COMPARATIVE STUDY OF INDUCIBLE AND CONSTITUTIVE CLINDAMYCIN RESISTANCE AMONG METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES

Dissertation Submitted in

Partial Fulfillment of the Regulations required for the award of

M.D.DEGREE

in

Microbiology – Branch IV

The Tamil Nadu

Dr.M.G.R.Medical University

Chennai

April – 2013

CERTIFICATE

This is to certify that the enclosed work **"COMPARATIVE STUDY OF INDUCIBLE AND CONSTITUTIVE CLINDAMYCIN RESISTANCE AMONG METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES"** submitted by Dr V Aruna to The Tamilnadu Dr.M.G.R Medical University is based on bonafide cases studied and analysed by the candidate in the Department of Microbiology, Coimbatore Medical College Hospital during the period of September 2011 – August 2012 under the guidance and supervision of **Dr. N.MYTHILY M.D.,** Associate Professor of Microbiology and the conclusions reached in this study are her own.

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DECLARATION

I, **Dr. V.ARUNA** solemnly declare that the dissertation entitled **"COMPARATIVE STUDY OF INDUCIBLE AND CONSTITUTIVE CLINDAMYCIN RESISTANCE AMONG METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES"** was done by me at Coimbatore Medical College Hospital, during the period of September 2011 to August 2012 under the guidance and supervision of **Dr. N.MYTHILY M.D.,** Associate Professor of Microbiology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr.M.G.R.Medical University towards the partial fulfillment of the requirement for the award of M.D. Degree (Branch - IV) in Microbiology.

I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

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LIST OF ABBREVIATIONS

S.aureus –Staphylococcus aureus

MRSA - Methicillin Resistant Staphylococcus aureus

MSSA- Methicillin Sensitive Staphylococcus aureus

CAMRSA-Community Acquired Methicillin Resistant Staphylococcus aureus

HAMRSA-Hospital Acquired Methicillin Resistant Staphylococcus aureus

PRSA- Penicillin Resistant Staphylococcus aureus

PBP - Penicillin Binding protein

SCCmec - Staphylococcal Cassette Chromosome

fem - factors essential for methicillin resistance

erm-erythromycin ribosome methylase

MLS_B resistance - Macrolide-Lincosamide-Streptogramin B resistance

$iMLS_{B-}$ resistance - Inducible clindamycin resistance

$cMLS_B$ resistance - Constitutive clindamycin resistance

S – Phenotype: Susceptible Phenotype

MS phenotype - Macrolide Streptogramin(type B) phenotype.

VISA - Vancomycin Intermediate Staphylococcus aureus.

VRSA - Vancomycin Resistant Staphylococcus aureus.

BORSA- Borderline Oxacillin Resistant Staphylococcus aureus.

CLSI-Clinical Laboratory Standard Institute

ABBREVIATIONS IN MASTER CHART

E-Erythromycin

CD-Clindamycin

CX-Cefoxitin

OX-Oxacillin

LZ-Linezolid

VAN-Vancomycin

AK-Amikacin

CIP-Ciprofloxacin

DO-Doxycycline

COT-Cotrimoxazole

P-Penicillin

AMC-Amoxyclavulanic acid

CN-Cephalexin

CTX-Cefotaxime

DRUG-S-sensitive,IS-Intermediate Sensitive,R-Resistant

OG-Obstetrics and Gynaecology

ORTHO – Orthopaedics.

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INTRODUCTION

INTRODUCTION

Staphylococcus aureus is the most frequently encountered pathogen isolated from clinical specimens. Staphylococcus aureus has the ability to asymptomatically colonize the normal population either persistently or transiently. 30% of humans are likely to be nasal carriers. Person to person contact or contact with fomites plays a role in its transmission. Loss of normal skin barrier & presence of predisposing factors such as diabetes and HIV complicates infection.

Staphylococcus aureus causes variety of human infections ranging from minor skin diseases such as furuncles, cellulitis, abscesses to life threatening infections like toxic shock syndrome, staphylococcal scalded skin syndrome, endocarditis, pneumonia & septicemia.

Penicillin was the drug of choice to which Staphylococcus aureus developed resistance by producing the enzyme betalactamase. So methicillin was introduced in1959. But methicillin resistant staphylococcus aureus (MRSA) fastly appeared in hospitals in 1961[1].

Prolonged hospitalization, indiscriminate use of antibiotics, and indwelling medical devices were the cause for the appearance and spread of MRSA. The nosocomial multidrug resistant MRSA(HA -MRSA) strains have a high effect on patient morbidity and mortality .Community associated MRSA(CA-MRSA) strains harbour Panton-Valentine

leucocidin gene associated with fulminant infections, such as necrotizing pneumonia .

Betalactam agents bind to PBP in cellwall of staphylococcus aureus resulting in disruption of peptidoglycan synthesis & bacterial cell death. The mecA gene coding for PBP2A in cell wall of MRSA harboured by mobile SCCmec chromosome is responsible for methicillin resistance.

CA-MRSA possess a small SCCmectype IV, V, or VII, which is transferred easily by transduction than the larger SCCmec types I, II, and III in HA-MRSA[2,7, 52].

Detection of MRSA can be performed by an oxacillin or cefoxitin disc diffusion test. Cefoxitin is a strong inducer of mec A gene and thus helps in detection of MRSA.

Alternatively the macrolide- lincosamide streptogramin B group of antibiotics can be used for treating MRSA infection. Clindamycin, a lincosamide antibiotic has become an attractive option for clinicians because of its bioavailability both in oral & intravenous formulations.

It has excellent tissue penetration.It is the treatment of option in individuals with penicillin allergy and renal impairement. Clindamycin has been used to treat pneumonia, soft-tissue and musculoskeletal infections due to MRSA .It can be used both in adults and children[3].

However, fear of appearance of clindamycin resistance during therapy has discouraged some clinicians prescribing it.

The mechanism of inducible clindamycin resistance ($iMLS_B$) is due to target site modification mediated by erm gene which can be expressed by an inducer like erythromycin or constitutively ($cMLS_B$). The overlapping binding sites of macrolides, lincosamides,and streptogramins B in 23S rRNA accounts for the cross resistance to the 3 classes of drugs[4] .

The D-test is performed by placing clindamycin and erythromycin discs at an edge-to-edge distance of 15 to 20mm and looking for flattening of the clindamycin zone nearest the erythromycin disc.[5] If D-test is positive it suggests the presence of an erm gene that could result in clindamycin resistance.

