observed in the study of Vijaya and Ananthan (1994) suggesting the absence of water soluble substance in the active constituent. In their study, neem leaf extracts did not show any activity by agar cup bore method. Rios et al (1998) stated that the water insoluble compounds of the plant *Azadirachta indica* might not have diffused in to the media. Hamsaveni and Purushothaman (1986) reported that *E.coli* was controlled by the leaves of *A.indica*.

Not much reports have been published regarding the antibacterial activity of *Emilia sonchifolia, Eupatorium urticifolium, Lawsonia inermis* and *Piper betle*.

Eucalyptus globulus showed inhibition against all the bacterial pathogens. The antibacterial properties of the essential oils of 24 different Eucalyptus species against 15 bacteria were reported by Kumar et al (1988). Antimicrobial activity for *Leucas aspera* was found in the study of Dhawan et al (1977). Naqvi et al (1991) reported the antibacterial, antifungal and anthelminthic activity of *Ocimum sanctum*. The antibacterial activity of *Tamarindus indicus* was reported by Bhat et al (1957).

The spices and their extracts act as microbial antagonists (Pruthi 1980, shelef 1983). Investigations have been undertaken to provide a scientific basis for understanding the medical use of *Syzygium aromaticum.* Several authors have reported its medicinal, insecticidal, antimicrobial, antiprotozoal and antitumour properties (Adetumbi 1986). Clove oil is useful in treatment of Catarrah, Cough,

Bronchitis, Skin diseases, Dyspepsia, Vomiting, Dental caries and Cephalalgia (Muthukrishnan 1997).

The antimicrobial activity of clove exceeds that of most broad spectrum antibiotics. It exhibits antibacterial, antifungal and antiviral properties. Susceptible bacteria include *S.aureus* (Huddleson et al 1944).

The volatle oil of cloves contains 40 to 90% of Eugenol (C10 H12 O2). Graphical formula (Jhon 1969).

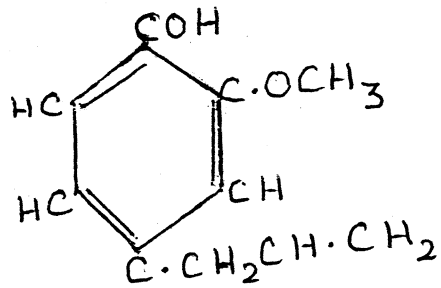


Fig (c)

The organic compounds that are reported to be present in the oil of cloves include Eugenol acetate, caryophyllene, methyl-n-amyl carbinol, methyl-n-hepty carbinol, methyl-n-heptyl-ketone, methyl benzoate, bezyl alcohol, furfuryl alcohol and vanillin (Winton 1939).

The antibacterial activity of *Cinnamomum zeylanicum* is due to the presence of cinnamaldehyde and eugenol (George et al 1958). High concentration of tannins are present in bark which are used in medicine to control haemorrhage, diarrhoea, heart disorders and also to some extent for antibacterial activity (Prasad and Bandyopadhyay 1993).

Black pepper is extensively used in several Ayurvedic preparations. But green pepper is found to be more bactericidal. According to Wang et al (1982) and Prasad and Bandyopadhyay (1993) compound A of pepper is more antioxidative and antimicrobial.

There are no reports on the antibacterial activity of *Brassica juncea, Capsicum annuum* and *Coriandrum sativum*. Hexane extract of *Cuminum cyminum* inhibited the growth of *S.aureus* and *E.coli* but does't had any activity against *Ps.aeruginosa* in the study of Shobha Agnihotri and Ashok Vaidya (1996).

Allium sativum (Garlic) is a member of Lilliaceae family. *Allium sativum* is one of the common spices that has long been recommended for cure for a number of ailments, viz, Pneumoniae, Foul ulcers, Bronchitis, Atopic dyspepsia and Gastro intestinal disorders (Jain and Konar 1977, Feltcher et al 1974 and Appleton and Tansey 1975, Jain et al 1973 and Konar and Jain 1978).

The crude extracts of *A.sativum* was found to be quite effective against Gram negative as well as Gram positive bacteria *in vitro* which inhibited the growth of some of the bacteria, which were resistant to commonly used antibiotics as in the study of Sharma et al (1977). He also reported that the antibacterial property of the extract was completely inactivated at 100°C within five minutes.

Bacteria resistant to eight main antibiotics showed a high response towards garlic extract, although *Ps.aeruginosa* showed resistance against garlic in the study of Sharma et al (1977). Garlic is found to be effective against *S.aureus* in the study of Huddleson et al 1944).

The antibacterial activity of garlic is attributed to the Allium (Farbman et al 1993) and Ajoena (Ankiri et al 1997) present in garlic. These two compounds have the potential to oxidise SH groups in bacteria (Naganawa et al 1996).

The *in vivo* studies on the Staphylococcal infections and the antibacterial effect of spices and plants have been conducted by various workers (Shibl 1982, Gold Schmidt 1972, Roy Choudhury et al 1993, Dat et al 1993). Intravenous and intraperitoneal infections were used to study various facets of Staphylococcal pathogenicity (Kapral 1980). But experiments have shown that experimental animals can tolerate large number of non capsulate *Staphylococcus aureus* strains given by this route as per various studies of Cohn and Yale and Koenig (Easmon and Good Fellow 1990).

The antibacterial activity exhibited by the Indian Medicinal plants and Spices stands promise for the future researchers to explore and to find out new, better, less toxic, cost-effective drugs from the nature itself.

CHAPTER - III

MATERIALS AND METHODS

3.1. Isolation of bacterial strains from throat, nose and sternal skin area of Cardiac Surgery Patients (CSP)

To study the carrier state of pathogenic bacteria at different stages of hospitalization, a total of four hundred patients admitted for cardiac surgery at Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST) hospital were screened for the carrier state of pathogenic bacteria in the nose, throat and sternal skin area.

Sterile cotton swabs were used for the collection of specimens from throat, nose and sternal skin area (Bannatyne et al 1979, Simor et al 1988). Cotton swabs were kept one each in a test tube, sterilized at 15 lb for 15 minutes before they were taken to the ward (Miller 1985). Saline dipped swabs (sterile) were used for skin specimens.

Pre-operatively swabs were collected on the day of admission. Post-operatively swabs were collected twice (1) on the day of transfer from ICU to ward and (2) on the day of discharge from the hospital. Thus samples were collected in the order of 9 swabs / patient to a grant total of 3600 samples in the present study.

3.2 Isolation of bacterial strains from throat, nose and skin of Health Care Personnel (HCP)

To find out the carrier state of bacterial harbouring in the throat, nose and skin area of health care personnel, hundred HCP were selected. Samples were isolated from throat, nose and skin (hands) and method of collection is as given in section 3.1. Thus a total of 300 swabs were collected from these subjects in the order of 3 swabs / HCP.

3.3 Isolation of bacterial strains from Infectious specimens

Urine, sputum samples and blood samples were collected if the patient was suffering from Urinary Tract Infection, Respiratory Infection or Bacteremia. Similarly wound swabs and pus discharges were also collected if the patient were suffering

from any post operative wound infection. The specimens collected were processed properly for isolation and identification of bacterial strains so as to find out the correlation between the rate of incidence of infectious type of pathogens and the carrier state.

3.4 Preparation of Culture Media

3.4.1 Nutrient Agar

Nutrient agar constitutes the base for blood agar.

Composition

Peptone	10 gm
Nacl	5 gm
Beef extract	3 gm
Agar	20 gm
Distilled water	1000 ml

pH was adjusted to 7.4 to 7.6. The mixture was autoclaved at 15 lb for 15 minutes and poured in to sterile petriplates and tubes as per the requirement.

3.4.2 Blood Agar

To the melted and cooled nutrient agar (about 50°C) sterile defibrinated sheep blood (8-10%) was added. Mixed well and poured into sterile petri dishes. Blood agar was used as an enriched media for growing bacteria and also to differentiate the type of hemolysis.

3.4.3 Chocolate Agar

Blood agar was heated to around 70°C so as to form the chocolate agar. This was used to check the growth of fastidious organisms.

3.4.4 Mac Conkey Agar

Composition

Lactose	10 gm
Bile salt	1.5 gm
Peptone	20 gm
Nacl	5 gm
Agar	20 gm
Distilled water	1000 ml

pH was adjusted to 7.2 to 7.4. Then added neutral red (0.03 gm) as indicator. It was then autoclaved at 15 lb for 15 minutes and poured into sterile petri dishes. This was used to differentiate between lactose fermenting and non lactose fermenting organisms and also as a selective medium for Enterobacteriaceae.

3.4.5 Semi Solid Nutrient Agar

0.5% of agar in nutrient broth constituted semi solid agar which was poured into penicillin vials and autoclaved at 15 lb for 15 minutes. This was used for stocking the bacterial strains for long periods (at room temperature or in the refrigerator).

3.4.6 Nutrient Broth

Composition

Peptone	10 gm
Nacl	5 gm
Beef extract	3 gm
Distilled water	1000 ml

The pH was adjusted to 7.5. The broth was dispersed (5 ml) to each tube, the tubes were plugged and then autoclaved at 15 lb for 15 minutes. This was used for subculturing for further identification tests as well as for antibiotic sensitivity tests.

3.4.7 Normal Saline

Composition

0.85% of Nacl.

Autoclaved at 15 lb for 15 minutes. This was used for dipping cotton swabs where ever needed.

3.5 Isolation of bacterial strains

The swabs collected from cardiac surgery patients, health care personnel, as well as from the infected patients were processed for the isolation and identification of bacterial strains.

The common medias employed for bacterial isolation includes blood agar, MacConkey agar and chocolate agar. The plates were incubated at 37°C for 15-18

hours. For further confirmation, the culture negative plates were reincubated further for a period of 24 hours.

3.6 Maintenance of bacterial strains

The isolated strains were maintained at room temperature in semi solid nutrient agar in penicillin vials over layered with sterile liquid paraffin. Subculturing was done periodically for further experimental procedures. The isolated colonies were subcultured into nutrient broth and subjected to bacterial identification as per standard procedures. (Cowan and Steel 1993, Arbeit 1995, Collee et al 1996).

3.7 Identification of bacterial strains

The strains were identified by colony morphology, Grams staining and different Biochemical tests (Arbeit 1995, Cowan and Steel 1993, Baird Parker 1963, Collee et al 1996).

3.7.1 Visual examination of the colony

Visual examination of the colony morphology of bacterial strains grown in blood agar, chocolate agar and MacConkey agar were noted. Lactose fermentation, surface of the colony, size of the colony, pigment production, hemolysis, opacity and shape of the colony were recorded as major distinguishing characteristics.

3.7.2 Gram's Staining

Gram's staining was done as per the Hucker modification method (Hucker 1988). A smear was prepared on a clean and grease free microslide, and fixed by mild heating by passing over the flame for two or three times. Staining of the fixed and dried smear was done as follows.

Primary staining was done with crystal violet solution for one minute. Slide was then rinsed with distilled water, flooded with Grams iodine solution and kept for one minute and then washed with distilled water. Then the slide was flooded with acetone for a few seconds until the violet colour stops coming and immediately washed with distilled water. After decolourisation, the smear was counterstained with safranin, kept for thirty seconds and rinsed subsequently with distilled water. The slide was then allowed to air dry.

Microscopic observation of Gram's stained smear was made under oil immersion (100x) for morphological characterisation of the bacterial strain. Gram positive bacteria were stained purple and Gram negative bacteria appeared red (Plate-1).

3.7.3 Biochemical tests

For the identification of different bacteria, various biochemical tests were performed (Cowan and Steel 1993, Baird Parker 1963, Arbeit 1995, Collee et al 1996).

3.7.3.1 Sugar-fermentation test

Sugar fermentation test is important in the identification of species of bacteria. Different bacterial species are capable of fermenting different sugars and some are capable of gas production.

Indicator (0.4%)

Composition

Dibromothymol sulphonaphthalein	- 0.1 gm
NaoH (0.02 N)	- 8 ml
Distilled water	- 242 ml.

Sugar Media.

Composition

Peptone	-	1 gm
Nacl	-	0.5 gm
Distilled water	-	100 ml
Sugar	-	1 gm

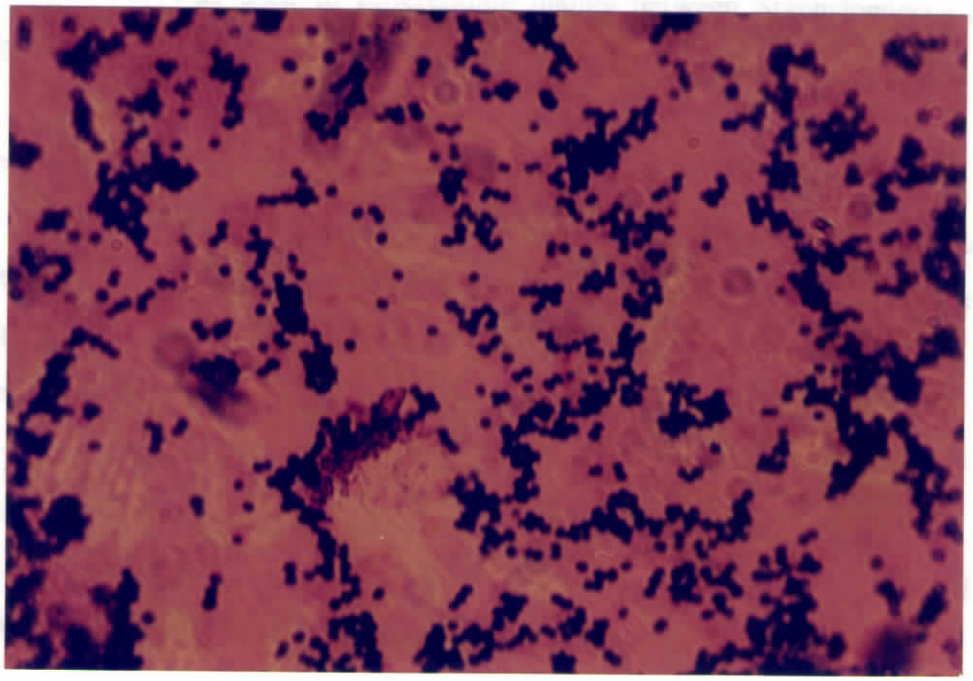
The pH was adjusted to 7.2. The different sugars tested include Lactose, Glucose, Sucrose, Maltose, Mannose, Mannitol, Trehalose and Xylose D (+). The indicator (blue at pH 7.0) was then added and poured to sterile test tubes. The indicator turns yellow (at pH 6.0) for positive sugar fermentation strains on overnight incubation.

To test the production of gas, Durham's tube was placed in each test tube and autoclaved at 10 lb for 10 minutes.

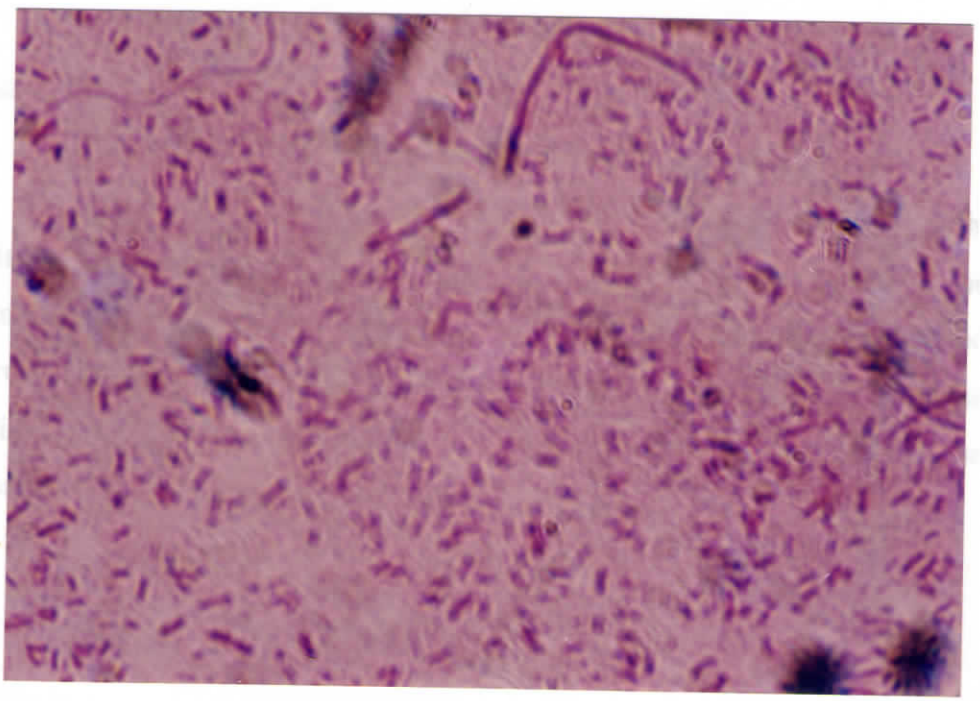
PHOTOGRAPHIC PLATE NO:1

GRAM STAINING

Gram Positive Organism *(S. aureus)*



Gram Negative Organism *(Klebsiella pneumoniae)*



Sugar fermentation method was used as a method to find out the strain variation and specifically for the speciation of CNS (Woods et al 1987, Kloos and Bannerman 1995).

3.7.3.2 Catalase test

The test was used to differentiate mainly between Staphylococcus and Streptococcus.

Procedure

The bacterial strain grown in nutrient agar overnight was introduced into a test tube containing 30% by volume hydrogen peroxidase solution. Effervescence (release of oxygen) observed in the hydrogen peroxidase after the insertion of bacteria, confirmed the organism as catalase positive.

3.7.3.3 Coagulase test

Coagulase test was employed for the identification of Staphylococcal strains as Coagulase Positive or Coagulase Negative Staphylococci.

Two methods were employed.

(a) Slide coagulase test and

(b) Tube coagulase test

Slide coagulase test

This test was done as a specific identification test for *s.aureus.*

The reagent used was human plasma. Dipped a flamed and cooled straight inoculating wire into the undiluted plasma at room temperature and stirred the adhering traces of plasma in to the Staphylococcal suspension on the slide. Flamed the wire and repeated for control suspension (Normal saline with plasma). A coarse clumping of cocci visible to the naked eye with in 10 seconds indicated a positive reaction confirming the organism as *s.aureus.*

Tube coagulase test

A colony of Staphylococcus was emulsified in a tube containing diluted plasma (1:6 dilution with saline). The tubes were incubated at 37°C and examined for clot at first, second and fourth hours.

The presence of the clot (gel) confirmed the presence of Coagulase Positive strain and its absence shows Coagulase Negative strain.

3.7.3.4 Motility test

Two methods were employed to test whether the bacterial strain isolated is motile or not

Hanging drop method

From two to three hour old culture, a loopful of culture was placed on a cover slip and was placed over a concavity slide. The motility was observed through the high power of an ordinary microscope.

Mannitol Motility method

Composition

Trypticase	-	20 gm
Agar	-	4 gm
Mannitol	-	2 gm
Potassium nitrate	-	1 gm
Distilled water	-	1000 ml
pH was adjusted to 7.4 to 7.6		
Phenol red (indicator)	-	100 mgs

The mixture was autoclaved and poured in tubes to form butts. Stab inoculation was made with a straight wire loop. Spreading growth on agar identified the organism as motile.

3.7.3.5 Urease test

The test was meant for detecting urease positive strain. The method is based on the colour change of the Christensen's urease medium from yellow to pink. This is because urease producing bacteria will reduce urea to ammonia.

Christensens Urea solution

Composition

Peptone	-	1 gm
Nacl	-	5 gm
Glucose	-	1 gm
Monobasic dipotassium hydrogen phosphate (K_2HPO_4)	-	2 gm
Urea	-	20 gm
Distilled water	-	100 ml
pH was adjusted to 6.8 to 6.9		
Phenol red (indicator)	-	0.012 gm

Agar base

Agar	-	20 gm
Distilled water	-	1000 ml

10 ml of urea solution (Sietz filter sterilized) was added to 100 ml of agar base (autoclaved) and poured in to tubes to form slopes. The slope was then heavily inoculated and incubated at 37°C. The medium was examined at the fourth hour and also after overnight incubation. Positive tubes changed the colour from yellow to pink.

3.7.3.6 Citrate Utilization test

The test was used to find out the bacterial species which can use citrate as a sole source of carbon and energy for growth and an ammonium salt as sole source of nitrogen. The test was widely used to differentiate *Klebsiella* spp., *E.coli, Enterobacter* spp. etc.

Composition :-

Ammonium dihydrogen phosphate	-	1 gm
Di potassium phosphate	-	1 gm
Nacl	-	5 gm
Mg So_4	-	0.2 gm
Sodium Citrate	-	2 gm
Agar	-	20 gm
Distilled water	-	1000 ml
pH was adjusted to 6.9		
Bromothymol blue (indicator)	-	0.08 gm

The mixture was autoclaved at 15 lb for 15 minutes. Positive test showed a change of blue colour from green.

3.7.3.7 Nitrate Reduction test

The test is based on the principle that the presence of the enzyme nitrate reductase reduces nitrate to nitrite and was used for differentiating bacteria belonging to Enterobacteriaceae family.

Preparation of nitrate medium

Composition

Tryptone	-	5 gm
Peptone	-	5 gm
Distilled water	-	1000 ml

The mixture was boiled and pH was adjusted to 7.3 to 7.4. To it added potassium nitrate 1 gm and dextrose. This was then autoclaved at 10 lb for 10 minutes.

Test reagents

Solution A

Dissolved 8 gm of sulphanilic acid in 1 litre 5 N acetic acid

Solution B

Dissolved 5 gm of alpha naphthylamine in 1 litre of acetic acid.

The test was conducted after growing the bacterium for five days at 37°C in nitrate medium. Equal volumes of solution A and B were mixed just before use (Test reagents). 0.1 ml of the test reagent was added to the culture. Positive reaction was indicated by the development of a red colour with in few minutes.

3.7.3.8 Amino Acid Decarboxylase and Arginine Dihydrolase tests

The test is based on the ability of some bacteria to decarboxylate an amino acid to the corresponding amine with the liberation of carbon dioxide. The production of these decarboxylases is induced by a low pH and as a result of their action, the pH rises to neutrality or above.

Lysine and Ornithine reactions are truly decarboxylase tests but arginine reaction is more a dihydrolase test.

Basal medium

Composition

Peptone	-	5 gm
Meat extract	-	5 gm
Glucose	-	0.5 gm
Pyridoxal	-	5 mg
Bromocresol purple (1 in 500 solution)	-	5 ml
Cresol red (1 in 500 solution)	-	2.5 ml
Distilled water	-	1000 ml

Dissolved the solids in water and pH was adjusted to 6.0 before the addition of the indicators.

Amino acids to be tested for decarboxylation was added to this basal medium. The basal medium was divided in to four portions.

(a) Added 1% L-lysine hydrochloride to one portion.

(b) Added 1% L-ornithine hydrochloride to second portion.

(c) Added 1% L-arginine hydrochloride to third portion and

(d) The portion with out any amino acid served as control.

The medium was distributed to 1 ml quantities in small tubes. Sterile paraffin layer (about 5 mm thick) was poured above the medium; autoclaved at 121°C for 15 min.

The test was performed by inoculating lightly through the paraffin layer with a straight wire. Incubated and read daily for 4 days.

Positive reaction was indicated, when the medium first become yellow due to acid production by glucose fermentation and then changed to violet due to decarboxylation. The control remaining as yellow confirmed the test.

3.7.3.9 Indole test

The method utilizes the ability of certain bacteria to decompose the aminoacid Tryptophan to Indole. Bacteria to be identified was inoculated into the sterile peptone

water and incubated at 37°C overnight. On adding Kovacs reagent a red ring formed confirmed a positive reaction.

Peptone Water

Composition

Peptone	-	10 gm
Nacl	-	5 gm
Distilled water	-	1000 ml

pH was adjusted to 7.4. The medium was dispersed in tubes and the mixture was sterilized by autoclaving at 121°C for 15 minutes.

Kovac's Reagent

Composition

Pure amyl alcohol	-	150 ml
Para dimethyl amino benzaldehyde	-	10 gm
Concentrated Hcl	-	50 ml

3.7.3.10 Methyl Red and Voges - Proskauer test

Methyl red test was employed to detect the production of sufficient acid due to the fermentation of glucose; such that pH of old culture is sustained below a value of about 4.5, which was shown by a colour change of methyl red indicator added at the end of incubation period.

The test is based on the ability of certain bacteria to produce acetyl methyl carbinol (Acetoin).

Glucose Phosphate Peptone Water

Composition

Peptone	-	7 gm
Dextrose	-	5 gm
Dipotassium hydrogen phosphate	-	5 gm
Distilled water	-	1000 ml
pH	-	6.9

The mixture was autoclaved at 10 lb for 10 minutes.

Methyl Red Indicator

Methyl red	-	0.1 gm
Ethanol	-	300 ml
Distilled water	-	200 ml

Voges Proskauer reagent

Solution A - 5% naphtholin and 95% ethane

Solution B - 40% KOH and 0.5% creatine.

Glucose phosphate medium prepared was poured 5 ml in to each of the sterile test tubes. For Methyl Red reaction, methyl red indicator was poured on to overnight incubated bacterial culture. positive reaction was indicated by the development of a red colour.

For Voges - Proskauer reaction, on addition of the VP reagent a crimson red colour developed in 2-5 minutes indicated a positive reaction.

3.7.3.11 Oxidation - Fermentation tests

The method depends on the use of a semi solid medium containing the carbohydrate (glucose) together with a pH indicator. If acid is produced at the surface of medium (development of yellow colour), where conditions are aerobic, the attack on sugars were considered as oxidative. If acid is formed through out the tube, including the lower layers where conditions are anaerobic the break down was taken as fermentative.

Composition

Trypticase or Tryptone	-	2.00 gm
Nacl	-	5.00 gm
K_2HPO_4	-	0.30 gm
Agar	-	20.00 gm
Distilled water	-	1000 ml
Bromothymol blue	-	0.03 gm

pH was adjusted to 7.1 before adding bromothymol blue and medium was autoclaved at 121°C for 15 minutes. The glucose was then added (sterilized separately) to a final concentration of 1%. The medium was then tubed to a depth of 4 cm.

Duplicate tubes of medium were inoculated by stabbing. One tube was covered with a layer of sterile melted petroleum jelly to a depth of 5-10 mm and both were incubated.

The fermenting organisms produced an acid reaction through out the medium in the tube covered as well as in the open one. The oxidising organism which produced an acid reaction only in the open tube.

3.7.3.12 10 % Lactose Agar

The test was specifically used to differentiate *Acinetobacter* species.

Composition

Nacl	-	5 gm
Peptone	-	10 gm
Meat extract	-	1 gm
Lactose	-	100 gm
Agar	-	20 gm
Distilled water	-	1000 ml
Phenol red	-	0.018 gm

pH was adjusted to 7.2 and the mixture was poured to tubes; autoclaved at 15 lb for 15 min. Slants were made after autoclaving. Inoculated heavily on to the slope and the positive reaction is indicated by change of colour of medium from red to yellow.

3.7.3.13 Oxidase reaction

A small strip of filter paper soaked in 1% solution of Tetra Methyl P-Phenylene Diamine Di-Hydrochloride, was laid in a petri dish and the colony to be tested was smeared on the paper in a line about 5 mm long. Positive reaction was indicated by the development of a deep purple colour in 10 to 60 seconds.

3.7.3.14 Hemolysis

The type of hemolysis was observed by streaking the bacterial strain in question to a blood agar and incubating it in an atmosphere rich in Co_2.

3.7.3.15 Triple Sugar Iron Agar

This was used to differentiate different bacteria based on fermentation of different sugars.

Composition

Beef extract	-	3 gm
Yeast extract	-	5 gm
Protease peptone	-	5 gm
Bacteriological peptone	-	15 gm
Ferrous sulphate	-	0.2 gm
Nacl	-	5 gm
Sodium thiosulphate	-	0.3 gm
Sucrose	-	10 gm
Lactose	-	1 gm
Dextrose	-	1 gm
Agar	-	20 gm
Distilled water	-	1000 ml
Phenol red (indicator)	-	0.03 gm

pH was adjusted to 7.4 to 7.6. Streaked a heavy inoculum over the surface of the slope and stabbed in to the butt. It was then incubated aerobically at 37°C for 24 hours.

The test was used as a first step in the identification of Enterobacteriaceae and the results were read as follows.

Slant/butt	Colour	Utilization
Alkaline/Acid	Red/Yellow	Glucose only fermented peptone utilized
Acid/Acid	Yellow/Yellow	Glucose, lactose and Sucrose fermented
Alkaline/Alkaline	Red/Red	No fermentation of glucose, lactose or Sucrose. Peptone utilized

3.7.3.16 DNAse test

DNAse agar

Composition

Tryptose	-	20 gm
Deoxy ribo nuclease	-	2 gm
Nacl	-	5 gm
Agar powder	-	12 gm
Distilled water	-	1000 ml

pH was adjusted to 7.3. The mixture was autoclaved at 121°C for 15 minutes. Each plate was divided into six sections. The strain to be tested was inoculated by spotting it on to a small area in the middle of one of the marked sections of this DNAse plate. Spot inoculated other strains to be tested on to other sections. A known DNAse positive strain (Control ATCC standard *S.aureus* strain 25923) was also included. Incubated the plate for 18-24 hours at 37°C. After proper incubation, the plate was flooded with 1 mol/litre (3.6%) HCl to precipitate the unhydrolysed DNA. After a few minutes a white cloudiness was seen in the agar. The plates were read under strong indirect light against dark back ground.

The results were read as follows. Spot cultures surrounded by a clear uncloudly zone comparable in width to that around control were taken as DNAse positive. Cultures with markedly smaller zone, or no clearing at all or having 3 mm in radial width from the edge of the colony was taken as negative.

3.8 Antibiotic Susceptibility Test

The antibiotic susceptibility tests of CNS strains, *S.aureus* strains and Klebsiella strains were carried out by disc diffusion techniques as per the method of Bauer-Kirby et al (1966) on Mueller Hinton Agar (MHA) plates. With each batch of testing ATCC strains were also tested as standard controls viz, ATCC *S.aureus* 25923, ATCC *Ps.aeruginosa* 27353.

The antibiotics discs used were manufactured and supplied by the M/s Pasteur Institute of Biologicals, Gujarat, India. Some were procured from M/s Hi Media, Mumbai.

The disc potency of each antibiotic used are as in Table 3.1.

TABLE - 3.1
ANTIBIOTICS USED FOR SUSCEPTIBILITY TEST AND THEIR INTERPRETATION

Sl. No.	Antibiotics used	Disc potency	Resistant R ≥ mm	Inter-mediate Ms≥mm	Sensitive S ≥mm
1	Ampicillin (AM)	10 mcg	20	21-28	29
2	Amikacin (AK)	30 mcg	14	15-16	17
3	Amoxycillin and Clavulanic Acid (AMC)	30 mcg	Staph-19 Other-13	- 14-17	20 18
4	Cloxacillin (CX)	1 mcg	9	10-14	15
5	Ceftriaxone (RP)	30 mcg	13	14-20	21
6	Cefuroxime (CB)	30 mcg	14	15-17	18
7	Clindamycin (CD)	2 mcg	14	15-16	17
8	Cephalexin (PR)	30 mcg	14	15-17	18
9	Cefazolin (CF)	30 mcg	14	15-17	18
10	Ciprofloxacin (RC)	5 mcg	15	16-20	21
11	Chloramphenicol (CH)	30 mcg	13	14-17	18
12	Co-trimoxazole (BA)	25 mcg	10	11-15	16
13	Erythromycin (ER)	15 mcg	13	14-17	18
14	Gentamicin (GM)	10 mcg	12	13-14	15
15	Methicillin (MC)	5 mcg	9	10-13	14
16	Norfloxacin (NX)	10 mcg	12	13-16	17
17	Nitrofurantoin (FD)	300 mcg	14	15-16	17
18	Netilmicin (NT)	30 mcg	16	-	17
19	Nalidixic Acid (NA)	30 mcg	13	14-18	19
20	Oxacillin (OC)	1 mcg	10	11-12	13
21	Penicillin-G (PG)	10 u	20	21-28	29
22	Rifampicin (R)	5 mcg	15	16-20	21
23	Tobramycin (TB)	10 mcg	12	13-14	15
24	Tetracycline (TE)	30 mcg	14	15-18	19
25	Vancomycin (VN)	30 mcg	9	10-11	12

Mueller Hinton Agar

Composition

Meat extract	-	3 gm.
Acid casein hydrolysate	-	17.5 gm
Starch (soluble)	-	1.5 gm
Agar	-	20 gm
Distilled water	-	1000 ml

pH was adjusted to 7.2 to 7.4. The mixture was autoclaved at 15 lbs for 15 minutes and poured into sterile petri dishes.

A single colony of a small fraction of culture of nutrient agar plate or a blood agar plate was inoculated into the nutrient broth and were incubated at 37°C for one to two hours thus making it ready for lawn culture.

Lawn culture

Mueller Hinton Agar plates to be used were kept in the incubator for drying and labelled with strain number, date of inoculation and the names of the antibiotics.

Sterile swabs dipped in the test strains grown in broth were squeezed on the sides of the test tubes and spreaded over the Mueller Hinton agar plates. Over flowing was avoided, since it would interfere with zones of inhibition by the neighbouring antibiotics discs.

Inoculated MHA plates were kept inside the incubator at 37°C for about 15-20 minutes for drying. Consequently antibiotic discs were placed in their respective places (marked earlier) inside the Mueller Hinton agar plate and incubated overnight at 37°C.

Reading of sensitivity test

The reading of the inhibitory zones around each antibiotic disc was done under bright light with dark background using a clear transparent plastic scale. The diameter of the inhibitory zones were noted down and recorded. The results of antibiotic susceptibility tests were recorded as (a) Sensitive (S) (b) Moderately sensitive (MS) and (c) Resistant (R) respectively with the help of interpretation chart Table 3.1. For typing procedure (antibiogram), exact diameter around each disc was also noted down.

Novobiocin (30 microgram) disc and Polymyxin (5 microgram) discs were used in the speciation of CNS where NB 'S' and PB 'R' were considered as *S.epidermidis* and NB 'R' and PB 'S' as *S.saprophyticus.*

3.9 Bacteriophage typing

Bacteriophage typing is based on the susceptibility of the test strain to get lysed by a panel of bacteriophages. The method has been applied to a number of bacteria associated with nosocomial infections and its epidemiological utility is mainly applicable for S.*aureus.*

An internationally accepted set of 23 Phages was used for typing. The Staphylococcal phage typing is by a pattern method. The strain to be typed was inoculated on a plate of nutrient agar to produce a lawn culture. After drying, the phages were applied over marked squares in a fixed dose (RTD). After overnight incubation, the culture was observed to be lysed by some phages but not by others. The phage type of the strain was expressed as the designations of the phages that lyse it.

Bacteriophage typing was also performed by RTDX 100 dilutions. But in this category also all cultures were not typable.

The following are the set of Staphylococcal phages used for RTD and RTD x 100 bacteriophage typing.

Group I	-	29, 52, 52A, 79, 80.
Group II	-	3A, 3C, 55, 71.
Group III	-	6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85.
Not Allocated Group	-	81, 94, 95, 96.

3.10 Bacteriocin typing

Bacteriocins are a group of highly specific antibiotic like substance produced by certain strains of bacteria and are active against other strains of the same or different species. These are mainly proteins and a cell producing bacteriocin is immune to it, but are sensitive to other bacteriocins. They have a very specific activity on bacteria.

Bacteriocin typing is mainly dependent on the ability of the bacteriocins produced by the test strain to kill standard indicator strains of bacteria. The usual method employed is the plate diffusion technique.

3.10.1 Pyocin Typing (Fyfe et al 1984, Shriniwas 1974)

The method followed was according to Fyfe et al (1984) with slight modification. This typing method was used to correlate pseudomonas strains isolated from the carrier state and the strains isolated from the infected patients.

Cross typing among the strains were carried out. The test bacterium (*Pseudomonas* spp.) was inoculated as a broad streak on the centre of culture medium and the plates were kept for overnight incubation at 37°C. The bacterial growth was then scrapped off and the remaining cells were killed by exposure to chloroform vapours. The other test strains of bacterium to be typed were then streaked at right angles to the original inoculum. After over night incubation, the pattern of inhibition was noted and results were recorded based on the fact that the bacteriocin produced by same strain will be immune to it and hence no inhibition pattern will be seen, where as with different strains, the inhibition pattern will be seen.

3.10.2 Klebocin typing (Buffermeyer et al 1976)

The cross typing among the Klebsiella strains were performed using the same procedure described above (section No.3.10.1) to find out the correlation between the Klebsiella strains isolated from the carrier state and that isolated from the infected patients.

3.10.3 Bacteriocin typing for Acinetobacter

The cross typing among Acinetobacter strains were performed using the same procedure as described in section No.3.10.1 to find out the correlation between the strains isolated from the carrier state and that isolated from the infected patients.

3.10.4. Bacteriocin typing for Staphylococcus strains

The cross typing among both Staphylococcus strains (CNS and *S.aureus*) was performed using the procedure as in (section No.3.10.1) to find out the correlation between the strains isolated from carrier state and that isolated from the infected patients.

3.11 Poly Acrylamide Gel Electrophoresis (PAGE)

Variation in proteins expressed by microorganisms was detected by electrophoretic protein typing.

SDS-PAGE (Sodium Dodecyl Sulfate -Poly Acrylamide Gel Electrophoresis) was performed after loading the gel with bacterial extracts to find out the correlation among the bacterial strains. The SDS-PAGE was performed as per the method of Mulligan et al (1988).

Reagents

3.11.1 Acrylamide concentrate

Acrylamide	-	29.2 gm
Bis acrylamide	-	0.8 gm

Dissolved in 70 ml deionized water and then made up to 100 ml, filtered and stored in amber bottle at 4° C.

3.11.2 Buffer I (Resolving buffer)

1.5 M Tris buffer	-	18.1 gm
Double Distilled Water	-	50 ml
1 N HCl	-	24 ml

Adjusted pH at 8.8 and made upto 100 ml and was stored at 4° C.

3.11.3 Buffer II (Stacking buffer)

0.5 M Tris	-	5.98 gm
Double Distilled Water	-	25 ml
IN HCl	-	48 ml

pH adjusted to 6.8 and made upto 100 ml and was stored at 4° C.

3.11.4 10% SDS (Sodium Dodecyl Sulfate)

SDS	-	1 gm
Double Distilled Water	-	10 ml

3.11.5 Catalyst (10% Ammonium Per Sulfate)

APS	-	100 mg
Double Distilled Water	-	1 ml

3.11.6 Accelerator

TEMED - N,N,N',N'-Tetra Methyl Ethylene Diamine

3.11.7 Chamber Buffer

0.025 M Tris	-	3 gms/litre
0.192 M Glycine	-	14 gms/litre.
SDS	-	1 gm/litre
Double Distilled Water	-	1000 ml

pH was adjusted to 8.3

3.11.8 Fixative (50% Methanol)

Methanol	-	50 ml
Distilled Water	-	50 ml
Formaldehyde	-	50 µl.

3.11.9 Staining Solution

Coomassie Brilliant Blue R-250	-	120 mg
Methanol	-	45 ml
Distilled Water	-	45 ml
Glacial Acetic acid	-	10 ml

3.11.10 Destaining Solution

Methanol	-	45 ml
Distilled Water	-	45 ml
Glacial Acetic acid	-	10 ml

3.11.11 Preparation of Resolving gel

Acrylamide	-	4 ml
Buffer I	-	2.5 ml
Double Distilled Water	-	3.35 ml
10% SDS	-	0.1 ml
10% APS	-	50 µl
TEMED	-	5 µl

3.11.12 Preparation of Stacking gel

Acrylamide	-	2 ml
Buffer II	-	2.5 ml
Double Distilled Water	-	10.35 ml
10% SDS	-	0.1 ml
10% APS	-	50 µl
TEMED	-	10 µl

3.11.13 Sample Buffer

Glycerol	-	0.5 ml
20% SDS	-	0.25 ml
Beta Mercapto Ethanol	-	0.05 ml
0.1% Bromo Phenol Blue	-	0.05 ml

3.11.14 Phosphate Buffered Saline

NaCl	-	43.5 gm
Na_2HPO_4	-	4.24 gm
KH_2PO_4	-	2.7 gm
Na Azide	-	1 gm
Distilled Water	-	5 ltrs.

pH was adjusted to 7.4

3.11.15 Preparation of Bacterial strains for loading on to the gel

Three colonies of the bacterial strain taken from an overnight incubated blood agar medium was emulsified in BHIB and was incubated at 35°C for 24 hours. After incubation, the cells were washed in 10 ml of 0.15 M PBS (7.4) for 3 times. After washing with PBS, EDTA (70 mM, 100 µl) was added to each tube of these washed cells. The tubes were then incubated at 45°C for 30 minutes. After proper incubation the tubes were centrifuged for 15 minutes at 1500 rpm and the supernatant was then stored at 20°C for further use.

For loading, a 50 µl portion of each extract was mixed with 12 µl of sample buffer (composition as in section 3.11.13) and were heated at 65°C for 15 minutes.

Resolving gel was poured and was allowed to set (20 to 30 min). Washed the top with distilled water and drained well. Prepared the stacking gel and poured above the separating gel. The comb was placed carefully and was allowed to set. Removed the comb and washed the wells with distilled water.

Chamber buffer was added to the top reservoir as well as to the bottom reservoir. Samples were loaded using a microliter syringe and gel was run at 20 mA till the dye front reaches the bottom.

After the dye front has reached the bottom of the gel, the power pack was turned off. Removed the gel plates and gently separated the plates apart. Molecular marker was also loaded simultaneously along with each run .

Fixing the gel

Fixing is done at room temperature by soaking the gel in the fixative.

Staining the gel

The gel was transferred to the staining solution and after proper staining it was destained and the band pattern obtained was noticed.

3.12. Detection of Beta-lactamases among Staphylococcus strains isolated from Cardiac Surgery Patient's and Health Care Personnel

3.12.1 **Iodometric Method** (Sykes and Mathew 1979)

In the iodometric method for the detection of beta-lactamases, four techniques were used for comparison (a) Test tube method (b) Microplate method (c) Filter paper method and (d) Agar plate method.

The detection of beta-lactamases by Iodometric technique was performed as follows.

3.12.1.1 Test-tube method

Iodine reagent

Iodine	-	2.03 gm
Potassium iodide	-	53.2 gm
Distilled water	-	100 ml

Other Reagents

Stock A

Contains 0.2 M solution of Monobasic sodium phosphate (27.8 gm in 1000 ml) of distilled water.

Stock B

Contains 0.2 M solution of Dibasic sodium phosphate (53.65 gm of Na_2HPO_4. $7H_2O$ or 71.7 gm of $Na_2 HPO_4$. $12H_2O$ in 1000 ml of distilled water).