Strains with inducible clindamycin resistance are difficult to detect in the routine laboratory as they appear erythromycin resistant and clindamycin sensitive in vitro when not placed adjacent to each other. In such cases, in vivo therapy with clindamycin may select constitutive erm mutants leading to clinical therapeutic failure. But mutations in the promoter region of erm gene allows the production of methylase without an inducer. These mutants are stably erythromycin and clindamycin resistant (Constitutive resistance).

MRSA constitute a major health care problem with a strong potential for dissemination and high rate of mortality and morbidity. So the availability of sensitive and specific methods for detecting antibiotic resistance in these pathogens accurately has become a significant tool in clinical diagnosis.

In PCR by amplification of the mecA gene, MRSA is detected.PCR is highly, sensitive, and specific. But it requires advanced equipments & moreover it is costly. So it is not possible for routine testing in clinical laboratories.Incidence of clindamycin resistance in MRSA isolates varies widely by hospital and geographic region [2].

Errors in the detection of methicillin resistance can have serious adverse clinical consequences. False susceptibility results may result in treatment failure and the spread of MRSA if appropriate infection control measures are not applied. Conversely, false resistance results may increase healthcare cost following unnecessary isolation precautions and may lead to overuse of glycopeptides.

For detection of methicillin & clindamycin resistance exactly and fastly disc diffusion can be used as a screening tool. It is important to treat the infected patients with correct antibiotic so that MRSA is controlled in the hospital environment.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

1. To isolate and identify staphylococcus aureus by gram staining, conventional culture methods and biochemical reactions.
2. To screen for Methicillin Resistant Staphylococcus aureus by disc diffusion method with cefoxitin and oxacillin discs.
3. To determine the prevalence of inducible Clindamycin resistance ($iMLS_B$) and constitutive clindamycin resistance ($cMLS_B$) in Methicillin Resistant Staphylococcus aureus isolates using erythromycin and clindamycin discs in‘D’test in our geographic area.
4. To compare inducible clindamycin resistance with constitutive resistance among MRSA.
5. To ascertain the relationship between MRSA and clindamycin resistance.

REVIEW OF LITERATURE

HISTORY

- Von Recklinghausen in 1871 observed Staphylococci in human pyogenic lesions[6].
- Alexander Ogston a Scottish surgeon was the first to publish the observations on Staphylococcal infections between 1880 and 1882. He recognized the role of staphylococci in abscess [7].
- Rosenbach in 1884 named Staphylococcal strains from pyogenic lesions as Staphylococcus aureus as it produced golden yellow pigment. He separated the genus Staphylococcus into Staphylococcus aureus and Staphylococcus albus.
- Staphylococci and Micrococci were positioned in genus Micrococcus by Zopf. But Flugge separated the genus Staphylococcus and genus Micrococcus [8].
- A French medical student, Ernest Duchesne, in 1886 found that Staphylococci aureus colonies could be lysed by the mold Penicillium notatum[7].
- The value of coagulase test to identify staphylococcus aureus was brought to attention by Von Daranyi in1925 [8].
- Bacteriologist Alexander Fleming in 1929 published his observation on lysis of staphylococcus aureus in the vicinity by Penicillium mold

which contaminated his culture at St. Marys hospital laboratory in London[9].

- In 1943, a large-scale production of the penicillin began in the United States of America[7].
- Kirby in 1944 described Penicillin resistant staphylococcus aureus (PRSA) for the first time [10].
- The drug vancomycin means "vanquished". In 1950 it was developed from soil samples in the jungles of Borneo island [7].
- Evans et al in 1955 proposed separating Staphylococci from Micrococci on the basis of oxidation fermentation test. The Staphylococci is aerobic and facultative anaerobic whereas Micrococci is an obligatory aerobe [8].
- In 1956 erythromycin resistance emerged [4].
- In 1959, world's first semi-synthetic penicillin, the methicillin was first marketed to counter the spread of PRSA following which different derivatives, like oxacillin were produced[7].
- Jevons[11] first reported MRSA in 1961 in England[2,7].
- Silvestry and Hill 1965 based on DNA composition clearly differentiated Staphylococci from Micrococci[8].
- McGehee et al in 1969, has reported the ineffectiveness of clindamycin when treating erythromycin resistant Staphylococci[12,15].

- Cato and Stackebrandt in 1989 tentatively placed Staphylococci in the family of Bacillaceae of the order Bacillales [8].
- In the 1990s, semi synthetic Macrolides with improved pharmacokinetics and tolerability developed [4].
- In1997, the Mu 50 first strain of Vancomycin intermediate Staphylococci aureus (VISA) was reported from Japan [13].
- Kuroda et al in 2001 first reported the whole genomic sequences of S.aureus [14] .
- A disc diffusion method was described by Feibelkorn et al in 2003 for detecting inducible clindamycin strains of staphylococcus aureus in clinical samples. This test was done by placing erythromycin and clindamycin discs in close proximity with the interdisc distance of 15-26mm in Mueller-Hinton agar [15].
- In 2005 Clincal Laboratories Standard Institute standardized the test as 'D' zone test[16].

In the pre antibiotic era mortality due to Staphylococcus aureus infection was high. The miracle drug penicillin when introduced had good impact. But this did not last long, because of the emergence of penicillinase producing Staphylococcus aureus. Methicillin was introduced in 1961.Soon both CA-MRSA and HA-MRSA became a growing problem to the general public in every region of the world.

Morphology

Staphylococcus aureus belongs to the family of Micrococcaceae. Staphylococci means cocci occuring in grape like clusters. (In Greek Staphyle means bunch of grapes).This gram positive bacteria is 1µm in diameter and is non motile. It is an aerobe and facultative anaerobe. The genome of Staphylococcus aureus is around 2.8 Mb and contains 2500 genes.

Staphylococcus aureus cell wall contains peptidoglycan and teichoic acid. Peptidoglycan has crosslinked polymers of N-acetyl glucosamine and N-acetyl muramic acid. The adherence of Staphylococcus aureus to mucosal surfaces is by teichoic acid. Moreover it provides rigidity to cell wall[17]. Some strains produce exopolysaccharide which helps in adherence of organism to host cell and prevents phagocytosis. It is occasionally capsulated.

Cultural characteristics

In nutrient agar, Staphylococcus aureus colonies are 1-3mm in diameter smooth, low convex, densely opaque with a entire edge and of butyrous consistency. Pigmentation ranges from cream through buff to gold and is characteristic. Pigmentation is enhanced by prolonged incubation as well as when culture plates are left at room temperature. Staphylococcus aureus tolerates the concentrations of sodium chloride at which other bacteria are inhibited.

In blood agar beta haemolysis is observed. In Mannitol salt agar colonies are of 1mm diameter surrounded by yellow zone due to acid production from mannitol. The selective media available for isolating staphylococcus aureus include Mannitol salt agar, Lipase– salt – mannitol agar, Phenyl ethyl alcohol agar, Columbia Colistin Nalidixic acid (CNA) agar and Baird –Parker agar base [18, 19].

Biochemical reactions

Catalase test, Slide coagulase and Tube coagulase tests are positive. Staphylococcus aureus is the only species of staphylococcus which ferments mannitol. Methyl Red and Voges Prosakeur tests are positive. Staphylococcus aureus hydrolyses DNA and produces phosphatase. It reduces tellurite to form black colonies in Potassium

tellurite medium. Urea is hydrolysed and gelatin is liquefied. Indole test is negative [18].

Pathogenesis

The Peptidoglycan and teichoic acid in cell wall are virulence factors. Staphylococcus aureus secretes toxins and enzymes which plays a role in virulence. Alpha, beta, gamma and delta toxins provokes cell destruction. Destruction of phagocytes is mediated by leucocidin.

Clumping factor, Coagulase and hyaluronidase helps in invasion and existence in tissues. These virulence factors are responsible for wound infections, as well as skin infections. Several exotoxins like Toxic shock syndrome toxin, exfoliative toxin, and enterotoxin are also produced. These potent toxins cause systemic effects [20].

Clinical syndromes

MRSA is defined as the strains of staphylococcus aureus resistant to the isoxazoyl penicillins such as methicillin, oxacillin, nafacillin and flucloxacillin. Staphylococcus aureus infections are classified as CA-MRSA infections and HA-MRSA infections. CA-MRSA secretes a toxin Panton-Valentine leucocidin causing infections in healthy individuals. CA-MRSA is frequently susceptible to a wide range of antibiotics than hospital strains. According to Center for disease control and prevention

people who satisfy the following criteria, are said to be infected with CA-MRSA.

1. Diagnosed in the outpatient setting as MRSA infected.
2. Culture for MRSA must be positive within 48 hours of admission in the hospital.
3. No medical history of colonization, hospitalisation, surgery or dialysis.
4. No permanent indwelling catheters or medical devices passing into the body through the skin [21].

Hospital acquired MRSA infection is defined as, occurring in a patient whose MRSA isolate was cultured more than 48 hours after admission or who has a history of hospitalization, surgery, dialysis or residence in a long term health care facility within six months prior to the culture date or had an indwelling intravenous line, catheter or any other percutaneous medical device present at the time of culture.

CA-MRSA infections occur both in healthy person and in those with known risk factors. Furuncles, impetigo, abscess and cellulitis are some of the common skin and soft tissue infections. Severe illness like necrotizing pneumonia is reported in patients who has undergone tracheostomy or in patients with prolonged intubation. Invasive procedures and use of resistant antibiotics results in bacteremia. Other

serious infections are endocarditis, septic arthritis, osteomyelitis, meningitis, and abscess in liver and spleen. Rarely dissemination to urinary tract occurs through bloodstream or by ascending infection from urethral meatus. Even pyelonephritis has been documented in some cases. Toxin mediated food poisoning, toxic shock syndrome and staphylococcal skin scalded syndrome can also occur.

HA-MRSA infections can include above diseases but surgical wound infections and bacteraemia associated with intravenous devices are common. Infections associated with cerebrospinal fluid shunts, prosthetic joints and vascular grafts and ventilator associated pneumonia are also seen with HA-MRSA infections [22].

Epidemiology

MRSA was 1st reported in United Kingdom and later on from Japan, Europe, and Australia. Waness A. 2010 has documented that MRSA has been prevalent in livestock animals and slaughter houses in countries like, Canada, Europe and Singapore. MRSA has been found in seawater in American beaches[7].

In Europe highest prevalence of HA-MRSA was reported in Portugal (54%), followed by Italy (43-58%) and Netherlands 2%.[1] Prevalence rate ranges from 2% in Netherlands and Switzerland to 70% in Japan and Honkong [23].

Lahari Saikia et al 2009 has reported that in India the Prevalence rate of MRSA is 31% and 38.56% in Tamil Nadu and New Delhi respectively. In some studies the rate is found to be low in Nagpur (19.56%) & high in Indore (80.89 %) comparatively[24].

Lakshman Swamy Parasa et al 2010 reported the incidence of MRSA in India as 32.8%-51.6% during the period of 1994-2001. Some studies have reported a prevalence of 39.50% in south Gujarat, 38.44% in a tertiary care hospital, North India. MRSA prevalence has been reported as 52.9% in Assam, 24% in Chandigarh, and 24% in Vellore.[23] The prevalence of HA-MRSA in South India hasbeen reported as 31.1% in the study of Poonam Sood Loomba et al 2010.[1] The prevalence varies by geographical location, patient age, and bacterial susceptibility profile.

30% patients colonise MRSA on nose. In Mathan et al study in 2009, out of 403 carriers the colonization site of MRSA were 78.5% in nose alae, 85.6% in nose and throat and 98% in perineum. Some, report that children and young adults affected by skin and soft tissue infections were likely to be carriers. The carriage rate of MRSA in health workers, inpatients, and outpatients was 1.8%, 15.6%, and 3.8% respectively. The carriage of MRSA was more in inpatients. Overall carriage rate was. 5%.

According to some studies 6-50% of health workers working in burns and intensive care units are nasal carriers[25].

William J Peppard et al 2009 has reported CA-MRSA in athletes, prison inmates, men who have sex with men, military people, drug users and children in day care centers, due to crowded living conditions & poor personal hygiene. Children less than 2 years, adults more than 65 years of age and homeless persons are prone for MRSA infection [21].

Tony Beavers May et al 2004 adds prior antimicrobial use, HIV infection and MRSA colonization of family members as some of the predisposing factors[26] .MRSA remains a major pathogen in nosocomial infections in developing countries. Shantala et al 2011 has documented 32.5% MRSA isolates with inducible clindamycin resistance. Different places of India have reported inducible clindamycin resistance in 30% to 64% of the MRSA isolates[27].

Mukesh Patel 2006 states diabetes mellitus, renal dysfunction, postsurgical status and malignancy, are some of predictors of inducible clindamycin resistance. Neutropenia, trauma, burns and organ transplant also adds to predictors of inducible clindamycin resistance. The prevalence of inducible clindamycin resistance in CA-MRSA in children is decreasing over time. This may be due to the expansion of MRSA clones lacking genes responsible for inducible clindamycin resistance [28].

The spread of MRSA between patients is called cross-infection. However, these patients may develop infections if the MRSA enter the body with breaks in their skin due to wounds, indwelling catheters, contaminated equipment or via environment. Epidemic MRSA may also spread between hospitals, presumably when colonised patients or staffs move from one hospital to another.