Phosphate buffered saline was prepared and adjusted to pH 7 by mixing 39 ml of stock solution A plus 61 ml of stock solution B. To this added 100 ml of distilled water and thus made up to 200 ml. Added 2 vials of penicillin to this so as to make the penicillin solution.

0.5 ml of penicillin solution containing 10,000/ml units of penicillin in phosphate buffered saline was taken in sugar tubes (10 mm x 75 mm) and heavy growth of 24 hour culture was mixed in this penicillin solution. The culture was mixed well and left at room temperature for 60 minutes. Before testing, added two drops of soluble starch solution followed by one drop of iodine solution. Again the tubes were mixed well and left at room temperature for 10 minutes.

Results were read as follows:

Instant discolouration	-	++++	(4+)
Discolouration in 2 minutes	-	+++	(3+)
Discolouration in 3-5 minutes	-	++	(2+)
Discolouration in 10 minutes	-	+	(1+)

The results obtained is as shown in (Plate No.2)

3.12.1.2 Microplate method

Procedure followed was as in test tube method except that the volume was reduced.

Penicillin solution	-	0.3 ml per well
Starch solution	-	0.1 ml
Iodine reagent	-	0.05 ml

Results were read as described in the test tube method and the result obtained is as shown in (Plate No.2)

3.12.1.3 Filter Paper method

1.5 cm x 10 cm Whatman No: 1 filter paper was soaked in 1% starch solution and dried in incubator for 4-5 hours. This starch paper was soaked in penicillin

By Test Tube Method

A. Negative
B. Weak Positive (1+)
C. Positive (2+)
D. Strong Positive (3+)

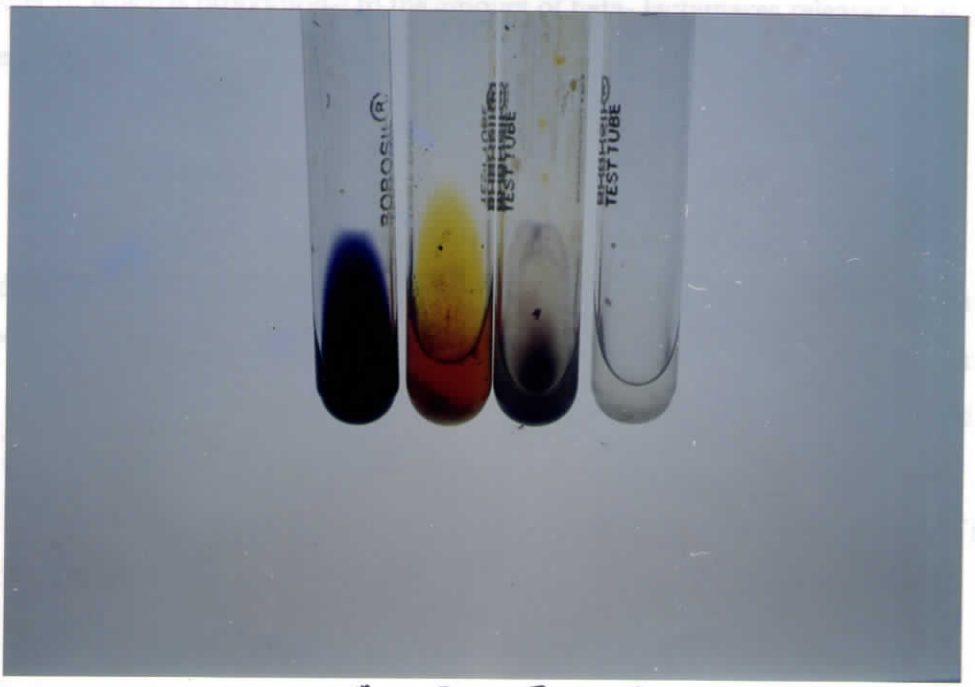
By Microplate Method

A. Negative
B. Weak Positive (1+)
C. Positive (2+)
D. Strong Positive (3+)

PHOTOGRAPHIC PLATE NO:2

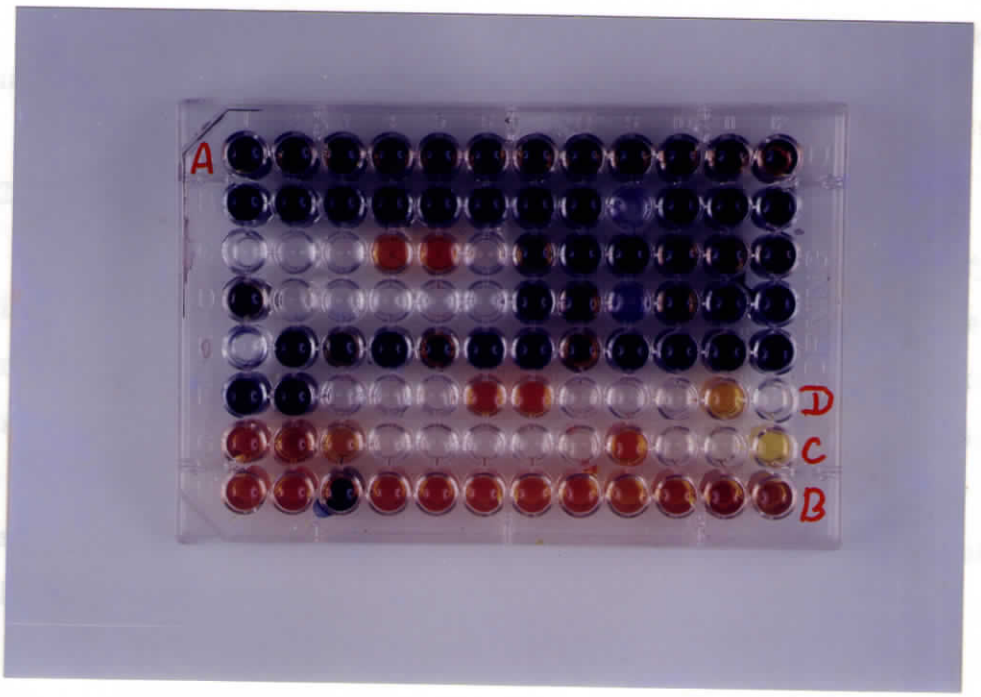
BETA-LACTAMASE DETECTION BY IODOMETRIC TECHNIQUES

By Test Tube Method



A C B D

By Microplate Method



solution before testing. Loop full of bacterial mass culture was kept in the centre of the filter paper at a distance of 15 mm. Incubated at 37°C for 30 minutes. The filter paper was then flooded with 1 in 5 dilution of Iodine solution (composition as given in section 3.12.1.1).

There was discolouration of filter paper around the bacterial mass, the diameter of which is proportional to the amount of beta- lactamases released from the bacterial strains. The result obtained is as shown in (Plate No.3).

3.12.1.4 Agar Diffusion Method

Nutrient agar (with 2% soluble starch) was prepared and the bacteria to be tested was inoculated as a single streak line on the plate. The plates were incubated at 37°C overnight and were then flooded with penicillin solution (10,000 units/ml) and kept at 37°C for 30 minutes. The solution was completely removed and the plates were flooded with 1 in 5 dilution of iodine solution.

Positive results were read as the discolouration around bacterial growth. The result obtained is as shown in (Plate No.3)

3.12.2 **Acidimetric method** (Sykes and Matthew 1979)

This method is based on the fact that the alkaline solution of Phenol Red (red) and Bromo Cresol Purple (purple) turn yellow in the presence of acid produced by Beta lactam hydrolysis.

Two indicators, Bromocresol purple and phenol red, were used both in filter strip method as well as in microplate method for comparison.

3.12.2.1 Filter Paper method (Slack et al 1977)

Filter strips were prepared by dissolving 600 mg of unbuffered benzyl penicillin in 4 ml of 0.1% aqueous solution of Bromocresol purple containing 0.05 ml of 0.1 M NaOH. Whatman No: 1 filter papers were immersed in this solution and immediately dried in a desiccator. After drying, the paper was cut in to strips. These were stored at 4°C. For testing, one drop of distilled water was placed over a microscopic slide and was covered with test paper. using an inoculation loop a streak of culture was made.

By Filter Paper Method

A. Strong Positive

B. Weak Positive

By Agar Diffusion Method

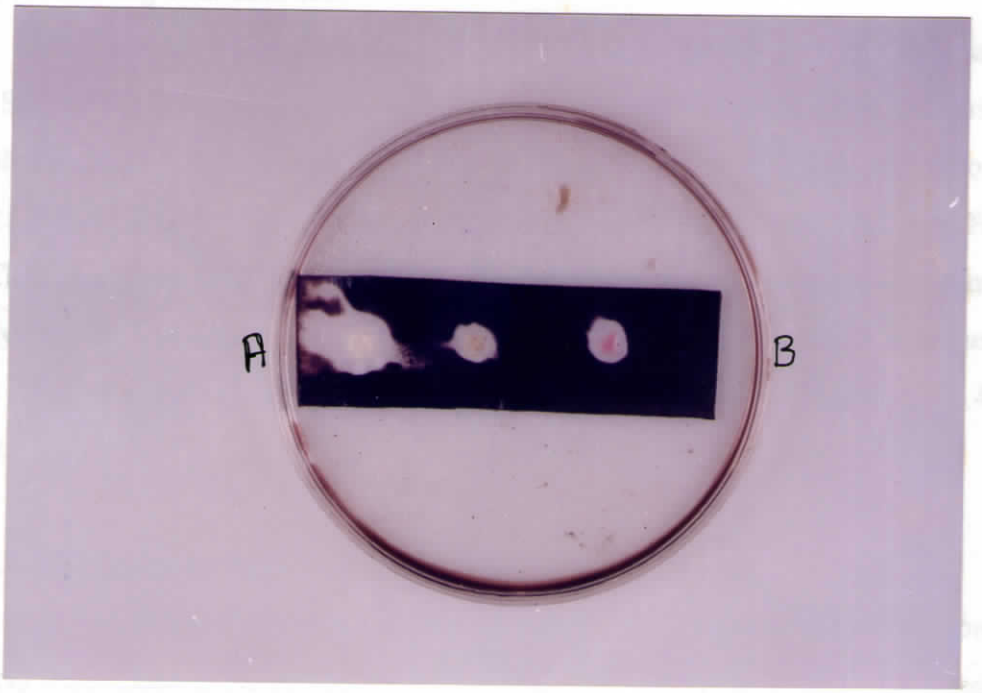
A. Strong Positive

B. Negative

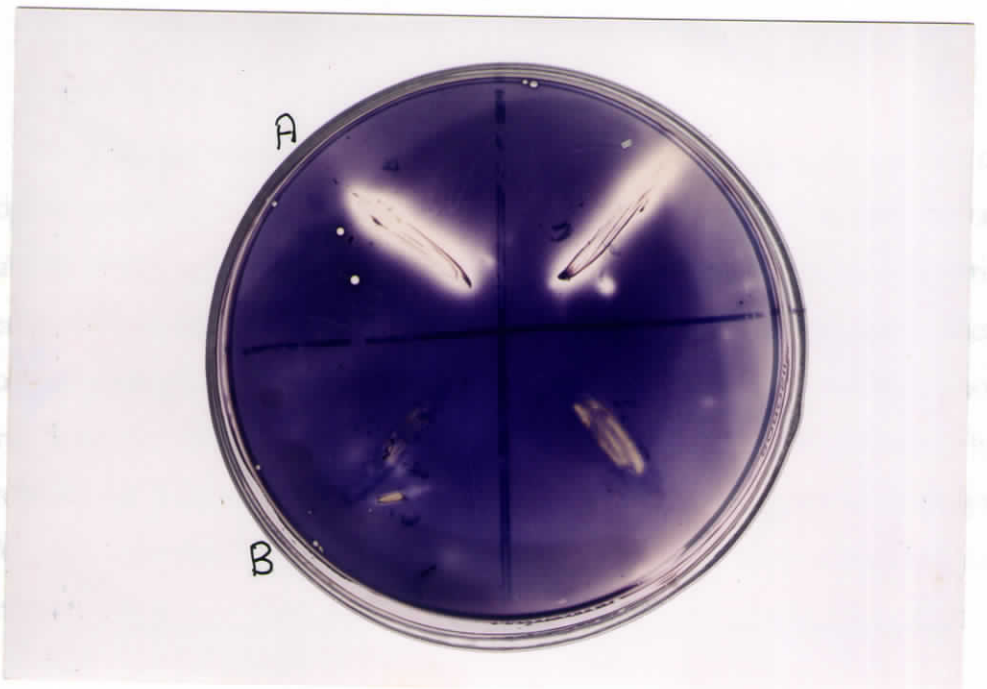
PHOTOGRAPHIC PLATE NO: 3

BETA-LACTAMASE DETECTION BY IODOMETRIC TECHNIQUES

By Filter Paper Method



By Agar Diffusion Method



Positive strains changed the colour of strip from purple to yellow with in 5 to 10 minutes which indicated the production of beta-lactamase. Negative strains that do not produce beta-lactamase does not changed the colour of strip. Known positive and negative National Culture Type Collection (NCTC) control *S.aureus* strains were also applied along with each test.

In the beta-lactamase detection using phenol red as the indicator (Rosen et al 1972, Scheifele et al 1976) the reagent was prepared by adding 2 ml of 0.5% phenol red solution to 16.6 ml of sterile distilled water containing 2 x 10^7 units (12 gm) of benzyl penicillin. 1 M NaoH was added drop wise until the solution turned violet (pH 8.5). The reagent was stored at - 70°C. Beta-lactamase producers turned the reagent bright yellow in 5-15 minutes. Known positive and negative NCTC control *S.aureus* strains were also applied along with the test. The results are as shown in (Plate No.4 and 5).

3.12.2.2 Microplate method

The Bromocresol purple and phenol red solutions (Composition as in section 3.12.2.1) were added to the microplate wells 100 µl each. Bacterial strain to be tested were added to this to test the production of Beta-lactamase. Yellow colour indicated positive reaction. Proper controls were also used. The results are as shown in (Plate No.4 and 5).

3.13 Determination of the MIC of antibiotics

The MIC for various antibiotics were found out according to the method of Aldridge et al (1983). Agar dilution method was used for the assay. The medium was prepared by adding appropriate dilution (with sterile distilled water) of the antibiotic in sterile, melted and cooled MHA (1 ml antibiotic solution for 9 ml agar). Each dilution was poured in to petridishes, mixed gently well and allowed to solidify. Two to three hour old nutrient broth cultures were inoculated in a line on each plate by means of a 2 mm diameter inoculation loop and the plates were incubated overnight at 37°C. MIC was taken as that concentration when no visible growth of the bacterial culture was seen in agar plate. A plate without antibiotic was used as the control.

By Phenol Red Filter Paper Method

A. Strong Positive

B. Negative

By Phenol Red Microplate Method

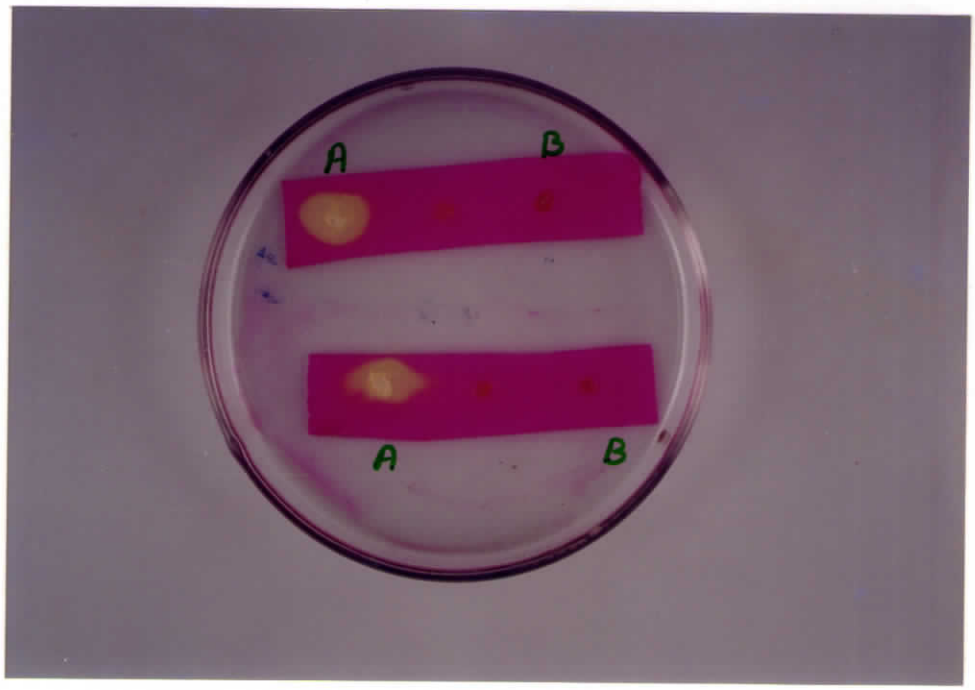
A. Positive

B. Negative

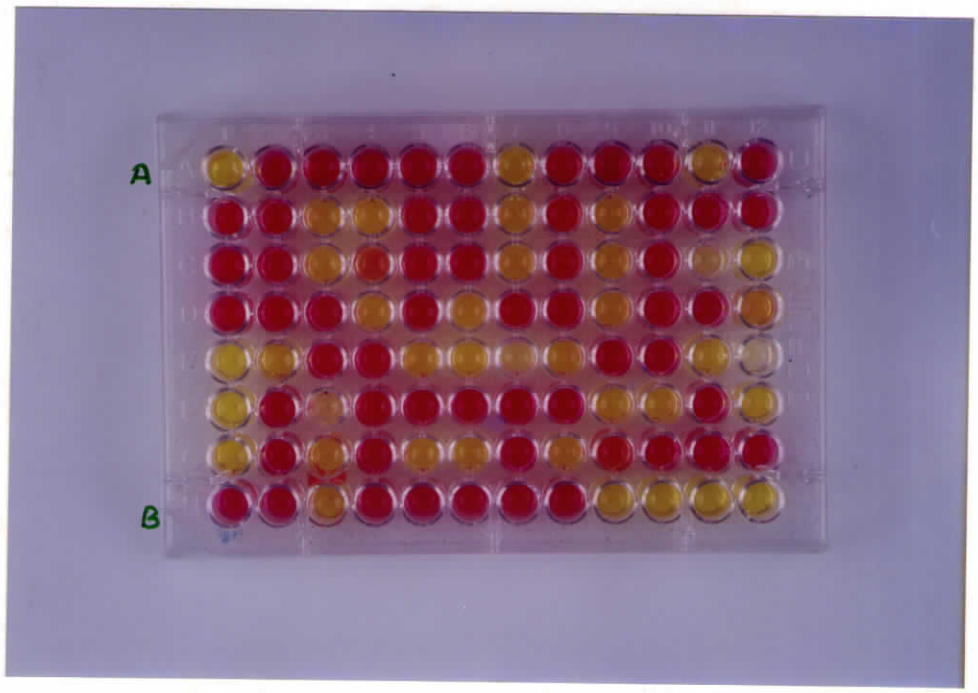
PHOTOGRAPHIC PLATE NO: 4

BETA-LACTAMASE DETECTION BY ACIDIMETRIC METHOD

A. By Phenol Red Filter Paper Method.



B. By Phenol Red Microplate Method



By Bromo Cresol Purple Filter Paper Method

A. Positive

B. Negative

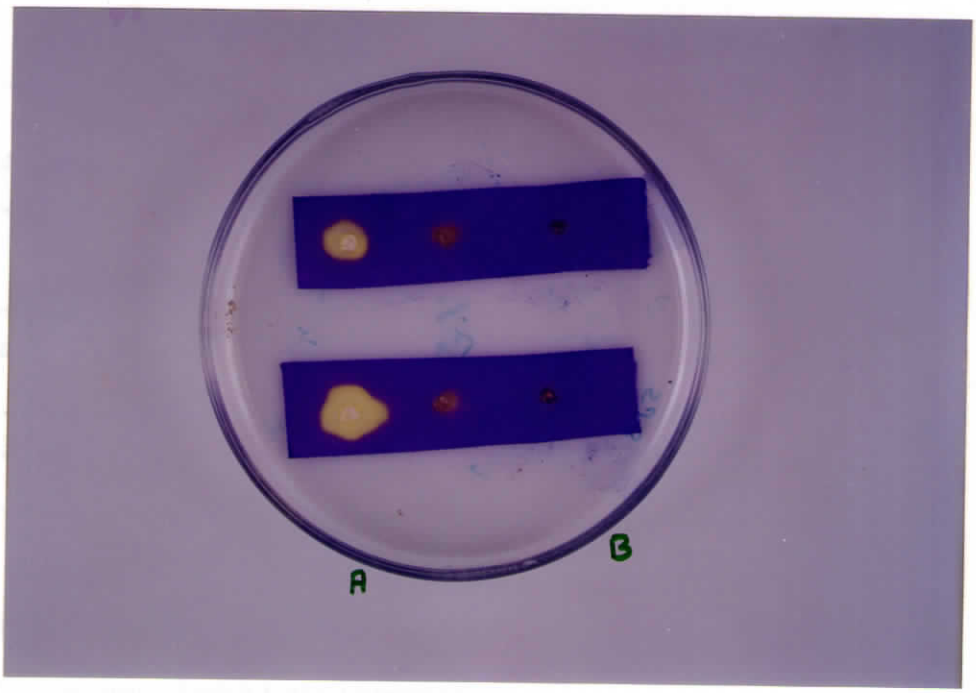
By Bromo Cresol Purple Microplate Method

A. Negative
B. Weak Positive (1+)
C. Positive (2+)
D. Strong Positive (3+)

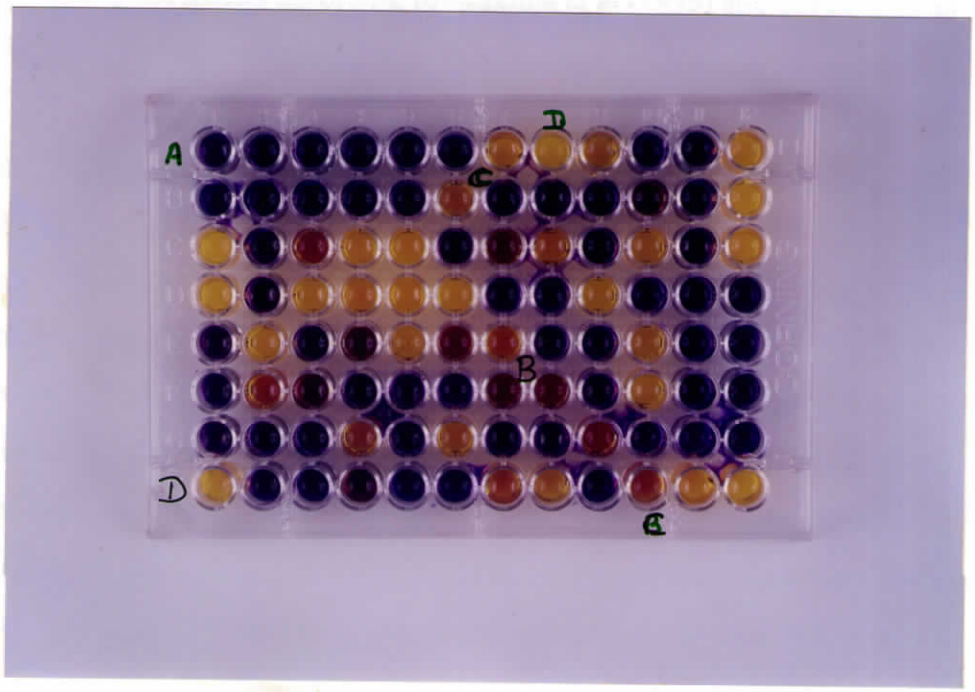
PHOTOGRAPHIC PLATE NO: 5

BETA-LACTAMASE DETECTION BY ACIDIMETRIC METHOD

A. By Bromo Cresol Purple Filter Paper Method.



B. By Bromo Cresol Purple Microplate Method



The MIC for Penicillin, Cloxacillin, Augmentin, Gentamicin, Ciprofloxacin, Cefuroxime, Tobramycin and Amikacin was determined in the same way. The dilution schedule for each antibiotic is given in separate tables (Table 3.2 to Table 3.9).

The details of the MIC determination for samples from cardiac surgery patients and health care personnel is as given below.

Bacterial Strain	No: of bacterial strains from		Antibiotics tested
	HCP	CSP	
CNS	50	50	PG, CX, RC, CB, TB, AK.
S.aureus	25	50	PG, CX, RC, CB, TB, AK.
Klebsiella	14	50	Aug, GM, RC, CB, TB, AK.

3.14 Slime detection in Staphylococcus strains isolated from Cardiac Surgery Patients and Health Care Personnel

Pathogenesis is multifactorial for the majority of diseases caused by Staphylococcus strains. But of the many potential virulence factors expressed, production of slime is a predictor of significance and an important virulence determinant among the species according to Makhija et al (1995) and can be used as an effective typing method according to Hedin (1996).

Procedure

3.14.1 Detection of slime by Christensen's test tube method

In the detection of slime using Christensens' test tube method, Trypticase Soya Broth (Soyabean casein digest medium) was taken uniformly in each test tube and the inoculum was added and was cultured for a visible film of adherent growth by keeping the tubes for incubation with out shaking. The broth was then decanted completely and was stained with 0.1% Safranin for 10 seconds (Christensens et al 1983). Test tubes were incubated in a tilted position to facilitate reading and slime production results were recorded. According to density of adherent film assessed

TABLE - 3 . 2

ANTIBIOTIC DILUTION SCHEDULE FOR MIC DETERMINATION OF PENICILLIN-G*

Sl. No.	Stock Solution units/ml	Stock Volume (in ml)	Diluent Volume in ml (Distilled water)	Dilution obtained in test tubes (U/ml)	Dilution obtained in MHA plates (U/ml)
1	2,00,000	2.0	2.0	1,00,000	10,000
2	1,00,000	0.5	4.5	10,000	1000
3	10,000	3.0	3.0	5,000	500
4	5,000	2.0	2.0	2,500	250
5	5,000	1.0	4.0	1,000	100
6	5,000	0.5	4.5	500	50
7	2,500	0.5	4.5	250	25
8	1,000	1.0	4.0	200	20
9	1,000	0.5	4.5	100	10
10	100	2.0	2.0	50	5
11	50	2.0	2.0	25	2.5
12	100	1.0	4.0	20	2.0
13	100	0.5	4.5	10	1.0
14	10	2.0	2.0	5	0.5
15	5	2.0	2.0	2.5	0.25
16	2.5	2.0	2.0	1.25	0.125

One plate without antibiotic was used as the control.

* One vial of penicillin : 10,00,000 units.
The initial stock was diluted to 2,00,000 units/ml.

TABLE - 3.3

ANTIBIOTIC DILUTION SCHEDULE FOR MIC DETERMINATION OF CLOXACILLIN*

Sl. No.	Stock Dilution	Stock Volume (in ml)	Diluent Volume in ml (Distilled water)	Dilution obtained in test tubes (µg/ml)	Dilution obtained in MHA plates (µg/ml)
1	1,00,000	1.0	1.0	50,000	5,000
2	50,000	1.0	4.0	10,000	1000
3	10,000	2.0	2.0	5,000	500
4	5,000	1.0	4.0	1,000	100
5	1,000	2.0	2.0	500	50
6	500	2.0	2.0	250	25
7	1000	0.5	4.5	100	10
8	100	2.0	2.0	50	5
9	50	2.0	2.0	25	2.5
10	25	2.0	2.0	12.5	1.25
11	12.5	2.0	2.0	6.25	0.625
12	6.25	2.0	2.0	3.125	0.3125
13	3.125	2.0	2.0	1.5625	0.15625
14	1.5625	2.0	2.0	0.781	0.0781

* One vial of Cloxacillin : 500 mg in 5 ml
The initial stock was diluted to 1,00,000 µg/ml.

TABLE - 3.4

ANTIBIOTIC DILUTION SCHEDULE FOR MIC DETERMINATION OF AUGMENTIN*

Sl. No.	Stock Dilution	Stock Volume (in ml)	Diluent Volume in ml (Distilled water)	Dilution obtained in test tubes (µg/ml)	Dilution obtained in MHA plates (µg/ml)
1	120,000	1.0	9.0	12,000	1200
2	12,000	1.0	1.0	6,000	600
3	6,000	1.0	1.0	3,000	300
4	6,000	1.0	5.0	1,000	100
5	1,000	1.0	1.0	500	50
6	500	1.0	1.0	250	25
7	1,000	1.0	4.0	200	20
8	200	1.0	1.0	100	10
9	100	1.0	1.0	50	5
10	50	1.0	1.0	25	2.5
11	100	0.5	4.5	10	1.0
12	10	1.0	1.0	5	0.5
13	5	1.0	1.0	2.5	0.25
14	2.5	1.0	1.0	1.25	0.125
15	1.25	1.0	1.0	0.625	0.0625
16	0.625	1	1	0.3125	0.03125

* One vial of Augmentin : 1.2 gm in 10 ml.
The initial stock was diluted to 120,000 µg/ml.

TABLE - 3.5

ANTIBIOTIC DILUTION SCHEDULE FOR MIC DETERMINATION OF GENTAMICIN*

Sl. No.	Stock Dilution	Stock Volume (in ml)	Diluent Volume in ml (Distilled water)	Dilution obtained in test tubes (µg/ml)	Dilution obtained in MHA plates (µg/ml)
1	40,000	1.0	9.0	4,000	400
2	4,000	1.0	3.0	1,000	100
3	1,000	1.0	1.0	500	50
4	500	1.0	1.0	250	25
5	1,000	1.0	4.0	200	20
6	200	1.0	1.0	100	10
7	100	1.0	1.0	50	5
8	50	1.0	1.0	25	2.5
9	100	0.5	4.5	10	1.0
10	10	1.0	1.0	5	0.5
11	5	1.0	1.0	2.5	0.25
12	2.5	1.0	1.0	1.25	0.125
13	1.25	1.0	1.0	0.625	0.0626
14	0.625	1.0	1.0	0.3125	0.03125
15	0.3125	1.0	1.0	0.15625	0.015625

* One vial of Gentamicin 80 mg in 2 ml.
The initial stock was diluted to 40,000µg/ml.

TABLE - 3 . 6

ANTIBIOTIC DILUTION SCHEDULE FOR MIC DETERMINATION OF CIPROFLOXACIN*

Sl. No.	Stock Dilution	Stock Volume (in ml)	Diluent Volume in ml (Distilled water)	Dilution obtained in test tubes (µg/ml)	Dilution obtained in MHA plates (µg/ml)
1	2,000	1.0	0	2,000	200
2	2,000	2.0	2.0	1,000	100
3	1,000	2.0	2.0	500	50
4	500	2.0	2.0	250	25
5	1,000	0.5	4.5	100	10
6	100	2.0	2.0	50	5
7	50	2.0	2.0	25	2.5
8	100	0.5	4.5	10	1.0
9	10	2.0	2.0	5	0.5
10	5	2.0	2.0	2.5	0.25
11	2.5	2.0	2.0	1.25	0.125
12	1.25	2.0	2.0	0.625	0.0625
13	0.625	2.0	2.0	0.3125	0.03125
14	0.3125	2.0	2.0	0.15625	0.015625

* One vial of ciprofloxacin : 200 mg in 100 ml.
The initial stock was diluted to 2000µg/ml.

TABLE - 3 . 7

ANTIBIOTIC DILUTION SCHEDULE FOR MIC DETERMINATION OF AMIKACIN *

Sl. No.	Stock Dilution	Stock Volume (in ml)	Diluent Volume in ml (Distilled water)	Dilution obtained in test tubes (µg/ml)	Dilution obtained in MHA plates (µg/ml)
1	250,000	1.0	9.0	25,000	2500
2	25,000	0.5	4.5	2500	250
3	2500	1.5	1.5	1250	125
4	2500	1.0	4.0	500	50
5	500	1.5	1.5	250	25
6	500	0.5	2.0	100	10
7	100	2.0	2.0	50	5
8	50	1.5	1.5	25	2.5
9	50	0.5	2.0	10	1.0
10	10	1.5	1.5	5	0.5
11	5	1.5	1.5	2.5	0.25
12	2.5	1.5	1.5	1.25	0.125
13	1.25	1.5	1.5	0.625	0.0625
14	0.625	1.5	1.5	0.3125	0.03125
15	0.3125	1.5	1.5	0.15625	0.015625

* One vial of Amikacin : 500 mg in 2 ml.
The initial stock was diluted to 250,000µg/ml.

TABLE - 3.8

ANTIBIOTIC DILUTION SCHEDULE FOR MIC DETERMINATION OF CEFUROXIME*

Sl. No.	Stock Dilution	Stock Volume (in ml)	Diluent Volume in ml (Distilled water)	Dilution obtained in test tubes (µg/ml)	Dilution obtained in MHA plates (µg/ml)
1	1,00,000	1.33	8.67	10,000	1000
2	10,000	2.0	2.0	5,000	500
3	5,000	2.0	2.0	2,500	250
4	10,000	0.5	4.5	1,000	100
5	1000	2.0	2.0	500	50
6	500	2.0	2.0	250	25
7	1000	0.5	4.5	100	10
8	100	2.0	2.0	50	5
9	50	2.0	2.0	25	2.5
10	25	2.0	2.0	12.5	1.25
11	12.5	2.0	2.0	6.25	0.625
12	6.25	2.0	2.0	3.125	0.3125
13	3.125	2.0	2.0	1.5625	0.15625
14	1.5625	2.0	2.0	0.78125	0.078125

* The initial stock was diluted to 100,000 µg/ml.

TABLE - 3 . 9

ANTIBIOTIC DILUTION SCHEDULE FOR MIC DETERMINATION OF TOBRAMYCIN *

Sl. No.	Stock Dilution	Stock Volume (in ml)	Diluent Volume in ml (Distilled water)	Dilution obtained in test tubes (µg/ml)	Dilution obtained in MHA plates (µg/ml)
1	10,000	1.0	4.0	2000	200
2	2,000	2.0	2.0	1000	100
3	1000	2.0	2.0	500	50
4	500	2.0	2.0	250	25
5	1000	0.2	1.8	100	10
6	100	2.0	2.0	50	5
7	50	2.0	2.0	25	2.5
8	100	0.2	1.8	10	1.0
9	10	2.0	2.0	5	0.5
10	5	2.0	2.0	2.5	0.25
11	2.5	2.0	2.0	1.25	0.125
12	1.25	2.0	2.0	0.625	0.0625
13	0.625	2.0	2.0	0.3125	0.03125
14	0.3125	2.0	2.0	0.15625	0.015625

* One vial of Tobramycin : 20 mg in 2 ml.
The initial stock was diluted to 10,000 µg/ml.

visually, the results were recorded as weak (+), moderate (++) or strong (+++). ATCC standard *S.epidermidis* strain (ATCC 35983) was included as positive control.

3.14.2 Microplate method

Sterile microplate wells were filled with 100 to 200 µl of sterile trypticase soya broth. The strains to be tested were inoculated and incubated overnight. After proper incubation, the contents of the microplate were removed without agitation using pasteur pipette. Subsequently 0.1% safranine was added through the side walls into each well and immediately aspirated gently. The plates were then dried for 10-30 minutes in an incubator. The results were recorded as in test tube method.

3.14.3 Congo Red Agar Plate method (Freeman et al 1989)

The Congo Red agar plates were prepared according to Freeman et al (1989) method.

Composition

Brain Heart Infusion Broth	-	3.7%
Sucrose	-	5%
Agar	-	1%
Congored	-	0.08%

The freshly prepared plate was inoculated with test strain and then incubated aerobically at 37°C for 24-48 hours. ATCC standard strain *S.epidermidis* 35983 was used as the control.

Observation

Dry, black, crystalline colonies	-	slime positive or slime producing strain.
Mucoid, pink/brown or bull eye colonies	-	Slime negative or non slime producing strain.
Dry, black without crystalline appearance	-	Intermediate or weak positive

The results are as shown in (Plate No.6).

3.41

A. Strong Positive

B. Weak Positive

C. Negative

PHOTOGRAPHIC PLATE NO: 6

SLIME DETECTION BY CONGO RED AGAR PLATE METHOD



One hundred and forty (140) and twenty seven (27) strains of *S.aureus* isolated and six hundred and seventy nine (679) and one hundred and eleven (111) strains of CNS isolated from cardiac surgery patients and health care personnel respectively were subjected for this study.

3.15 Screening of Indigenous medicinal plants and spices for the Antibacterial activity

The plant material and spices that were used to study the antibacterial activity in the present study are shown in Table 3.10 and 3.11.

The plant materials were collected from Trivandrum district, Kerala state, India. The plant materials were dried thoroughly, powdered and extract of appropriate concentration were prepared in distilled water and used.

3.15.1 Preparation of the filter sterile aqueous extract

Powdered plant material (50 gm) were grinded with 100 ml of sterile distilled water to a complete mash to get the final concentration of 50%, and then filtered through a muslin cloth. The clear filtrate was then filter sterilized using a millipore filter (Sietz filter).

The filtrate so obtained was stored at 4°C and was used up to a weak for further testing procedures.

Similarly 20% aqueous extract were also made. From this, 10%, 5% and 2.5% dilutions were made as in Table 3.12.

3.15.2 Preparation of the autoclaved aqueous extract

The autoclaved extracts were prepared to find out whether the antibacterial elements in the extract is heat stable. Powdered plant materials (50 gm) were grinded thoroughly with 100 ml of distilled water to a complete mash to get the final concentration of 50% and then filtered through a muslin cloth. The extract so obtained was autoclaved at 15 lbs for 15 minutes. The autoclaved extract was stored for a week at 4°C for further tests. The required further dilutions were made as in (Table 3.12).

TABLE - 3 . 10

LIST OF PLANT MATERIAL USED TO STUDY THE ANTIBACTERIAL ACTIVITY

Sl. No.	Latin name	Local Name	Part Used	Types of extracts tested
1	*Moringa oleifera*	Muringa	Seed	Filter Sterile aqueous extract. Autoclaved aqueous extract.
2	*Azadriachta indica*	Veppu	Leaves	Filter sterile aqueous extract. Autoclaved aqueous extract.
3	*Ocimum sanctum*	Tulsi	Leaves	Filter sterile aqueous extract. Autoclaved aqueous extract.
4	*Leucas aspera*	Thumba	Leaves	Filter sterile aqueous extract. Autoclaved aqueous extract.
5	*Piper betle*	Vetta	Leaves	Filter sterile aqueous extract. Autoclaved aqueous extract.
6	*Camelia sinensis*	Theyila	Tea dust	Filter sterile aqueous extract. Autoclaved aqueous extract.
7	*Lawsonia inermis*	Mayilanchi	Leaves	Filter sterile aqueous extract. Autoclaved aqueous extract.
8	*Eucalyptus globulus*	Eucalyptus	Leaves	Filter sterile aqueous extract. Autoclaved aqueous extract.

(Cont'd...)

9	*Phyllanthes niruri*	Keezharnelli	Leaves	Filter sterile aqueous extract. Autoclaved aqueous extract.
10	*Murraya koenigii*	Kariveppu	Leaves	Filter sterile aqueous extract. Autoclaved aqueous extract.
11	*Adhatoda vasica*	Adalodaka	Leaves	Filter sterile aqueous extract Autoclaved aqueous extract.
12	*Eupatorium urticifolium*	C.Pacha	Leaves	Filter sterile aqueous extract Autoclaved aqueous extract.
13	*Clerodendron infortunatum*	Perimala	Leaves	Filter sterile aqueous extract. Autoclaved aqueous extract.
14	*Emilia sonchifolia*	Muyal cheviyan	Leaves	Filter sterile aqueous extract. Autoclaved aqueous extract.
15	*Tamarindus indicus*	Pulie	Pulp of fruit.	Filter sterile aqueous extract. Autoclaved aqueous extract.

TABLE - 3 . 11

LIST OF INDIGENOUS SPICES USED TO STUDY THE ANTIBACTERIAL ACTIVITY

Sl. No.	Latin name	Malayalam name	English name	Part used	Types of extracts tested
1	*Allium sativum*	Veluthulli	Garlic	Bulb	Filter sterile aqueous extract. Autoclaved aqueous extract.
2	*Zingiber officinale*	Inchi	Ginger	Rhizome	Filter sterile aqueous extract. Autoclaved aqueous extract.
3	*Syzygium aromaticum*	Grambu	Clove	Buds	Filter Sterile aqueous extract. Autoclaved aqueous extract.
4	*Cinnamomum zeylanicum*	Pattah	Cinnamon	Bark	Filter sterile aqueous extract. Autoclaved aqueous extract.
5	*Capsicum annuum*	Mulaku	Chilly	Fruit	Filter sterile aqueous extract. Autoclaved aqueous extract.
6	*Brassica juncea*	Kaduku	Mustard	Seed	Filter sterile aqueous extract. Autoclaved aqueous extract.
7	*Curcuma aromatica*	Kasthuri mangal	Turmeric	Rhizome	Filter sterile aqueous extract. Autoclaved aqueous extract.
8	*Piper nigrum*	Kurumulaku	Pepper	Seed	Filter sterile aqueous extract. Autoclaved aqueous extract.

(Contd...)

9	*Coriandrum sativum*	Mallie	Coriander	Seed	Filter Sterile aqueous extract. Autoclaved aqueous extract.
10	*Cuminum cyminum*	Geerah	Cumin	Seed	Filter sterile aqueous extract. Autoclaved aqueous extract.

TABLE - 3 . 12

PLANT AND SPICE EXTRACT DILUTION SCHEDULE FOR M.I.C. DETERMINATION.

Sl. No.	Stock Dilution	Stock Volume	Diluent Volume	Final concentration of extract
1	20%	10 ml	10 ml	10%
2	10%	10 ml	10 ml	5%
3	5%	10 ml	10 ml	2.5%

* Grams / 100 ml powdered extract.

Test organisms

Twenty five strains of CNS; *S.aureus*, *Klebsiella* spp. and *Pseudomonas* spp. isolated from cardiac surgery patients and twenty five strains of CNS, *S.aureus* and fourteen strains of *Klebsiella* spp. isolated from health care personnel were used for testing the antibacterial activity of various indigenous plants and spices in the present study.

Method of testing

Agar plate well diffusion method (Perez et al 1990)

Agar plate well diffusion method was done as per the method of Perez et al (1990). Mueller Hinton Agar was used for culturing the bacteria and four hour culture organism (10^6 cfu/ml) were seeded on the top of agar so as to obtain a lawn culture. Wells were bored (10 mm diameter) using sterile cork borer and the ditches were removed. Using sterile pipettes, 100 µl of the extract was added aseptically to these wells. The plates were kept in the refrigerator (2 hes) for proper diffusion of the fractions (Rios et al 1988) and later incubated for 24 hours at 37°C. The zones of inhibition were measured and recorded. The Zone diameter of 13 mm and below was considered as Resistant (R); those between 14 and 16 mm was taken as Moderately sensitive and that having 17 mm and above was taken as sensitive strain.

Minimum Inhibitory Concentration was defined as the concentration of the plant extract at which no sensitivity zone is observed around the well and the growth of culture inoculated is observed till the rim of the well. The results are as shown in (Plate No.7).