In India, spreading of CA-MRSA was probably due to overcrowding and poor personal hygiene. Although it mainly manifests in severe soft tissue and skin infections requiring surgical drainage, it is now becoming pronounced in bacteremia affecting neonates, especially from lower economic sections, and breast abscesses in lactating mothers. It is becoming increasingly common in urban areas. Sheetel Verma et al 2000 reports that MRSA is common in intensive care units and burns unit[29] .MRSA accounts for 40% to 70% of staphylococcus aureus infections in intensive care units[10]. P.U.Krishnan et al 2002, has documented 65 isolates of MRSA from patients and health workers working in burns units, at St.Johns Medical college Hospital Bangalore, India[11].

In addition to the United States CA-MRSA strains have been reported from Canada, Asia, South America, Australia Europe, Norway, Netherlands, Denmark, and Finland. Globally, CA-MRSA strains show

remarkable diversity in the number of different clones that have been identified. The resistance to penicillin by CA-MRSA was the first wave of antibiotic resistance which began in mid 1940s. USA 400 clone, causes community onset disease among Indigenous populations in Alaska and the Pacific Northwest and was isolated from the paediatric cases prior to 2001. A second epidemic clone, USA300, emerged between 1999 and 2001, and now causes the vast majority of CA-MRSA infections in the United States. Among the MRSA clinical isolates is the archetypal strain COL, isolated from a patient in Colindale, United Kingdom in 1960.Iberian and Rome clones constituted the third wave of antibiotic resistance[30] .Marta aires et al in 2001 describes Iberian, Brazilian, Pediatric, and Newyork-Tokyo clones in addition at international level[31]. Phage typing is an important epidemiological investigation to identify MRSA, which is done by 23 internationally accepted phages [32].

Mechanism of resistance:

Staphylococcus aureus is susceptible to most antibiotic. Resistance to antibiotics is acquired by transfer of genes from outside sources, and chromosomal mutation. Antibiotic selection is also to be considered. Genes responsible for resistance mechanism is either present on the chromosome or on a plasmid. Antibiotic resistant genes can be

transferred by Plasmid. Conjugation is the most common method of transfer of resistant genes. Transposon is a genetic factor that carries portions of plasmid from one organism to another.

The resistance mechanism is called constitutive if it is expressed continuously even if an inciting challenge is available or not. But in some genes it must be induced by exposure to the challenging substance. Uniform expression of resistance is homogenous expression. But if only a small fraction of bacteria expresses the resistance it is called heterogenous resistance. It is very difficult to identify this kind of resistance in the clinical laboratory.

Methicillin:

MRSA is resistant to all currently used betalactam antibiotics. Betalactam antibiotics are penicillins,cephalosporins and carbapenams. They constitute same structure and mechanism of action. Betalactum antibiotics inhibit bacterial cellwall peptidoglycan synthesis [9].

Methicillin, oxacillin and flucloxacillin are semisynthetic penicillins derived from 6-aminopenicillanic acid and they are penicillinase resistant[33]. These bactericidal drugs are administered by parenteral route and can’t be administered to patients with a history of hypersensitivity reaction to penicillin.

Cefoxitin is a cephamycin produced by Streptomyces lactum durans[34] .The cephamycins are similar to cephalosporins but have a methoxy group at position 7 of the betalactum ring of the 7 amino cephalosporanic acid nucleus. This is a potent inducer of mecA gene[35] . Cefoxitin is a surrogate marker of methicillin resistance. Cefoxitin disc diffusion tests and PCR have similar sensitivity and specificity. The simple 'D'test is mandatory for all clinical laboratories to detect clindamycin resistance[2].

The expression of methicillin resistance in S.aureus due to acquired penicillin binding protein PBP2a which is 78 KDa with 668 aminoacids possessing both transglycosylase, transpeptidase enzymes involved in disruption of final step of peptidoglycan synthesis of bacterial cellwall[36]. PBP2a is encoded by mecA gene,origin of which is not known. mec A gene is located within larger region of chromosome, the staphylococcal cassette chromosome mec region(SCCmec)(21-67kb).The basic elements of SCCmec are the mecRI- mec I-Pbp2a region and ccrA.Mobility of SCCmec is conferred by ccrA and ccrB genes.Nosocomial isolates are multidrug resistant due to accumulation of plasmids & transposons in SCCmec .As they are larger in size not transfered by bacteriophages [1].

SCCmec is classified into types I, II, III, IVa, IVb and V. Types I, II, III are found in nosocomial infections. Type IV is found in CA-

MRSA. Namita D' Souza et al 2010 has classified SCCmec additionaly into type VI, VII,which are also found rarely. The mec A gene complex, cassette chromosome recombinase complex (ccr complex) and junkyard variation results in characterization of SCCmec element [37].

Other genes like fem (factor essential for methicillin resistance), aux (auxillary gene), BlaI gene which are involved in the formation of Staphylococcus aureus cellwall also influence the expression of methicillin resistance [1].

Disc diffusion test for detection of Methicillin resistance:

For detection of Methicillin resistance, cefoxitin disc diffusion test and oxacillin disc diffusion test are used.0.5 McFarland standard suspension of the staphylococcus aureus isolate is made and lawn culture done on Muller Hinton Agar plate .A 30 µ of cefoxitin and 1 µ of oxacillin are placed and plates are incubated at 37º C for 24 hours and zone size are measured . Oxacillin disc diffusion test must be read in transmitted light[38].

In January 2007 CLSI published inhibition zone diameter for Cefoxitin as follows. Zone diameter of ≥ 22mm is reported methicillin susceptible and ≤ 21mm considered methicillin resistant and for oxacillin of ≥13mm is reported methicillin susceptible and ≤10 mm is considered as methicillin resistant [34]. Poonam Sood Loombaetal 2010 explains that

disc diffusion test using cefoxitin is easy to read. It gives clearer end points than oxacillin[1]. Oxacillin is frequently misinterpreted as susceptible due to haziness. False susceptibility of 4.4% has been reported with oxacillin disc diffusion test [34].

Environmental condition like P^H, temperature and salt concentration also decides the expression of methicillin resistance[39,40]. In R.Skov et al 2006 suggest that incubation temperature influences zone diameter and MIC for staphylococcal strains that are methicillin resistant .Detection of MRSA by cefoxitin disc is not much affected by temperature variation. But for oxacillin the temperature should not exceed 37ºC. Incubation at 30 º C was associated with lower accuracy. Increasing the duration of incubation from 18 hours to 24 hours did not improve accuracy[41]. Incubation temperature of 37ºC for 24 hours in disc diffusion test is trustworthy[1]. Isolates resistant to both cefoxitin and oxacillin had an MIC 0.5-2µg/ml. As per CLSI criteria MIC less than 2µg/ml is interpreted as MSSA[35].

So results of either cefoxitin disc diffusion or MIC tests can be used to predict mec A mediated oxacillin resistance. Based on cefoxitin results, oxacillin should be reported susceptible or resistant. Susceptibility

or resistance to betalactam antibiotics may be deduced from testing only penicillin and either cefoxitin or oxacillin[42].

K.B. Anand et al 2009 describes that the mecA gene positive staphylococcus aureus isolates are expressed either as homogenous or heterogenous resistant strains. mecA gene is expressed in low level in heterogenous resistance . On disc diffusion testing these strains appears as susceptible to oxacillin[43]. The gene mecA is expressed only in 1in 10^5cells and its expression is rapid if betalactam bind to the surface receptors for the derepression of mecA. (Henneth H, Randetal 2004)[44] .

Cefoxitin disc diffusion test is a superior test to oxacillin disc diffusion test as it has higher sensitivity and specificity[38]. Anila A. Mathews et al describes methicillin resistance detected by oxacillin diffusion test could be false positive due to hyperbetalactamase production. These isolates were sensitive to cefoxitin and negative for mecA gene. These isolates were named BORSA (Borderline oxacillin resistant Staphylococcus aureus). These strains may evolve to fully resistant ones in due course of time under antibiotic pressure [35].

Oxacillin resistant strains are resistant to all penicillins, cephalosporins, monobactam, other betalactams /betalactamase inhibitor combinations, and carbapenams. Penicillin susceptible staphylococcus are

also susceptible to other penicillins,beta lactam/beta lactam inhibitor combinations, and carbapenam. Oxacillin resistant staphylococci are resistant to all currently available betalactam antibiotics with exceptions of newer cephalosporins with anti-MRSA activity[42,45].

Other than disc diffusion method, different methods are available for the detection of MRSA namely broth dilution method, agar dilution method and epsilometry test[46]. Mannitol salt agar medium supplemented with oxacillin and MRSA select medium, are some of the culture media available for MRSA detection[47,48]. In Oxacillin resistant screening agar medium**,** Mueller Hinton agar supplemented with 4% Nacl and 6mg/ml of oxacillin is used for detecting MRSA[49]. Immunochromatographic test and Latex agglutination test is also used for detecting PBP2. PCR detects mec Agene but it is very costly. So it is not possible to perform it as a routine procedure in clinical laboratories[36]. Serhat Unal states that mecA can be detected by DNA hybridization. Rapid cell lysis technique was established for the release of DNA from staphylococcus isolates[39].

Macrolide and Lincosamide

MRSA has left as with few therapeutic alternatives to treat.The major alternative to penicillins and cephalosporins are Macrolide – Lincosamide- Streptogramin B (MLS_B) antibiotics for the treatment

staphylococcus. Macrolides have been known for many years. The evolution of the macrolide class has been marked, in 1990s, especially with production of semisynthetic macrolides with improved pharmacokinetics and tolerability.

Macrolide and lincosamide antibiotics are chemically different but have a similar mode of action. They are active against gram-positive staphylococci. Macrolides have two or more amino or neutral sugars.The sugars are attached to a lactone ring whereas lincosamides (eg.,clindamycin and lincomycin) are devoid of a lactone ring. Clindamycin and macrolides act at sites which are in close proximity.Increasing knowledge of the molecular mechanisms of resistance to macrolides has led to the design of ketolides which are active against certain types of erythromycin resistant organisms. Macrolides and lincosamide antibiotics are bacteriostatic. They inhibit protein synthesis by binding to 50s ribosomal units of the organism reversibly.

Clindamycin is used to treat staphylococcus aureus infection as it has excellent pharmacokinetic properties. James et al 2oo5 states that clindamycin is an attractive option for skin and soft tissue infections because this drug is available in oral (90% bioavailability) and intravenous formulations. Unlike beta lactam it is not impeded by high

bacterial burden at infection site. Staphylococcal toxins and virulence factors are inhibited by this drug[12] .It is advisable to use clindamycin in necrotizing skin and soft tissue infections as it has the capacity to reduce toxin expression [50]. Clindamycin has good penetration into various tissues including bones except CSF[1.] .

Clindamycin is a congener of lincosamin. It is a derivative of the aminoacid trans L- 4 –n-propyl hygrinic acid attached to octose which has sulfur. It binds to the 50 s ribosomal unit of bacterial ribosomes and thus inhibiting bacterial synthesis. It is completely absorbed following oral administration. Clindamycin palmitate is an oral preparation for paediatric use.The phosphate ester of clindamycin when given parenterally is hydrolyzed in vivo to an active drug. Drug crosses the placental barrier.90% is bound to plasma proteins. This accumulates in inflammatory cells like leucocytes and macrophages. It is metabolized as N-dimethyl clindamycin, and sulfoxide and finally excreated in urine and bile.MIC of clindamycin 0.25-8g/ml. MIC of clindamycin ≤0.5 g/ml was considered sensitive and MIC ≥ 4g/ml was taken as resistant[3].

Mechanism of Clindamycin resistance:

Resistance of staphylococcus aureus to MLS_B antibiotics can occur by different mechanisms.

1. Macrolides Streptogramin resistance

The first involves macrolide active efflux and is relatively common. A specific efflux pump is encoded by the gene msrA in staphylococci .This energy dependent pump effectively expels macrolides from the bacterial cell before they can bind to their target site on the ribosome. This mechanism of resistance creates resistance, but only to macrolides, azalides (e.g., Azithromycin and group B streptogramins - quinupristin) . Lincosamides (e.g., clindamycin and lincomycin) are not a substrate to this macrolide efflux pump[4,12].

2. MLS_B resistance (Macrolide-Lincosamide-Streptogramin B)

The second mechanism of resistance to macrolides in staphylococci involves modification of the drug binding site on the ribosome. This results in resistance to macrolides (and azalides),lincosamides, and group B streptogramins and is commonly referred to as "MLS_B resistance" coded by the erm gene.MLS_B resistance can be either constitutive ($cMLS_B$) or inducible ($iMLS_B$). In vitro staphylococcal isolates with constitutive resistance are resistant to both erythromycin and clindamycin , while isolates with inducible resistance are resistant to, but appear to be susceptible to clindamycin [4,12,27].

3. P. Sireesha et al 2012 states that third mechanism of resistance to lincosamides in staphylococci is by rare lnu gene which causes chemical alteration resulting in inactivation of the drug [3,4].

The inducible Clindamycin resistance and constitutive clindamycin resistance occurs through the second mechanism of resistance involving modification of the drug binding site on the ribosome, as mentioned above.