Antibacterial activity of Tamarind

In the case of tamarind, since the extract is very viscous, the agar plate well diffusion method was very difficult to perform and hence agar incorporation method was followed to find out the antibacterial activity of tamarind.

Agar Incorporation method

Agar incorporation method was done as described by Vanden Berghe and Vlietinck (1991). Different concentration viz, 2.5%, 5% and 10% were prepared and autoclaved. For preparing these concentrations, 5, 10, and 20 gm of tamarind was

ANTIBACTERIAL ACTIVITY OF

A. Autoclaved aqueous extract of *Lawsonia inermis*

(1) 50% (2) 20% (3) 10% (4) 5% (5) 2.5%

B. Filter sterile aqueous extract of *Allium sativum*

(1) 50% (2) 20% (3) 10% (4) 5% (5) 2.5%

C. Filter sterile aqueous extract of *Moringa oleifera*

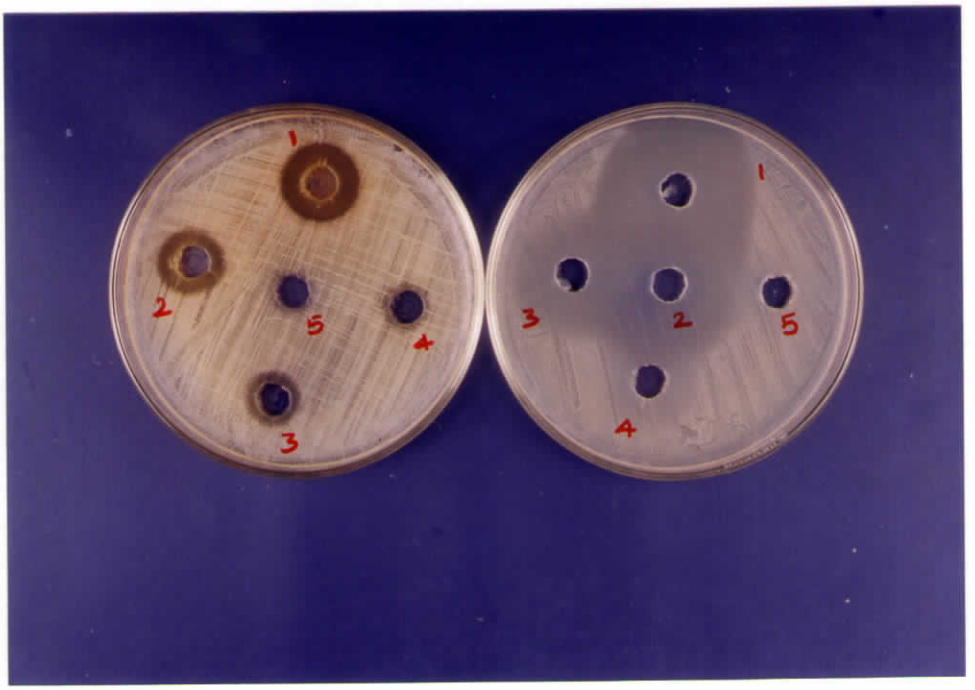
(1) 50% (2) 20% (3) 10% (4) 5% (5) 2.5%

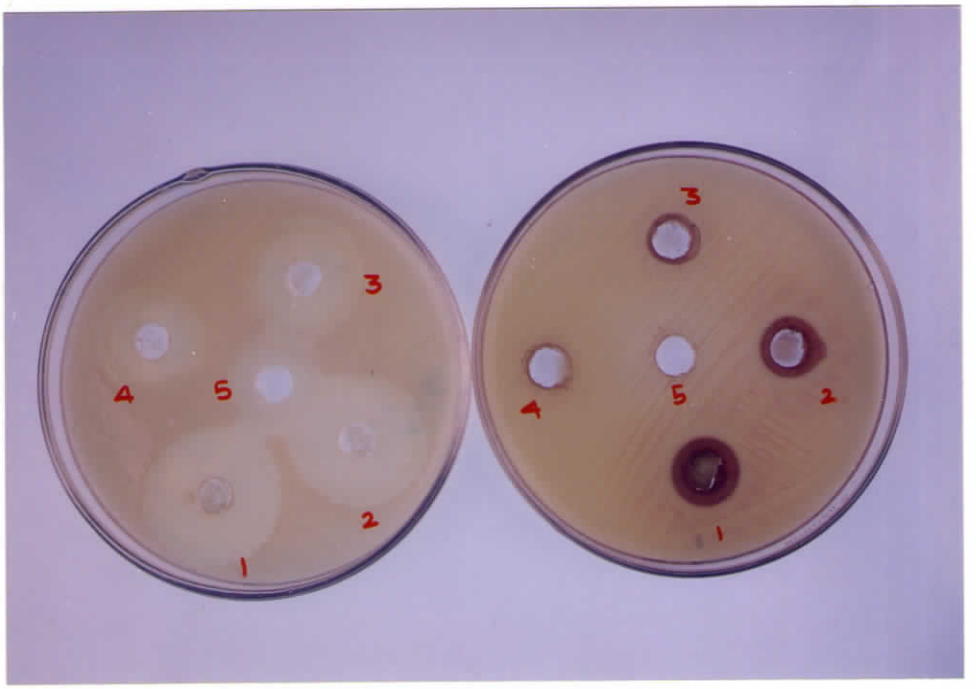
D. Autoclaved aqueous extract of *Eupatorium urticifolium*

(1) 50% (2) 20% (3) 10% (4) 5% (5) 2.5%

PHOTOGRAPHIC PLATE NO: 7

DETECTION OF ANTIBACTERIAL ACTIVITY BY AGAR PLATE WELL DIFFUSION METHOD.





A) Control MHA plate without Tamarind *(Tamarindus indicus)* inoculated with *S.aureus* strains.

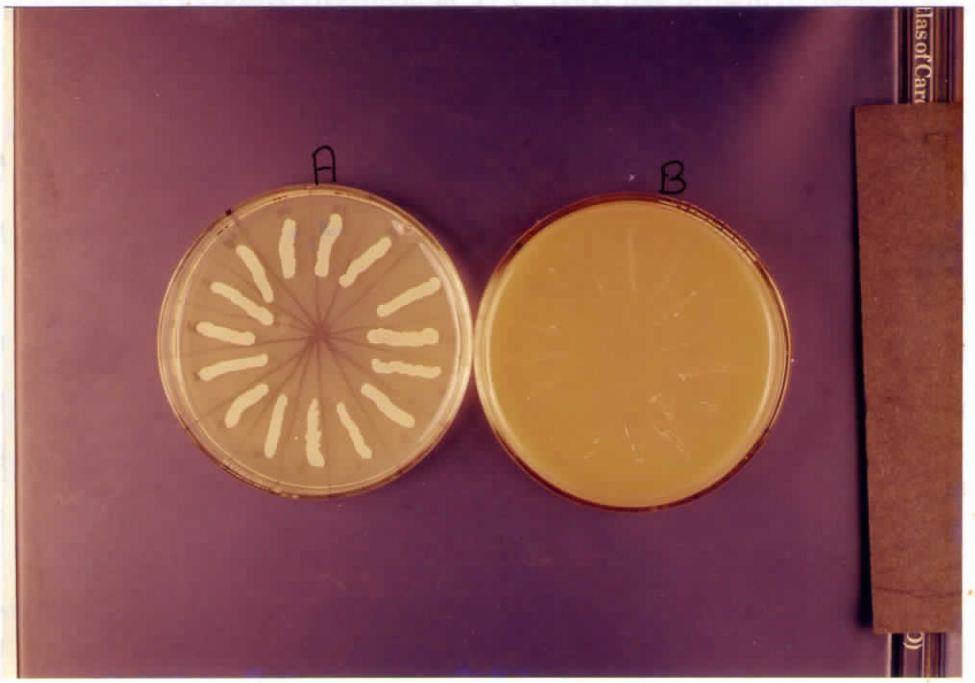
B) MHA plate incorporated with 2.5% concentration of autoclaved aqueous extract of Tamarind *(Tamarindus indicus)* and inoculated with the same *S.aureus* strains as in control plate.

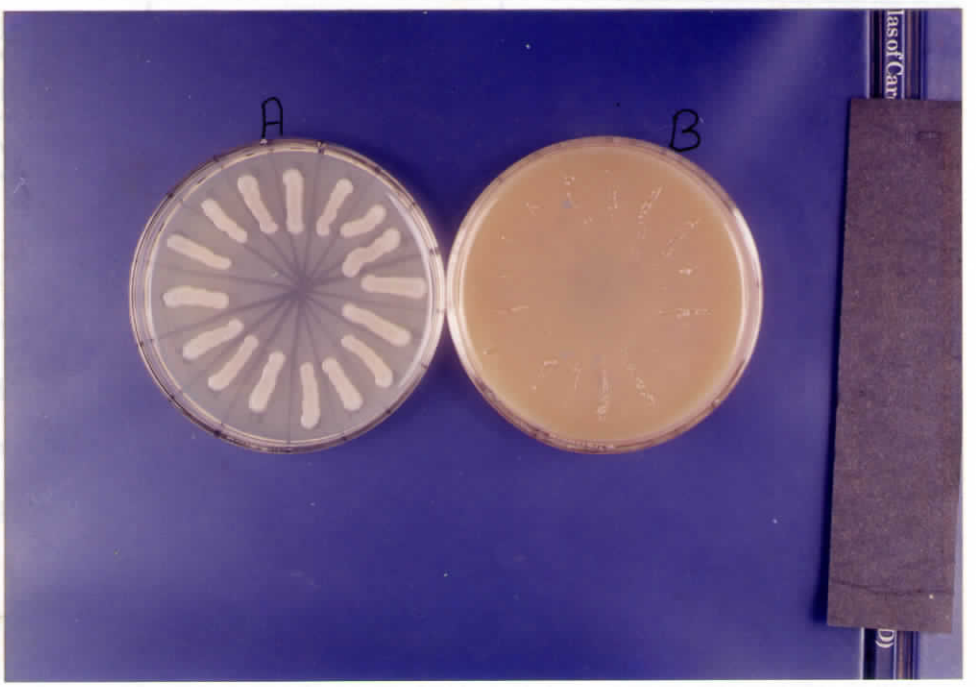
A) Control MHA plate without Tamarind *(Tamarindus indicus)* inoculated with *Klebsiella* spp.

B) MHA plate incorporated with 2.5% concentration of autoclaved aqueous extract of Tamarind *(Tamarindus indicus)* and inoculated with the same *Klebsiella* spp. as in control plate.

PHOTOGRAPHIC PLATE NO: 8

DETECTION OF ANTIBACTERIAL ACTIVITY BY AGAR INCORPORATION METHOD.





added to 200 ml each of MHA made in different flasks. Increased concentration of agar (4%) were added in MHA for the medium to get solidified.

A control plate without tamarind was also prepared. The bacterial strains tested is same as that tested for Agar plate well diffusion method. The bacteria were inoculated in all plates by drawing a small line with a loopfull of bacteria over the plate. The plates were incubated overnight at 37°C. Next day growth of bacteria was tested visually (Plate No: 8).

3.16 Study on *In vivo* anti Staphylococcal effect of Garlic and Tamarind in experimental mice

To find out the anti-Staphylococcal effect of Garlic and Tamarind in experimental mice. In the present study, a total of 30 male mice having body weight between 22 and 35 grams were selected and divided in to five groups as shown below. Each group was having six mice in each and was marked as per the method of Festing (1992).

Groups	No: of mice employed in each group.	Treatment /Control
Group I	6	Treatment with Garlic
Group II	6	Treatment with Tamarind
Group III	6	Treatment with cefuroxime
Group IV	6	Control
Group V	6	Vehicle Control

Each mouse in all the six groups was given 0.2 ml of $2x10^7$ Colony Forming Units(CFU) of ATCC *S.aureus* 25923 intraperitonially based on the work of Darling and Herbert (1996) and Simmons et al (1963). Handling anaesthetising and inoculation of these mice were done as per the method of Darling and Herbert (1996). 45 mg/Kg of Penta barbitone sodium was given intraperitonially as the anaesthetising agent.

The first group of animals received 1.0 ml of 20% of filter sterile aqueous extract of Garlic orally. The second group received 1.0 ml of 2.5% of autoclaved aqueous

extract of Tamarind orally.Third group of animals were given 0.5 ml of antibiotic Cefuroxime intraperitonially. Fourth group served as control with out receiving any antibacterial agent and the fifth group served as the vehicle control receiving 1.0 ml of sterile distilled water orally. The dose were given for 3 consecutive days and on the seventh day all mice were sacrificed. Swabs were taken from the intraperitoneal region of each animal for the qualitative and quantitative assessment of the bacterial strain injected.

RESULTS AND DISCUSSIONS

CHAPTER - IV

ANALYSIS OF DATA

A. Results

Four hundred patients admitted for cardiac surgery at Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum were screened for understanding the carrier state of bacterial infection. Table 4.1 shows the rate of isolation of different bacterial species from throat, nose and sternal skin area of the subjects. A total of 1160 strains were isolated belonging to seven species (Table 4.1).

The Table 4.2 shows the percentage of isolates from the throat, nose and sternal skin area. It is clear that more than half of the isolates were from the nose. The major isolate from throat is Klebsiella whereas from nose and skin area it was the CNS which was predominant. The number of isolates of *S.aureus* was greater in nose when compared to the other two swabs.

The number of isolates on admission (pre-operative period), during the post-operative period after shifting from ICU to ward and on the date of discharge of the patient, from the hospital are separately given in Table 4.3. It is quite clear from the data that the percentage of possible pathogenic strains in all cases on admission is significantly higher prior to antibiotic administration. But on post-operative period there is a tremendous reduction almost to a level of total elimination in the case of post-operative skin swabs, except with the CNS. The tendency of reduction continued to the date of discharge in all cases. With nasal swabs, when each bacterial species is considered individually the tendency is slightly reversed, though not substantial.

The influence of age on bacterial colonisation in nose, throat and sternal skin area is given in Table 4.4. CNS and *S.aureus* are more common in nose and Klebsiella in throat for all age groups and is predominant in the middle age group. Beta-hemolytic Streptococci is predominant in children (below 10 years of age). However CNS has no age preference.

Table – 4.1

ISOLATION RATE OF DIFFERENT BACTERIAL SPECIES FROM THROAT, NOSE AND STERNAL SKIN AREA OF CARDIAC SURGERY PATIENTS (CARRIER STATE)

Sl. No.	Name of bacteria	Number Isolated	Percentage
1.	Coagulase Negative *Staphylococci*	766	66.03
2.	*S.aureus*	147	12.67
3.	*Klebsiella* spp.	117	10.08
4.	*Diphtheroids* spp.	55	4.74
5.	β-hemolytic *Streptococci*	33	2.84
6.	Nonfermentors	22	1.89
7.	*Pseudomonas aeruginosa*	20	1.72
TOTAL		1160 strains	100.00

Number of patients studied = 400

Table - 4.2

THE BACTERIAL ISOLATES FROM THROAT, NOSE AND STERNAL SKIN AREA OF CARDIAC SURGERY PATIENTS

Name of bacteria	Number of isolates from		
	Throat (%)	Nose (%)	Skin (%)
CNS	63 (28.5)	459 (69.6)	244 (87.14)
S.aureus	17 (7.7)	111 (16.8)	19 (6.78)
Klebsiella spp.	90 (40.7)	27 (4.09)	NI
Diphtheroids spp.	1 (0.45)	47 (7.13)	7 (2.50)
Pseudomonas aeruginosa	12 (5.43)	8 (1.21)	NI
Nonfermentor	5 (2.26)	7 (1.06)	10 (3.57)
β-hemolytic *Streptococci*	33 (14.9)	NI	NI
TOTAL	221 (19.05)	659 (56.8)	280 (24.14)

Total number of strains isolated = 1160

CNS - Coagulase Negative *Staphylococci*

NI - Not isolated.
The percentage from the given group is given in brackets.

Table - 4.3

ISOLATION RATE OF BACTERIA FROM THROAT, NOSE AND STERNAL SKIN AREA OF CARDIAC SURGERY PATIENTS ON ADMISSION, DURING POST OPERATIVE PERIOD AND ON DISCHARGE

Name Of Bacteria	THROAT SWAB			NASAL SWAB			SKIN SWAB		
	Adm	Post	Dis	Adm	Post	Dis	Adm	Post	Dis
CNS	35	12	16	235	118	106	207	24	13
%	24.31	30.00	43.24	67.70	73.75	69.70	85.89	100	86.60
S.aureus	9	5	3	62	24	25	18	NI	1
%	6.25	12.50	8.11	17.86	15.00	16.44	7.46	-	6.66
Klebsiella Spp.	55	19	16	16	4	7	NI	NI	NI
%	38.19	47.50	43.24	4.61	2.50	4.61	-	-	-
Diphtheroids spp.	1	NI	NI	27	8	12	7	NI	NI
%	0.69	-	-	7.78	5.00	7.89	2.90	-	-
Ps.aeruginosa	8	2	2	3	5	NI	NI	NI	NI
%	5.55	5.00	5.41	0.86	3.13	-	-	-	-
Nonfermentors	3	2	NI	4	1	2	9	NI	1
%	2.08	5.00	-	1.15	0.63	1.31	3.73	-	6.66
β-hemo *Streptococci*	33	NI	NI	NI	NI	NI	NI	NI	NI
%	22.90	-	-	-	-	-	-	-	-
TOTAL	144	40	37	347	160	152	241	24	15
%	65.16	18.10	16.74	52.65	24.22	23.06	86.07	8.57	5.36

Total number of strains isolated = 1160; Total strains from throat = 221, from nose = 659, from sternal skin = 280.

NI - Not isolated

Adm - On the day of admission to the hospital

Post - Post-operatively after shifting from ICU to ward.

Dis - On the day of discharge from the hospital

Table - 4.4

ISOLATION RATE OF BACTERIA FROM THROAT, NOSE AND STERNAL SKIN AREA OF CARDIAC SURGERY PATIENTS IN DIFFERENT AGE GROUPS.

Age in years

Name of Bacteria	0-10			11-20			21-30			31-40			41-50			Above 50			Total
	TS	NS	SS	TS	NS	SS	TS	NS	SS	TS	NS	SS	TS	NS	SS	TS	NS	SS	
CNS	18	80	50	14	81	49	11	84	49	5	51	30	10	81	46	9	68	30	766
S.aureus	6	27	6	5	32	5	2	19	4	2	13	2	1	4	2	3	13	1	147
Klebsiella spp.	17	2	NI	12	8	NI	22	7	NI	12	2	NI	17	5	NI	11	2	NI	117
Diphtheroids	NI	9	2	NI	12	1	NI	10	2	1	5	1	NI	3	1	NI	8	NI	55
Ps.aeruginosa	NI	NI	NI	2	2	NI	3	3	NI	2	NI	NI	1	1	NI	4	2	NI	20
Nonfermentor	2	2	3	NI	4	NI	NI	NI	2	1	1	1	NI	NI	2	1	NI	3	22
β-hemo *Strep.*	15	NI	NI	7	NI	NI	2	NI	NI	4	NI	NI	3	NI	NI	2	NI	NI	33
Total	58	120	61	40	139	55	40	123	57	27	72	34	32	94	51	30	93	34	
Grand Total	239			234			220			133			177			157			

Total number of strains isolated - 1160

CNS - Coagulase Negative *Staphylococci*

TS - Throat swab

NS - Nasal swab

SS - Sternal skin swab

N1 - Not isolated

An analysis was made to know whether there is any relation between bacterial harbouring and sex. The results of the comparison of bacterial strains isolated from throat, nose and sternal skin area of cardiac surgery patients of both sexes is shown in Table 4.5. Table 4.6 and Table 4.7 elaborate the gender based isolation rate of bacteria at admission, in post-operative period and at discharge. The total bacterial harbouring of pathogenic bacteria is more with male sex as compared to that of females though not significant.

There is increasing occurrence of post-operative nosocomial infection by cross-contamination through hospital personnel (Perdreau-Reming et al 1995, Berlau et al 1999 and Stubbs et al 1994). Hence hundred health care personnel were screened for the study of their carrier state. The results are given in Tables 4.8 and 4.9.

One hundred and seventy five (175) strains were isolated from 300 swabs belonging to 5 different species (Table 4.8). Beta-hemolytic Streptococci and *Ps. aeruginosa* could not be isolated from any of these personnel.

The isolates from throat, nose and skin showed similar patterns to those seen in cardiac surgery patients (Table 4.9 and 4.2).

Since some of the species seen in health care perosonnel are potentially pathogenic and were also isolated from cardiac surgery patients, the question of source of infection gains importance. Thus to know whether the post-operative infection is from exogenous or endogenous source, samples from post-operative infected cases were further analysed for correlation. The results are as given in Tables 4.10, 4.11 and 4.12.

It is quite evident (Table 4.10) that most of the pathogenic species isolated from both sources were also isolated from infected post-operative specimens (Table 4.10).

The types of infection developed and their frequency are given in Table 4.11. The major organisms isolated from these patients are as given in Table 4.12.

18.25% of patients developed postoperative infection (Table 4.11), which includes Urinary Tract Infection (UTI); Bacteremia, Respiratory Tract Infection (RTI) and Wound Infection. Table 4.11 indicates the prevalence of post-operative wound infection, the highest of all types of post-operative infections.

Table – 4.5

COMPARISON OF BACTERIAL STRAINS ISOLATED FROM THROAT, NOSE AND STERNAL SKIN AREA OF CARDIAC SURGERY PATIENTS (BOTH SEXES)

Name of bacteria	THROAT SWAB		NASAL SWAB		SKIN SWAB	
	Male	Female	Male	Female	Male	Female
CNS	34 (29.31)	29 (27.62)	231 (68.90)	228 (70.37)	128 (90.78)	116 (83.45)
S.aureus	10 (8.62)	7 (6.66)	57 (17.01)	54 (16.66)	9 (6.38)	10 (7.19)
Klebsiella spp.	43 (37.06)	47 (44.76)	11 (3.28)	16 (4.94)	NI -	NI -
Diphtheroids spp.	NI -	1 (0.95)	26 (7.76)	21 (6.48)	3 (2.13)	4 (2.87)
Ps.aeruginosa	7 (6.03)	5 (4.76)	5 (1.49)	3 (0.93)	NI -	NI -
Nonfermen-tors	4 (3.45)	1 (0.95)	5 (1.49)	2 (0.62)	1 (0.71)	9 (6.47)
β-hemo-*Streptococci*	18 (15.52)	15 (14.29)	NI -	NI -	NI -	NI -
TOTAL	116 (10.00)	105 (9.05)	335 (28.87)	324 (27.93)	141 (12.16)	139 (11.98)

Total number of strains tested - 1160

Total number of male patients - 204

Total number of female patients - 196

NI - Not isolated.

Table - 4.6

ISOLATION RATE OF BACTERIA FROM THROAT, NOSE AND STERNAL SKIN AREA ON ADMISSION, DURING POST OPERATIVE PERIOD AND ON DISCHARGE DATE IN MALE PATIENTS

Name Of Bacteria	THROAT SWAB			NASAL SWAB			SKIN SWAB		
	Adm	Post	Dis	Adm	Post	Dis	Adm	Post	Dis
CNS	20	6	8	121	61	49	110	12	6
%	25.30	28.57	50.00	68.36	71.76	67.12	89.43	100	100
S.aureus	6	3	1	29	13	15	9	NI	NI
%	7.59	14.28	6.25	16.38	15.29	20.54	7.32	-	-
Klebsiella Spp.	28	9	6	6	2	3	NI	NI	NI
%	35.44	42.85	37.5	3.39	2.35	4.11	-	-	-
Diphtheroids spp.	NI	NI	NI	16	5	5	3	NI	NI
%	-	-	-	9.04	5.88	6.85	2.44	-	-
Ps.aerugi-nosa	5	1	1	2	3	NI	NI	NI	NI
%	6.33	4.76	6.25	1.13	3.52	-	-	-	-
Nonfermentors	2	2	NI	3	1	1	1	NI	NI
%	2.53	9.52	-	1.69	1.18	1.37	0.81	-	-
β-hemo *Streptococci*	18	NI	NI	NI	NI	NI	NI	NI	NI
%	22.78	-	-	-	-	-	-	-	-
TOTAL	79	21	16	177	85	73	123	12	6
%	68.10	18.10	13.79	52.99	25.44	21.86	87.23	8.51	4.26
GRAND TOTAL	116			335			141		

NI - Not isolated
Adm - On the day of admission to the hospital
Post - Post-operatively after shifting from ICU to ward.
Dis - On the day of discharge from the hospital

Table - 4.7

ISOLATION RATE OF BACTERIA FROM THROAT, NOSE AND STERNAL SKIN AREA ON ADMISSION, DURING POST OPERATIVE PERIOD AND ON DISCHARGE DATE IN FEMALE PATIENTS

Name of Bacteria	THROAT SWAB			NASAL SWAB			SKIN SWAB		
	Adm	Post	Dis	Adm	Post	Dis	Adm	Post	Dis
CNS	15	6	8	114	57	57	97	12	7
%	23.08	31.58	38.09	66.66	76.00	73.07	82.20	100	77.77
S.aureus	3	2	2	34	11	9	9	NI	1
%	4.62	10.53	9.52	19.88	14.66	11.54	7.63	-	11.11
Klebsiella spp.	27	10	10	10	2	4	NI	NI	NI
%	41.54	52.63	47.62	5.85	2.66	5.13	-	-	-
Diphtheroids spp.	1	NI	NI	11	3	7	4	NI	NI
%	1.54	-	-	6.43	4.00	8.97	3.39	-	-
Ps.aeruginosa	3	1	1	1	2	NI	NI	NI	NI
%	4.62	5.26	4.76	0.58	2.66	-	-	-	-
Nonfermentors	1	NI	NI	1	NI	1	8	NI	1
%	1.54	-	-	0.58	-	1.28	6.77	-	11.11
β-hemo *Streptococci*	15	NI	NI	NI	NI	NI	NI	NI	NI
%	23.08	-	-	-	-	-	-	-	-
TOTAL	65	19	21	171	75	78	118	12	9
%	61.90	18.09	20.00	52.77	23.15	24.07	84.28	8.63	6.47
GRAND TOTAL	105			324			139		

NI - Not isolated
Adm - On the day of admission to the hospital
Post - Post-operatively after shifting from ICU to ward.
Dis - On the day of discharge from the hospital

Table – 4.8

ISOLATION RATE OF DIFFERENT BACTERIAL SPECIES FROM THROAT, NOSE AND SKIN OF HEALTH CARE PERSONNEL (CARRIER STATE)

Sl. No.	Name of bacteria	Number Isolated	Percentage
1.	Coagulase Negative *Staphylococci*	111	63.40
2.	*S.aureus*	27	15.40
3.	*Klebsiella* spp.	14	8.00
4.	*Diphtheroids* spp.	19	10.80
5.	*Acinetobacter* spp.	4	2.30
TOTAL		175 strains	100.00

Number of health care personnel studied = 100

Table - 4.9

THE BACTERIAL ISOLATES FROM THROAT, NOSE AND SKIN AREA OF HEALTH CARE PERSONNEL

Name of bacteria	Number of isolates from		
	Throat (%)	Nose (%)	Skin (%)
CNS	11 (36.6)	61 (66.3)	39 (73.5)
S.aureus	6 (20.0)	20 (21.7)	1 (1.9)
Klebsiella spp.	13 (43.3)	1 (1.08)	NI
Diphtheroids spp.	NI	10 (10.8)	9 (16.9)
Acinetobacter spp.	NI	NI	4 (7.5)
TOTAL	30 (17.14)	92 (52.57)	53 (30.20)

Total number of strains isolated = 175

CNS - Coagulase Negative *Staphylococci*

NI - Not isolated.
The percentage from the given group is given in brackets

Table - 4.10

STRAINS ISOLATED FROM POST-OPERATIVE SPECIMENS OF CARDIAC SURGERY PATIENTS DURING INFECTED STATE

Name	Number of isolates	Percentage
CNS	18	18.18
S.aureus	13	13.13
Klebsiella spp.	13	13.13
Diphtheroids spp.	3	3.03
Nonfermentor	26	26.26
Ps.aeruginosa	8	8.08
Enterobacter spp.	10	10.10
Citrobacter freundii	1	1.01
Non hemo *Streptococci*	2	2.02
E.coli	1	1.01
Candida	4	4.04

CNS - Coagulase Negative *Staphylococci*

Total number of patients studied - 400

Number of patients who developed post-operative infections - 73 (18.25%)

Table – 4.11

ANALYSIS OF RATE OF INFECTION

Sl. No.	Type of infection	Infection Rate		Infection Male		Female	
		No.	%*	No.	%**	No.	%**
1	U.T.I.	10	2.50	5	50.00	5	50.00
2	Bacteremia	18	4.50	12	66.66	6	33.33
3	R.T.I.	27	6.75	17	62.96	10	37.04
4	W.I.	31	7.75	21	67.74	10	32.26

Total number of patients studied - 400

Number of Female patients in the study - 196 (49.00%)

Number of male patients in the study - 204 (51.00%)

Number of patients who developed post -operative infections - 73 (18.25%)

U.T.I. - Urinary tract infection

R.T.I. - Respiratory tract infection

W.I. - Wound infection

* **The rate is given is number of patients infected out of 400 patients**

** **The percentage is given as the number of patients (Male/Female) infected out of total number infected by the respective infection.**

TABLE – 4.12

STRAINS ISOLATED FROM POST-OPERATIVE INFECTED SPECIMENS

Sl. No.	Type of infection	Leading isolate	Per-centage	Other organisms Isolated
1	U.T.I.	*Candida* CNS	30 20	Non-fermentors *Ps.aeruginosa* Non-haem *Streptococci*
2	Bacteremia	Non-fermentor CNS	55.55 16.66	*Diphtheroids,* *Ps.aeruginosa,* *Candida,* *Enterobacter* spp.
3	R.T.I.	*K.pneumoniae* Non-fermentor *Enterobacter* spp.	44.44 29.62 22.22	*Ps.aeruginosa* CNS, *S.aureus* *E.coli*
4	W.I.	CNS *S.aureus* Non-fermentor	38.70 32.25 19.35	*Ps.aeruginosa* *Cit.freundii* *K.pneumoniae* *enterobacter* spp.

U.T.I. - Urinary Tract Infection
R.T.I. - Respiratory Tract Infection
W.I. - Wound Infection

Of the various organisms isolated from the specimens, the most common bacteria in Urinary Tract Infection was CNS; in bacteremia it is the Non-fermentor followed by CNS, in Respiratory Tract Infection it is *K.pneumoniae* followed by Non-fermentor and *Enterobacter* spp. and in Wound infection it is the CNS followed by *S.aureus*. (Table 4.12). The other organisms isolated in various cases are also given in Table 4.12.

The identification of the source of infection and strains of bacteria is the prime requirement to prevent the spread of infection. This requires some methods to distinguish between the strains and to point to the source of infection. Different typing methods were adopted and the results are given in Plates 9, 10, 11, 12, 13,14 and 15 and the result of phage typing is as in Table 4.13.

The different typing methods employed were **Antibiogram, Biotyping, Bacteriocin typing, Bacterio-phage typing, Poly Acrylamide Gel Electrophoresis** and **Slime production** for Gram positive organisms and **Antibiogram, Biotyping** and **Bacteriocin typing** for Gram negative organisms.

In the **Antibiogram** pattern, with the bacterial strains isolated from the carrier state and post-operative infected state of the same patient, the zone diameter observed with the same antibiotic discs was found to be similar in many cases as shown in Plate 9 A and B; 10 D and E. Hence they may belong to the same strain. The dissimilar pattern as in Plate 9 A and C; 10 D and F showed that the strains are dissimilar.

Biotyping, based on differences in biochemical reactions (Tenover et al 1997, and Pfaller 1995) was done. The results of biotyping with the bacterial strains CNS, *S.aureus*, *Klebsiella* spp., *Pseudomonas* spp. and Non-fermentors from carrier state and infected state of patients and also from health care personnel were compared. The two strains isolated from the same patient (ie, during the carrier state and post operative infected state) were found to be similar when same biochemical reactions are shown by both strains (Plate 11A and 12A). Strains which do not give the same biochemical reactions are different strains (Plates 11 B and 12 B).

Bacteriocin typing (Fyfe et al 1984) was done with *Pseudomonas* spp., *Klebsiella* spp., *S.aureus,* CNS as well as with *Acinetobacter* spp. isolated from the carrier state and post-operative infected state of the same patient and also from health care personnel. The results are as in Plates 13 A, B, C and D. No inhibition

PHOTOGRAPHIC PLATE NO: 9

ANTIBIOGRAM PATTERN FOR THE IDENTIFICATION OF STRAINS

Antibiotics discs were tested against the bacterial strains isolated from pre-operative carrier state (A) and post-operative infected state (B) from the same patient. The method adopted is as given in materials and methods section 3.8.

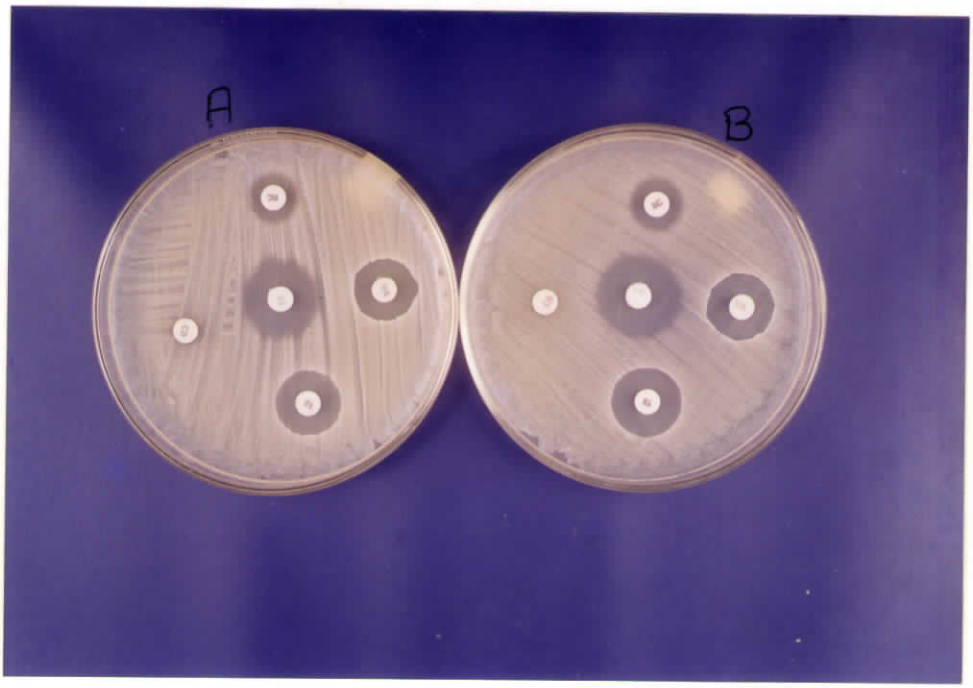
Similarity in zone diameter is observed with all discs.

Antibiotics discs were tested against the bacterial strains isolated from pre-operative carrier state (A) and post-operative infected state (C) from the same patient. The method adopted is as given in materials and methods section 3.8.

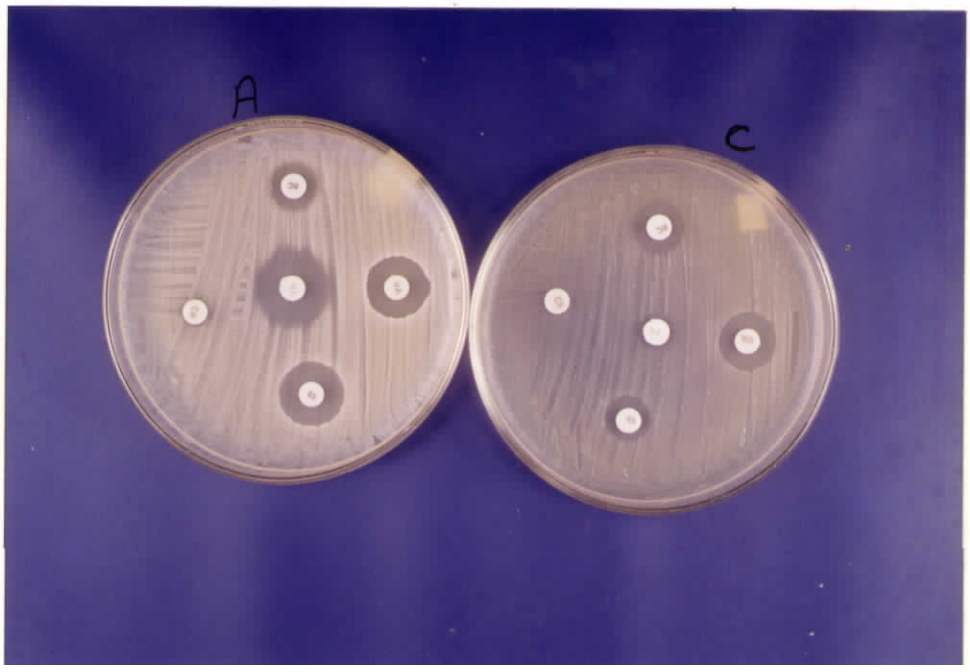
Dissimilarity in zone diameter is observed with all discs except ciprofloxacin (RC).

PHOTOGRAPHIC PLATE NO: 9

A typical antibiogram pattern showing strain similarity in Gram Positive Organism



A typical antibiogram pattern showing strain dissimilarity in Gram Positive Organism



PHOTOGRAPHIC PLATE NO: 10

ANTIBIOGRAM PATTERN FOR THE IDENTIFICATION OF STRAINS

Antibiotics discs were tested against the bacterial strains isolated from pre-operative carrier state (D) and post-operative infected state (E) from the same patient. The method adopted is as given in materials and methods section 3.8.

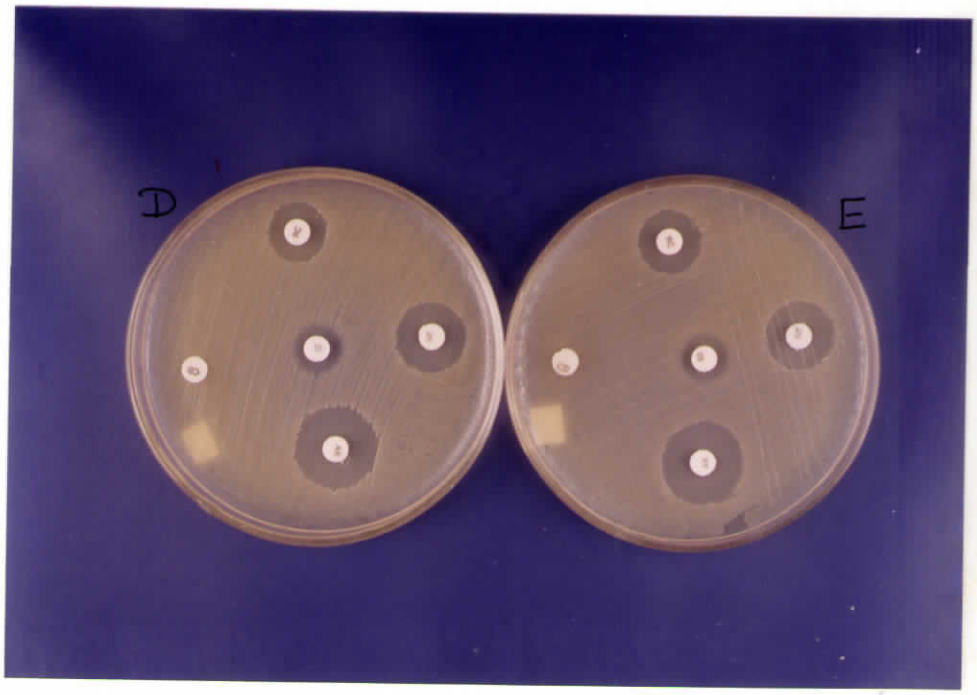
Similarity in zone diameter is observed with all discs.

Antibiotics discs were tested against the bacterial strains isolated from pre-operative carrier state (D) and post-operative infected state (F) from the same patient. The method adopted is as given in materials and methods section 3.8.

Dissimilarity in zone diameter is observed with all discs except Tobramycin (TB).

PHOTOGRAPHIC PLATE NO: 10

A typical antibiogram pattern showing strain similarity in Gram Negative Organism



A typical antibiogram pattern showing strain dissimilarity in Gram Negative Organism



PHOTOGRAPHIC PLATE NO: 11

IDENTIFICATION OF STRAINS USING BIOCHEMICAL TESTS (BIOTYPING)

The alternate tubes in (A) represents the biochemical reactions shown by the bacterial strains isolated from pre-operative carrier state and that of post-operative infected state from the same patient. The procedure is as given in materials and methods section 3.7.3.

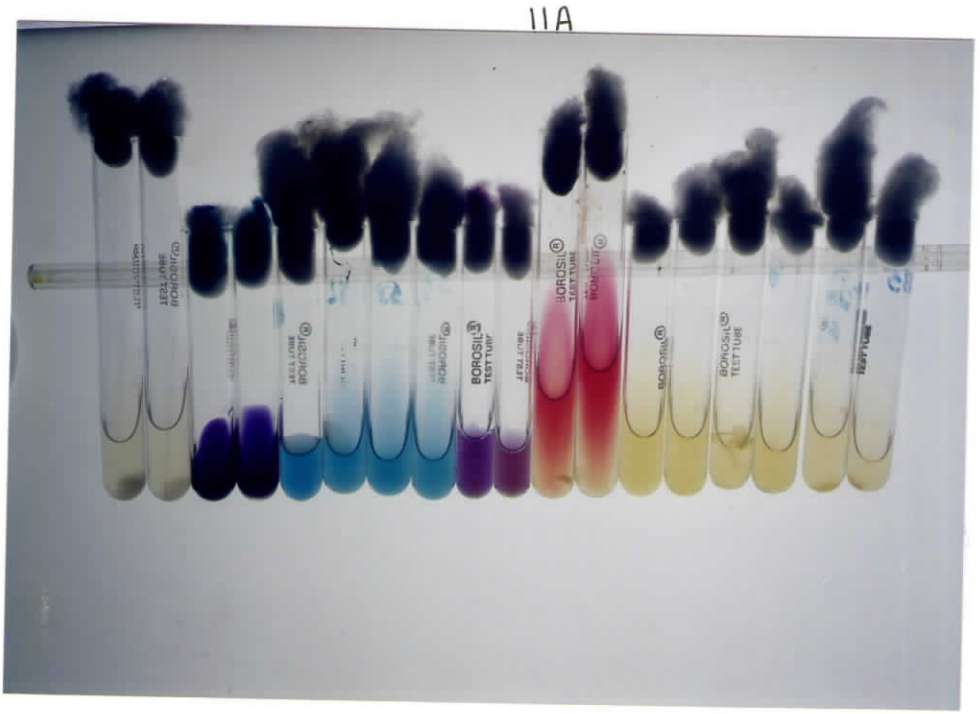
Similarity in biochemical reactions is observed with all set of tubes.