The MLS_B phenotype is encoded by erm (erythromycin ribosome methylase) genes in staphylococci. The erm(A) genes are mostly present in methicillin resistant staphylococcus aureus and are borne by transposons related to Tn554, whereas erm (C) genes which are present in methicillin-susceptible staphylococcus aureus are borne by plasmids [4]. There is a single adenine in nascent 23S rRNA, which is part of the large (50S) ribosomal subunit. erm protein dimethylates the adenine. The A2058 residue is located within a conserved region of domain V of 23S ribosomal RNA. It plays a role in the binding of MLS_B antibiotics. As a outcome of methylation, binding of erythromycin to its target is impaired. The overlapping binding sites of macrolides, lincosamides,and streptogramins B in 23S rRNA explains the cross-resistance to the 3 classes of drugs [12,51].

Expression of MLS_B resistance can be constitutive or inducible. In inducible resistance, the bacteria produce inactive mRNA which is unable to encode methylase .It is activated in the presence of macrolides which are inducers ,but not by lincosamides and Streptogramin B which are non inducers. This leads to rearrangements of mRNA, which allow ribosomes to translate the methylase coding sequence[4].

The messanger RNA in its 5' end constitutes leader peptide along with a set of inverted repeats. This forms a hairpin like structure. This, by base pairing sequesters the initiating sequences (initiating codon) for methylation.The inducer macrolide binds to the ribosome when leader peptide is translated. Now there is destabilization of hairpin like structure. The initiating sequences are exposed to the ribosomes and there is translation of methylase[52]. According to Claire Daurel et al 2008 the regulatory region of ermA is longer.There is one leader peptide and four inverted repeats in the regulatory region of ermC.The regulatory region of ermA has two leader peptide and six inverted repeats .This leads to the difference in the structure of attenuator leads and therefore different patterns of MLS_B inducible resistance are observed[4,52]. In constitutive expression, active methylase mRNA is produced in the absence of an inducer [53] .Additional changes in the 5' upstream sequences by deletion, duplication and mutation leads to constitutive resistance[12].

Method of detection of Clindamycin resistance

D-test (Double-disk diffusion test)

The inducible Clindamycin resistance is not detected by standard broth microdilution method, automated susceptibility testing devices, standard disc diffusion test or E test[12]. So the procedure for clindamycin resistance testing was introduced in January 2004 by National committee for Clinical Laboratory Standards Institute. (Now CLSI)[54].

In this disk diffusion test, for detecting clindamycin resistance, the truncated zone of inhibition to the drug clindamycin resembles the letter 'D'. So this test was called as 'D- test' (James et al 2oo5)[12].

Procedure:

Clindamycin (2 µ) and erythromycin (15µ) discs are placed 15 mm (edge to edge) apart on Mueller-Hinton agar that has been inoculated with a standardized (0.5 MacFarland) suspension of staphylococcus aureus and incubated overnight at 37 ºC. D test was read in reflected light[55].

Following were observed in disc diffusion results:

Inducible Clindamycin resistance ($iMLS_B$):

It is very important to find out the emerging Clindamycin resistance patterns to institute proper management to HA-MRSA and CA-MRSA .

Staphylococcal isolates showing resistance to erythromycin (zone size ≤ 13mm) but sensitive to clindamycin (zone size ≥ 21 mm) shows two distinct induction phenotypes.

1. Sensitivity to clindamycin results in a D-shaped blunting of the circular zone of inhibition around the clindamycin disc on the side facing the erythromycin disc. A clear, D-shaped zone of inhibition round the clindamycin disc was designated as the D phenotype.
2. D-shaped zone containing inner colonies growing up to the clindamycin disc was designated as $D^{+56,3}$.

   N.Pal 2010 states that both D and D^{+} were considered positive for inducible clindamycin resistance[56].

MS phenotype:

The msrA gene confers the so called MS phenotype (resistance to erythromycin, inducible resistance to streptogramins and susceptibility to clindamycin) by efflux. Erythromycin resistant (zone size ≤ 13mm) but sensitive to clindamycin showing circular zone of inhibition around clindamycin with the zone size of ≥ 21mm was called as MS phenotype[55].

Constitutive resistance ($cMLS_B$):

In constitutive resistance Staphylococcal isolates shows erythromycin resistance (zone size ≤ 13mm) & clindamycin resistance

(zone size ≤ 14 mm). Clindamycin leads to selection of constitutive mutants at frequency of 10^7CFU[3] .According to P.Sireesha et al 2012 D and D^+ were considered as constitutive resistance[3].

S (susceptible) phenotype:

Staphylococcal isolates sensitive to both erythromycin & clindamycin. Strains showing higher MIC inspite of being sensitive to both erythromycin & clindamycin shows heteroresistance as possibility. Further studies are done to find out other mechanisms of resistance involved.

HD phenotype (Hazy D zone) [56,3]

This type shows 2 different zones, one zone is a light hazy growth around clindamycin disc and the other is with heavy growth in the shape of letter ‘D’.

So at present, disc diffusion test is the preferred method for testing staphylococcus aureus isolates for inducible clindamycin resistance.

Feibelkorn et al 2003 has reported 100% sensitivity in detecting $iMLS_B$ resistance on performing disc diffusion test using 15- 26 mm interdisc distance between erythromycin 30µg and clindamycin 2 µg

discs.Whereas only 97% sensitivity has been documented with 26-28 mm interdisc distance [15].

Mathew V.N.O. Sullivan et al 2006 recommended an edge to edge distance of 15mm in disc diffusion test for detecting $iMLS_B$ resistance. This is because , on performing D-test an error rate of 18.2% was found with 22mm interdisc spacing between erythromycin and clindamycin discs in MRSA isolates[51].

G.S.Ajanta et al 2008 informs that ideal interdisc spacing between the erythromycin and clindamycin is not yet clear. But false positivity was not reported with 15mm of spacing. Clinical and Laboratory Standards Institute has suggested 15-26 mm of interdisc spacing.[57]According to Clarece J. Fernandes 2007 when compared to genotypic analysis disc diffusion test have high sensitivity and specificity but if the disc separation distance is too wide false negative results may occur[58].

Christine D Steward et al 2005 has stated that clindamycin resistance is effectively induced by erythromycin[59]. Mukesh Patel et al 2006 states that inducible clindamycin resistance exihibiting strains have high rate of undergoing mutation to constitutive resistance spontaneously. D-test was done in 402 staphylococcal isolates, in which 280 were MRSA

and 122 were MSSA. Out of 280 MRSA, 139 MRSA showed inducible clindamycin resistance. Likewise Out of 122 MSSA, 73 MSSA showed inducible clindamycin resistance. 56% HA-MRSA and 41 % CA-MRSA have contributed to positive D test. There was low prevalence of $iMLS_B$ resistance in CA-MRSA which has favoured the use of clindamycin as outpatient treatment [28].

Angel et al have not found any constitutive MLS_B resistance in staphylococcus aureus strain[60]. Sireesha et al 2012 states HD phenotype is considered as constitutive MLS_B resistance.[1] Shailesh kumar et al reported 2.9% of constitutive resistance in MRSA. [61] This contrasts with the Korean study where constitutive resistance was reported in majority (79%) in MRSA[62].

Dr.R.Vasanthi et al 2012 reported sensitivity of D test performed at 15 mm distance spacing correlated 100% with detection of erm & msr genes by PCR. Moreover $iMLS_B$ resistance is higher than constitutive resistance in HAMRSA. Inducible clindamycin resistance is higher in MRSA(1.88%) than MSSA (3.5%)[63] .In the study by Adebayo et al 2006 constitutive MLS_B resistance was absent in MRSA and one was identified in MSSA [54]. Dr.Mohanasundaram et al 2011, highlights that $iMLS_B$ resistance is higher in MRSA (28%) than MSSA (11%)[64]. This is

supported by the Study of Shantala et al 2011 who has documented 32.3% of $iMLS_B$ resistance in MRSA isolates & 15.38% in MSSA isolates[27]. Vidyapai et al reported 18.8% of $iMLS_B$ resistance in MRSA while in MSSA it was 3.5%[65].

V.Gupta et al 2009 has documented 66.67% of $iMLS_B$ resistance from community and 33.33% from hospital[66]. Since higher incidence of CAMRSA is being reported in outpatient clinic, the Clindamycin available in oral formulations has been frequently prescribed. In India Gadepelli et al has documented higher rate of constitutive resistance than $iMLS_B$ resistance. 30% $iMLS_B$ resistance in MRSA 10% $iMLS_B$ resistance in MSSA 38% constitutive resistance in MRSA a15% constitutive resistance in MSSA .In the study by V.Gupta et al 2009, 46% of constitutive resistance and 20% of $iMLS_B$ resistance has been reported in MRSA[66]. But in MSSA, $iMLS_B$ resistance (17.3%) was in higher percentage than constitutive resistance (10%).Todd P Levin etal 2005 reports that in Houstan,among the children infected with MRSA 2.2% of D test positivity was reported. But children in Chicago infected with MRSA showed 94% of positive result in D-test [53].

Clarece J. Fernandes 2007 has described an agar dilution method for the detection of inducible clindamycin resistance in staphylococcus

aureus [58] .Broth microdilution is also used as a method to detect inducible clindamycin resistance [67].

Other antibiotics of choice in MRSA

James S. Lewis etal 2005observed that multiple outpatient antibiotic regimen of CA-MRSA had a narrow antibiotic resistance profile. It is sensitive to non betalactam drugs like clindamycin, trimethoprim-sulfamethoxazole and tetracycline, doxycycline, minocycline and fluroquinolones[12].

Shaileshkumar et al states majority of MRSA isolates are susceptible to clindamycin,vancomycin and linezolid but most of them are resistant to trimethoprim -sulfamethoxazole and ciprofloxacin[61].

Tetracycline resistance is exhibited in staphylococcus aureus through plasmid mediated tetk, gene encoding efflux mechanism. staphylococcus aureus can also be resistant to aminoglycosides due to modification of aminoglycosides by enzymes so that they cant bind to ribosomes [68].

Usage of clindamycin & trimethoprim-sulfamethoxazole is necessary as there is increase in vancomycin resistance. (Hwan sublime et al 2006)[62].

Wei Qi et al 2005 have reported that resistance to trimethoprim is by mutation of chromosomal gene for dihydrofolate reductase or by transposon Tn4003 borne dfr gene [69].

In the study of Adebayo O Shittu et al, E test macrodilution method was performed to find the resistance to vancomycin and teicoplanin among MRSA .But none of the MRSA isolates were resistant to both the drugs. It was found penicillin and ampicillin were the least effective drugs to treat staphylococcus aureus which is a stumbling block for antibiotic therapy[54].

67% of $iMLS_B$ resistant isolates were susceptible to ciprofloxacin.28% of $iMLS_B$ resistance isolates were susceptible to linezolid and van[55]. Vancomycin is a glycopeptides which binds to the D-alanyl- D-Alanine of the peptidoglycan precursor at the cell membrane thus inhibiting crosslinking and polymerization of peptidoglycan. First strain of Vancomycin-intermediate S. aureus (VISA) was identified in Japan in 1996[10]. The second strain of Vancomycin-intermediate S. aureus (VISA) JH 9 was isolated from a bacteremic patient in United States in 2000[13]. Both Mu 50 the homogenous strain and JH 9 heterogenous strain were resistant to oxacillin [13] .Following that two additional cases were reported from United States First clinical isolate of vancomycin-resistant

S. aureus (VRSA) was reported from United States in 2002 from a patient in Michigan [10].

Marilyn chung et al 2008 observed that MRSA isolates are often resistant to penicillin,tetracycline and erythromycin.VRSA isolates were resistant to betalactams and glycopeptides. Ceftobiprole was effective against vancomycin resistant MRSA.This new cephalosporin is the active form of the prodrug Ceftobiprole medocaril[13]. Linezolid is a oxazolidinone which is a bacteriostatic. PVL cytotoxin is inhibited by linezolid and clindamycin .Chromososmal mutation in gene encoding DNAgyrase and topoisomerase IV enzymes is responsible for fluroquinolone resistance. Dalbavancin is a semisynthetic lipo glycopeptide with a long half life .So once weekly dosage is advised.

Skin and soft tissue infections can be treated with clindamycin,trimethoprim-sulfamethoxazole,doxycycline, tetracycline or linezolid. HA-MRSA is treated with intravenous clindamycin, vancomycin and linezolid. Clindamycin and linezolid are not advised if there is infective endocarditis, or if there is a source of endovascular infection. (Catherine etal2011)[70.]

Quinopristin-dalfopristin are streptogramin antibiotic used in bacteremia and in complicated skin and soft tissue infection. But its use is

limited because of the adverse reactions and is given only when conventional therapy is not used. In the sameway tigecycline is also used in complicated skin and soft tissue infection. Daptomycin is a lipopeptide. It is bactericidal by disrupting bacterial cytoplasmic membrane in the presence of calcium ions. Combination of daptomycin with oxacillin & Betalactam acts in synergy and so may be useful in treating MRSA (Henneth H, Randetal 2004)[44]. Vancomycin, linezolid, quinopristin-dalfopristin, daptomycin, tigecycline and teicoplanin are used parenteraly. Multiple antibiotics which are active against MRSA like telavancin, and oritavancin are under development. Iclaprim a dihydrofolate reductase inhibitor is also under trial[21].