The alternate tubes in (B) represents the biochemical reactions shown by the bacterial strains isolated from pre-operative carrier state and that of post-operative infected state from the same patient. The procedure is as given in materials and methods section 3.7.3.

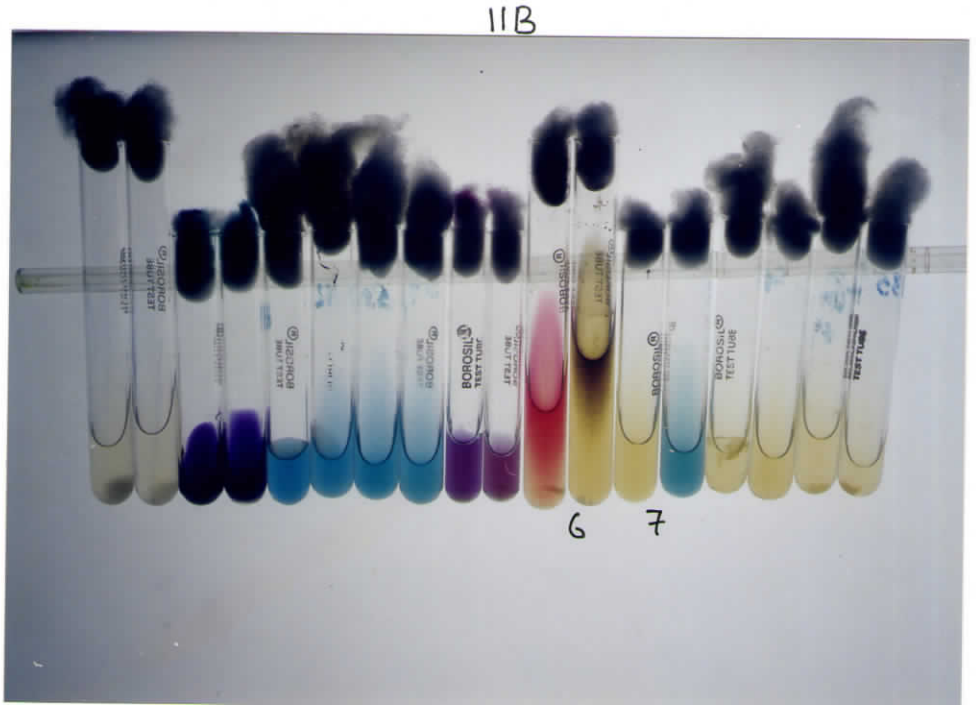
Dissimilarity in biochemical reactions can be seen in 6th and 7th set of tubes.

PHOTOGRAPHIC PLATE NO: 11

Biotyping result showing strain similarity in Gram Positive Organism



Biotyping result showing strain dissimilarity in Gram Positive Organism



PHOTOGRAPHIC PLATE NO: 12

IDENTIFICATION OF STRAINS USING BIOCHEMICAL TESTS (BIOTYPING)

The alternate tubes in (A) represents the biochemical reactions shown by the bacterial strains isolated from pre-operative carrier state and that of post-operative infected state from the same patient. The procedure is as given in materials and methods section 3.7.3.

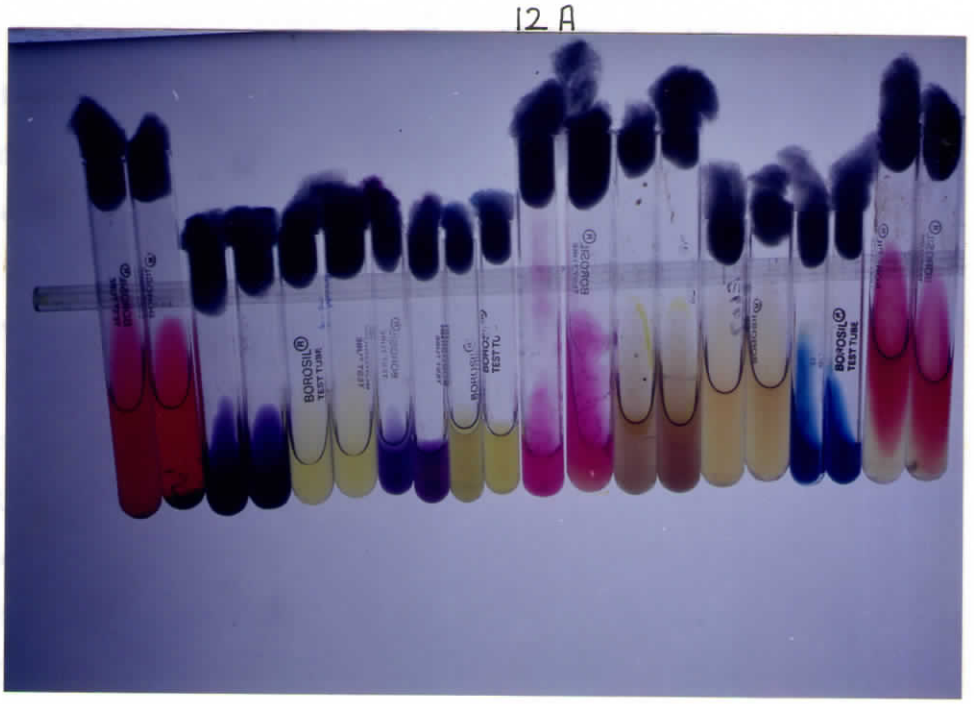
Similarity in biochemical reactions is observed with all set of tubes.

The alternate tubes in (B) represent the biochemical reactions shown by the bacterial strains isolated from pre-operative carrier state and that of post-operative infected state from the same patient. The procedure is as given in materials and methods section 3.7.3.

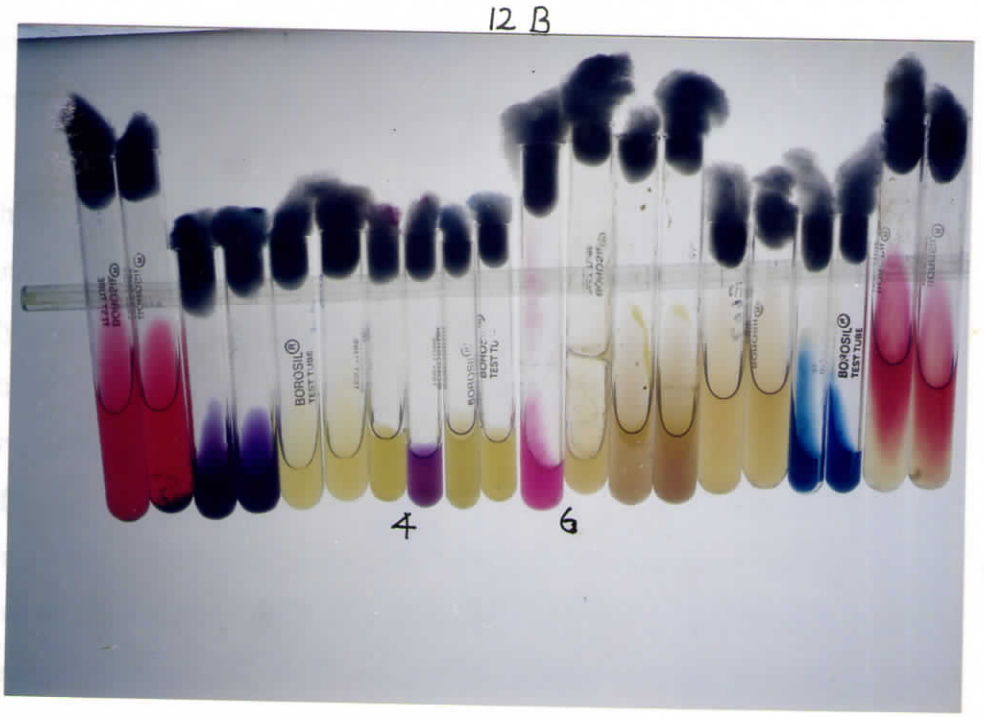
Dissimilarity in biochemical reactions can be seen in 4th and 6th set of tubes.

PHOTOGRAPHIC PLATE NO: 12

Biotyping result showing strain similarity in Gram Negative Organism



Biotyping result showing strain dissimilarity in Gram Negative Organism



pattern was observed in cases of strains isolated from carrier state and post operative infected state of the same patient indicating that the strains are similar in many cases (Plates 13 A, B, C and D). In the case of strains from health care personnel and post-operative infected state in patients, inhibition pattern was observed showing that the strains are not similar (Plates 13 A, B, C and D).

Bacterio-phage typing of *Staphylococcus aureus* was done and the results are given in Table 4.13. *S.aureus* strains isolated from the carrier state and post-operative infected state of the same patient were performed. Of the total thirteen *S.aureus* strains isolated froms specimens in the post-operative infected state, six of them showed correlation with the carrier isolates of the respective patients where as three of them doesn't showed correlation with the carrier state isolate. Four of the isolates were not typable.

Results of typing pattern of **PAGE** also conforms to the results obtained with other typing methods. The band patterns obtained are as in Plate 14.

Strain similarity was also tested by **Slime production** for the Staphylococcal strains (CNS and *S.aureus*), which is considered as an important typing method for these Gram positive organisms (Hedin 1996). Similarity in slime production among strains were considered as belonging to same strain (Plate 15A and 15B) and dissimilarity as belonging to different strains.

There was full correlation between organisms isolated during the carrier state and infected state of the same patient with all the typing methods employed proving that the organism from the patients normal flora is causing infection upto a certain extent.

The results of all typing methods used are summarized in Table 4.14.

Among ten patients (2.5%) who developed Urinary Tract Infection post-operatively, no correlation was found with the bacteria isolated from the carrier state and the infected state (Table 4.14). Also, no correlation was found with bacteria isolated from health care personnel.

Of the total 27 patients who developed Respiratory Tract Infection (RTI), no correlation was found either with the carrier state in 17 patients or with the health care personnel. But in 10 patients, correlation was found in strains isolated from specimens in the post-operative state and with samples isolated from carrier state

PHOTOGRAPHIC PLATE NO: 13

BACTERIOCIN TYPING FOR THE DIFFERENTIATION OF STRAINS

Bacteriocin typing (procedure as in materials and methods section 3.10) for *Pseudomonas* spp. (A), *Klebsiella* spp. (B), Gram Positive organism (C) as well as for *Acinetobacter* species (D) isolated from pre-operative carrier state, post-operative infected state of patients as well as from health care personnel.

Strains where inhibition pattern was not observed belongs to same strains as in A (2 &3), B (1 & 3), C (1) and D (1 & 2).

Strains where inhibition pattern was observed belongs to dissimilar strains as in A (1), B (2), C (2 & 3) and D (3).

PHOTOGRAPHIC PLATE NO: 13

BACTERIOCIN TYPING FOR THE DIFFERENTIATION OF STRAINS

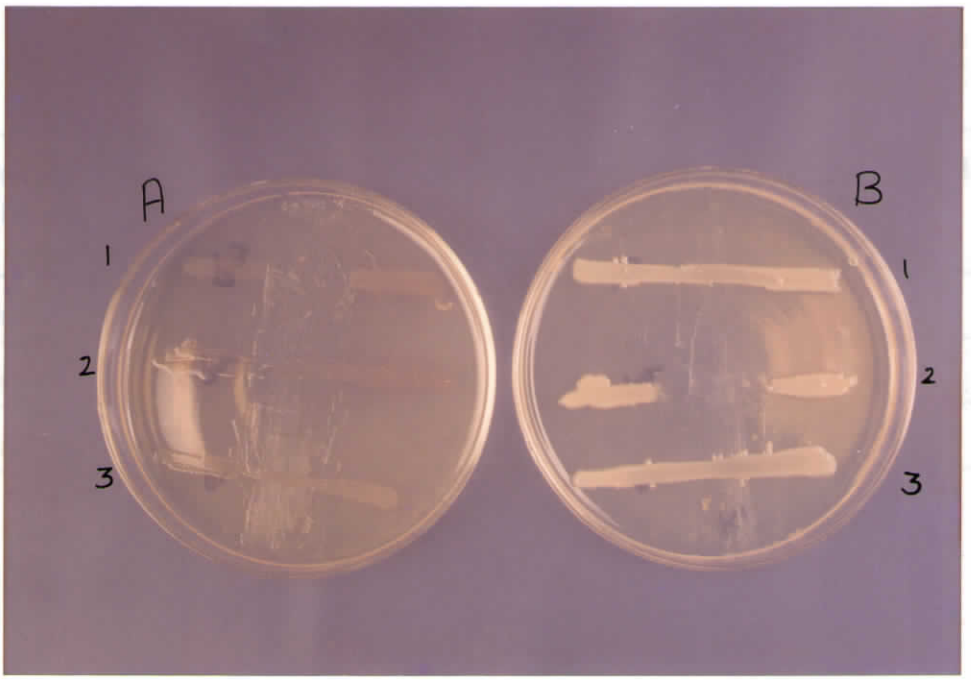
Bacteriocin typing (procedure as in materials and methods section 3.10) for *Pseudomonas* spp. (A), *Klebsiella* spp. (B), Gram Positive organism (C) as well as for *Acinetobacter* species (D) isolated from pre-operative carrier state, post-operative infected state of patients as well as from health care personnel.

Strains where inhibition pattern was not observed belongs to same strains as in A (2 &3), B (1 & 3), C (1) and D (1 & 2).

Strains where inhibition pattern was observed belongs to dissimilar strains as in A (1), B (2), C (2 & 3) and D (3).

PHOTOGRAPHIC PLATE NO: 13

Bacteriocin typing for the differentiation of strains



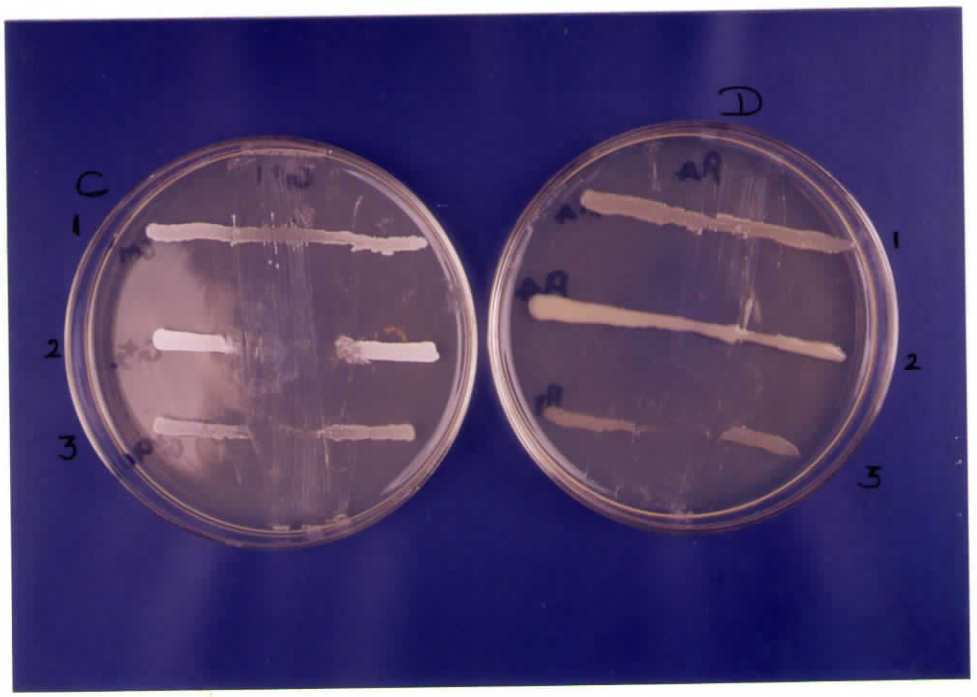


Table – 4.13

RESULTS OF BACTERIOPHAGE TYPING OF *S.aureus* STRAINS ISOLATED FROM INFECTIOUS SPECIMENS AND ITS CORRELATION WITH THE CARRIER STATE ISOLATES OF PATIENTS AND HEALTH CARE PERSONNEL

Source of isolate	Total isolate	Correlation with patient isolate	Correlation not found	Not typable
Sputum	1	0	1	-
Wound	12	6	2	4
Total	13	6	3	4

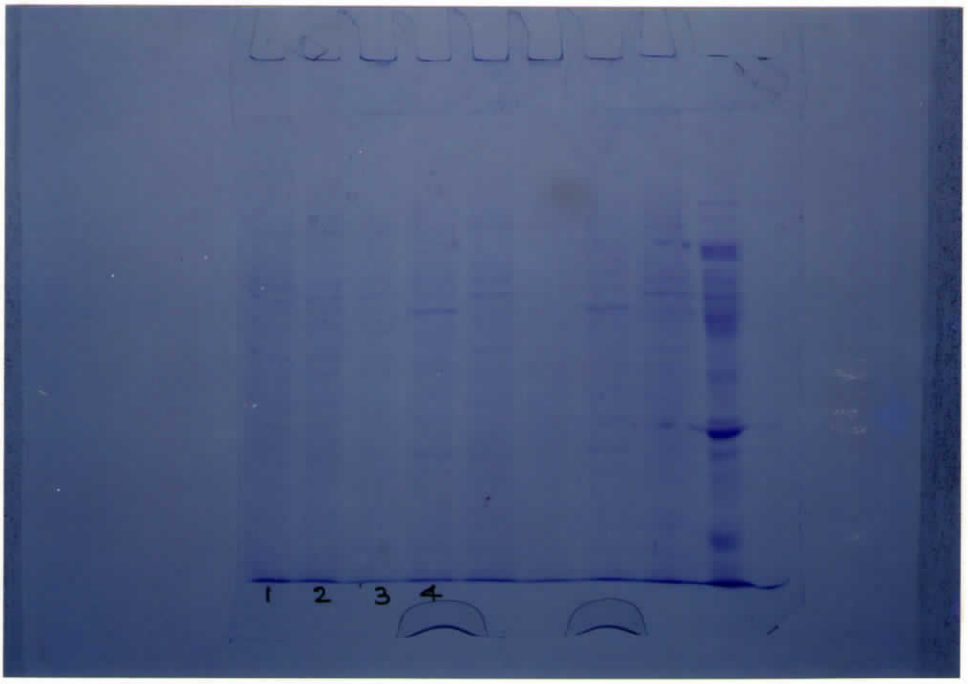
PHOTOGRAPHIC PLATE NO: 14

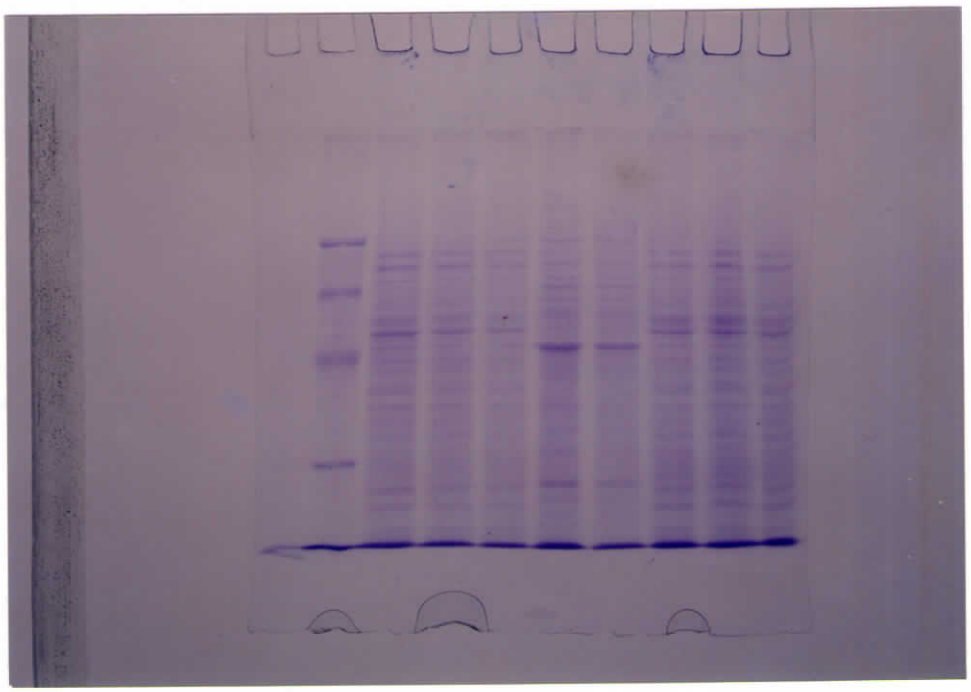
Bacterial strain in Lane 1 & 2 showed similar band pattern revealing that they belong to same strain.

Bacterial strain in Lane 3 & 4 showed dissimilar band pattern revealing that they are different strains.

PHOTOGRAPHIC PLATE NO: 14

ELECTROPHORETIC PATTERN FOR THE ANALYSIS OF ENVELOPE PROTEINS FOR IDENTIFICATION OF STRAIN SIMILARITY





PHOTOGRAPHIC PLATE NO: 15

STRAIN IDENTIFICATION USING SLIME PRODUCTION

Similarity in slime production is shown in both plates [among CNS strains (15A) and *S.aureus* strains (15B)] revealing that the strains are similar. The procedure adopted is as given in materials and methods section 3.14.

PHOTOGRAPHIC PLATE NO: 15

Strain identification using Slime Production

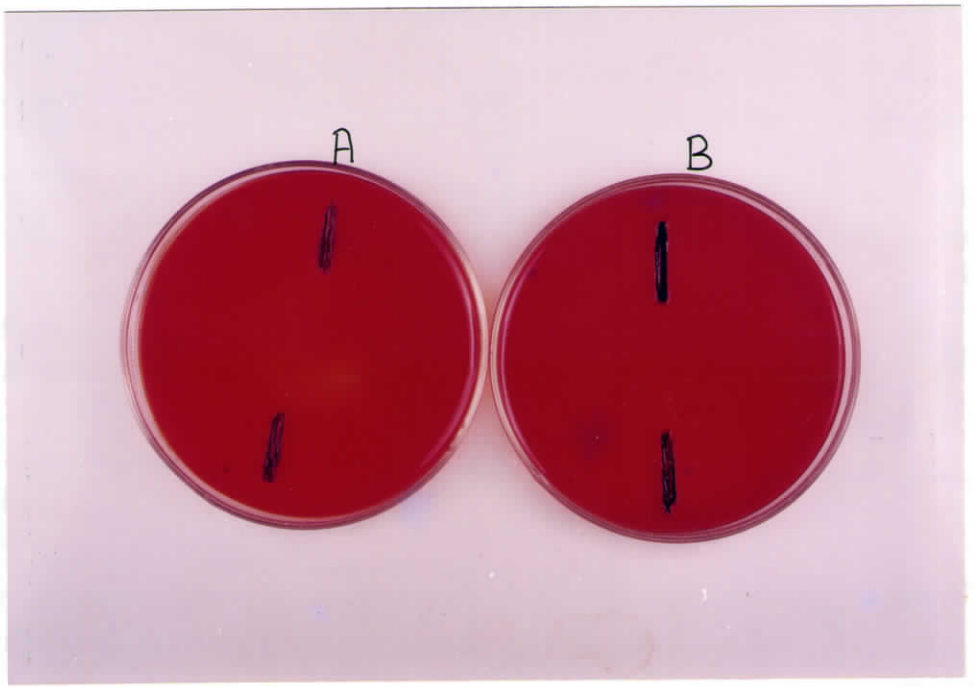


Table – 4.14

CORRELATION OF THE BACTERIAL STRAINS ISOLATED FROM POST-OPERATIVE INFECTED SPECIMENS WITH PRE-OPERATIVE AND POST-OPERATIVE CARRIER STATE AND WITH HEALTH CARE PERSONNELS

Sl. No.	Infection/ Sample	Number /Rate of Infection	Total Correlation with carrier state	Correlation of Bacterial strain From specimen with		Correl-ation not found with carrier state	Correl-ation with health care personnel
				Pre-op Carrier State	Post-op carrier state		
1	U.T.I./ Urine	10 (2.50%)	0	0	0	10	0
2	R.T.I./ Sputum	27 (6.75%)	10	7	3	17	0
3	Bacteremia/blood	18 (4.50%)	7	4	3	11	0
4	W.I/Pus	31 (7.75%)	21	14	7	10	0

U.T.I. - Urinary Tract Infection

R.T.I. - Respiratory Tract Infection

W.I. - Wound Infection

(Table 4.14). Of this, in seven patients correlation was found with bacteria isolated from pre-operative carrier state whereas with three patients correlation was found with bacteria isolated from post-operative carrier state.

In case of bacteremia, of the total of 18 cases, correlation with carrier state was found in 7 patients and no correlation in 11 patients. Similarly, correlation was not observed with strains isolated from health care personnel. But of the 7 patients where correlation was present, 4 patients showed correlation with pre-operative culture samples. 3 patients showed correlation with post-operative carrier state (Table 4.14).

Wound Infection

Of the total 31 patients who developed wound infection (7.75%) correlation of bacterial strains isolated from carrier state and post-operative infected state was found in 21 patients whereas in 10 patients and with health care personnel, no correlation was found. Of the 21 patients, where correlation was found, correlation with pre-operative carrier state was observed in the case of 14 patients. With 7 patients, correlation was observed with strains isolated from post-operative carrier state (Table 4.14).

VIRULENCE AND DRUG RESISTANCE

The pre and post-operative administration of antibiotics in adequate level require specific informations regarding the targeted species, their virulence and antibiotic susceptibility patterns.

A. Speciation of bacterial strains isolated from Cardiac Surgery Patients and Health Care Personnel

Recent observations have shown that some of the species of the normal flora (non-pathogenic) sometimes becomes opportunists and cause serious threat to patients undergoing surgery (Patrick et al 1990, Foster and Dashner 1998, Bergogne and Towner 1996). Thus species identification is important to discriminate the pathogenic opportunists.

CNS is one of the major pathogens isolated from specimens in the post-operative infected state (Table 4.12). It is also to be noted that CNS is the major

species isolated from pre and post-operative carrier state of patients (Table 4.1 and 4.3) and also from health care personnel (Table 4.8). Thus a systematic speciation of bacterial strains isolated from the carrier state and from health care personnel were carried out (Table 4.15, 4.16 and 4.17) and the results were compared with the strains isolated from the post-operative infected state of patients (Table 4.18).

750 strains of CNS, 113 strains of Klebsiella and 19 strains of Acinetobacter isolated from Cardiac Surgery patients and 111 strains of CNS, 14 strains of Klebsiella and 4 strains of Acinetobacter from the health care personnel were subjected for speciation using different biochemical tests. From Table 4.15 it is clear that *S.epidermidis* is the leading isolate of CNS in both carrier state of patients and health care personnel followed by *S.hominis*. Other species of isolation are shown in Table 4.15. But *S.xylosus* and *S.warneri* were isolated only from carrier state of patients.

In the speciation of Klebsiella, it could be seen that *K. pneumoniae* is the leading species among patients (Table 4.16) while with health care personnel, the leading species is *K.aerogenes*. *K.ozanae* could not be isolated from health care personnel (Table 4.16). *A . calcoaceticus* was only isolated from the carrier state of the patients and health care personnel as shown in Table 4.17

Among CNS strains only *S.epidermidis* and among Klebsiella strains only *K.pneumoniae* were isolated from the post-operative infected specimens while *A.Calcoaceticus* and *A.anitratus* were isolated among Acinetobacter species Table 4.18.

B. Antibiotic susceptibility test

Determination of resistance or sensitivity to antibiotics is of use in selecting a specific or a group of antibiotics to be used prophylactically or in case of infection. (Jacques et al 1994, Pegues et al 1998, Lyyttikainen et al 1996). Thus 689 strains of CNS, 132 strains of *S.aureus* and 104 strains of *Klebsiella* species isolated from cardiac surgery patients and 111 strains of CNS, 27 strains of *S.aureus* and 14 strains of *Klebsiella* species isolated from health care personnel were tested for their susceptibility to various antibiotics. The results are as shown in Table 4.19 and 4.20.

It could be noted that multiple antibiotic resistance was shown by Klebsiella and *S.aureus* strains isolated from patients (Table 4.19). But in case of CNS, the number of resistant strains is slightly less when compared to other two bacterial species (Table 4.19). Interestingly, the strains of all three species, isolated from health care personnel showed greater sensitivity when compared to the strains from patients (Table 4.20). The *S.aureus* strains isolated from carrier state of both patients and health

Table - 4.15

PREVALENCE RATE OF CNS SPECIES FOUND AMONG THE ISOLATES OF CARRIER STATE OF CARDIAC SURGERY PATIENTS AND HEALTH CARE PERSONNEL

Species	Cardiac surgery patients Isolates		Health care personnel isolates	
	Number	Percentage	Number	Percentage
S.epidermidis	306	40.80	49	44.14
S. hominis	175	23.33	26	23.42
S.cohnii	118	15.73	11	9.9
S.capitis	92	12.27	24	21.62
S.saprophyticus	41	5.47	1	0.9
S.xylosus	8	1.06	0	-
S.warneri	10	1.33	0	-
TOTAL	750	100.00	111	100.00

Table - 4.16

PREVALENCE RATE OF KLEBSIELLA SPECIES FOUND AMONG THE ISOLATES OF CARRIER STATE OF CARDIAC SURGERY PATIENTS AND HEALTH CARE PERSONNEL

Species	Cardiac surgery patients Isolates		Health care personnel isolates	
	Number	Percentage	Number	Percentage
K.pneumoniae	57	50.44	5	35.7
K.aerogenes	41	36.28	9	64.3
K.ozanae	15	13.27	0	-
TOTAL	113	100.00	14	100.00

Table - 4.17

PREVALENCE RATE OF ACINETOBACTER SPECIES FOUND AMONG THE ISOLATES OF CARRIER STATE OF CARDIAC SURGERY PATIENTS AND HEALTH CARE PERSONNEL

Species	Cardiac surgery patients Isolates		Health care personnel isolates	
	Number	Percentage	Number	Percentage
A.calcoaceticus	19	100.00	4	100.00
TOTAL	19	100.00	4	100.00

Table - 4.18

PREVALENCE RATE OF SPECIES FOUND AMONG THE ISOLATES FROM POST-OPERATIVE INFECTED SPECIMENS

Species	Number	Percentage	Total
S.epidermidis	18	100.00	18
K.pneumoniae	13	100.00	13
A.calcoaceticus	17	77.26	22
A.anitratus	5	22.72	22

Table – 4.19

RESISTANCE/SENSITIVITY PERCENTAGE OF LEADING BACTERIAL STRAINS ISOLATED FROM THROAT, NOSE AND STERNAL SKIN OF CARDIAC SURGERY PATIENTS

Antibiotic	Bacterial Strain	R %	S %	Ms%
Ampicillin	CNS	47.75	49.78	2.46
	S.aureus	82.24	16.66	1.10
	Klebsiella	99.03	0.96	-
Ceftriaxone	CNS	27.86	70.39	1.74
	S.aureus	71.06	23.63	5.30
	Klebsiella	28.84	52.88	18.26
Cloxacillin	CNS	38.89	59.07	2.03
	S.aureus	68.63	31.36	-
	Klebsiella	97.11	2.88	-
Ciprofloxacin	CNS	29.59	67.35	3.04
	S.aureus	61.21	25.75	13.03
	Klebsiella	38.46	35.57	25.96
Co-trimoxazole	CNS	27.28	69.66	3.04
	S.aureus	76.51	21.21	2.27
	Klebsiella	34.61	53.84	11.53
Erythromycin	CNS	32.06	63.58	4.35
	S.aureus	64.39	31.06	4.54
	Klebsiella	94.23	4.80	0.96
Gentamicin	CNS	27.70	68.51	3.77
	S.aureus	57.57	40.15	2.27
	Klebsiella	38.46	45.19	16.34
Tobramycin	CNS	15.38	83.74	0.87
	S.aureus	49.54	42.89	7.57
	Klebsiella	48.07	37.50	14.42

(Cont'd)

		R%	S%	Ms%
Cefuroxime	CNS	22.64	75.32	2.03
	S.aureus	61.96	35.75	2.27
	Klebsiella	61.53	25.96	12.50
Netilmicin	CNS	18.71	80.71	0.58
	S.aureus	55.30	41.66	3.03
	Klebsiella	63.46	36.53	-
Amikacin	CNS	16.38	82.74	0.87
	S.aureus	45.45	52.27	2.27
	Klebsiella	27.88	41.34	30.76
Tetracycline	CNS	26.56	60.82	12.61
	S.aureus	66.66	23.48	9.84
	Klebsiella	51.92	23.07	25.00
Penicillin – G	CNS	81.27	14.94	3.77
	S.aureus	100.00	-	-
	Klebsiella	ND	ND	ND
Methicillin/ Oxacillin	CNS	12.04	87.81	0.14
	S.aureus	37.12	56.06	6.81
	Klebsiella	ND	ND	ND
Amoxycillin & Clavulanic acid	CNS	15.38	84.03	0.58
	S.aureus	71.06	25.90	3.03
	Klebsiella $	88.23	11.76	-
Chloramphenicol	CNS	3.19	95.79	1.01
	S.aureus	15.90	82.57	1.51
	Klebsiella	24.03	64.42	11.53
Nitrofurantoin	CNS*	14.23	82.20	3.55
	S.aureus# #	43.28	28.35	28.35
	Klebsiella	76.92	17.30	5.76

		R%	S%	Ms%
Cephalexin	CNS**	24.95	73.46	1.58
	S.aureus ###	76.20	23.79	-
	Klebsiella$$	65.82	20.25	13.92
Norfloxacin	CNS*	28.47	67.96	3.55
	S.aureus ##	67.16	22.38	10.44
	Klebsiella	35.57	45.19	19.23
Vancomycin	CNS***	-	100.00	-
	S.aureus@	-	100.00	-
	Klebsiella	ND	ND	ND
Nalidixic Acid	CNS	ND	ND	ND
	S.aureus	ND	ND	ND
	Klebsiella$$	39.24	37.97	22.78
Rifampicin	CNS***	-	94.40	5.60
	S.aureus #	4.5	95.45	-
	Klebsiella	ND	ND	ND
Clindamycin	CNS***	-	99.2	0.8
	S.aureus #	4.50	95.45	-
	Klebsiella	ND	ND	ND

Number of strains tested - CNS - 689; *S.aureus* - 132
Klebsiella sp. -104

*	CNS	-	Tested for 309 strains
**	CNS	-	Tested for 505 strains
***	CNS	-	Tested for 125 strains
#	*S.aureus*	-	Tested for 22 strains
##	*S.aureus*	-	Tested for 67 strains
###	*S.aureus*	-	Tested for 87 strains
@	*S.aureus*	-	Tested for 100 strains
$	*Klebsiella*	-	Tested for 34 strains
$$	*Klebsiella*	-	Tested for 79 strains

R - Resistant; S - Sensitive; Ms-Moderate sensitive; ND - Not done

Table – 4.20

RESISTANCE/SENSITIVITY PERCENTAGE OF LEADING BACTERIAL STRAINS ISOLATED FROM THROAT, NOSE AND SKIN OF HEALTH CARE PERSONNEL

Antibiotic	Bacterial Strain	R %	S %	Ms%
Ampicillin	CNS	35.14	55.80	9.01
	S.aureus	77.99	14.60	7.41
	Klebsiella	100.00	-	-
Ceftriaxone	CNS	11.71	79.28	9.01
	S.aureus	33.33	51.85	14.80
	Klebsiella	28.57	50.00	21.42
Cloxacillin	CNS	19.82	72.97	7.21
	S.aureus	66.19	30.11	3.70
	Klebsiella	100.00	-	-
Ciprofloxacin	CNS	19.09	80.90	-
	S.aureus	14.81	77.77	7.41
	Klebsiella	21.43	50.00	28.50
Co-trimoxazole	CNS	12.60	77.47	9.90
	S.aureus	22.20	59.26	18.50
	Klebsiella	21.40	50.00	28.60
Erythromycin	CNS	9.01	76.57	14.41
	S.aureus	37.04	48.15	14.80
	Klebsiella	64.30	21.42	14.28
Gentamicin	CNS	16.21	70.18	13.60
	S.aureus	18.52	81.48	-
	Klebsiella	21.43	57.14	21.42

(Cont'd)

		R%	S%	Ms%
Tobramycin	CNS	8.11	86.48	5.41
	S.aureus	18.50	66.66	14.80
	Klebsiella	42.80	42.80	14.20
Cefuroxime	CNS	10.80	85.58	3.60
	S.aureus	9.91	79.26	10.83
	Klebsiella	35.70	42.80	21.42
Netilmicin	CNS	10.00	87.30	2.70
	S.aureus	-	96.29	3.70
	Klebsiella	35.70	42.85	21.42
Amikacin	CNS	6.31	93.69	-
	S.aureus	-	92.59	7.41
	Klebsiella	14.28	78.57	7.14
Tetracycline	CNS	16.20	70.18	13.60
	S.aureus	14.81	77.77	7.41
	Klebsiella	85.70	14.28	-
Penicillin - G	CNS	54.04	12.60	33.33
	S.aureus	100.00	-	-
	Klebsiella	ND	ND	ND
Methicillin/ Oxacillin	CNS	-	100.00	-
	S.aureus	-	100.00	-
	Klebsiella	ND	ND	ND
Amoxycillin & Clavulanic acid	CNS	25.20	74.77	-
	S.aureus	11.11	70.37	18.50
	Klebsiella	57.14	28.57	14.28
Chloramphenicol	CNS	3.60	96.40	-
	S.aureus	-	100.00	-
	Klebsiella	21.43	64.28	14.30

(Cont'd)

		R%	S%	Ms%
Nitrofurantoin	CNS	4.50	88.28	7.21
	S.aureus	22.22	40.74	37.04
	Klebsiella	50.00	28.57	21.42
Cefazolin	CNS	7.21	81.98	10.80
	S.aureus	22.22	66.66	11.11
	Klebsiella	28.57	42.80	28.57
Norfloxacin	CNS	11.71	83.78	4.50
	S.aureus	65.93	16.66	17.41
	Klebsiella	35.70	57.14	7.14
Vancomycin	CNS*	-	100.00	-
	*S.aureus***	-	100.00	-
	Klebsiella	ND	ND	ND

Number of strains testes - CNS - 111
S.aureus - 27
Klebsiella sp. - 14

* CNS - Tested for 40 strains
** *S.aureus* - Tested for 18 strains

R - Resistant; S - Sensitive; Ms-Moderate sensitive; ND - Not done

care personnel showed greater sensitivity when compared to the strains from patients (Table 4.20). The *S.aureus* strains isolated from carrier state of both patients and health care personnel showed total resistance to the antibiotic penicillin (Table 4.19 and 4.20) and the CNS showed least sensitivity to this antibiotic (Table 4.19 and 4.20).

C. Detection of Beta-lactamase production by different Iodometric and Acidimetric techniques.

The result with antibiotic susceptibility test was further confirmed with different acidimetric and iodometric techniques. 644 strains of CNS and 134 strains of *S.aureus* from cardiac surgery patients and 111 strains of CNS and 27 strains of *S.aureus* from health care personnel were subjected for the detection of beta-lactamase using different acidimetric techniques and iodometric techniques (Tables 4.21, 4.22, 4.23 and 4.24).

From all four acidimetric methods such as Bromocresol purple filter paper, Bromocresol purple microplate, Phenol red filter paper and Phenol red microplate methods, it has been shown that *S.aureus* is almost totally resistant, while CNS is resistant to a greater *extend* in both cases (Table 4.21 and 4.22). A Comparison of methods clearly revealed that, of the four methods, the phenol red filter paper method is the best for detection of beta-lactamase (Table 4.21 and 4.22).

All the iodometric methods support the interpretation of the results of the acidimetric methods (Table 4.23 and 4.24). However iodometric methods seem to be slightly more sensitive, as evidenced from the slight increase in the percentage of positivity observed (Table 4.23 and 4.24). The iodometric test tube and filter paper methods were found to be very sensitive.

D. Detection of slime for Staphylococcal strains isolated from Cardiac Surgery Patients and Health Care Personnel

The production of slime is known to be a marker of virulence. Since all the CNS and *S.aureus* strains isolated from specimens from post-operative infected state of patients (Table 4.25) are found to be highly slime producers, it is worthwhile to test the rate of slime production by these strains in the carrier state of patients and health care personnel to reveal the hidden clinical significance of the strains. The

Table – 4.21

COMPARISON OF BETA LACTAMASE PRODUCTION AMONG CNS & *S.aureus* STRAINS ISOLATED FROM CARDIAC SURGERY PATIENTS - BY DIFFERENT ACIDIMETRIC TECHNIQUES

Indicator used	Method employed	Positive (%)		Negative (%)	
		CNS	*S.aureus*	CNS	*S.aureus*
B.cresol purple	Filter paper	468 (72.67)	127 (94.77)	176 (27.33)	7 (5.22)
B.cresol purple	Micro plate	414 (64.28)	127 (94.77)	230 (35.71)	7 (5.22)
Phenol Red	Filter paper	516 (80.12)	129 (96.27)	128 (19.87)	5 (3.73)
Phenol Red	Micro plate	464 (72.05)	127 (96.27)	180 (27.95)	7 (5.22)

Number of strains tested	-	CNS	-	644 strains
		S.aureus	-	134 strains
Total positivity	-	CNS	-	516 (80.12)
		S.aureus	-	129 (96.27)
Total Negative	-	CNS	-	128 (19.87)
		S.aureus	-	5 (3.73)

Table – 4.22

COMPARISON OF BETA LACTAMASE PRODUCTION AMONG CNS & *S.aureus* STRAINS SOLATED FROM HEALTH CARE PERSONNEL - BY DIFFERENT ACIDIMETRIC TECHNIQUES

Indicator used	Method employed	Positive (%)		Negative (%)	
		CNS	*S.aureus*	CNS	*S.aureus*
B.cresol purple	Filter paper	93 (83.78)	26 (96.30)	18 (16.20)	1 (3.70)
B.cresol purple	Micro plate	77 (69.36)	26 (96.30)	34 (30.63)	1 (3.70)
Phenol Red	Filter paper	97 (87.38)	26 (96.30)	14 (12.60)	1 (3.70)
Phenol Red	Micro plate	85 (76.57)	25 (92.60)	26 (23.42)	2 (7.41)

Number of strains tested - CNS - 111 strains
S.aureus - 27 strains

Total positivity - CNS - 97 (87.38)
S.aureus - 26 (96.30)

Total Negative - CNS - 14 (12.60)
S.aureus - 1 (3.70)

Table – 4.23

COMPARISON OF BETA LACTAMASE PRODUCTION AMONG CNS & *S.aureus* STRAINS ISOLATED FROM CARDIAC SURGERY PATIENTS BY IODOMETRIC METHODS

Method	Positive (%)		Negative (%)	
	CNS	*S.aureus*	CNS	*S.aureus*
Test Tube	527 (81.83)	130 (97.01)	117 (18.17)	4 (2.98)
Agar Plate	468 (72.67)	127 (94.77)	176 (27.32)	7 (5.22)
Filter paper	508 (78.88)	130 (97.01)	136 (21.12)	4 (2.98)
Micro plate	468 (72.67)	126 (94.03)	176 (27.33)	8 (5.97)

Number of strains tested	-	CNS	-	644 strains
		S.aureus	-	134 strains
Total positivity		CNS	-	527 (81.83)
	-	*S.aureus*	-	130 (97.01)
Total Negative	-	CNS	-	117 (18.17)
		S.aureus	-	4 (2.98)

Table – 4.24

COMPARISON OF BETA-LACTAMASE PRODUCTION AMONG CNS & *S.aureus* STRAINS FROM HEALTH CARE PERSONNEL BY IODOMETRIC METHODS

Method	Positive (%)		Negative (%)	
	CNS	*S.aureus*	CNS	*S.aureus*
Test Tube	96 (86.48)	26 (96.29)	15 (13.5)	1 (3.70)
Agar Plate	94 (84.68)	25 (92.59)	17 (15.3)	2 (7.41)
Filter paper	102 (91.89)	26 96.29)	9 (8.11)	1 (3.70)
Micro plate	95 (85.58)	25 (92.59)	16 (14.4)	2 (7.41)

Number of strains tested	-	CNS	-	111 strains
		S.aureus	-	27 strains
Total positivity	-	CNS	-	105 (94.59)
		S.aureus	-	26 (96.29)
Total Negative	-	CNS	-	6 (5.40)
		S.aureus	-	1 (3.70)

Table – 4.25

RATE OF SLIME POSITIVE CNS AND *S.AUREUS* STRAINS IN POST OPERATIVE INFECTED SPECIMENS

Method	Positive (%)		Negative (%)	
	CNS	*S.aureus*	CNS	*S.aureus*
Test tube method using 0.1% Safranine	18 (100.00)	13 (100.00)	0 -	0 -
Micro plate method using 0.1% Safranine	18 (100.00)	13 (100.00)	0 -	0 -
Congored agar plate method	18 (100.00)	13 (100.00)	0 -	0 -

Total Number of strain tested - CNS - 18
***S.aureus* - 13**

results are as given in Tables 4.26 and 4.27. From them, it is evident that approximately 63% of CNS and 92% of *S.aureus* were slime producers in patient isolates and 69% of CNS and 77% of *S.aureus* were slime producers in health care personnel isolates. It is quite clear that patient isolates are more slime producing than those from health care personnel indicating that the virulence is more with these isolates. The Congo Red Agar plate method as described by Freeman et al (1989) is found to be more sensitive than the Christensen et al (1983) test tube method.

E. **Determination of MIC against different antibiotics.**

Wound infection, a major risk factor in cardiac surgery patients has been found to be mainly endogenous (Table 4.14) as also supported by the works of Klutyman et al (1995) and Calia et al (1969). Thus a previous knowledge about the carrier state of the patient admitted for surgery will be helpful in deciding the antibiotic prophylaxis. Administration of the correct doze of specific antibiotic or a group of antibiotic(s) has to be decided for each pathogen. In this context, an estimation of the MIC for major pathogens is of great advantage. It is evident that CNS, *S.aureus* and Klebsiella are the major potential pathogens isolated from the specimen in the post-operative infected state (Table 4.10) and from the carrier state of patients and health care personnel (Table 4.1 and 4.8). Hence finding out the apt antibiotic(s) in their optimum concentration and administering them prophylactically against these strains will reduce the risk of wound infection to a greater extent. Therefore the susceptibility of these strains to various antibiotics such as Cloxacillin, Penicillin, Cefuroxime, Ciprofloxacin, Tobramycin, Amikacin, Gentamicin and Augmentin and their MIC was found out. The results are as shown in Graphs 1to 18.

From the figures, it is clear that for CNS, the least MIC values are obtained with the antibiotic Amikacin followed by Cefuroxime where as with *S.aureus* it is the Cloxacillin followed by Tobramycin and Cefuroxime. In the case of Klebsiella, the least MIC value was obtained with Amikacin followed by Tobramycin and Augmentin.

Another observation was that the bacterial strains isolated from health care personnel are having less MIC values as compared to that isolated from cardiac surgery patients, which in turn indicates that the strains isolated from health care personnel are more sensitive than the strains isolated from cardiac surgery patients.

Table – 4.26

RATE OF SLIME POSITIVE CNS AND *S.AUREUS* STRAINS ISOLATED FROM CARRIER STATE OF CARDIAC SURGERY PATIENTS

Method	Positive (%)		Negative (%)	
	CNS	*S.aureus*	CNS	*S.aureus*
Test tube method using 0.1% Safranine	412 (60.67)	128 (91.43)	267 (39.32)	12 (8.57)
Micro plate method using 0.1% Safranine	423 (62.29)	129 (92.14)	256 (37.70)	11 (7.86)
Congored agar plate method	431 (63.47)	129 (92.14)	248 (36.52)	11 (7.86)

Total Number of strains tested — **CNS** - **679**; ***S.aureus*** - **140**

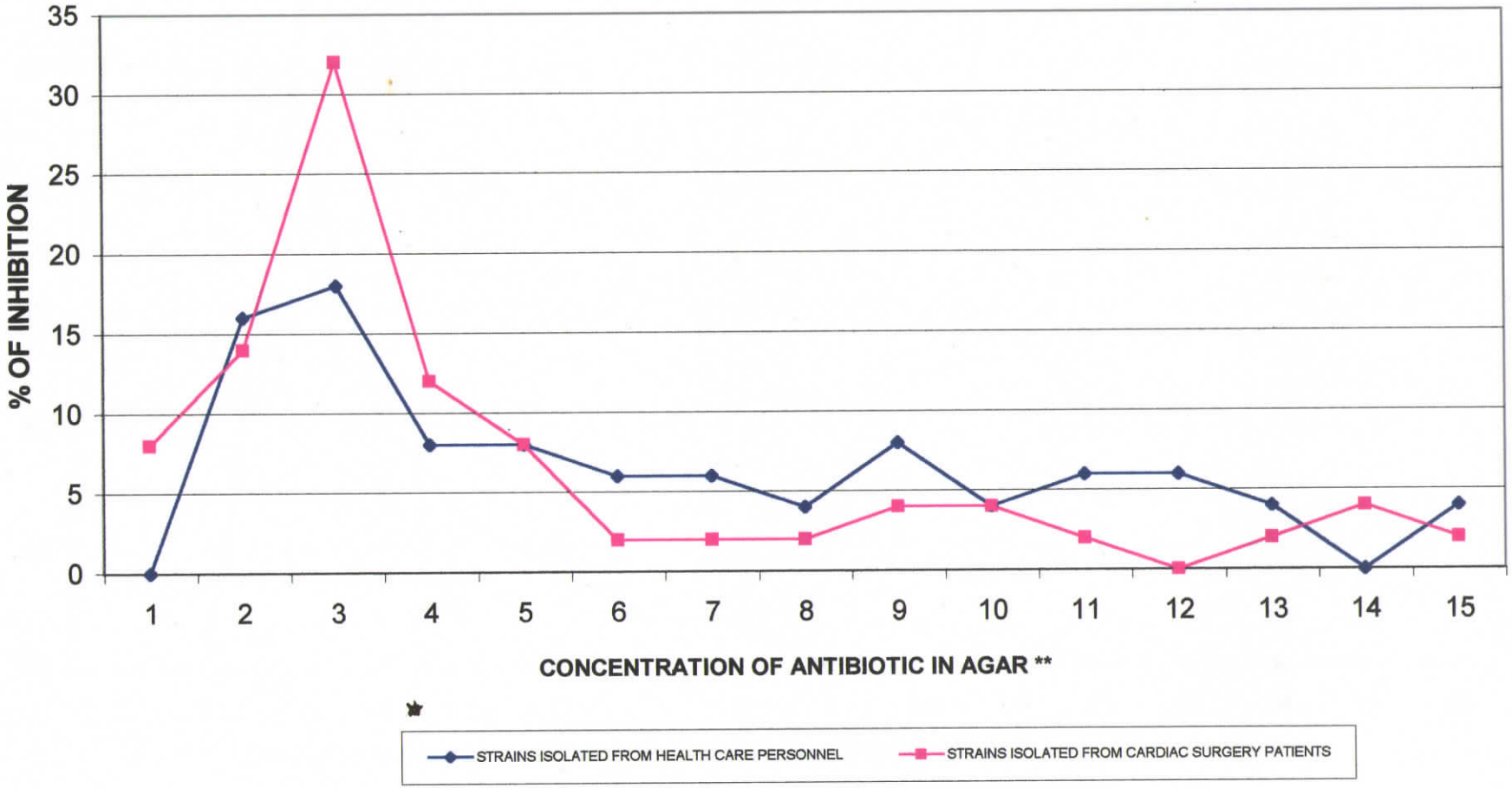
Table – 4.27

RATE OF SLIME POSITIVE CNS AND *S.AUREUS* STRAINS ISOLATED FROM CARRIER STATE OF HEALTH CARE PERSONNEL

Method	Positive (%)		Negative (%)	
	CNS	*S.aureus*	CNS	*S.aureus*
Test tube method using 0.1% Safranine	66 (59.45)	21 (77.7)	45 (40.54)	6 (22.20)
Micro plate method using 0.1% Safranine	75 (67.56)	21 (77.7)	36 (32.43)	6 (22.20)
Congored agar plate method	77 (69.36)	22 (77.7)	34 (30.63)	6 (22.20)

Total Number of strains tested — **CNS** - **111**; ***S.aureus*** - **27**

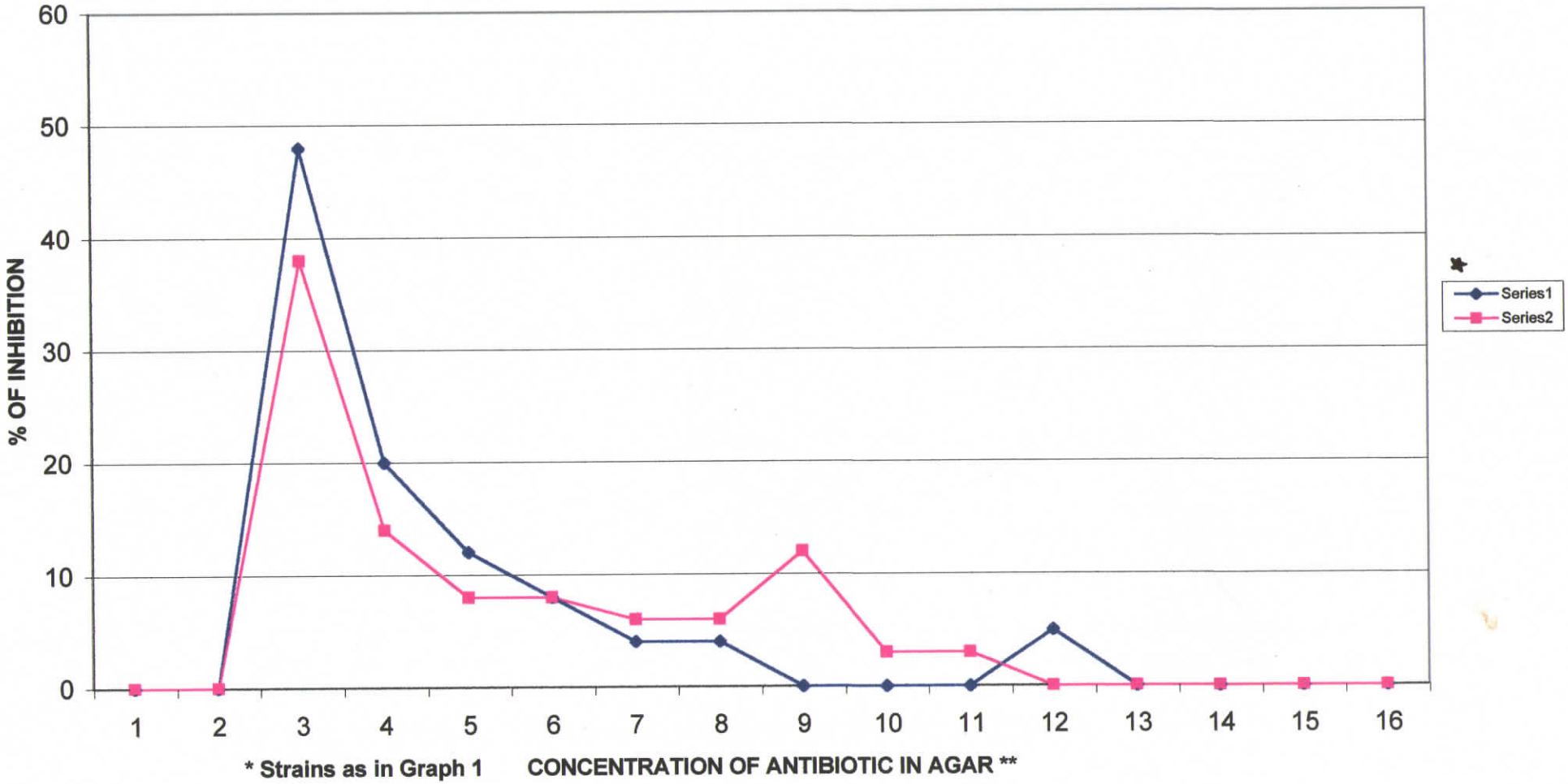
Graph 1
DETERMINATION OF MIC OF PENICILLIN FOR CNS



** Respective Conc: is given in Table 3.2

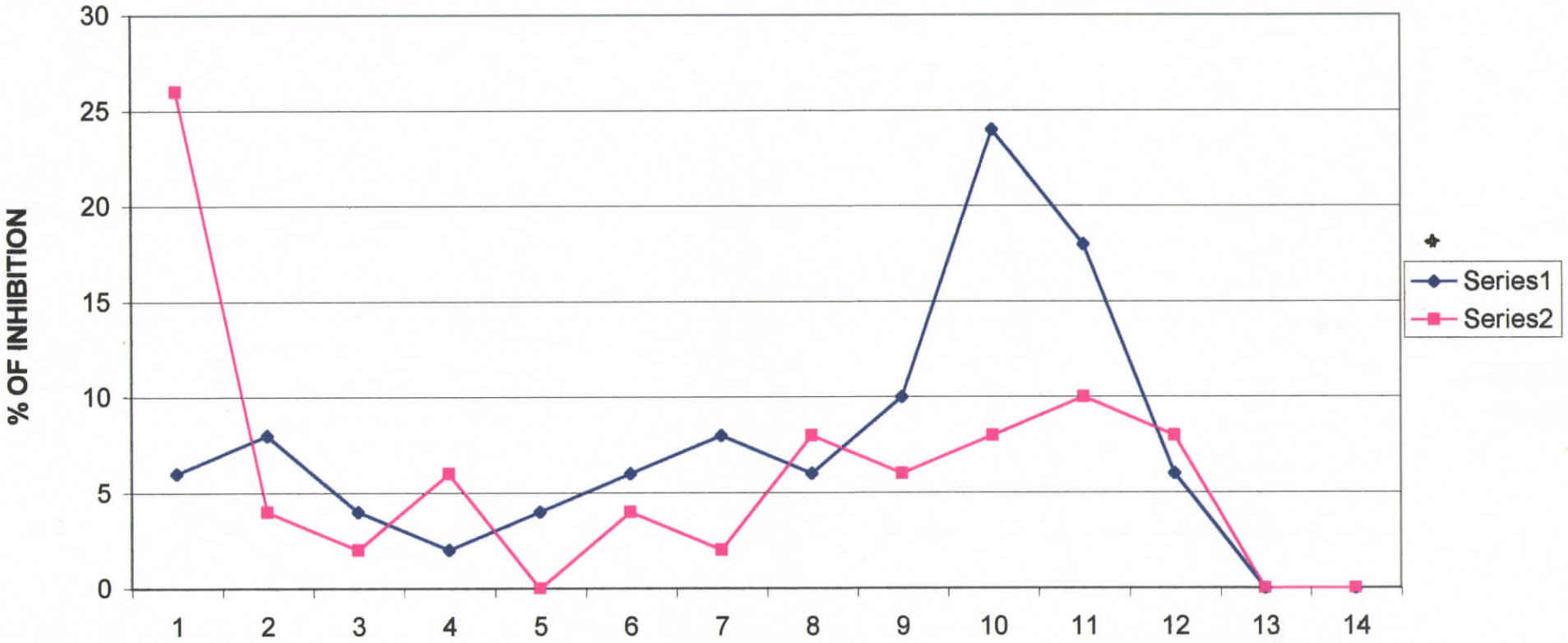
Graph 2

DETERMINATI0N OF MIC OF PENICILLIN FOR *S.aureus*



** Respective Conc: is given in Table 3.2

Graph 3
DETERMINATION OF MIC OF CLOXACILLIN FOR CNS

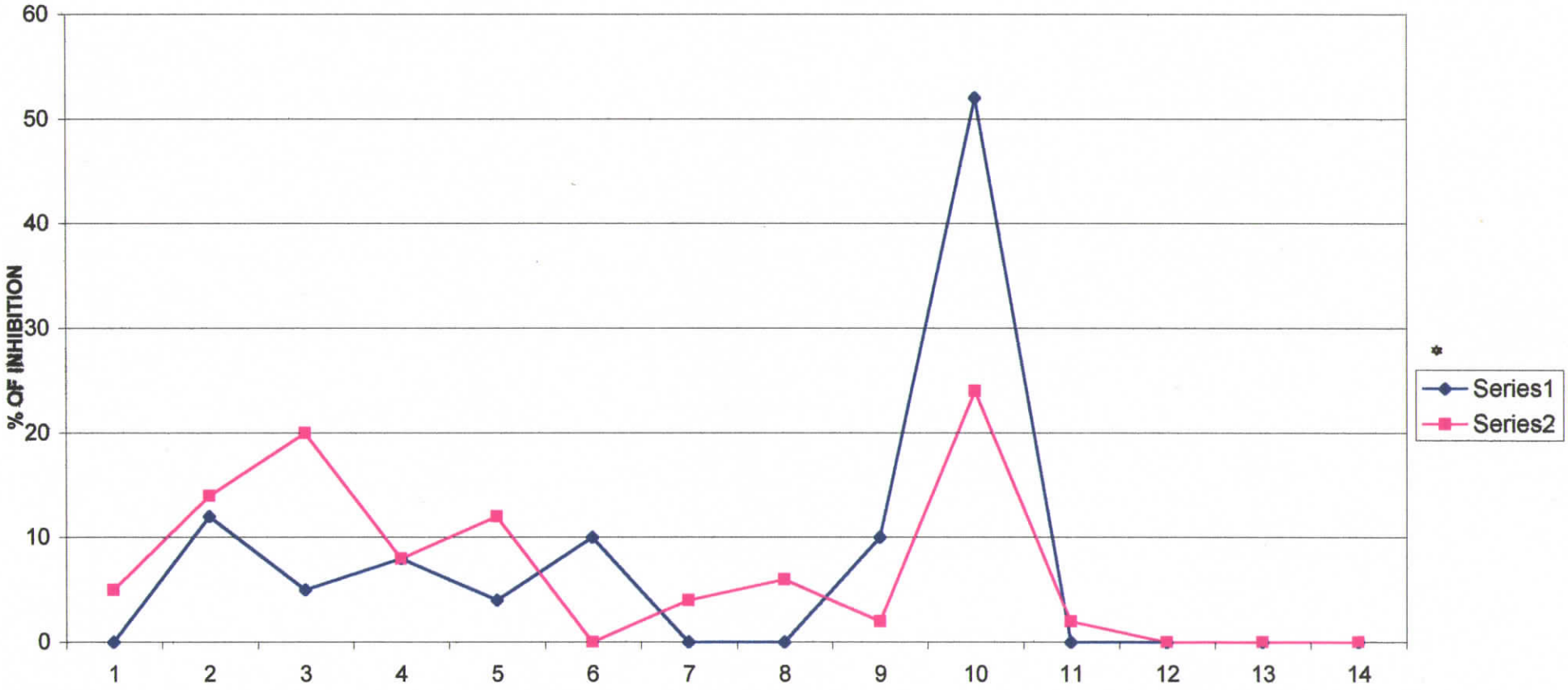


*Strains as in Graph 1 CONCENTRATION OF ANTIBIOTIC IN AGAR **

** Respective Conc: is given in Table 3.3

Graph 4

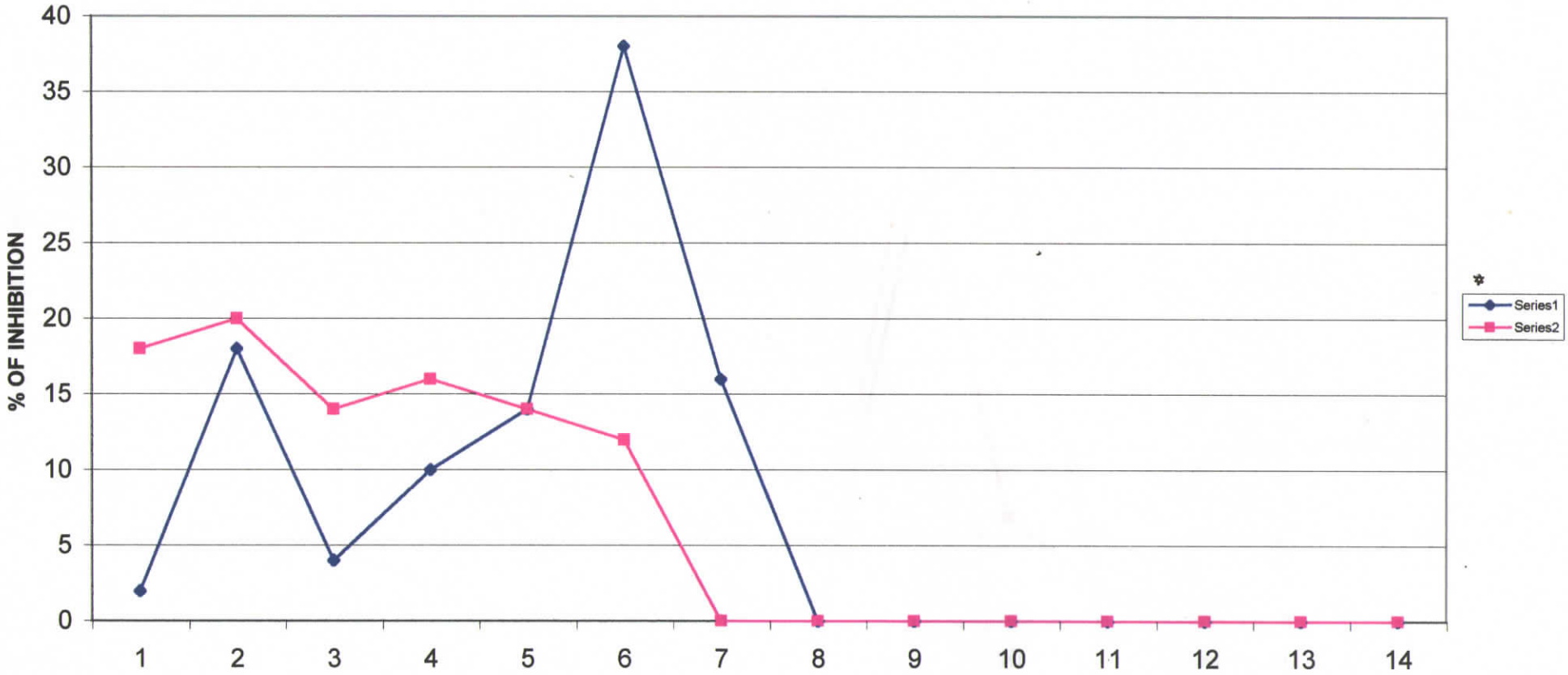
DETERMINATION OF MIC OF CLOXACILLIN FOR *S.aureus*



*** Strains as in Graph 1** **CONCENTRATION OF ANTIBIOTIC IN AGAR ****

**** Respective Conc. is given in Table 3.3**

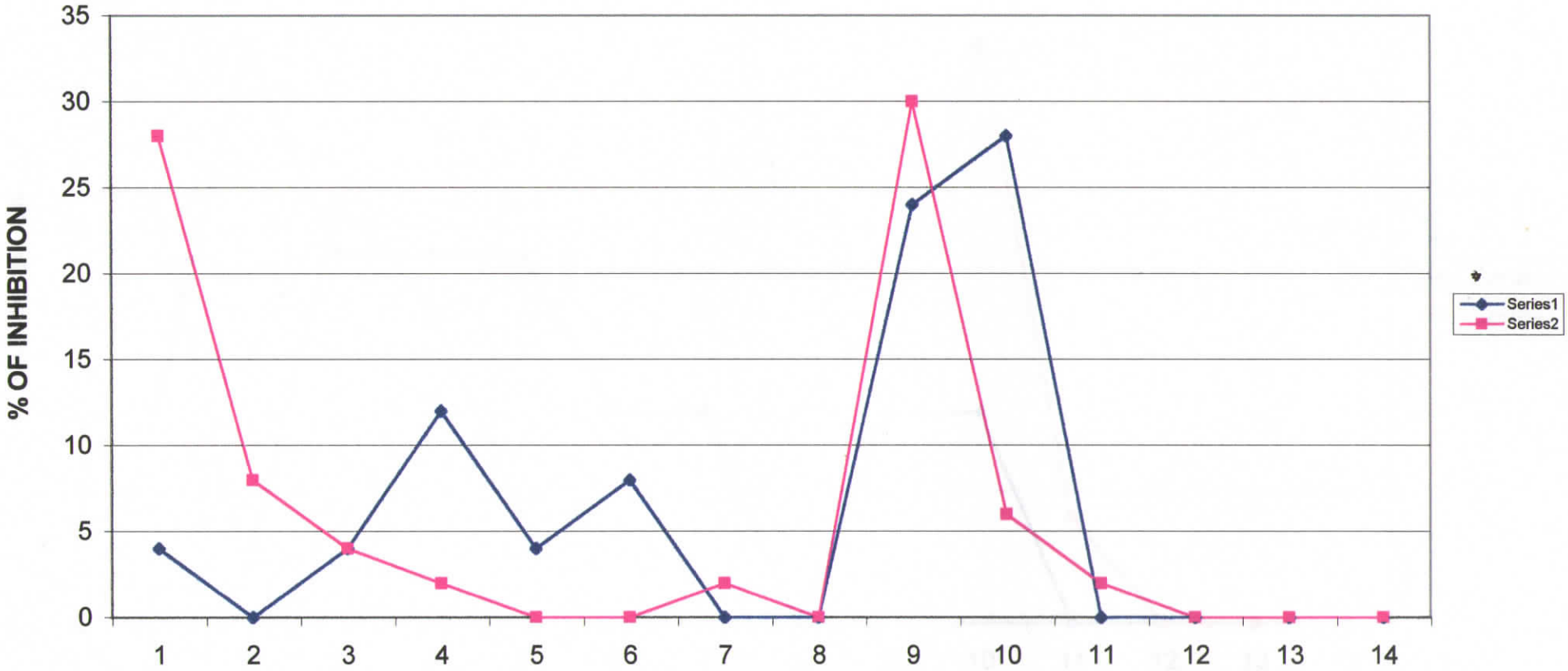
Graph 5
DETERMINATION OF MIC OF TOBRAMYCIN FOR CNS



* Strains as in Graph 1 CONCENTRATION OF ANTIBIOTIC IN AGAR **

** Respective Conc. is given in Table 3.9

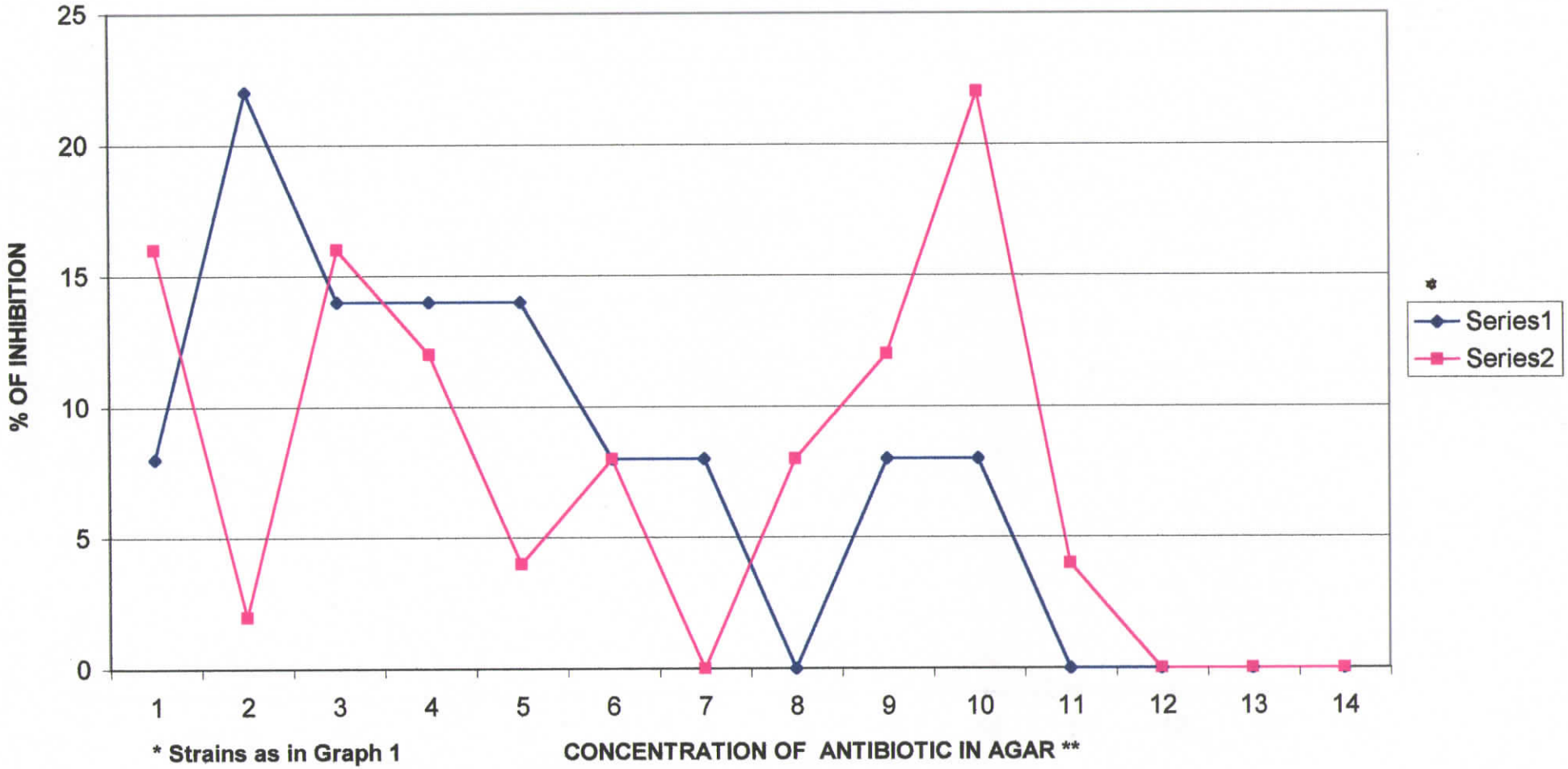
Graph 6
DETERMINATION OF MIC OF TOBRAMYCIN FOR *S.aureus*



** Respective Conc: is given in Table 3.9

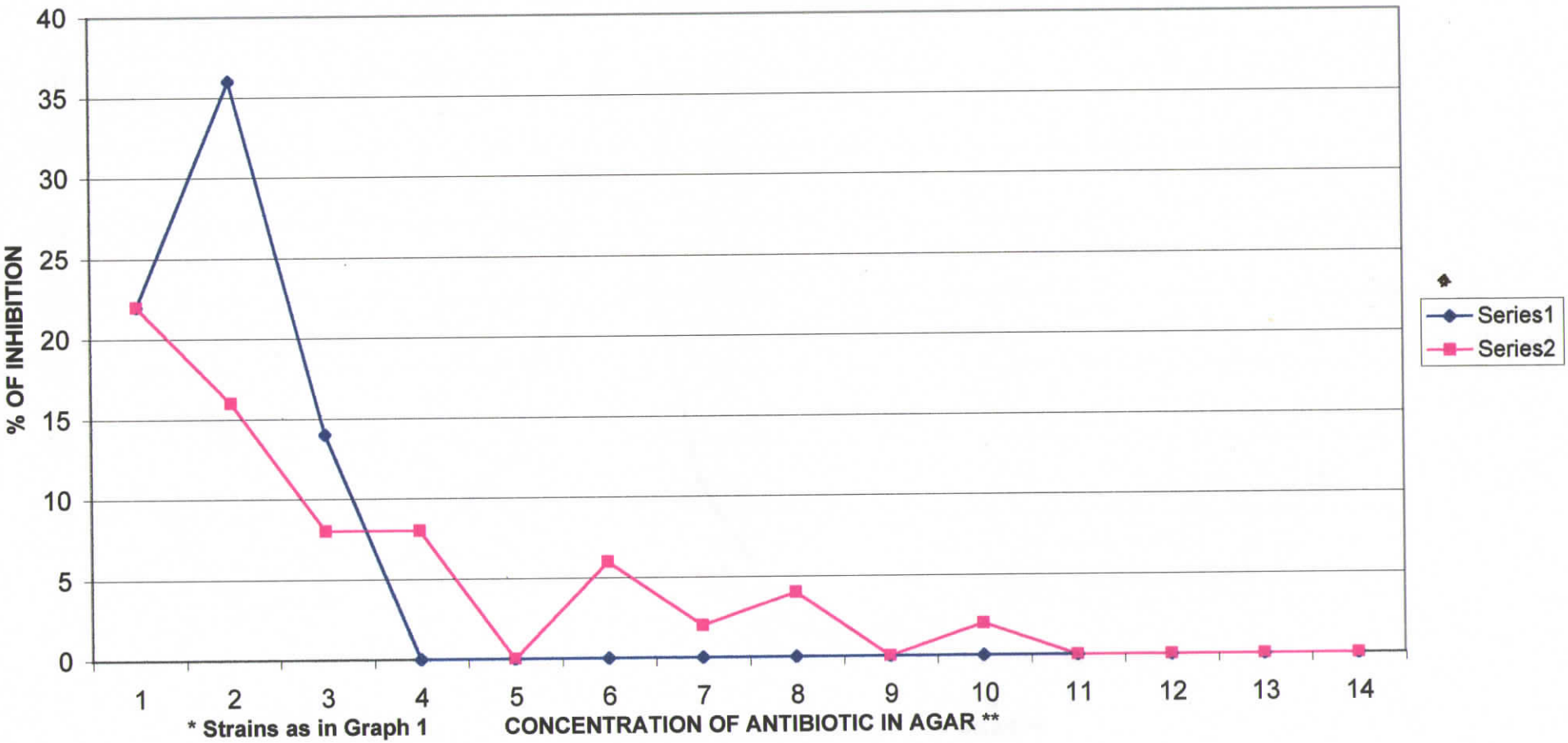
Graph 7

DETERMINATION OF MIC OF TOBRAMYCIN FOR KLEBSIELLA SPECIES



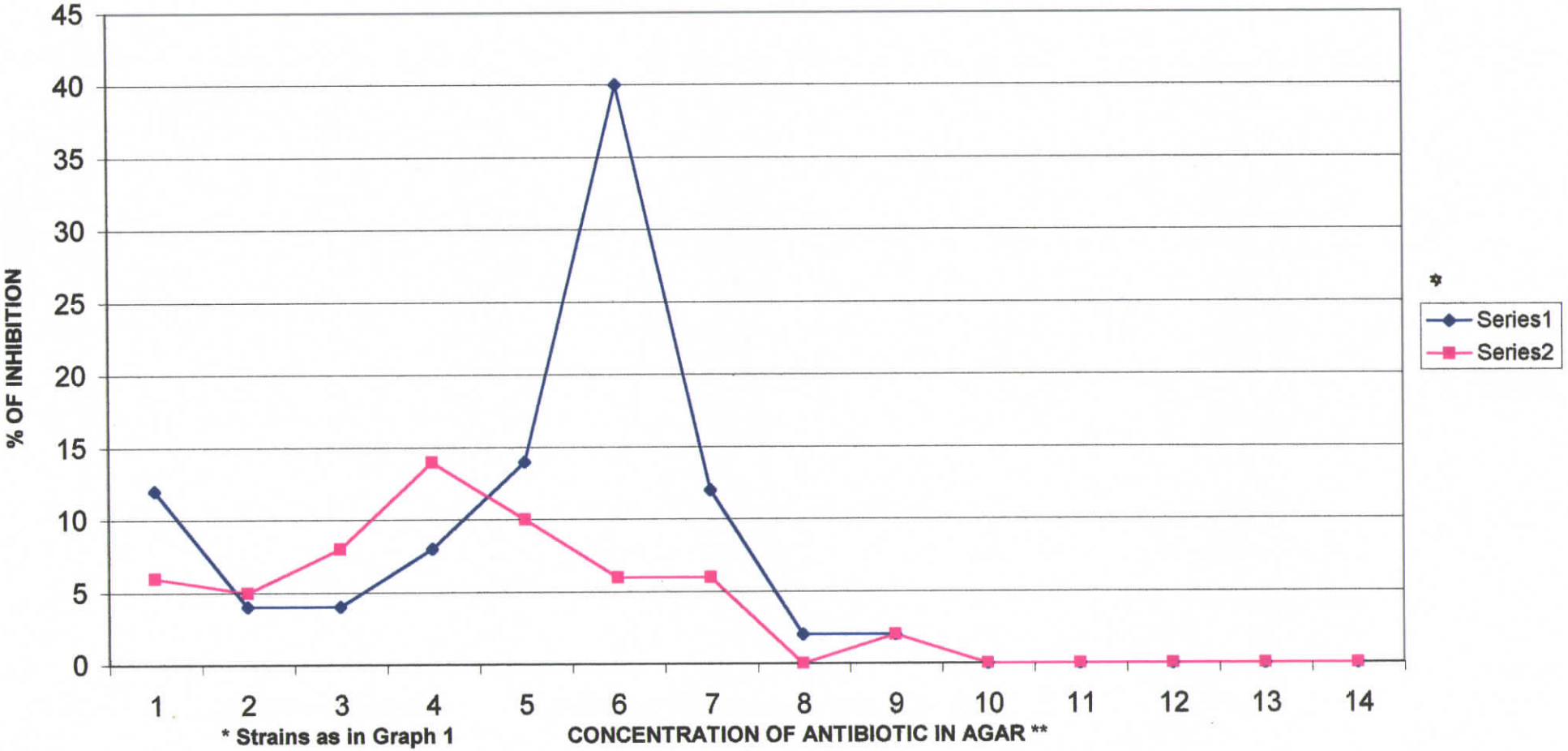
** Respective Conc. is given in Table 3.9

Graph 8
DETERMINATION OF MIC OF CEFUROXIME FOR KLEBSIELLA SPECIES



** Respective Conc. is given in Table 3.8

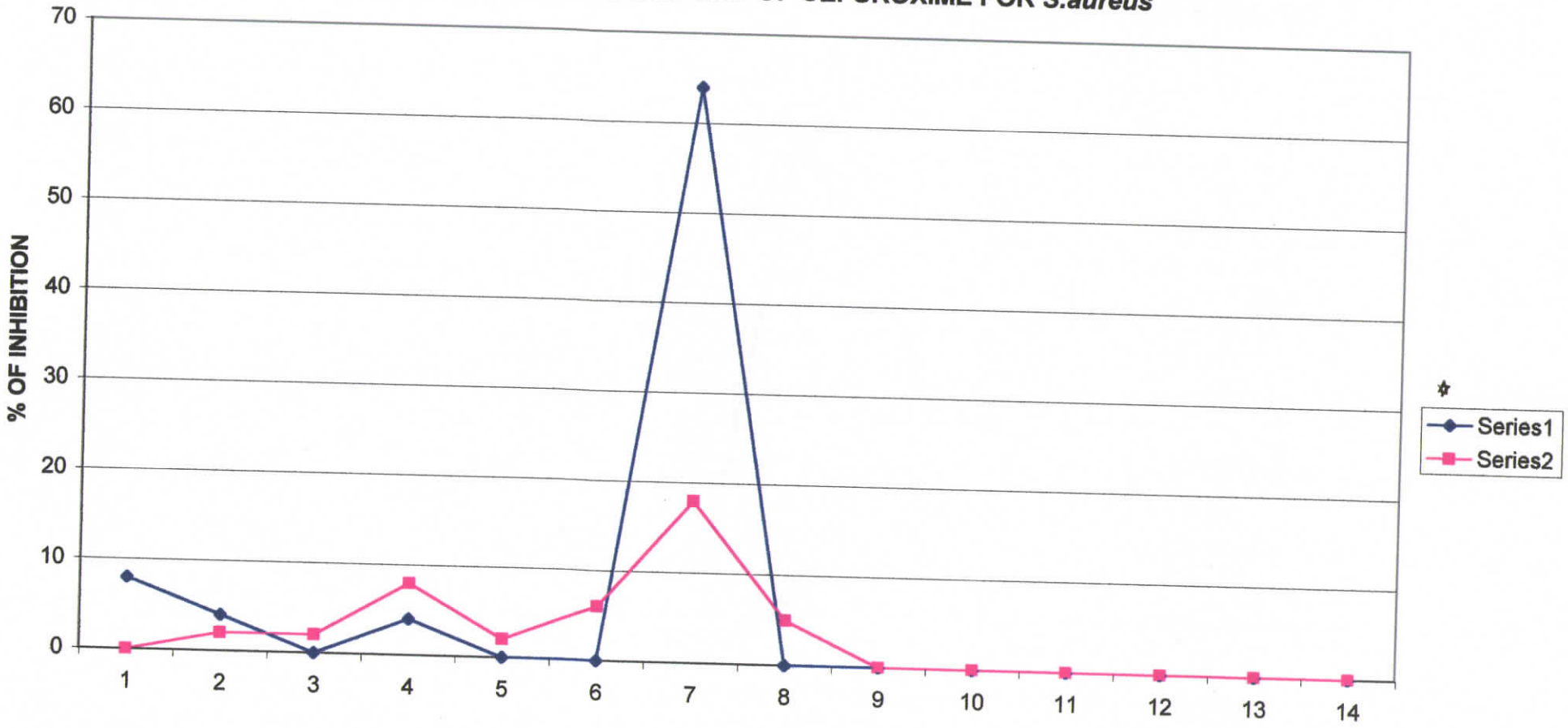
Graph 9
DETERMINATION OF MIC OF CEFUROXIME FOR CNS



** Respective Conc. is given in Table 3.8

Graph 10

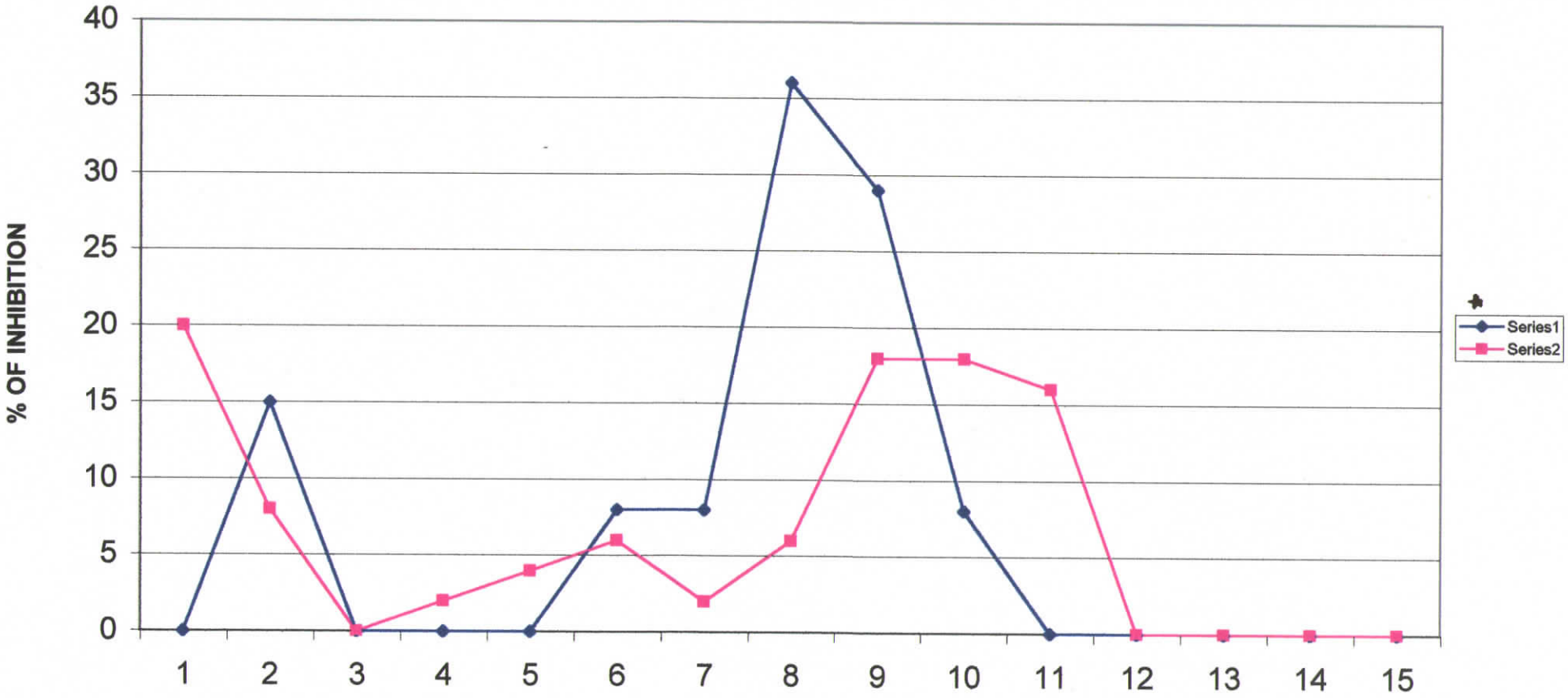
DETERMINATION OF MIC OF CEFUROXIME FOR *S.aureus*



* Strains as in Graph 1 CONCENTRATION OF ANTIBIOTIC IN AGAR **

** Respective Conc. is given in Table 3.8

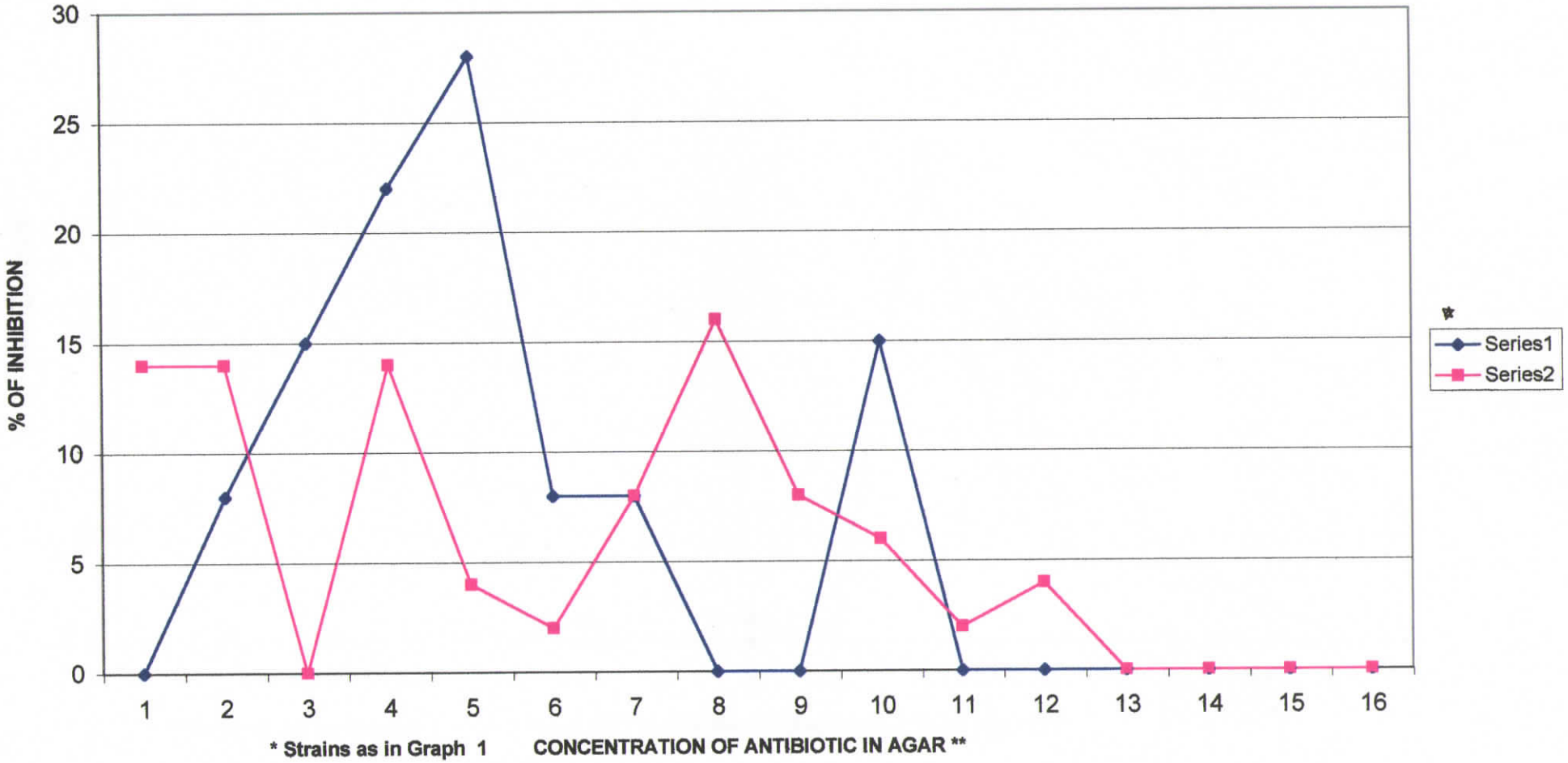
Graph 11
DETERMINATION OF MIC OF GENTAMICIN FOR KLEBSIELLA SPECIES



** Respective Conc.is given in Table 3.5

Graph 12

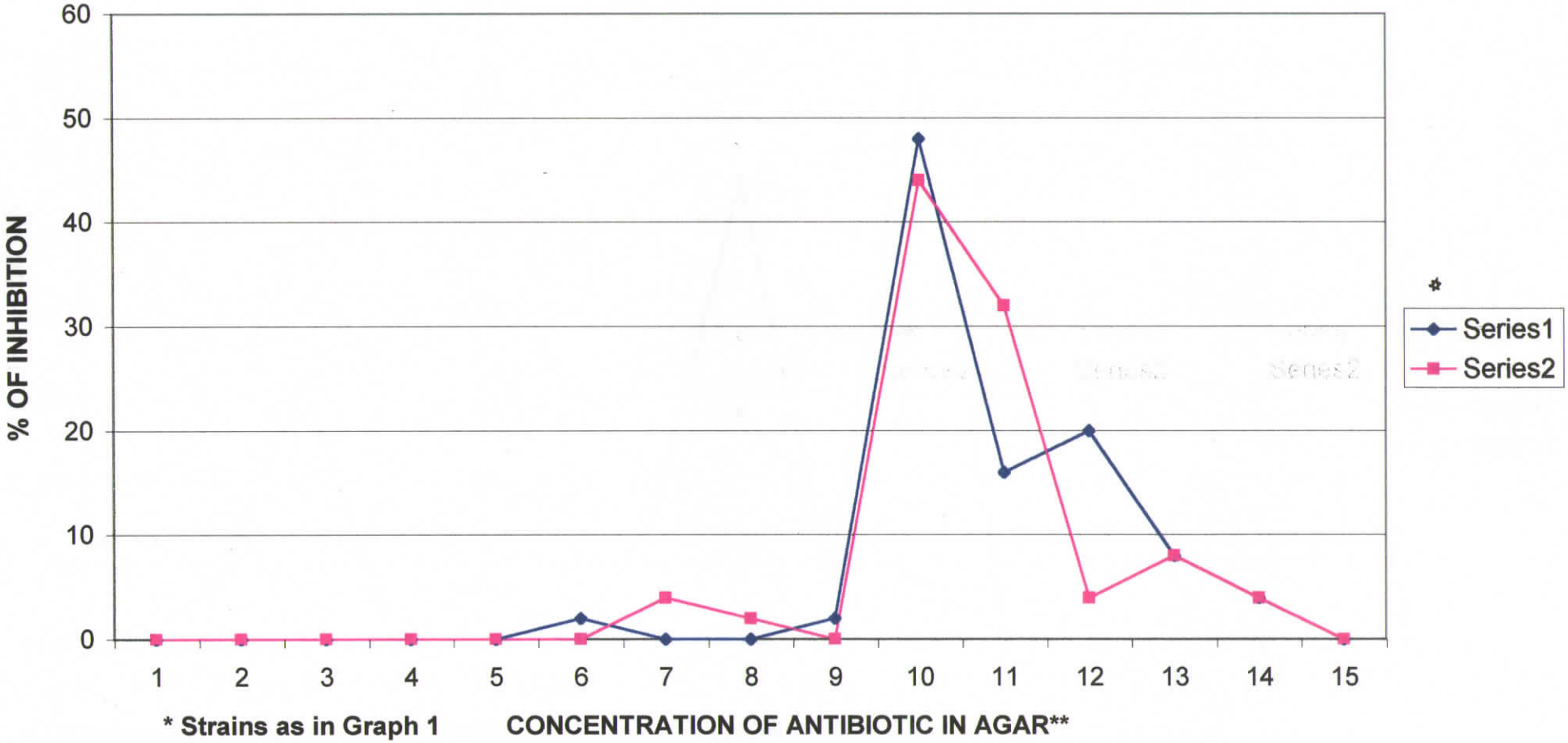
DETERMINATION OF MIC OF AUGMENTIN FOR KLEBSIELLA SPECIES



* Strains as in Graph 1 CONCENTRATION OF ANTIBIOTIC IN AGAR **

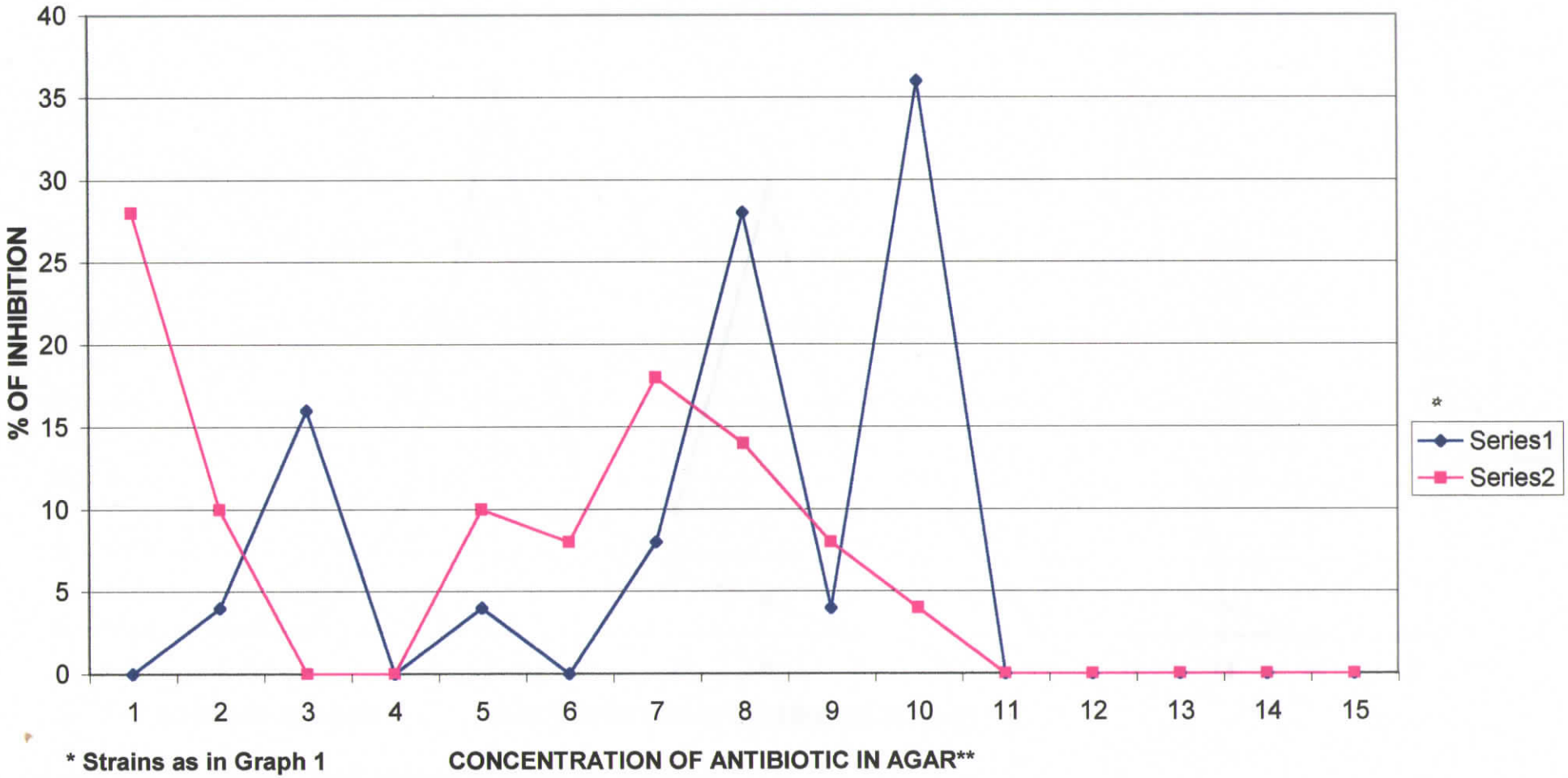
** Respective Conc. is given in Table 3.4

Graph 13
DETERMINATION OF MIC OF AMIKACIN FOR CNS



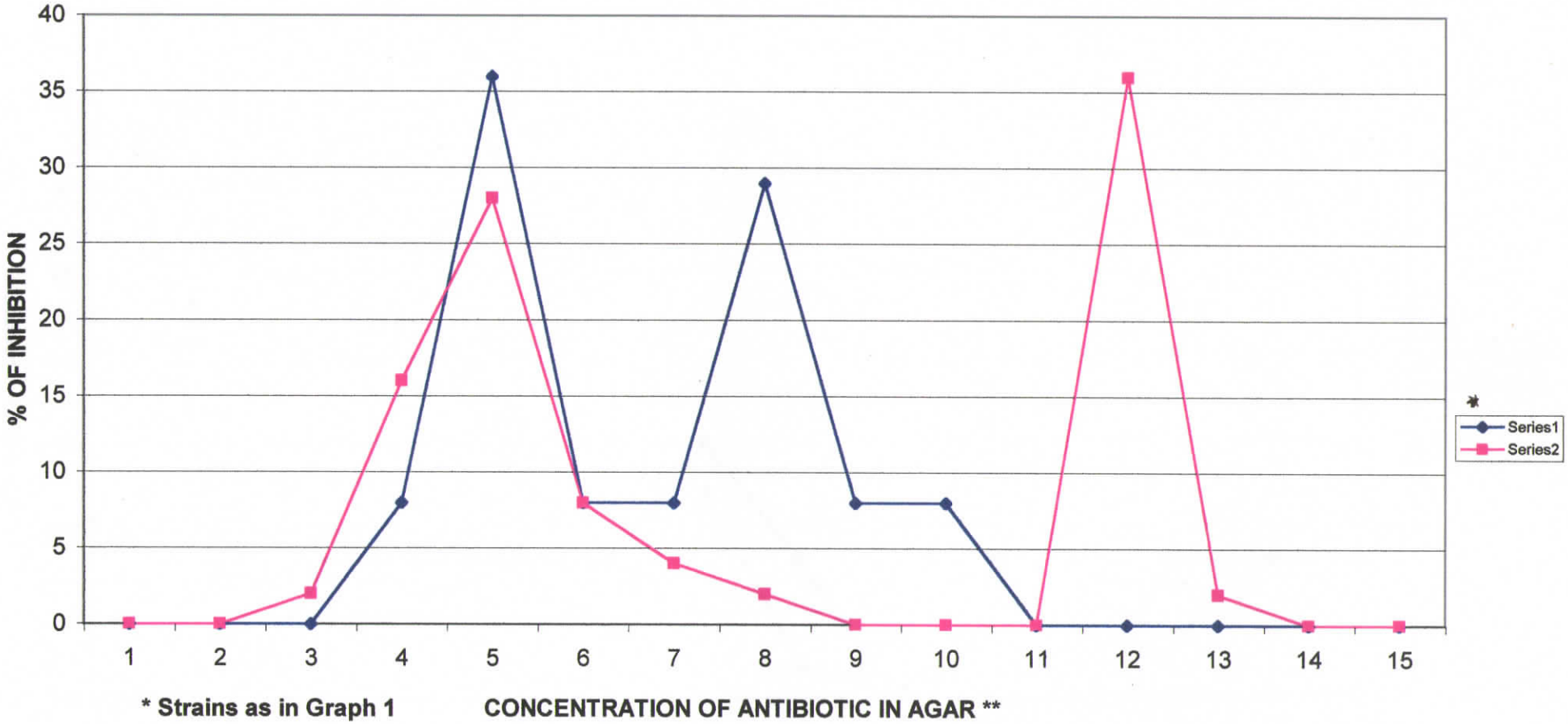
** Respective Conc: is given in Table 3.7

Graph 14
DETERMINATION OF MIC OF AMIKACIN FOR *S.aureus*



** Respective Conc: is given in Table 3.7

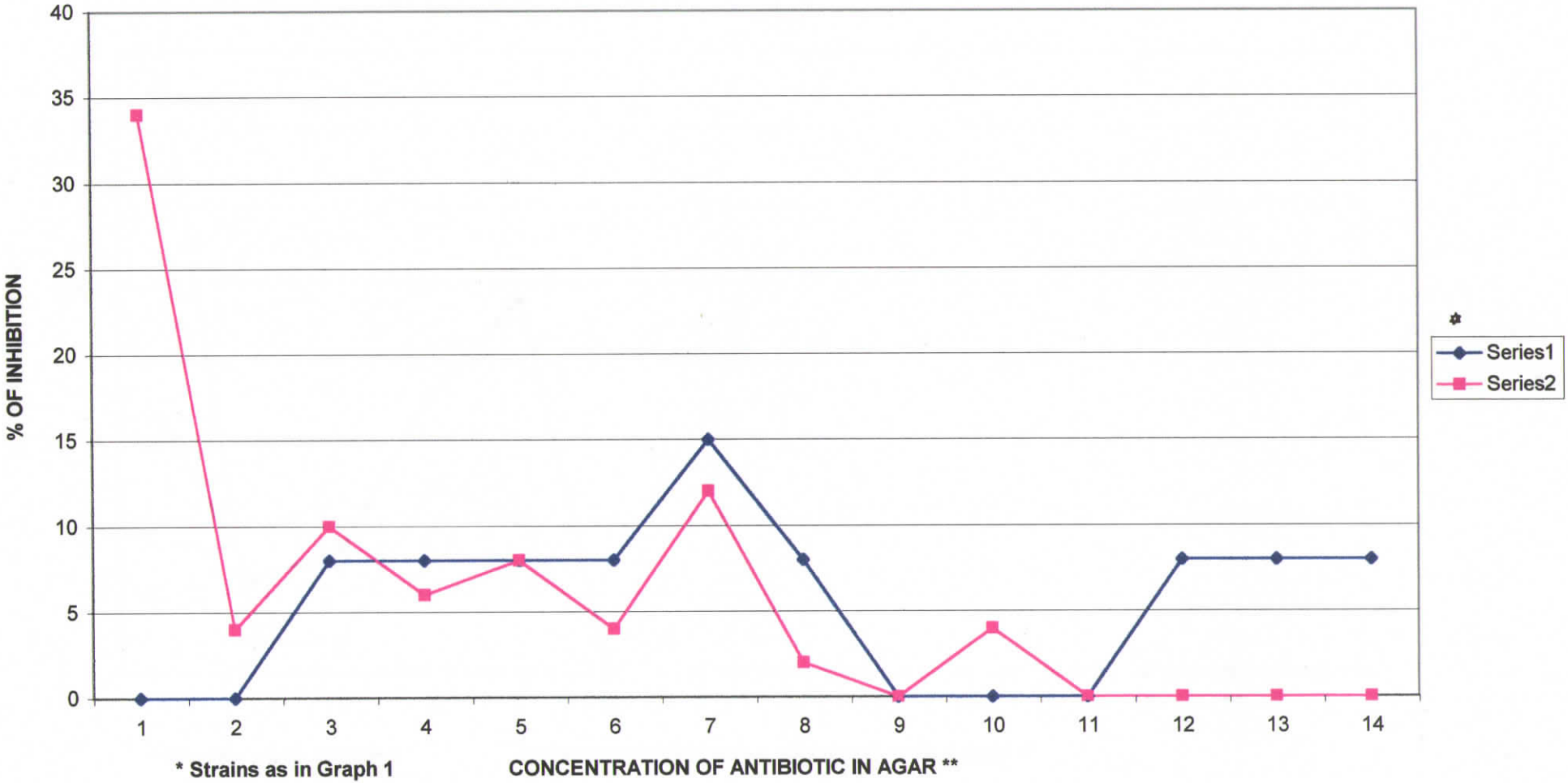
Graph 15
DETERMINATION OF MIC OF AMIKACIN FOR KLEBSIELLA SPECIES



* Strains as in Graph 1 CONCENTRATION OF ANTIBIOTIC IN AGAR **

** Respective Conc: is given in Table 3.7

Graph 16
DETERMINATION OF MIC OF CIPROFLOXACIN FOR KLEBSIELLA SPECIES

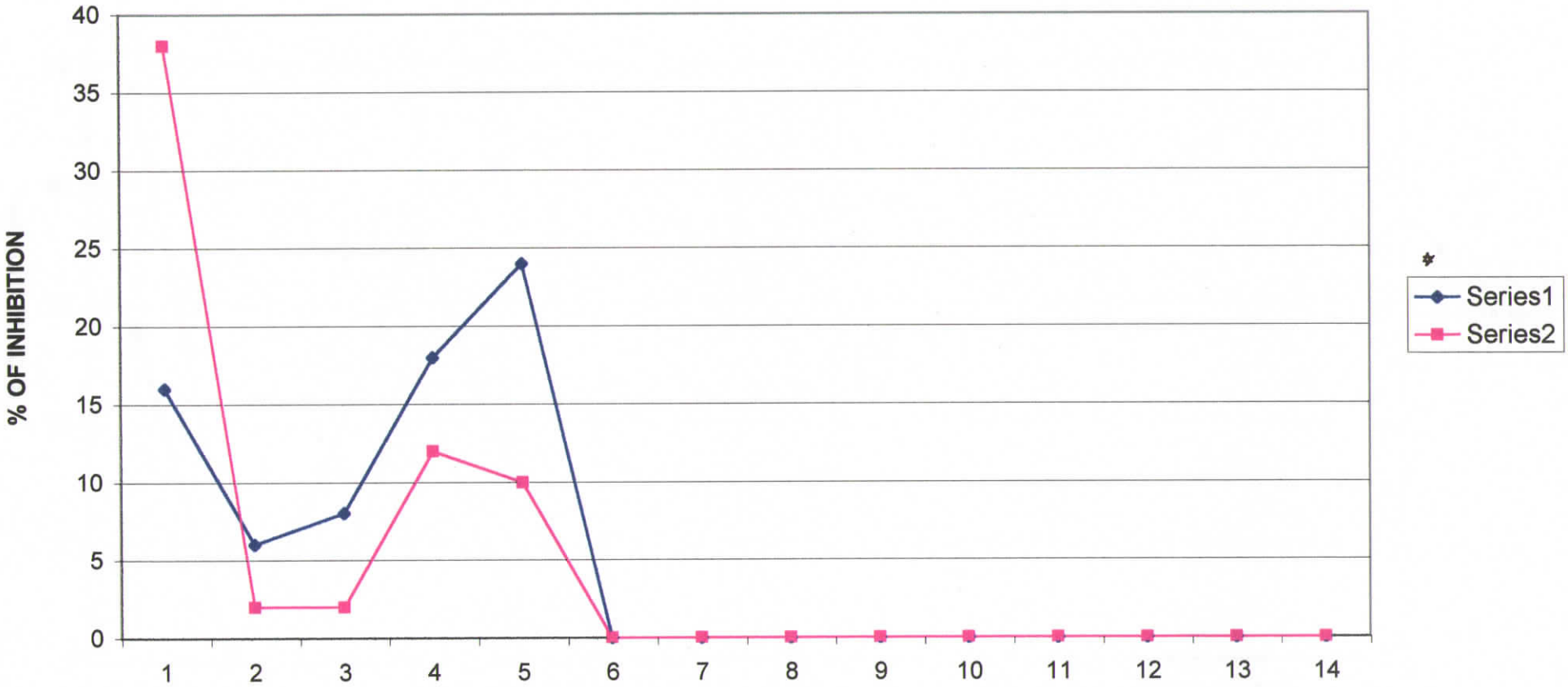


* Strains as in Graph 1

CONCENTRATION OF ANTIBIOTIC IN AGAR **

** Respective Conc: is given in Table 3.6

Graph 17
DETERMINATION OF MIC OF CIPROFLOXACIN FOR CNS



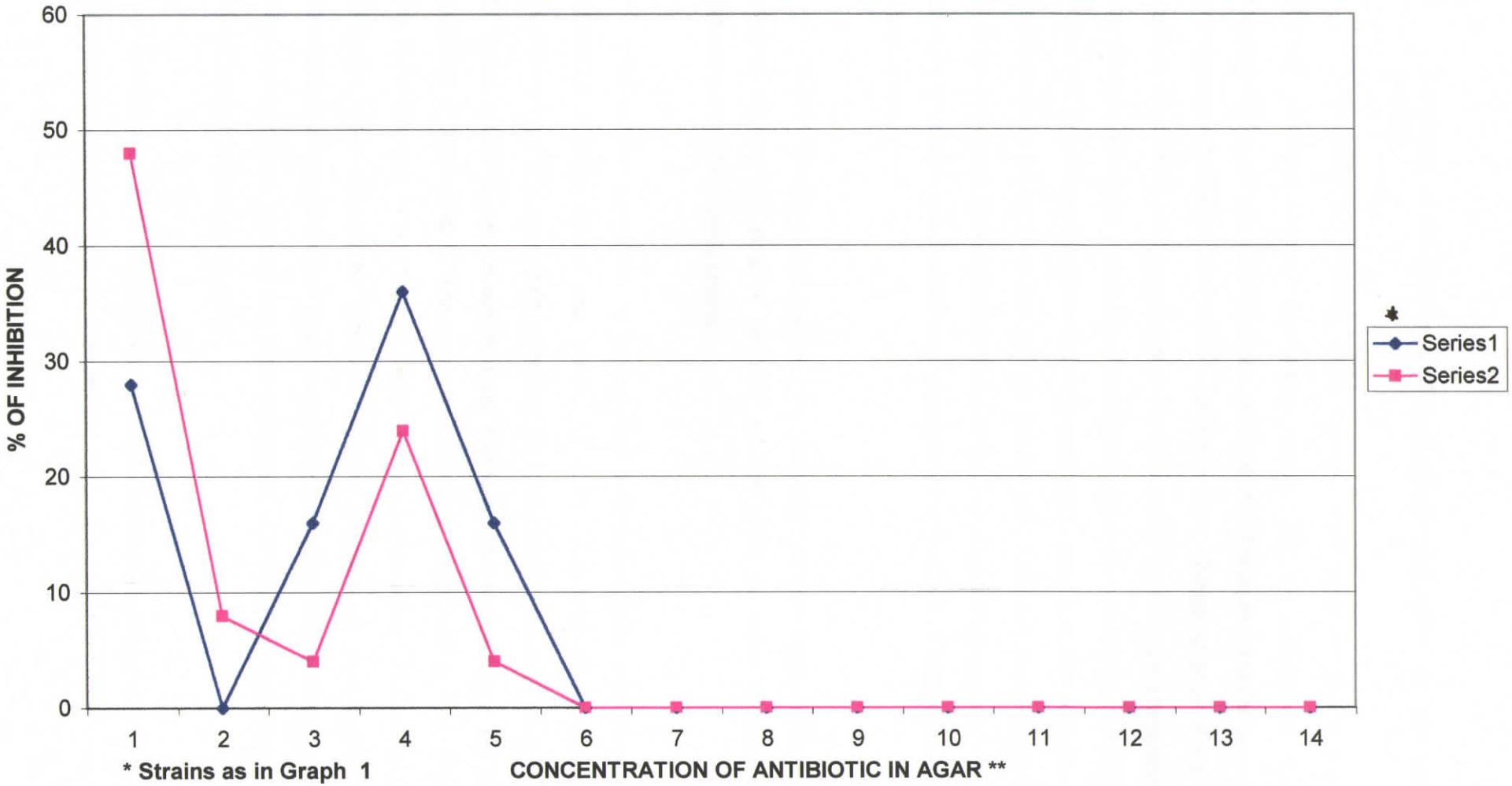
*** Strains as in Graph 1**

CONCENTRATION OF ANTIBIOTIC IN AGAR **

**** Respective Conc: is given in Table 3.6**

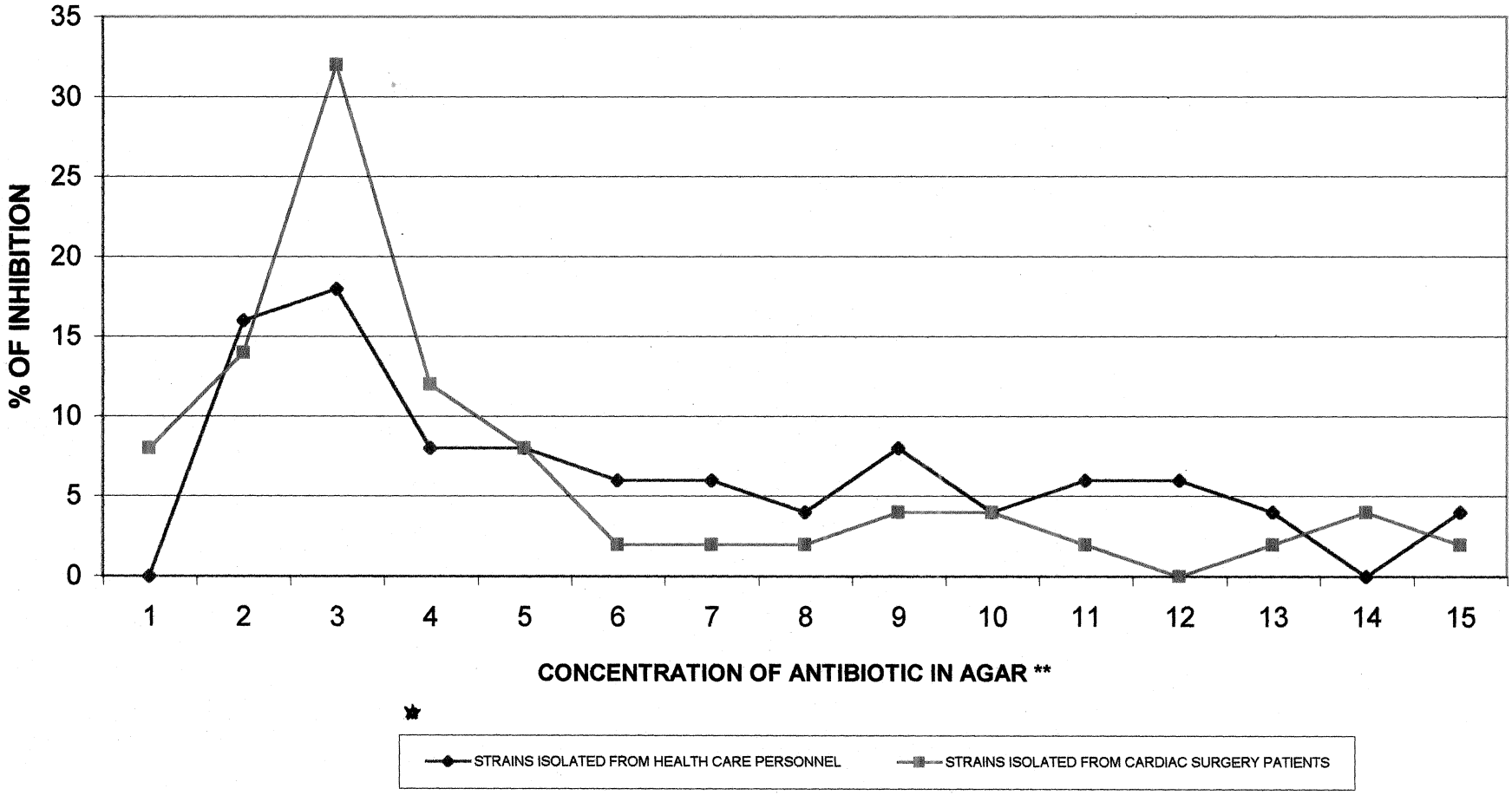
Graph 18

DETERMINATION OF MIC OF CIPROFLOXACIN FOR S.aureus



** Respective Conc: is given in Table 3.6

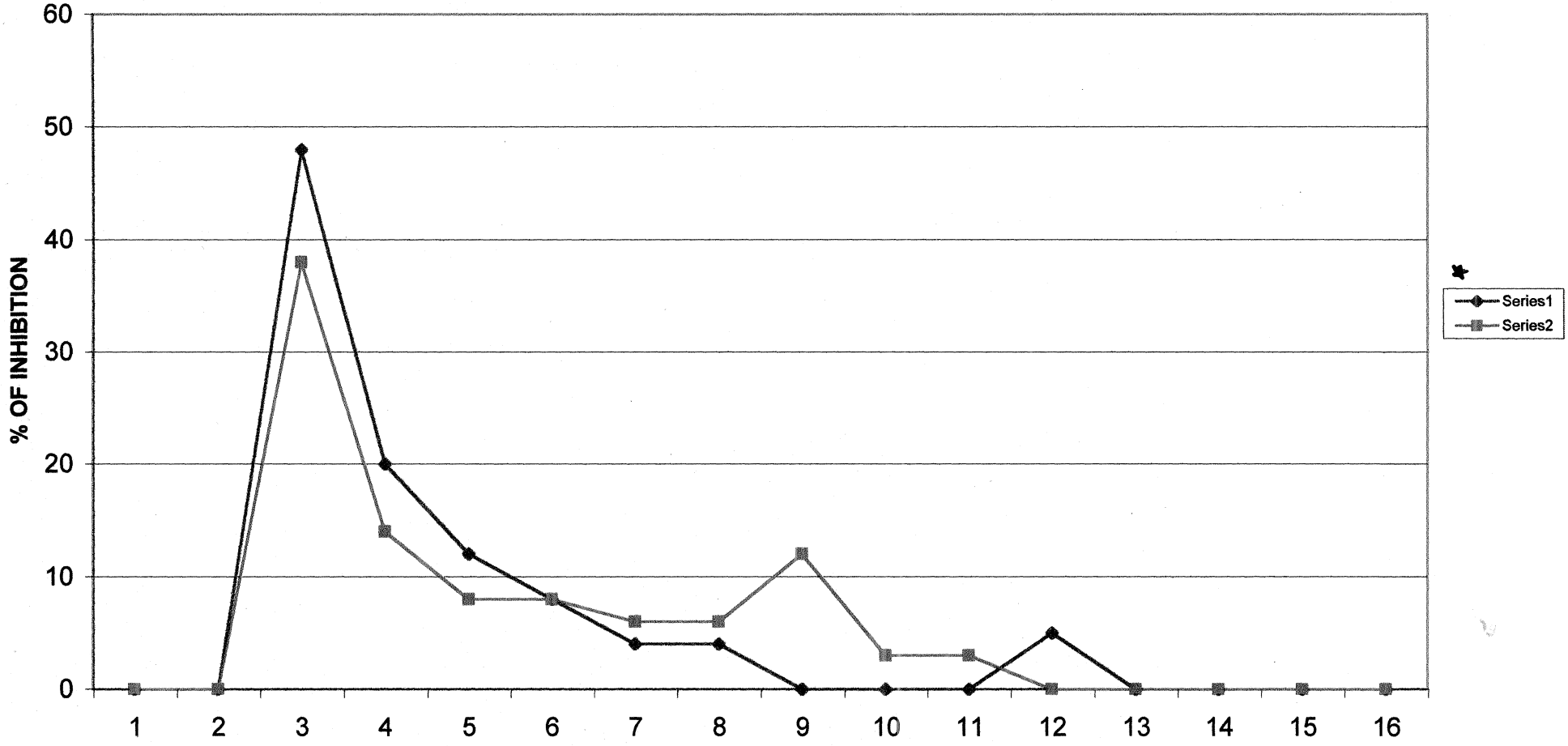
Graph 1
DETERMINATION OF MIC OF PENICILLIN FOR CNS



** Respective Conc: is given in Table 3.2

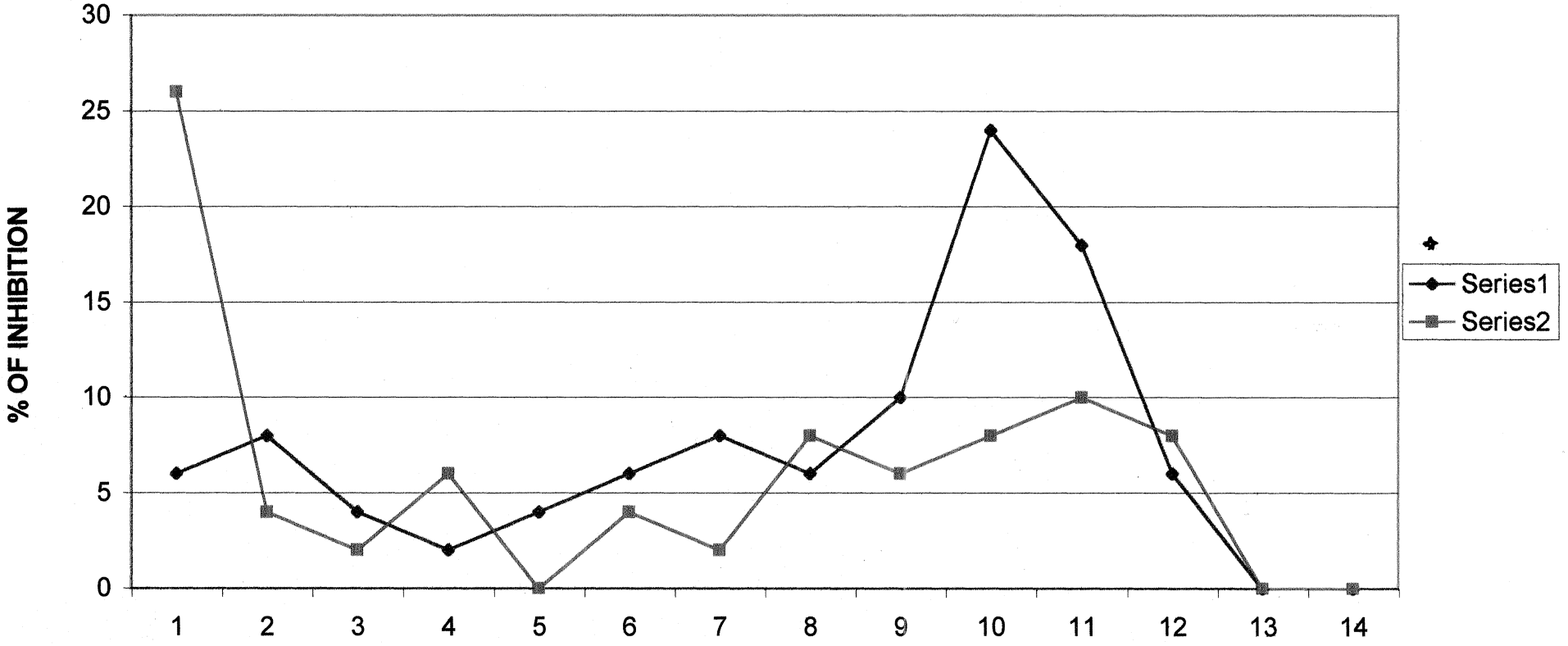
Graph 2

DETERMINATI0N OF MIC OF PENICILLIN FOR *S.aureus*



** Respective Conc: is given in Table 3.2

Graph 3
DETERMINATION OF MIC OF CLOXACILLIN FOR CNS



*Strains as in Graph 1 CONCENTRATION OF ANTIBIOTIC IN AGAR **

** Respective Conc: is given in Table 3.3

F. Screening of indigenous medicinal plants and spices for the antibacterial activity.

Since Alexander Fleming, many attempts were made for finding out new and effective antibiotics against pathogenic strains. But an unfortunate complication accompanied the use of antibiotics is the development of resistance against many antibiotics and consequent proliferation of the new resistant strains. Though an array of antibiotics are available, most of the existing pathogenic bacterial strains have acquired resistance towards most of them. Moreover excess use of antibiotics paved the way to other infections caused by viral and fungal organisms. The high cost required for treatment with antibiotics which is not affordable to the common man and the fact that new antibiotics are not forthcoming as fast as in early years have shifted the focus to the use of natural substances. WHO reports that 80% of people around world even now depend on traditional medicines for the cure of various ailments.

Thus an attempt to find out new, less costly and low toxic antibacterial agents from traditional origin is the need of the hour to over come the threat of increasing drug resistance among pathogenic strains.

An attempt was made to screen some indigenous plants and spices for their antibacterial activity against the strains of CNS, *S.aureus, Klebsiella* spp. and *Pseudomonas* spp. In a preliminary screening, the effects of the extracts of plants and spices were tested against the above strains using filtered sterile aqueous forms and also after autoclaving of these extracts. The results are shown in Tables 4.28 to 4.41. From these tables, it is interesting to note that the filtered sterile aqueous extract of *Moringa oleifera, Phyllanthes niruri, Eucalyptus globulus, Ocimum sanctum* are showing fairly good antibacterial activity against Gram positive organisms, while the autoclaved aqueous extract of *Tamarindus indicus* showed very high antibacterial activity against both Gram positive and Gram negative leading pathogens.

Similarly, it is quite evident from the results that the antibacterial activity of filtered sterile aqueous extract of Garlic (*A.sativum*) also holds promise.

Table - 4.28

PERCENTAGE OF ANTISTAPHYLOCOCCAL ACTIVITIES OF FILTER STERILE AQUEOUS EXTRACT OF VARIOUS PLANTS

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Phyllanthes niruri* *	Keezha rnelli	CNS/ CSP	12	32	4	20	0	8	0	0	0	0
2	*Phyllanthes niruri* *	Keezha rnelli	CNS/ HCP	16	32	8	24	0	4	0	0	0	0
3	*Moringa Oleifera*	Muri- nga	CNS/ CSP	12	52	0	36	0	0	0	0	0	0
4	*Moringa Oleifera*	Muri- nga	CNS/ HCP	8	56	8	44	0	4	0	0	0	0
5	*Eucalyptus globulus*	Eucaly	CNS/ CSP	16	40	12	20	0	8	0	0	0	0
6	*Eucalyptus globulus*	Eucaly	CNS/ HCP	24	40	20	24	4	20	0	4	0	0
7	*Lawsonia inermis*	Maylan chi	CNS/ CSP	4	20	0	12	0	8	0	0	0	0
8	*Lawsonia inermis*	Maylan chi	CNS/ HCP	8	32	0	16	0	4	0	0	0	0
9	*Eupatorium urticifolium*	C.pa- cha	CNS/ CSP	4	12	4	4	0	0	0	0	0	0
10	*Eupatorium urticifolium*	C.pa- Cha	CNS/ HCP	8	12	0	8	0	0	0	0	0	0
11	*Ocimum Sanctum*	Thulasi	CNS/ CSP	12	20	0	12	0	4	0	0	0	0
12	*Ocimum Sanctum*	Thulasi	CNS/ HCP	16	24	4	16	0	8	0	0	0	0

(Cont'd....)

Sl No	Botanical name	Local Name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Emilia sonchifolia*	Muyal chevian	CNS/ CSP	4	20	0	16	0	0	0	0	0	0
14	*Emilia sonchifolia*	Muyal chevian	CNS/ HCP	4	20	0	12	0	0	0	0	0	0
15	*Azadriacht a indica*	Veppu	CNS/ CSP	4	20	0	12	0	0	0	0	0	0
16	*Azadriacht a indica*	Veppu	CNS/ HCP	4	32	0	20	0	4	0	0	0	0
17	*Adhatoda vasica*	Adaloda kam	CNS/ CSP	8	12	0	12	0	0	0	0	0	0
18	*Adhatoda vasica*	Adaloda kom	CNS/ HCP	8	16	0	16	0	4	0	0	0	0
19	*Leucas aspera*	Thumba	CNS/ CSP	0	16	0	4	0	0	0	0	0	0
20	*Leucas aspera*	Thumba	CNS/ HCP	0	20	0	12	0	0	0	0	0	0
21	*Camelia sinensis*	Theyila	CNS/ CSP	0	12	0	12	0	4	0	0	0	0
22	*Camelia sinensis*	Theyila	CNS/ HCP	0	16	0	8	0	0	0	0	0	0
23	*Clerodend ron infort- unatum*	Perimala	CNS/ CSP	0	8	0	4	0	0	0	0	0	0
24	*Clerodend ron infort- unatum*	Perimala	CNS/ HCP	0	8	0	4	0	0	0	0	0	0

(Cont'd....)

Sl No	Botanical name	Local Name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
25	*Piper betle*	Vettila	CNS/ CSP	0	8	0	8	0	0	0	0	0	0
26	*Piper betle*	Vettila	CNS/ HCP	0	12	0	8	0	0	0	0	0	0
27	*Murraya koenigii*	Karive-ppu	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
28	*Murraya koenigii*	Karive-ppu	CNS/ HCP	0	8	0	0	0	0	0	0	0	0

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel
S - Sensitive
Ms - Moderately Sensitive

Table - 4.29

PERCENTAGE OF ANTISTAPHYLOCOCCAL ACTIVITIES OF AUTOCLAVED AQUEOUS EXTRACT OF VARIOUS PLANTS

Sl No	Botanical Name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Phyllanthes niruri*	Keezhar nelli	CNS/ CSP	20	40	20	40	12	16	0	12	0	0
2	*Phyllanthes Niruri*	Keezhar nelli	CNS/ HCP	12	52	4	24	0	8	0	0	0	0
3	*Moringa Oleifera*	Muri-nga	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
4	*Moringa Oleifera*	Muri-nga	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
5	*Eucalyptus Globulus*	Eucaly	CNS/ CSP	12	28	0	12	0	0	0	0	0	0
6	*Eucalyptus Globulus*	Eucaly	CNS/ HCP	16	36	4	16	0	4	0	0	0	0
7	*Lawsonia inermis*	Maylan chi	CNS/ CSP	24	24	8	16	4	4	4	4	0	0
8	*Lawsonia Inermis*	Maylan chi	CNS/ HCP	20	24	4	12	0	4	0	0	0	0
9	*Eupatoriu m urtici-folium*	C.pacha	CNS/ CSP	12	12	12	12	0	12	0	4	0	0
10	*Eupatoriu m urtici-Folium*	C.pacha	CNS/ HCP	16	32	8	12	0	12	0	0	0	0
11	*Ocimum Sanctum*	Thulasi	CNS/ CSP	8	28	0	8	0	0	0	0	0	0
12	*Ocimum Sanctum*	Thulasi	CNS/ HCP	8	28	0	24	0	4	0	0	0	0

(Cont'd....)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Emilia sonchifolia*	Muyal chevian	CNS/ CSP	16	20	4	24	0	4	0	0	0	0
14	*Emilia sonchifolia*	Muyal chevian	CNS/ HCP	20	16	4	8	0	4	0	0	0	0
15	*Azadriacht a indica*	Veppu	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
16	*Azadriacht a indica*	Veppu	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
17	*Adhatoda vasica*	Adaloda kam	CNS/ CSP	8	16	0	0	0	0	0	0	0	0
18	*Adhatoda vasica*	Adaloda kom	CNS/ HCP	8	20	0	4	0	4	0	0	0	0
19	*Leucas aspera*	Thumba	CNS/ CSP	0	24	0	0	0	0	0	0	0	0
20	*Leucas aspera*	Thumba	CNS/ HCP	8	20	0	12	0	4	0	0	0	0
21	*Camelia sinensis*	Theyila	CNS/ CSP	0	12	0	0	0	0	0	0	0	0
22	*Camelia sinensis*	Theyila	CNS/ HCP	0	12	0	0	0	0	0	0	0	0
23	*Clerodend ron infort- unatum*	Perimala	CNS/ CSP	0	16	0	0	0	0	0	0	0	0
24	*Clerodend ron infort- unatum*	Perimala	CNS/ HCP	0	12	0	0	0	0	0	0	0	0

(Cont'd....)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
25	*Piper betle*	Vettila	CNS/ CSP	0	16	0	0	0	0	0	0	0	0
26	*Piper betle*	Vettila	CNS/ HCP	0	16	0	0	0	0	0	0	0	0
27	*Murraya koenigii*	Karive-ppu	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
28	*Murraya koenigii*	Karive-Ppu	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
29	*Tamarind us indicus*	Puli	CNS/ CSP	100	-	100	-	100	-	100	-	100	-
30	*Tamarind us indicus*	Puli	CNS/ HCP	100	-	100	-	100	-	100	-	100	-

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel

Table - 4.30

PERCENTAGE OF ANTISTAPHYLOCOCCAL ACTIVITIES OF FILTER STERILE AQUEOUS EXTRACT OF VARIOUS PLANTS

Sl No	Botanical Name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Phyllanthes niruri*	Keezharnelli	*S.aureus* CSP	0	28	0	8	0	0	0	0	0	0
2	*Phyllanthes Niruri*	Keezharnelli	*S.aureus* HCP	0	28	0	20	0	0	0	0	0	0
3	*Moringa Oleifera*	Muri-nga	*S.aureus* CSP	4	60	0	20	0	0	0	0	0	0
4	*Moringa Oleifera*	Muri-nga	*S.aureus* HCP	8	64	0	36	0	0	0	0	0	0
5	*Eucalyptus globulus*	Eucaly	*S.aureus* CSP	4	28	0	8	0	0	0	0	0	0
6	*Eucalyptus globulus*	Eucaly	*S.aureus* HCP	28	40	16	32	4	16	0	4	0	0
7	*Lawsonia inermis*	Maylanchi	*S.aureus* CSP	4	20	4	8	0	0	0	0	0	0
8	*Lawsonia inermis*	Maylanchi	*S.aureus* HCP	8	20	0	12	0	0	0	0	0	0
9	*Eupatorium urtici-folium*	C.pacha	*S.aureus* CSP	8	28	0	20	0	4	0	0	0	0
10	*Eupatorium urtici-folium*	C.pacha	*S.aureus* HCP	12	44	0	8	0	0	0	0	0	0
11	*Ocimum sanctum*	Thulasi	*S.aureus* CSP	0	16	0	0	0	0	0	0	0	0
12	*Ocimum sanctum*	Thulasi	*S.aureus* HCP	8	16	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical Name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Emilia sonchifolia*	Muyal chevian	*S.aureus* CSP	0	16	0	8	0	0	0	0	0	0
14	*Emilia sonchifolia*	Muyal chevian	*S.aureus* HCP	0	16	0	0	0	0	0	0	0	0
15	*Azadriachta indica*	Veppu	*S.aureus* CSP	0	16	0	12	0	0	0	0	0	0
16	*Azadriachta indica*	Veppu	*S.aureus* HCP	0	16	0	12	0	0	0	0	0	0
17	*Adhatoda vasica*	Adaloda kam	*S.aureus* CSP	0	16	0	0	0	0	0	0	0	0
18	*Adhatoda Vasica*	Adaloda kom	*S.aureus* HCP	8	16	0	0	0	0	0	0	0	0
19	*Leucas aspera*	Thumba	*S.aureus* CSP	0	4	0	0	0	0	0	0	0	0
20	*Leucas aspera*	Thumba	*S.aureus* HCP	0	12	0	0	0	0	0	0	0	0
21	*Camelia sinensis*	Theyila	*S.aureus* CSP	0	4	0	0	0	0	0	0	0	0
22	*Camelia sinensis*	Theyila	*S.aureus* HCP	0	12	0	0	0	0	0	0	0	0
23	*Clerodendron infortunatum*	Perimala	*S.aureus* CSP	0	16	0	12	0	4	0	0	0	0
24	*Clerodendron infortunatum*	Perimala	*S.aureus* HCP	0	16	0	8	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
25	*Piper betle*	Vettila	*S.aureus* CSP	0	4	0	0	0	0	0	0	0	0
26	*Piper betle*	Vettila	*S.aureus* HCP	0	8	0	0	0	0	0	0	0	0
27	*Murraya Koenigii*	Karive-ppu	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
28	*Murraya koenigii*	Karive-ppu	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0

CSP - **Cardiac Surgery Patients**
HCP - **Health Care Personnel**
S - **Sensitive**
Ms - **Moderately Sensitive**

Table - 4.31

PERCENTAGE OF ANTISTAPHYLOCOCCAL ACTIVITIES OF AUTOCLAVED AQUEOUS EXTRACT OF VARIOUS PLANTS

Sl No	Botanical Name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Phyllanthes niruri*	Keezhar nelli	*S.aureus* CSP	4	44	4	32	0	0	0	0	0	0
2	*Phyllanthes Niruri*	Keezhar nelli	*S.aureus* HCP	12	40	4	36	0	4	0	0	0	0
3	*Moringa Oleifera*	Muri-nga	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
4	*Moringa Oleifera*	Muri-nga	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
5	*Eucalyptus Globulus*	Eucaly	*S.aureus* CSP	4	24	4	8	0	0	0	0	0	0
6	*Eucalyptus Globulus*	Eucaly	*S.aureus* HCP	0	48	0	0	0	0	0	0	0	0
7	*Lawsonia inermis*	Maylan chi	*S.aureus* CSP	12	28	8	20	0	0	0	0	0	0
8	*Lawsonia Inermis*	Maylan chi	*S.aureus* HCP	16	32	8	32	0	16	0	0	0	0
9	*Eupatoriu m urtici-folium*	C.pacha	*S.aureus* CSP	8	24	0	16	0	8	0	0	0	0
10	*Eupatoriu m urtici-Folium*	C.pacha	*S.aureus* HCP	12	32	4	8	0	4	0	0	0	0
11	*Ocimum Sanctum*	Thulasi	*S.aureus* CSP	0	16	0	0	0	0	0	0	0	0
12	*Ocimum Sanctum*	Thulasi	*S.aureus* HCP	12	16	0	8	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Emilia sonchifolia*	Muyal chevian	*S.aureus* CSP	0	16	0	8	0	0	0	0	0	0
14	*Emilia sonchifolia*	Muyal chevian	*S.aureus* HCP	0	20	0	0	0	0	0	0	0	0
15	*Azadriachta indica*	Veppu	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
16	*Azadriachta indica*	Veppu	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
17	*Adhatoda vasica*	Adaloda kam	*S.aureus* CSP	0	4	0	0	0	0	0	0	0	0
18	*Adhatoda vasica*	Adaloda kom	*S.aureus* HCP	0	8	0	0	0	0	0	0	0	0
19	*Leucas aspera*	Thumba	*S.aureus* CSP	0	4	0	0	0	0	0	0	0	0
20	*Leucas aspera*	Thumba	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
21	*Camelia sinensis*	Theyila	*S.aureus* CSP	0	12	0	0	0	0	0	0	0	0
22	*Camelia sinensis*	Theyila	*S.aureus* HCP	0	12	0	0	0	0	0	0	0	0
23	*Clerodendron infortunatum*	Perimala	*S.aureus* CSP	0	16	0	16	0	4	0	0	0	0
24	*Clerodendron infortunatum*	Perimala	*S.aureus* HCP	0	16	0	8	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
25	*Piper betle*	Vettila	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
26	*Piper betle*	Vettila	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
27	*Murraya koenigii*	Karivepu	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
28	*Murraya koenigii*	Karivepu	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
29	*Tamarindus indicus*	Puli	*S.aureus* CSP	100	-	100	-	100	-	100	-	100	-
30	*Tamarindus indicus*	Puli	*S.aureus* HCP	100	-	100	-	100	-	100	-	100	-

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel
S - Sensitive
Ms - Moderative Sensitive

Table - 4.32

PERCENTAGE OF ANTIBACTERIAL ACTIVITIES OF FILTER STERILE AQUEOUS EXTRACT OF VARIOUS PLANTS AGAINST KLEBSIELLA

Sl No	Botanical Name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Phyllanthes niruri*	Keezharnelli	*Klebsiella* CSP	0	4	0	0	0	0	0	0	0	0
2	*Phyllanthes niruri*	Keezharnelli	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
3	*Moringa Oleifera*	Murin-ga	*Klebsiella* CSP	0	12	0	0	0	0	0	0	0	0
4	*Moringa Oleifera*	Murin-ga	*Klebsiella* HCP	0	14	0	0	0	0	0	0	0	0
5	*Eucalyptus globulus*	Eucaly	*Klebsiella* CSP	0	24	0	4	0	0	0	0	0	0
6	*Eucalyptus globulus*	Eucaly	*Klebsiella* HCP	0	16	0	0	0	0	0	0	0	0
7	*Lawsonia inermis*	Maylan chi	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
8	*Lawsonia inermis*	Maylan chi	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
9	*Eupatoriu m urtici-folium*	C.pacha	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
10	*Eupatoriu m urtici-folium*	C.pacha	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
11	*Ocimum sanctum*	Thulasi	*Klebsiella* CSP	0	12	0	0	0	0	0	0	0	0
12	*Ocimum sanctum*	Thulasi	*Klebsiella* HCP	14	0	0	14	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Emilia sonchifolia*	Muyal chevian	*Klebsiella* CSP	0	8	0	0	0	0	0	0	0	0
14	*Emilia sonchifolia*	Muyal chevian	*Klebsiella* HCP	0	7	0	0	0	0	0	0	0	0
15	*Azadriacht a indica*	Veppu	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
16	*Azadriacht a indica*	Veppu	*Klebsiella* HCP	0	7	0	0	0	0	0	0	0	0
17	*Adhatoda vasica*	Adaloda kam	*Klebsiella* CSP	0	8	0	0	0	0	0	0	0	0
18	*Adhatoda vasica*	Adaloda kom	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
19	*Leucas aspera*	Thumba	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
20	*Leucas aspera*	Thumba	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
21	*Camelia sinensis*	Theyila	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
22	*Camelia sinensis*	Theyila	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
23	*Clerodend ron infort-unatum*	Perimala	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
24	*Clerodend ron infort-unatum*	Perimala	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
25	*Piper betle*	Vettila	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
26	*Piper betle*	Vettila	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
27	*Murraya koenigii*	Karive-ppu	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
28	*Murraya koenigii*	Karive-ppu	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel
S - Sensitive
Ms - Moderatively Sensitive

Table - 4.33

PERCENTAGE OF ANTIBACTERIAL ACTIVITIES OF AUTOCLAVED AQUEOUS EXTRACT OF VARIOUS PLANTS AGAINST KLEBSIELLA

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Phyllanthes niruri*	Keezhar nelli	*Klebsiella* CSP	0	8	0	0	0	0	0	0	0	0
2	*Phyllanthes Niruri*	Keezhar nelli	*Klebsiella* HCP	0	14	0	0	0	4	0	0	0	0
3	*Moringa Oleifera*	Muri-nga	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
4	*Moringa Oleifera*	Muri-nga	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
5	*Eucalyptus Globulus*	Eucaly	*Klebsiella* CSP	0	20	0	4	0	0	0	0	0	0
6	*Eucalyptus Globulus*	Eucaly	*Klebsiella* HCP	0	28	0	0	0	0	0	0	0	0
7	*Lawsonia inermis*	Maylan chi	*Klebsiella* CSP	0	4	0	0	0	0	0	0	0	0
8	*Lawsonia Inermis*	Maylan chi	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
9	*Eupatoriu m urtici-folium*	C.pacha	*Klebsiella* CSP	0	8	0	0	0	0	0	0	0	0
10	*Eupatoriu m urtici-folium*	C.pacha	*Klebsiella* HCP	0	7	0	0	0	0	0	0	0	0
11	*Ocimum sanctum*	Thulasi	*Klebsiella* CSP	0	16	0	0	0	0	0	0	0	0
12	*Ocimum sanctum*	Thulasi	*Klebsiella* HCP	0	14	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local Name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	M %
13	*Emilia sonchifolia*	Muyal chevian	*Klebsiella* CSP	0	16	0	0	0	0	0	0	0	0
14	*Emilia sonchifolia*	Muyal chevian	*Klebsiella* HCP	0	14	0	0	0	0	0	0	0	0
15	*Azadriacht a indica*	Veppu	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
16	*Azadriacht a indica*	Veppu	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
17	*Adhatoda vasica*	Adaloda kam	*Klebsiella* CSP	0	4	0	0	0	0	0	0	0	0
18	*Adhatoda vasica*	Adaloda kom	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
19	*Leucas aspera*	Thumba	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
20	*Leucas aspera*	Thumba	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
21	*Camelia sinensis*	Theyila	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
22	*Camelia sinensis*	Theyila	*Klebsiella* HCP	0	14	0	0	0	0	0	0	0	0
23	*Clerodend ron infort-unatum*	Perimala	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
24	*Clerodend ron infort-unatum*	Perimala	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
25	*Piper betle*	Vettila	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
26	*Piper betle*	Vettila	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
27	*Murraya koenigii*	Karive-ppu	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
28	*Murraya koenigii*	Karive-ppu	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
29	*Tamarind us indicus*	Puli	*Klebsiella* CSP	100	-	100	-	100	-	100	-	100	-
30	*Tamarind us indicus*	Puli	*Klebsiella* HCP	100	-	100	-	100	-	100	-	100	-

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel
S - Sensitive
Ms - Moderatively Sensitive

Table - 4.34

PERCENTAGE OF ANTIBACTERIAL ACTIVITIES OF FILTER STERILE AND AUTOCLAVED AQUEOUS EXTRACT OF VARIOUS PLANTS AGAINST PSEUDOMONAS ISOLOATED FROM CARDIAC SURGERY PATIENTS

Sl No	Botanical name	Local name	Type of extract	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Phyllanthes niruri*	Keezhar nelli	SAE	0	8	0	8	0	0	0	0	0	0
2	*Phyllanthes niruri*	Keezhar nelli	AAE	0	8	0	4	0	0	0	0	0	0
3	*Moringa Oleifera*	Muri-nga	SAE	0	8	0	0	0	0	0	0	0	0
4	*Moringa Oleifera*	Muri-nga	AAE	0	0	0	0	0	0	0	0	0	0
5	*Eucalyptus globulus*	Eucaly	SAE	0	0	0	0	0	0	0	0	0	0
6	*Eucalyptus globulus*	Eucaly	AAE	0	8	0	0	0	0	0	0	0	0
7	*Lawsonia inermis*	Maylan chi	SAE	0	0	0	0	0	0	0	0	0	0
8	*Lawsonia inermis*	Maylan chi	AAE	0	0	0	0	0	0	0	0	0	0
9	*Eupatoriu m urtici-folium*	C.pacha	SAE	0	0	0	0	0	0	0	0	0	0
10	*Eupatoriu m urtici-folium*	C.pacha	AAE	0	12	0	4	0	0	0	0	0	0
11	*Ocimum sanctum*	Thulasi	SAE	0	8	0	0	0	0	0	0	0	0
12	*Ocimum Sanctum*	Thulasi	AAE	0	12	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Type of extract	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Emilia sonchifolia*	Muyal chevian	SAE	0	0	0	0	0	0	0	0	0	0
14	*Emilia sonchifolia*	Muyal chevian	AAE	0	8	0	4	0	0	0	0	0	0
15	*Azadriacht a indica*	Veppu	SAE	0	0	0	0	0	0	0	0	0	0
16	*Azadriacht a indica*	Veppu	AAE	0	0	0	0	0	0	0	0	0	0
17	*Adhatoda vasica*	Adalod akam	SAE	0	0	0	0	0	0	0	0	0	0
18	*Adhatoda vasica*	Adalod akom	AAE	0	0	0	0	0	0	0	0	0	0
19	*Leucas aspera*	Thum-ba	SAE	0	0	0	0	0	0	0	0	0	0
20	*Leucas aspera*	Thum-ba	AAE	0	0	0	0	0	0	0	0	0	0
21	*Camelia sinensis*	Theyila	SAE	0	0	0	0	0	0	0	0	0	0
22	*Camelia sinensis*	Theyila	AAE	0	0	0	0	0	0	0	0	0	0
23	*Clerodend ron infort-unatum*	Perim-ala	SAE	0	0	0	0	0	0	0	0	0	0
24	*Clerodend ron infort-unatum*	Perim-ala	AAE	0	0	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Type of extract	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	
25	*Piper betle*	Vettila	SAE	0	0	0	0	0	0	0	0	0	
26	*Piper betle*	Vettila	AAE	0	0	0	0	0	0	0	0	0	
27	*Murraya koenigii*	Karive-ppu	SAE	0	0	0	0	0	0	0	0	0	
28	*Murraya koenigii*	Karive-ppu	AAE	0	0	0	0	0	0	0	0	0	
29	*Tamarind us indicus*	Puli	AAE	100	-	100	-	100	-	100	-	100	

SAE - Filter Sterile Aqueous Extract
AAE - Autoclaved Aqueous Extract
S - Sensitive
Ms - Moderatively Sensitive

Table - 4.35

PERCENTAGE OF ANTISTAPHYLOCOCCAL ACTIVITIES OF FILTER STERILE AQUEOUS EXTRACT OF VARIOUS INDIGENOUS SPICES

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Allium sativum*	Veluth-ulli	CNS/ CSP	100	-	84	12	52	32	12	20	-	16
2	*Allium sativum*	Veluth-ulli	CNS/ HCP	100	-	100	-	92	4	64	8	20	20
3	*Eugenia caryophyllata*	Gram bu	CNS/ CSP	8	32	4	36	0	8	0	4	0	0
4	*Eugenia caryophyllata*	Gram bu	CNS/ HCP	24	40	8	52	4	20	4	0	0	4
5	*Piper nigrum*	Kurum ulaku	CNS/ CSP	0	12	0	8	0	0	0	0	0	0
6	*Piper nigrum*	Kurum ulaku	CNS/ HCP	0	12	0	8	0	0	0	0	0	0
7	*Curcuma longa*	Kasthur imangal	CNS/ CSP	4	20	4	8	0	4	0	0	0	0
8	*Curcuma longa*	Kasthur imangal	CNS/ HCP	12	20	8	24	0	12	0	0	0	0
9	*Zingiber officinale*	Inchi	CNS/ CSP	4	12	0	4	0	0	0	0	0	0
10	*Zingiber officinale*	Inchi	CNS/ HCP	12	20	4	28	4	8	0	8	0	0
11	*Coriandrum sativum*	Malli	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
12	*Coriandrum sativum*	Malli	CNS/ HCP	0	0	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Cuminum cyminum*	Geerah	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
14	*Cuminum cyminum*	Geerah	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
15	*Brassica juncea*	Kaduk	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
16	*Brassica juncea*	Kaduk	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
17	*Capsicum annum*	Mulaku	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
18	*Capsicum annum*	Mulaku	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
19	*Cinnamo mum zeyl-anicum*	Pattah	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
20	*Cinnamo m um zeyl-anicum*	Pattah	CNS/ HCP	0	8	0	20	0	0	0	0	0	0

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel
S - Sensitive
Ms - Moderatively Sensitive

Table - 4.36

PERCENTAGE OF ANTISTAPHYLOCOCCAL ACTIVITIES OF AUTOCLAVED AQUEOUS EXTRACT OF VARIOUS INDIGENOUS SPICES

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	M %
1	*Allium sativum*	Veluth-ulli	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
2	*Allium sativum*	Veluth-ulli	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
3	*Eugenia caryophyllata*	Gram bu	CNS/ CSP	12	24	8	20	4	4	0	0	0	0
4	*Eugenia caryophyllata*	Gram bu	CNS/ HCP	20	32	8	28	4	20	0	0	0	0
5	*Piper nigrum*	Kurum ulaku	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
6	*Piper nigrum*	Kurum ulaku	CNS/ HCP	0	4	0	4	0	0	0	0	0	0
7	*Curcuma longa*	Kasthur imangal	CNS/ CSP	24	16	0	36	0	8	0	0	0	0
8	*Curcuma longa*	Kasthur imangal	CNS/ HCP	20	28	8	36	0	20	0	0	0	0
9	*Zingiber officinale*	Inchi	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
10	*Zingiber officinale*	Inchi	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
11	*Coriandrum sativum*	Malli	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
12	*Coriandrum sativum*	Malli	CNS/ HCP	0	0	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Cuminum cyminum*	Geerah	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
14	*Cuminum cyminum*	Geerah	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
15	*Brassica juncea*	Kaduk	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
16	*Brassica juncea*	Kaduk	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
17	*Capsicum annum*	Mulaku	CNS/ CSP	0	0	0	0	0	0	0	0	0	0
18	*Capsicum annum*	Mulaku	CNS/ HCP	0	0	0	0	0	0	0	0	0	0
19	*Cinnamomum zeyl-anicum*	Pattah	CNS/ CSP	8	16	0	20	0	0	0	0	0	0
20	*Cinnamomum zeyl-anicum*	Pattah	CNS/ HCP	8	24	0	24	0	0	0	0	0	0

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel
S - Sensitive
Ms - Moderatively Sensitive

Table - 4.37

PERCENTAGE OF ANTISTAPHYLOCOCCAL ACTIVITIES OF FILTER STERILE AQUEOUS EXTRACT OF VARIOUS INDIGENOUS SPICES

Sl No	Botanical Name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Allium sativum*	Veluth-ulli	*S.aureus* CSP	100	-	96	4	84	12	64	12	8	40
2	*Allium sativum*	Veluth-ulli	*S.aureus* HCP	100	-	100	-	96	4	64	4	24	20
3	*Eugenia caryophyllata*	Gram bu	*S.aureus* CSP	20	28	8	40	4	20	0	8	0	0
4	*Eugenia caryophyllata*	Gram bu	*S.aureus* HCP	4	36	4	32	4	4	0	4	0	0
5	*Piper Nigrum*	Kurum ulaku	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
6	*Piper Nigrum*	Kurum ulaku	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
7	*Curcuma Longa*	Kasthur imangal	*S.aureus* CSP	0	12	0	8	0	0	0	0	0	0
8	*Curcuma Longa*	Kasthur imangal	*S.aureus* HCP	0	32	0	28	0	4	0	0	0	0
9	*Zingiber Officinale*	Inchi	*S.aureus* CSP	0	8	0	8	0	0	0	0	0	0
10	*Zingiber Officinale*	Inchi	*S.aureus* HCP	4	16	0	0	0	0	0	0	0	0
11	*Coriandrum sativum*	Malli	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
12	*Coriandrum sativum*	Malli	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Cuminum cyminum*	Geerah	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
14	*Cuminum cyminum*	Geerah	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
15	*Brassica juncea*	Kaduk	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
16	*Brassica juncea*	Kaduk	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
17	*Capsicum annum*	Mulaku	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
18	*Capsicum annum*	Mulaku	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
19	*Cinnamomum zeyl-anicum*	Pattah	*S.aureus* CSP	0	24	0	24	0	0	0	0	0	0
20	*Cinnamom um zeyl-anicum*	Pattah	*S.aureus* HCP	0	24	0	24	0	0	0	0	0	0

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel
S - Sensitive
Ms - Moderatively Sensitive

Table - 4.38

PERCENTAGE OF ANTISTAPHYLOCOCCAL ACTIVITIES OF AUTOCLAVED AQUEOUS EXTRACT OF VARIOUS INDIGENOUS SPICES

Sl No	Botanical Name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Allium sativum*	Veluth -ulli	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
2	*Allium sativum*	Veluth -ulli	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
3	*Eugenia caryophyllata*	Gram bu	*S.aureus* CSP	24	28	16	36	12	4	0	4	0	0
4	*Eugenia caryophyllata*	Gram bu	*S.aureus* HCP	24	28	20	36	4	8	0	4	0	0
5	*Piper Nigrum*	Kurum ulaku	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
6	*Piper Nigrum*	Kurum ulaku	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
7	*Curcuma Longa*	Kasthu riman-gal	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
8	*Curcuma Longa*	Kasthu riman-gal	*S.aureus* HCP	0	12	0	12	0	0	0	0	0	0
9	*Zingiber Officinale*	Inchi	*S.aureus* CSP	0	16	0	16	0	0	0	0	0	0
10	*Zingiber Officinale*	Inchi	*S.aureus* HCP	0	16	0	16	0	0	0	0	0	0
11	*Coriandrum sativum*	Malli	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
12	*Coriandrum sativum*	Malli	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Cuminum cyminum*	Geerah	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
14	*Cuminum cyminum*	Geerah	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
15	*Brassica juncea*	Kaduk	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
16	*Brassica juncea*	Kaduk	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
17	*Capsicum annum*	Mulaku	*S.aureus* CSP	0	0	0	0	0	0	0	0	0	0
18	*Capsicum annum*	Mulaku	*S.aureus* HCP	0	0	0	0	0	0	0	0	0	0
19	*Cinnamo mum zeyl-anicum*	Pattah	*S.aureus* CSP	4	20	0	12	0	0	0	0	0	0
20	*Cinnamo mum zeyl-anicum*	Pattah	*S.aureus* HCP	8	24	0	20	0	4	0	0	0	0

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel
S - Sensitive
Ms - Moderatively Sensitive

Table - 4.39

PERCENTAGE OF ANTIBACTERIAL ACTIVITIES OF FILTER STERILE AQUEOUS EXTRACT OF VARIOUS INDIGENOUS SPICES AGAINST KLEBSIELLA

Sl No	Botanical Name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Allium sativum*	Veluth-ulli	*Klebsiella* CSP	52	36	44	28	8	24	0	8	0	0
2	*Allium sativum*	Veluth-ulli	*Klebsiella* HCP	50	36	7	14	0	7	0	0	0	0
3	*Eugenia caryophyllata*	Gram bu	*Klebsiella* CSP	0	20	0	16	0	8	0	0	0	0
4	*Eugenia caryophyllata*	Gram bu	*Klebsiella* HCP	0	7	0	7	0	0	0	0	0	0
5	*Piper Nigrum*	Kurum ulaku	*Klebsiella* CSP	0	8	0	8	0	0	0	0	0	0
6	*Piper Nigrum*	Kurum ulaku	*Klebsiella* HCP	0	21	0	14	0	0	0	0	0	0
7	*Curcuma Longa*	Kasthur imangal	*Klebsiella* CSP	0	8	0	0	0	0	0	0	0	0
8	*Curcuma Longa*	Kasthur imangal	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
9	*Zingiber Officinale*	Inchi	*Klebsiella* CSP	0	4	0	4	0	0	0	0	0	0
10	*Zingiber Officinale*	Inchi	*Klebsiella* HCP	0	7	0	7	0	0	0	0	0	0
11	*Coriandrum sativum*	Malli	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
12	*Coriandrum sativum*	Malli	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Cuminum cyminum*	Geerah	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
14	*Cuminum cyminum*	Geerah	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
15	*Brassica juncea*	Kaduk	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
16	*Brassica juncea*	Kaduk	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
17	*Capsicum annum*	Mulaku	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
18	*Capsicum annum*	Mulaku	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
19	*Cinnamo mum zeyl-anicum*	Pattah	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
20	*Cinnamo m um zeyl-anicum*	Pattah	*Klebsiella* HCP	0	21	0	0	0	0	0	0	0	0

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel
S - Sensitive
Ms - Moderatively Sensitive

Table - 4.40

PERCENTAGE OF ANTIBACTERIAL ACTIVITIES OF AUTOCLAVED AQUEOUS EXTRACT OF VARIOUS INDIGENOUS SPICES AGAINST KLEBSIELLA

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Allium sativum*	Veluth-ulli	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
2	*Allium sativum*	Veluth-ulli	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
3	*Eugenia caryophyllata*	Gram-bu	*Klebsiella* CSP	0	24	0	24	0	8	0	0	0	0
4	*Eugenia caryophyllata*	Gram-bu	*Klebsiella* HCP	0	14	0	14	0	0	0	0	0	0
5	*Piper nigrum*	Kurum ulaku	*Klebsiella* CSP	0	12	0	12	0	0	0	0	0	0
6	*Piper nigrum*	Kurum ulaku	*Klebsiella* HCP	0	14	0	14	0	0	0	0	0	0
7	*Curcuma longa*	Kasthur imangal	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
8	*Curcuma longa*	Kasthur imangal	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
9	*Zingiber officinale*	Inchi	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
10	*Zingiber officinale*	Inchi	*Klebsiella* HCP	0	4	0	4	0	0	0	0	0	0
11	*Coriandrum sativum*	Malli	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
12	*Coriandrum sativum*	Malli	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0

(Cont'd)

Sl No	Botanical name	Local name	Bacterial strain and Source of Isolation	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Cuminum cyminum*	Geerah	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
14	*Cuminum cyminum*	Geerah	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
15	*Brassica juncea*	Kaduk	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
16	*Brassica juncea*	Kaduk	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
17	*Capsicum annum*	Mulaku	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
18	*Capsicum annum*	Mulaku	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0
19	*Cinnamomum zeyl-anicum*	Pattah	*Klebsiella* CSP	0	0	0	0	0	0	0	0	0	0
20	*Cinnamom um zeyl-anicum*	Pattah	*Klebsiella* HCP	0	0	0	0	0	0	0	0	0	0

CSP - Cardiac Surgery Patients
HCP - Health Care Personnel
S - Sensitive
Ms - Moderatively Sensitive

Table - 4.41

PERCENTAGE OF ANTIBACTERIAL ACTIVITIES OF FILTER STERILE AND AUTOCLAVED AQUEOUS EXTRACT OF VARIOUS INDIGENOUS SPICES AGAINST PSEUDOMONAS STRAINS ISOLATED FROM CARDIAC SURGERY PATIENTS

Sl No	Botanical Name	Local Name	Type of extract	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
1	*Allium sativum*	Veluthulli	SAE	12	16	8	8	0	8	0	0	0	0
2	*Allium sativum*	Veluthulli	AAE	0	0	0	0	0	0	0	0	0	0
3	*Eugenia caryophyllata*	Grambu	SAE	0	12	0	8	0	0	0	0	0	0
4	*Eugenia caryophyllata*	Grambu	AAE	0	12	0	8	0	0	0	0	0	0
5	*Piper Nigrum*	Kurumulaku	SAE	0	0	0	0	0	0	0	0	0	0
6	*Piper Nigrum*	Kurumulaku	AAE	0	0	0	0	0	0	0	0	0	0
7	*Curcuma Longa*	Kasthuri mangal	SAE	0	0	0	0	0	0	0	0	0	0
8	*Curcuma Longa*	Kasthuri mangal	AAE	0	0	0	0	0	0	0	0	0	0
9	*Zingiber Officinale*	Inchi	SAE	0	0	0	0	0	0	0	0	0	0
10	*Zingiber Officinale*	Inchi	AAE	0	4	0	0	0	0	0	0	0	0
11	*Coriandrum sativum*	Malli	SAE	0	0	0	0	0	0	0	0	0	0
12	*Coriandrum sativum*	Malli	AAE	0	0	0	0	0	0	0	0	0	0

LIBRARY

(Cont'd)

Sl No	Botanical name	Local name	Type of extract	Concentration									
				50%		20%		10%		5%		2.5%	
				S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %	S %	Ms %
13	*Cuminum cyminum*	Geerah	SAE	0	0	0	0	0	0	0	0	0	0
14	*Cuminum cyminum*	Geerah	AAE	0	0	0	0	0	0	0	0	0	0
15	*Brassica juncea*	Kaduk	SAE	0	0	0	0	0	0	0	0	0	0
16	*Brassica juncea*	Kaduk	AAE	0	0	0	0	0	0	0	0	0	0
17	*Capsicum annum*	Mulaku	SAE	0	0	0	0	0	0	0	0	0	0
18	*Capsicum annum*	Mulaku	AAE	0	0	0	0	0	0	0	0	0	0
19	*Cinnamomum zeyl-anicum*	Pattah	SAE	0	0	0	0	0	0	0	0	0	0
20	*Cinnamom um zeyl-anicum*	Pattah	AAE	0	0	0	0	0	0	0	0	0	0

SAE - Filter Sterile Aqueous Extract
AAE - Autoclaved Aqueous Extract
S - Sensitive
Ms - Moderatively Sensitive

G. ***In vivo experiment***

In the experiment to study the *in vivo* anti-Staphylococcal effect of *A.Sativum* and *Tamarindus indicus* in comparison with the antibiotic Cefuroxime in experimental mice, it could be seen that in the first three groups which received Garlic, Tamarind and the antibiotic respectively, only one animal each in a group showed scanty growth of *S.aureus* strains in the intraperitoneal region (<10 CFU). The vehicle control which received distilled water, instead of the antibacterial agents, showed heavy growth of the same organism injected from intraperitoneal area of 2 animals. However in the control group heavy growth of the same organism inoculated were recovered from the intraperitoneal region of three animals (>100 CFU) (Graph 19).

The strain similarity of the injected and the reisolated strains were confirmed using the biochemical tests and the antibiogram pattern as per the standard procedures (Kirby-Bauer et al 1966, Tenover et al 1994 and Balows and Isenberg 1978). The bacterial strains isolated from the control and vehicle control groups are shown in Photographic Plate No: 16.

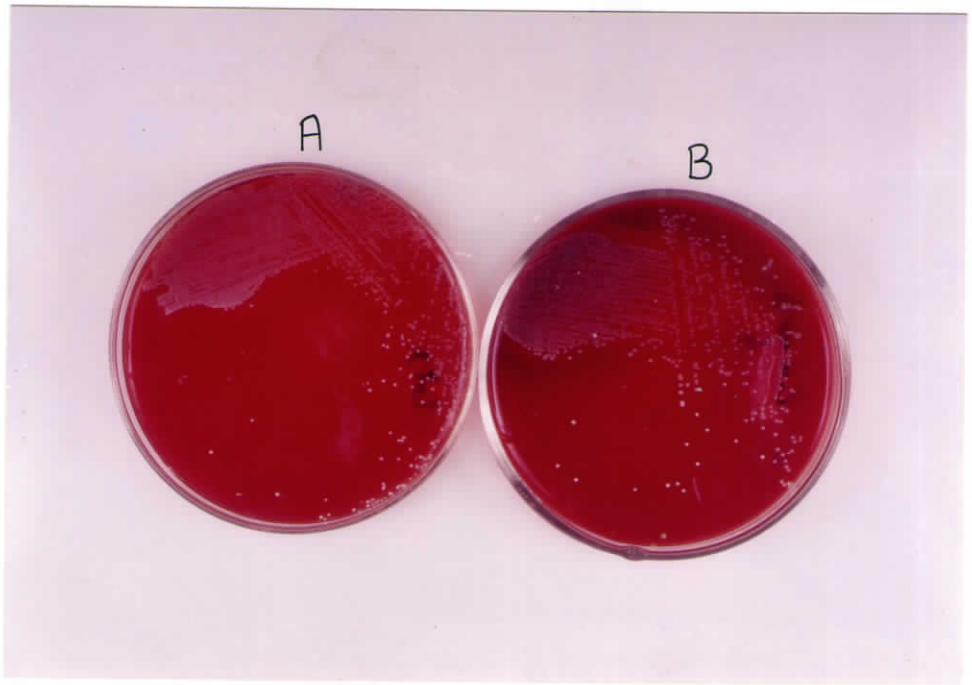
PHOTOGRAPHIC PLATE NO: 16

Re-isolated *S.aureus* strain from intra peritoneal area of mice in

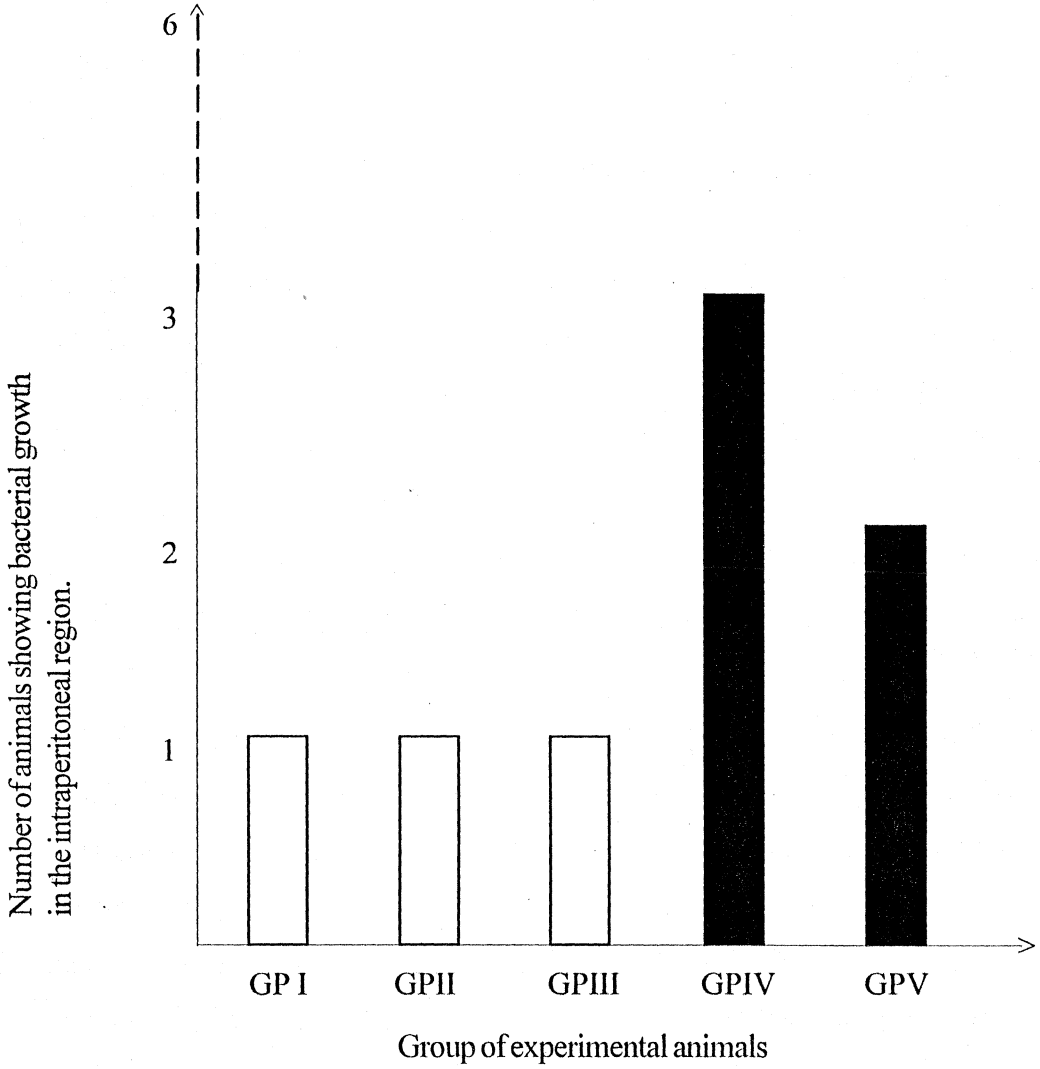
A. control group
B. Vehicle control group

PHOTOGRAPHIC PLATE NO: 16

Reisolated *S.aureus* strains from the Intra Peritoneal region in control group of experimental Mice



Graph - 19


6
3
2
1
Number of animals showing bacterial growth in the intraperitoneal region.
GP I
GPII
GPIII
GPIV
GPV
Group of experimental animals
Scanty growth of S.aureus strain in the I.P region.
Heavy growth of S.aureus strain in the I.P region

B. Discussion

It is well known that better management of hospital conditions will reduce the risk of hospital acquired infections. It is essential to identify the possible pathogens in the hospital environment, those from the patients, the health care personnel and the general environment. A proper and systematic screening of patients and health care personnel is indispensable to analyse the carrier state of pathogenic bacteria.

The results of this study on the carrier state of bacteria harboured in patients admitted for cardiac surgery clearly revealed that bacterial species isolated from throat, nose and sternal skin area of these patients are possible pathogens (Table 4.1). Of the seven species isolated *S.aureus*, Klebsiella, Beta-hemolytic Streptococci and *Ps.aeruginosa* are potential pathogens and others are opportunists. The major share of the 1160 strains isolated is Coagulase Negative Staphylococci followed by *S.aureus* and Klebsiella (Table 4.1)

Nearly 60% of the total isolate is from the nose (Table 4.2). This may be because of inaccessibility of the prophylactic antibiotic to reach the secretions in sufficient concentration. From Table 4.2 it is clear that CNS is the major isolate from all sites except throat. This observation is well in agreement with its existence as the major normal flora. Works conducted by Shanmugam et al (1994) and Needham and stempsey (1984) have also reported CNS as the major bacterial strain in the human normal flora. The incidence and distribution of various species of microorganisms on normal human skin have been described in detail in the symposium of Marples (1973). Woodroffe and Shaw (1976) have described that the majority of strains of skin Staphylococci are coagulase negative.

The reason why Klebsiella is the major isolate from throat is unknown. Similar to our study, Naseema et al (1988) and Mengistu and Gedebou (1986) have also reported the predominance of *Klebsiella* species in the throat. It is also interesting to note that *Klebsiella* species, *Ps.aeruginosa* and Beta-hemolytic Streptococci were not found in skin swabs in this study and the latter was found only in the throat swab (Table 4.2). The absence of these strains from the skin region correlates well with the study of Naseema et al (1988).

The fact that the percentage of possible pathogenic strains is significantly high in all patients on admission (Table 4.3) increases the probability of post-operative

endogenous infection and therefore reinforces the need for pre-operative screening.

It has to been noted that there is a tremendous decrease in the percentage of strains isolated in the post-operative period. This may be due to the prophylactic administration of antibiotics (Kriaras et al 1997 and Herbst et al 1989). In this context, it is also interesting to remind the observations of Kluge et al (1973) and Eigel et al (1978) that the bactericidal serum concentrations of antibiotics are maintained throughout the peri-operative period only if the drug is administered immediately before operation.

The total elimination of colonies from sternal skin swab is in perfect correlation with the application of povidone-iodine (a strong antibacterial agent) or other antiseptics at the sternal skin area. Studies of Farington (1988) and Gilmore (1977) have also shown such reduction with the use of such antibacterial agents.

It is worthwhile to analyse the relation of the rate of bacterial harbouring with age in the wake of contrasting reports. Age has been found to be an insignificant risk factor by Rutledge et al (1985) and Miholic et al (1986). But the study of Ulicny et al (1990) has found that age is a significant factor for development of sternal infection. The increased occurrence of bacterial harbouring in the younger age group is also suggestive of susceptibility to infection as with old age groups (Table 4.4).

The predominance of Beta-hemolytic Streptococci in throat swabs (Table 4.4) is coinciding well with the reports of Ruoff (1995), Cimolai et al (1991) and Hoffman et al (1990). This observation is of relevance as there is a possible threat of developing rheumatic heart disease in children as reported by Wessels and Kasper (1993) and Bisno (1991). In this study also the predominance of this strain is in children below 10 Years of age.

Demmy et al (1990) pointed out that male sex is a statistically independent predictor of sternal wound infection, but was opposed by Loop et al (1990) and Ulicny et al (1990). It is a known fact that smokers are more likely to get rid of the normal flora replacing them with the pathogenic bacteria which will proliferate and colonize the throat region. This is more commonly seen with males as compared to females as males are more used to smoking habit. In this study also (Tables 4.5,4.6 and 4.7), it is to be noted that the pathogenic bacterial harbouring in male subjects

is more than that of females, though the difference is not substantial.

It is interesting to note that 175 strains were isolated from 300 swabs from health care personnel against 1160 strains isolated from 3600 swabs from cardiac surgery patients making it clear that health care personnel are also carriers of these strains. Thus there is always a greater chance of cross infection from this exogenous source (health care personnel) as has been shown by various studies (Perdreau-Reming et al 1995, Berlau et al 1999 and Ayliffe et al 1998).

As revealed by several studies of El Oakley et al (1997), Kriaras et al (1997), Munoz et al (1997) and Marroni et al (1999) wound infection is a predominant post-operative infection in cardiac surgery patients. In this present study, 7.75% of post-operative wound infection was observed. (Table-4.11).

The Gram positive cocci were the major organisms found to be responsible for post operative wound infection which includes the CNS followed by *S.aureus* (Table-4.12). This correlates with the results of the carrier state study of same group of patients where nearly 79% of total organisms isolated were Gram positive cocci (Table 4.1)

It have been shown by several authors that the Gram positive cocci are the predominant pathogens responsible for post-operative wound infection. Sarr et al (1984) implicate Staphylococcus as the most common pathogen causing post-operative mediastinal infection which coincides well with our results where a major share of post-operative wound infection were found to be due to the Gram positive coccal organisms. Loop et al (1990) also got a similar result where Staphylococcus species were the leading organism in causing post-operative wound infection. Similarly in several other studies also Gram positive cocci were found to be the predominant pathogen. CNS was responsible for one half of sternal wound infection in the study of Kohman et al (1990), while *S.aureus* was the most commonly cultured organism in the study of Pairolero and Arnold (1984).

The Gram positive infections are more likely to result from intra operative contamination which could be derived mainly from patient, operating room personnel, instruments, Intravenous infusions and blood transfusions (Stiver et al 1979 and Weinstein et al 1976).

Since the same species of bacteria were isolated from pre-operative carrier state of patients (Table 4.1); Post operative carrier state of patients, carrier state of

health care personnel (Table 4.8) and also from specimens in the post-operative infected state of patients (Table 4.10), further studies are absolutely essential to establish the source.

The identification of source of infection and strains of bacteria to prevent the spread of infection requires typing methods to distinguish between the strains and to point to the source of infection. Typing systems are based on the principle that clonally related isolates share characteristics by which they can be differentiated from unrelated isolates (Arbeit 1995).

There are several evidences to show that the antibiogram pattern has been used as an important typing method in the epidemiologic studies to verify the strain similarity (Tenover et al 1994 and Jacques et al 1994). According to Mickelsen et al (1985), the relevance of antibiogram as a typing method increases especially when it is used in conjunction with other typing methods. According to Hedin (1996), the use of antibiogram for epidemiological typing is a natural first step in clinical practice. The antibiogram pattern of the bacterial strain from the carrier state and post-operative infection cases of the same patient in some cases indicated that they belong to the same strain (Plate 9 A and B; 10 D and E). However in some other cases, they gave different patterns revealing that the carrier strains and strains isolated from the specimens in the post-operative infected state of the same patient belonged to different strains (Plate 9 A and C; 10 D and F). The pattern of the bacterial strains isolated from the health care personnel showed dissimilarity with strains from post-operative infected specimens confirming that the health care personnel studied have not acted as the source of these strains.

Biotyping has been used as an important typing method in the epidemiological studies to verify the strain similarity (Aber and Mickel 1981, Christensen et al 1983, Maslow et al 1993, Coia et al 1990 and Philippe et al 1990). Biotyping in the broad sense, refers to any systematic method which will demonstrate phenotypic similarities or dissimilarities between two or more isolates that have been previously identified as belonging to same genus and species. Biotyping strengthens the validity of identification with antibiogram pattern. Biotyping involves comparison of biochemical or substrate utilization pattern and qualitative or quantitative measurement of metabolic end products. (Balows and Isenberg 1978). The biotyping results of this study clearly supports the interpretation derived from the antibiogram pattern. In

cases where the antibiogram pattern showed correlation between the carrier strain isolate and strain isolated from specimens in the post-operative infected state of the same patient, biotyping also showed similarity (Plate 11 A and 12 A) and in those strains where dissimilarity was observed, biotyping also showed dissimilarity (Plate 11 B and 12 B).

Bacteriocin typing increases the sensitivity of epidemiologic typing when used in combination with other typing systems such as antibiogram or biotyping as per the study of Ivannovics et al (1962), Fyfe et al (1984), Pitt (1988) and Pfaller (1995). The patterns of the bacteriocin typing (Plate 13) also confirmed that the strains which were found to be similar by antibiogram and biotyping belonged to same strain. With strains isolated from the carrier state of health care personnel and post-operative infected state of patient, the typing method gave inhibition pattern revealing that the strains are dissimilar.

Bacteriophage typing has been employed as an important typing method in epidemiologic studies especially the studies with strains of *S.aureus*. Several works are there employing bacteriophage typing as one of the typing method (Mulligan and Arbeit 1991, Blair and Williams 1961, Ten over et al 1994, Mulligan et al 1988, Mathew et al 1983). From the phage typing result (Table-4.13), it is quite obvious that, of the typable strains isolated from post-operative infected specimens, six showed correlation with the carrier isolates of the respective patients, three did not show correlation with carrier state isolates while four of the isolates were not typable.

Although phage typing has been used to type *S.aureus* for over 40 years, the emergence of Methicillin Resistance in *S.aureus* has resulted in limitations of phage typing for Methicillin Resistant *S.aureus* (Rossney et al 1994, Marples and Cooke 1988, and Witte et al 1988).

Poly Acrylamide Gel Electrophoresis has been used by several workers to find out the strain similarity in both Gram positive as well as in Gram negative organisms (Arbeit 1995 and Mulligan et al 1988). The band pattern observed with this typing method (Plate 14) has also helped to find out the strain similarity with the strains isolated from carrier state and post-operative infected state of the patient.

(Hedin 1996) is of the opinion that while typing the Gram positive isolates, Slime production can also be considered as an important typing method to confirm the strain similarity. Similarity in slime production among strains isolated from the

carrier state and post-operative infected stated of the same patient were considered as belonging to same strain (Plate15A and 15B).

It has been proposed by Arbeit (1995) that similarity in three or more typing methods can be considered as belonging to same strain. From the summary of the results of various typing methods (Table 4.14), it is quite clear that there is absolutely no strain correlation with carrier state and post-operative infected state in the case of Urinary Tract Infection . In the wake of the observation that there is no correlation with the strains from infected state of patients and those from carrier state of health care personnel, it can be concluded that the source of the strains responsible for UTI in post-operative infections is entirely exogenous, other than the hospital health care personnel.

In case of Respiratory Tract Infection and Bacteremia, Correlation / no-correlation studies have shown that the infection was only partly exogenous. Of the Correlation observed with strains isolated from carrier state and post-operative infected state, a major share was totally endogenous and the source of the rest could either be exogenous or endogenous (Table 4.14).

It is well known that ICU environment is heavily laden with pathogens as compared to other areas in the hospital. Reports by Mc Gowan et al (1989) and Bryce and Smith (1995) also support this view. Respiratory therapy equipment, prolonged use of *in situ* invasive devices, devices for assessing respiratory functions especially those having water reservoir which may harbour the hospital pathogens-all make the ICU environment an ideal area for developing infections in the hospitalized patients. This has been further proved by studies of Donowitz et al (1982), Lennox et al (1997) and Jarvis et al (1991).

Sternal wound infection is the most threatening of all hospital acquired infections in case of cardiac surgery patients. It is now well known that patient's own flora form a major factor in case of wound infection associated with surgery which has been supported by many studies (Klutyman et al 1995 and Wenzel and Perl 1995). One notable point from the correlation/ no-correlation study is that most of the strains isolated were found to have correlation with the carrier state suggestive of high endogenous chance. However a minor share showed correlation with post-operative carrier state suggestive of the source of organism as either exogenous or endogenous (Table- 4.14).

It is interesting to note that none of the specimens showed correlation with strains isolated from health care personnel (Table-4.14). Though there is a high chance of exogenous infection from health care personnel, as shown by various studies (Perdreaiu - Reming et al 1995, Berlau et al 1999 and Stubbs et al 1994) our data shows that the cross contamination has not occurred from the health care personnel studied. This may be because of the good hospital policy and strict precautionary measures.

Thus it can be concluded that though there are pathogenic bacteria present in samples from carrier state and post-operative infected state of patients and in health care personnel, the correlation / no-correlation study of the strains have revealed that health care personnel are not responsible for these infections. A strict vigil on the carrier state of patients is of atmost importance since the infection is found to be partly endogenous. This is especially true in the case of wound infection, where the chance of infection is found to be mostly endogenous. Thus pre-operative screening and subsequent antibiotic administration is a pre-requisite to prevent the outbreak of post-operative infections.

VIRULENCE AND DRUG RESISTANCE

The control of spread of infection requires an understanding of the species responsible, its virulence and its antibiotic susceptibility pattern. Thus species identification from the carrier state and infected state of patient and from health care personnel is inevitable. This will further help in finding out the exact source of infection which will inturn help in totally erradicating it within the minimum possible time. Therefore a screening was done accordingly (Tables 4.15, 4.16 and 4.17). Though different species of CNS and Klebsiella were isolated from carrier state of patients and health care personnel, only one species of CNS (*S.epidermidis*) and one species of Klebsiella (*K.pneumoniae*) were isolated from the specimens in the post-operative infected state of the patients studied (Table 4.18).

CNS once considered as the nonpathogenic normal flora of humans are gaining importance nowadays, as reports are there, regarding increasing cases of infections caused due to certain species of CNS. This is especially true in cardiac surgery patients where sternal wound infections are known to be caused by the CNS species. Studies which have showed the CNS as a pathogen causing serious threat

to patients includes the work of Patrick (1990), Boyce et al (1990) and Patricia et al (1985).

Narayani et al (1988) who worked on the speciation of CNS have found that 49.1% of healthy carriers and 39% of patient isolates belonged to *S.epidermidis* whereas Shanmugam et al (1994) have found it as 49% and 32.1% respectively. Naseema et al (1988) reported that among CNS, the leading species in the carrier state of hospital personnel in their study were *S.epidermidis*. According to Nystrom (1992) the increased rate of prevalence of this strain among patients gives an idea on the continuous persistence of them in the hospital environment. This is evident from the repeated isolation and increased multiple drug resistance, prevailing among the strains isolated from clinical specimens and also from carrier state. The prevalence of *S.epidermidis* as the leading species in our study is in agreement with the reports of Thurn et al (1992) who found the same species as the leading one 89.1% in his study.

An elaborate study on the antibiotic susceptibility of the strains isolated from carrier state of patients and health care personnel have revealed that the major strains such as Klebsiella, *S.aureus* and CNS to some extent have shown resistance to several antibiotics used. (Table 4.19 and 4.20). However a pertinent point to be noted is that the strains from the health care personnel showed greater sensitivity when compared to that from patients indicative of the fact that the highly drug resistant strains in patients might have developed due to the previous administration of antibiotics. It is a known fact that the administration of antibiotics will increase the chance of resistant bacteria to get colonized removing the normal sensitive strains (Sindhu et al 1997).

The CNS strains isolated from carrier state of health care personnel and cardiac surgery patients showed more sensitivity than the *S. aureus* and Klebsiella strains as evident from Tables 4.19 and 4.20. The high rate of sensitivity shown towards Vancomycin is indicative that Vancomycin resistant strains have not yet appeared in our hospital. On the contrary the high rate of resistance shown towards Penicillin by the strains of CNS and *S.aureus* (Tables 4.19 and 4.20) may be accounted for by many reasons including the production of beta-lactamase or penicillinase (Bauern-feind 1986 and Antone 1997).

The premier antibiotic Penicillin unfortunately is the first among antibiotics

which became ineffective to most of the bacterial strains due to widespread and prolonged usage. It has already been demonstrated by many workers (Rathinam and shanmugam 1994 and Lowry and Hammer 1983) that penicillin is the least effective antibiotic to treat CNS species.

The Beta-lactamase production of CNS species from health care personnel was found to be slightly more than cardiac surgery patients (Tables -4.21, 4.22, 4.23, and 4.24). This may be because chances of the hospital strains to come in contact with lower doses of drugs are more than the rest of the community leading to a substantial increase in the potency of the enzyme produced. In the study of Shanmugam and Beena Panicker (1996), 95.5% positivity of beta-lactamase production was obtained with CNS strains isolated from health care personnel which is comparable with the present study where 94.57% of positivity was obtained with CNS strains isolated from health care personnel (Table 4.24). Bhatia et al (1982) have found 96% of strains tested by them as beta-lactamase producers. Horton et al (1982) have found all the Penicillin resistant strains in their study to be beta-lactamase producers. According to Elizabeth et al (1970) and Horton et al (1982), the beta-lactamase negative strains on exposure to small amount of certain drugs may transform to strongly beta-lactamase producing strains.

It is clear that, of the four different acidimetric methods adopted (Tables 4.21 and 4.22) Phenol Red filter paper method is found to be the most sensitive for the detection of beta-lactamase and hence this method is advisable for routine screening of penicillin resistance. But it has to be kept in mind that the exact amount proportional to the resistance could not be accounted with phenol red as indicator and hence grading is not possible; while bromocresol purple indicator can be used for grading although the latter is found to be the less sensitive than the previous one.

Of the various methods employed for the detection of beta-lactamases among *S.aureus* and CNS strains isolated from carrier state of cardiac surgery patients and health care personnel, the iodometric method seemed to be slightly better in terms of sensitivity when compared to other methods. This is true especially in iodometric test tube and filter paper method (Table 4.23 and 4.24).

The prevalence of virulent strain is relevant in any infection. The production of slime is a reliable marker of virulence according to Davenport et al (1986). Etessi Carssenti et al (1990) have reported that slime producers get colonized more than

non-slime producers.

The slime offers protection from host's immune response, acts as a protective layer against the attack of antimicrobial agents and protects the bacteria from being phagocytosed (Gray et al 1984 and Johnson et al 1986). Since all the *S.aureus* strains isolated from the specimens in the post-operative infected state are totally slime producers (Table 4.25), and in the wake of the observation that the rate of the slime positive *S.aureus* strains is considerably higher in carrier state of patients when compared to health care personnel (Tables 4.26 abd 4.27), it is reasonable to assume that there might be a chance of antibiotic induced slime production in these strains (Sindhu et al 1999).

Nayak et al (1990), Rathinam et al (1994) and Makhija et al (1995) found that most of the species of CNS in their study were slime positive except *S.xylosus* which showed negative responses to slime. Rathinam and Shanmugam (1995) detected slime production by 3 methods; viz, test tube, microplate and Congo Red Agar plate method. Maximum positivity for slime detection was found with Congored Agar Plate method in their study similar to the results of our study. Freeman et al (1989) Congo Red Agar Plate method proved to be more sensitive method than the Christensen et al (1983) test tube and micro plate method (Table 4.26 and 4.27). This may be due to potential possibility of the removal of slime while emptying the culture medium initially in test tube method or after staining and/or due to failure of slime sticking to well or wall surface of test tube or floor surface of microwells. In Congo Red Agar plate methods these draw backs won't happen. Moreover different degrees of positives can be recorded. Again contamination if any can easily be recognized in this method and the reproducibility is far better than the other techniques.

Opportunistic pathogens among the patients own body flora (endogenous) is found to be a major threat in cardiac surgery patients especially in wound infection. Pre-operative screening and subsequent prophylactic antibiotic administration is the only way out to avoid such endogenous infections. This require previous knowledge about the appropriate dose of the specific antibiotics to be used. Thus determination of the minimum concentration of antibiotic required for the bactericidal activity becomes essential. Therefore in the wake of the findings that CNS, *S.aureus* and Klebsiella are the major potential pathogens in the carrier state as well as in infected cases, Minimum Inhibitory Concentration (MIC) for various antibiotics against these

strains were found out.

From the MIC values, it can be inferred that for CNS, the best antibiotic of choice is Amikacin followed by Cefuroxime (Graph 13 and 9) while for *S.aureus* it is Cloxacillin followed by Tobramycin and Cefuroxime (Graph 4,6 and 10). In case of Klebsiella, the antibiotic of choice is Amikacin followed by Tobramycin and Augmentin (Graph 15,7 and 12). Thus it can be suggested that Amikacin and Tobramycin can be used as the potential prophylactic antibacterial agents. But it has to be kept in mind that they cannot be administered to the patients prophylactically as these drugs have high nephrotoxicity (Joseph and Robert 1995). Therefore the best antibiotic of choice available is Cefuroximes as also supported by various studies of Kriaras et al (1997), Gorbasch (1982), Vuorisalo et al (1997) and Ariano and Zhand (1991). For Gram positive organisms Cloxacillin has also proved to be a promising drug especially for *S.aureus.*

Various studies have been conducted to find out an appropriate antibiotic that could be administered as a prophylactic means in order to reduce patient morbidity and mortality, to shorten the hospital stay and also to lower hospitalisation costs. A second generation cephalosporin is usually selected because of its low toxicity, increased stability to beta-lactamase enzymes, broad antibacterial spectrum and good tissue penetration. Works by Hall et al (1993) ,Gorbasch et al (1982) and Nooyen et al (1994), also support the above statement. Ariano and Zhand (1991) stated that although clinical studies have not demonstrated any significant differences with in the cephalosporin class of antimicrobials, the trend has seemed to favour Cefuroxime that can be administered for preventing the post-operative wound infections in patients undergoing open surgery.

The observation that bacterial strains isolated from health care personnel are having less MIC values as compared to that isolated from cardiac surgery patients, inturn means that the strains isolated from health care personnel are more sensitive to antibiotics than the strains isolated from cardiac surgery patients. This observation is well in agreement with results of the antibiotic susceptibility test using kirby-Bauer method. (Table 4.19 and 4.20).

Thus it can be concluded that prophylactic administration of Cefuroxime and Cloxacillin can be advised for patients undergoing open heart surgery to reduce the endogenous infection to a certain extent, there by reducing the rate of morbidity and

mortality, reducing the cost for patient care and shortening the hospital stay of the patient.

Most of the existing pathogenic strains of bacteria have acquired resistance towards most of the antibiotics in current use and has resulted in the subsequent proliferation of the new resistant strains. Since the major nosocomial pathogens had developed very high resistance towards many antibiotics available; to overcome the threat of this increasing drug resistance among pathogenic strains, it is the need of the hour to find out a new effective antibacterial agent which is costeffective, and less toxic from traditional origin.

WHO reports that nearly 80% of people around world even now depend on traditional medicines for cure of various ailments (Vijaya and Ananthan 1994). Several works have been conducted to find out the antibacterial activity of a few plants and spices (Atal et al 1978, Cacerer et al 1991, Valsaraj et al 1997, Pushpangadan and Atal 1984, Rios et al 1998, Kumar et al, 1998, Naqvi et al 1991, Ankiri et al 1997, Farbman et al 1993).

From the Screening study of a few medicinal plants and spices of Indian origin, to find out their antibacterial activity in the present study, promising results are obtained with the filtered sterile aqueous extract of *Allium sativum, Moringa oleifera, Eucalyptus globulus, Phyllanthes niruri (amarus*) and the autoclaved extract of *Tamarindus indicus, Lawsonia inermis* (Table 4.28 to 4.41). All of these hold great promise for the future researchers to explore in this field so as to find new effective, less toxic, cost effective antibacterial agents that can be given safely to these patients as prophylactic means.

***In vivo* experiment**

Intraperitoneal region is a sterile region where no bacteria could be isolated in the healthy state (Wilson 1992). From the *in vivo* experiment to find out the antibacterial activity of *A.Sativum* and *T.indicus* in comparison with the antibiotic Cefuroxime, it is clear that the group where no antibacterial agents were given, the injected strain had shown a tendency to establish themselves in the intraperitoneal region which may cause further complications. But in the groups, where the antibacterial agents such as *A.sativum* and *T.indicus* and the antibiotic Cefuroxime were given, do not favoured the growth of these bacterial strains injected indicating that these are having good anti Staphylococcal activity again confirming the *in vitro* study.

CHAPTER V

SUMMARY

The proposed study mainly aimed at

a. Understanding the rate of carrier state of potential pathogenic bacteria in patients undergoing open heart surgery

b. To correlate the incidence of post-operative wound infections with the carrier state and

c. To suggest effective control measures in order to reduce such endogenous infections.

To reduce the risk of hospital acquired infections and for better management of hospital conditions, an understanding of the possible pathogens in the hospital environment is essential. Thus a proper and systematic screening of patients and health care personnel is indispensable for understanding the carrier state of pathogenic bacterial harbouring.

Since post-operative wound infection has been found as the most serious complication associated with patients undergoing cardiac surgery and in the wake of the fact that patients own flora is a high risk factor in case of wound infection associated with surgery, systematic screening of pathogenic bacteria can provide essential information necessary for reducing the risk of infection.

Of the total four hundred patients screened for understanding the carrier state of bacterial harbouring, a total of 1160 bacterial strains belonging to seven species were isolated. More than half of the total isolate were from nose. The majority of the bacterial strain isolated belonged to CNS followed by *S.aureus* and Klebsiella.

The fact that the percentage of possible pathogenic strains in all cases of admission is significantly higher increases the probability of post-operative endgenous infection and therefore the observation reinforces the need for pre-operative screening. But on post-operative periods, towards the day of discharge there is a tremendous reduction with almost to a level of total elimination especially in the case of all post-operative skin swabs except the CNS.

Since health care personnel studied also harboured comparatively higher rate of bacteria in the carrier state, and in the wake of the fact that some of the species are pathogenic and was also isolated from cardiac surgery patients, the question of source of infection is gaining importance. Thus to examine whether the risk of post-operative infection is predominantly from exo or endogenous source, samples were collected from post-operative infection cases of the patient and strains isolated were compared for correlation with the carrier state isolate of the same patient.

The Gram positive cocci were the major organism found to be responsible for post-operative wound infection which includes the CNS followed by *S.aureus.*

Since the same species of bacteria were isolated from pre-operative carrier state of patients, post-operative carrier state of patients, carrier state of health care personnel and also from post-operative infected specimens, further studies are absolutely essential to establish the source; which requires the use of discriminatory typing systems to understand the dynamics of the problem and also to find out the source and similarity of the strain.

The different typing systems employed in the study includes **Antibiogram, Biotyping, Bacteriocin typing, Bacteriophage typing, Slime production** and **Poly Acrylamide Gel Electrophoresis.**

Of the four hundred patients studied 73 (18.25%) developed post-operative infections which includes UTI (2.5%), RTI (6.75%), Bacteremia (4.5%) and Post-operative Wound infection (7.75).

From the correlation/no-correlation study, the source of strain responsible for UTI is found to be entirely exogenous. In case of RTI and Bacteremia, correlation/no correlation studies have shown that the infection is partly exogenous and partly endogenous where as in the case of post-operative wound infection, from the correlation/no- correlation study it could be seen that most of the strains isolated were found to have correlation with carrier state suggestive of high endogenous chance and least exogenous chance.

None of the strains isolated from the post-operative infected state of the patient showed correlation with strains isolated from health care personnel. However a strict vigil on the carrier state of patients is of atmost importance since infection is found to be partly endogenous in RTI and Bacteremia and mostly endogenous in post-operative wound infection.

Thus pre-operative screening and subsequent antibiotic administration is a pre-requisite to prevent the outbreak of post-operative infections.

The control of outbreak of infection requires an understanding of the species responsible, its virulence and its antibiotic susceptibility pattern. Thus species identification from carrier state and from infected state is inevitable to find out the exact source of infection. More over speciation has gained much importance now a days, as many bacterial species, which were once considered as non pathogenic and constituent of normal flora of man, are gaining importance causing serious infections to patients due to the emergence of multiple antibiotic resistance among them.

S.epidermidis was the leading species isolated among CNS, *K.pneumoniae* among Klebsiella and *A.calcoaceticus* among Acinetobacter strains isolated from carrier state of patients.

The strains isolated from health care personnel showed more sensitivity than the strains from cardiac surgery patients. The *S.aureus* and Klebsiella strains isolated from cardiac surgery patients showed increased resistance to antibiotics.

The beta-lactamase production in health care personnel were found to be slightly more than cardiac surgery patients in this study. Acidimetric phenol red filter paper method, Iodometric test tube and filter paper methods were found to be the most sensitive methods for detection of beta-lactamase based on the study and hence the methods are advisable for routine screening.

From the studies on MIC of six antibiotics against the leading pathogens, the results concluded that the best antibiotic of choice is the Amikacin or Tobramycin. But due to its nephrotoxicity they cannot be administered prophylactically to patients. Therefore the best antibiotic of choice that can be given is Cefurorime and for *S.aureus* Cloxacillin also stands good that can be given prophylactically for patients undergoing open heart surgery to reduce the endogenous infection to a certain extend there by reducing the rate of morbidity and mortality, cost of patient care and shortening the hospital stay of the patient.

Since most of the existing pathogenic strains of bacteria have acquired resistance towards most of the antibiotics in current use, it is the need of the hour to find out a new effective antibacterial agent which is cost effective and less toxic from the traditional origin.

In this context, the results obtained with the preliminary screening of some Medicinal plants and Indigenous spices to find out their antibacterial activity holds promise. Fairly good antibacterial activity of filtered sterile aqueous extracts of *Allium sativum, Moringa oleifera, Eucalyptus globulus, Phyllanthes niruri* and autoclaved extract of *Tamarindus indicus, Lawsonia inermis* - all holds great promise for the future researchers to explore in this field so as to find out a new effective, less toxic, cost effective antibacterial agent that can be given safely to these patients as a prophylactic means.

CONCLUSION

1. A total of 1160 bacterial strains belonging to seven species were isolated from the throat swab, nasal swab and sternal skin swab of 400 cardiac surgery patients screened to understand the rate of bacterial harbouring.
2. The leading bacterial strains identified from cardiac surgery patients were CNS followed by *S.aureus* and *Klebsiella* species, of which CNS and *S.aureus* were mainly isolated from nose and Klebsiella from throat.
3. During hospitalization, a marked reduction of bacteria in skin carriage was observed (from 86.07% on the day of admission to 5.36% on the day of discharge from the hospital).
4. β-hemolytic *Streptococci* and *Ps.aeruginosa* were not isolated from health care personnel.
5. 18.25% of patients developed post-operative infections including UTI (2.5%), Bacteremia (4.5%), RTI. (6.75%) and Wound Infection (7.75%).
6. Gram positive organisms (70%) were mainly responsible for causing post-operative wound infection of which 38% belonged to CNS strains and 32% belonged to *S.aureus.*
7. From correlation / no correlation study, based on different typing methods, the source of strain causing UTI were found to be purely exogenous, partly endogenous in case of bacteremia and RTI and mainly endogenous in case of post-operative wound infection.
8. *S.epidermidis* was the leading isolate among the CNS strains isolated from both groups (40.80% and 44.14% from cardiac surgery patients and health care personnel respectively.
9. Unlike the carrier state, only one species of CNS and one species of Klebsiella could be isolated from specimens in the post-operative infected state.
10. The strains isolated from carrier state of health care personnel showed more "sensitive" than the strains isolated from carrier state of cardiac surgery patients.
11. 80.12% of CNS and 96.27% of *S.aureus* strains isolated from patients and 87.38% of CNS and 96.30% of *S.aureus* strains isolated from health care personnel were beta-lactamase producers.

12. Acidimetric phenol red filter paper method and Iodometric filter paper and test tube methods are the most sensitive methods for beta-lactamase detection.

13. 63.47% of CNS and 92.14% of *S.aureus* isolated from patients and 69.36% of CNS and 77.77% of *S.aureus* isolated from health care personnel were slime producers.

14. Of the six antibiotics tested for MIC, Cefuroxime and Cloxacillin proved to be the drug of choice for prophylaxis.

15. A correlation of the incidence of post-operative wound infection with carrier state clearly revealed that over half the incidence of post-operative infection is endogenous. Thus screening the patients pre-operatively and subsequently prophylactic administration of proper antibiotic is of great importance to reduce the chances of post-operative infection. Thus it can be suggested that the antibiotic susceptibility screening of the potential pathogenic bacteria pre-operatively is preferable in deciding the specific antibiotic in its proper dose. This will help in ensuring the reduction in hospital stay and expenses with lower morbidity and mortality which is the ultimate aim of medical science.

16. In the wake of increasing multiple drug resistance among the nosocomial pathogens, screening for new antibacterial agents is of utmost relevance. In this context, the promising antibacterial activity of filtered sterile aqueous extract of *Allium sativum*, *Moringa oleifera*, *Eucalyptus globulus*, *Phyllanthes niruri* and the autoclaved extract of *Tamarindus indicus* and *Lawsonia inermis* offers promise.

17. In the *in vivo* experiment to find out the anti-Staphylococcal activity of *A. Sativum* and *T. indicus* in comparison with the antibiotic Cefuroxime in experimental mice, it could be seen that in groups where these antibacterial agents were not given had shown a tendency to establish themselves in the intraperitoneal region, where as in test groups where these agents were given do not favoured the growth of the bacterial strains injected indicating that *A. Sativum* and *T.indicus* are having good anti-Staphylococcal activity again confirming the *in vitro* study.

RECOMMENDATIONS

1. Since the percentage of possible pathogenic strains in all cases of admission has been found to be significantly higher, in turn indicates increased probability of developing post-operative endogenous infection and therefore the observation reinforces the need for pre-operative screening.
2. A strict vigil on the carrier state of patients is of utmost importance since infection is found to be partly endogenous in RTI and Bacteremia and mostly endogenous with case of W.I.
3. Since the control of outbreak of infection requires an understanding of the species responsible its virulence and its antibiotic susceptibility pattern the species identification is inevitable and should be done routinely in the clinical laboratories to find out the exact source of infection.
4. The study indicates the alarming rate of beta-lactamase positive Staphylococcal species prevalent among the patients and health care personnel, indicating the problem of treatment by beta-lactamase susceptible antibiotics. Hence the study demands the need for routine testing of betalactamase in microbiology laboratories.
5. Acidimetric phenol red filter paper method, Iodometric filter paper and test tube method are the most sensitive methods found for detecting beta-lactamase.
6. Detection of slime will help to get a knowledge about the prevalence of virulent strains and the Congo Red Agar plate method is the best method for detection of slime.
7. Based on the study it is advisable to give Cefuroxime or Cloxacillin as a prophylatic drug to the patient undergoing cardiac surgery so as to reduce post-operative wound infection.
8. The promising results obtained from some medicinal plants and indigenous spices on screening for their antibacterial activity is an opening door for the future researchers to explore and to find out better less toxic cost effective new drugs of traditional and natural origin that can be given to these patients.

9. Studies carried out in connection with this thesis work recommend strongly for the interdisciplinary research activity involving the expertise of microbiologists, clinicians and health care personnel associated with cardiac surgery ward/ICU's in order to reduce the hospital stay and cost involved for the patient care to a certain extend as well as to reduce the rate of morbidity and mortality caused due to such endogenous infection which is ultimate aim of medical science.

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APPENDIX - A

LIST OF PUBLISHED PAPERS

1. SINDHU CP, SHANMUGAM J, SANKAR KUMAR R, MOLLY ANTONY, MOHANSINGH MP, VIJITH KC : " ANTIBIOGRAM PATTERN OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM CARDIAC SURGERY PATIENTS DURING PRE AND POST OPERATIVE PERIODS". *BIOMEDICINE*, 1997; 17 : 63-70.

2. SINDHU CP and SHANMUGAM J : "BETA-LACTAMASE PRODUCTION BY *STAPHYLOCOCCUS AUREUS* AND COAGULASE NEGATIVE STAPHYLOCOCCI-DETECTION BY DIFFERENT IODOMETRIC AND ACIDIMETRIC TECHNIQUES". *BIOMEDICINE*, 1998; 18 : 74-80.

3. SINDHU CP and SHANMUGAM J : "CORRELATION BETWEEN SLIME PRODUCTION AND MULTIPLE ANTIBIOTIC RESISTANCE IN *STAPHYLOCOCCUS AUREUS*". *BIOMEDICINE*, 1999; 19 : 171-179.

APPENDIX - B

LIST OF PAPERS PRESENTED IN CONFERENCES

1. SINDHU CP, SHANMUGAM J, MOLLY ANTONY, SANKAR KUMAR R, MOHANSINGH MP.
"CARRIER STATE OF PATHOGENIC BACTERIA AMONG CARDIAC SURGERY PATIENTS ON ADMISSION, AFTER SURGERY AND AT DISCHARGE FROM THE HOSPITAL".
FIRST INTERNATIONAL CONFERENCE OF THE "FEDERATION OF ASIA-PACIFIC MICROBIOLOGY SOCIETIES" (FAPMS), MAY 24-27. 1998, SINGAPORE. ABST NO: 96; P : 123.

2. SINDHU CP and SHANMUGAM J.
"COMPARISON OF BETA-LACTAMASE PRODUCTION BY STAPHYLOCOCCAL SPECIES ISOLATED FROM CARDIAC SURGERY PATIENTS AND HEALTHY PERSONNELS BY DIFFERENT IODOMETRIC AND ACIDIMETRIC TECHNIQUES".
19th ANNUAL CONFERENCE OF "THE INDIAN ASSOCIATION OF BIOMEDICAL SCIENTISTS ON 30-31st OF OCT AND 1st OF NOV: 1998 AT KASTURBA MEDICAL COLLEGE, MANGALORE. ABST NO: Fg1, p.28.

3. SHANMUGAM J, SINDHU CP and SANKAR KUMAR R.
"STUDY OF CARRIER STATE OF PATHOGENIC BACTERIA IN CARDIAC SURGERY PATIENTS AND HOSPITAL PERSONNELS".
XXII NATIONAL CONGRESS OF THE INDIAN ASSOCIATION OF MEDICAL MICROBIOLOGISTS NOV 7-9, 1998 AT MANIPAL, INDIA. ABST NO: 006, p:3.

APPENDIX - B

LIST OF SEMINAR/SYMPOSIA ATTENDED

1. PARTICIPATED IN THE **WORK SHOP** CONDUCTED BY THE NATIONAL INSTITUTE OF COMMUNICABLE DISEASES (GOVT: OF INDIA) - WHO TRAINING COURSE ON "ANTIMICROBIAL RESISTANCE MONITORING" HELD AT SCTIMIST, THIRUVANANTHAPURAM FROM 8/6/99 TO 12/6/99.

2. ATTENDED THE **NATIONAL CONFERENCE** ON "MOLECULAR DIAGNOSTICS" ORGANISED BY DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF KERALA FROM 27th TO 29th JUNE 1998.

3. ATTENDED THE **REGIONAL SYMPOSIUM** "AN UPDATE ON ANTIBIOTICS-CURRENT STRATEGIES AND FUTURE PERSPECTIVES" ORGANISED UNDER THE AUSPICES OF THE KERALA CHAPTER OF THE INTERNATIONAL MEDICAL SCIENCES ACADEMY IMSA ON 14th OF JUNE 1997 AT SCTIMST, THIRUVANANTHAPURAM-695011, INDIA.

4. ATTENDED THE **SEMINAR** ON "MEDICINAL PLANTS" SPONSORED BY STED GOVT: OF KERALA PHARMACY GRADUATES ASSOCIATION HELD ON 24th APRIL 1997 AT COLLEGE OF PHARMACEUTICAL SCIENCES, THIRUVANANTHAPURAM.

5. ATTENDED THE **INDO-UK SYMPOSIUM** ON "HOSPITAL INFECTIONS IN ICUs AND CONTROL", HELD AT SCTIMST, THIRUVANANTHAPURAM, INDIA ON 17th AND 18th OF JANUARY 1996.