Nasal decolonization of MRSA in carriers is by applying mupirocin, body decolonization is done with chlorhexidine soap along with oral antibiotics rifampicin in combination with trimethoprim-sulfamethoxazole or ciprofloxacin [1].

Therapeutic options for MRSA have been limited due to emergence and spread of multidrug resistant organisms. Therefore sensible use of antibiotics is essential. Knowledge of prevalence of MRSA & their antimicrobial susceptibility pattern is very important in treating patients appropriately. In India where molecular methods are not

feasible as routine, the disc diffusion tests helps in detecting drug resistance.

MATERIALS AND METHODS

MATERIALS AND METHODS

Place of study

The present study was conducted in Coimbatore Medical college hospital, Coimbatore.

Study period

The study period was for one year from September 2011 to August 2012.

Ethical consideration

Before starting the study the Ethical and Research clearance was obtained from Ethical committee of Coimbatore Medical college hospital, Coimbatore.

Sample

A total of 200 staphylococcus aureus isolates from clinical samples including, pus, sputum, blood, vaginal swab and urine were included in the study. Samples were received from outpatients and in patients who attended Coimbatore Medical college hospital, Coimbatore.

Processing of samples

The received samples were checked for proper labelling with Name, Age, Sex and I.P/ O.P No. of the patient, date and time of collection of the sample and processed immediately. Direct smears were prepared from sample material like pus, sputum, urine and vaginal swab on a clean glass slide. Gram staining was done and examined under microscope. The findings were recorded.

Blood samples sent in brain heart infusion broths were incubated for18 -24hours and then subcultured. All the above specimens were inoculated on to the nutrient agar plate ,blood agar, and MacConkey agar, and incubated at 37^{o} C for 18-24 hours aerobically and observed after incubation.

All the suspected colonies were identified by colony morphology, gram staining was done and the organism subjected to various biochemical tests to identify and characterize them. Further confirmation was done by slide and tube coagulase test, and growth on Mannitol Salt Agar.

Identification tests

Microscopy

Gram stain

Colonies from 18 to 24 hour culture was taken from the agar plate and a smear was prepared on a clean glass slide. Then it was air dried and heat fixed.

The smear was overlaid with primary stain 0.5% methylviolet and kept for 1 minute and then washed with water. The mordant gram's iodine was applied to the smear and washed with water after1 minute. This was decolorized with few drops of acetone and washed with water immediately.

The counter stain, 1:20 dilute carbol fuchsin was flooded on the smear , kept for 1 minute and then washed with water. The smear was air dried and then viewed under oil immersion objective. Gram positive cocci arranged in clusters were observed.

Culture[18]

Colony morphology

Nutrient agar

Golden yellow pigmented colonies, 1 to 3mm in diameter, circular, smooth, low convex and densely opaque with butyrous consistency was seen.

Blood agar

Colonies surrounded by narrow zone of beta hemolysis was identified.

Mac Conkey agar

No Growth

Mannitol salt agar

This is a selective and indicator medium. The organism was inoculated in mannitol salt agar which consists of 1% mannitol, 7.5% sodium chloride, and phenol red. The plates were incubated at 37 ºC for 18-24 hours and then examined. Staphylococcus aureus produced yellow colonies surrounded by yellow zone due to acid formation (Mannitol fermentation). Staphylococcus aureus ATCC 25923was used as positive control.

Biochemical reactions

Catalase test[71]

The Staphylococcus aureus produces catalase enzyme which will split hydrogen peroxide into water and oxygen. Release of oxygen produces the effervescence.

Procedure

One ml of 3% hydrogen peroxide was taken in a clean test tube. Few colonies of the test organism were taken from the agar plate with a sterile glass rod and immersed in the hydrogen peroxide solution.

Interpretation

Catalase test is positive if immediate and sustained effervescence is produced. In staphylococcus aureus Catalase test is positive.

Coagulase test[72]

This test confirms staphylococcus aureus isolates. Staphylococcus aureus produces the enzyme Coagulase which converts fibrinogen to fibrin that causes plasma to clot. Two types of coagulase are produced by staphylococcus aureus. The free coagulase which converts fibrinogen to fibrin by activating coagulase reacting factor present in plasma is detected by tube coagulase test.

Bound coagulase (clumping factor) which converts fibrinogen to fibrin with no involvement of clotting factor.It can be detected by clumping as seen in the slide test.

Slide coagulase test

Two circles were drawn on a clean glass slide with wax pencil. With the help of the bacteriological loop the test organism was emulsified in drops of saline kept on both the circles to form a smooth milky suspension. One suspension was kept as the control and to the other, trace of plasma was added by a flamed, cooled, straight inoculating wire.

Coarse clumping of organisms in suspension, visible to naked eye within 10 seconds was considered positive. Absence of clumping in both the suspensions was considered as negative.

Tube coagulase test

Staphylococcus aureus to be tested was grown in brain heart infusion broth and was incubated overnight at 37º C .To 1ml of this culture, 0.5 ml of undiluted plasma was added. Positive control ATCC Staphylococcus aureus and Negative controls ATCC CONS were included. All tubes were incubated at 37ºC. Tubes were examined at 1,2 and 4 hrs for coagulam formation by tilting the tube at 90º. If no coagulam is formed at the end of 4 hours, the tubes were reincubated at

room temperature for the next 12-16 hours and reexamined for the presence of a coagulam. Any degree of coagulam formation was considered as positive. If otherwise the the test was considered as negative.

Sugar fermentation test[73]

The test is used to determine the ability of an organism to ferment a specific carbohydrate which is incorporated in a basal medium and to produce acid or acid with visible gas sugar medium with 1% mannitol with, bromothymol blue as indicator was used.

Procedure

The test media was inoculated with the cultural isolate and subsequently incubated at 37ºC for 24 hours.

Interpretation

A positive test was shown by yellow colouration of the medium due to acid production.

Methyl red test (MR test) [74]

This test is used to determine the ability of organisms to produce acids by glucose fermentation through the mixed acid fermentation pathway.

Procedure

One drop from 24 hour brain heart infusion broth culture was inoculated in 5ml of MRVP broth.Incubated at 37◦Cfor 48 hrs. After incubation 5 drops of methyl red reagent was added to 5ml of broth.

Interpretation

Bright red colour indicates positive MR test. All Staphylococcus aureus isolates were MR positive.

Voges proskauer test: (VP test) [74]

VP test is used to determine the ability of organisms to produce acetoin by glucose fermentation.

Procedure

The organism isolated from primary culture plate was inoculated in glucose phosphate peptone water and incubated for 48 hrs at 37° C. To 1ml of MRVP broth 0.6ml of 5% alpha naphthol and 0.2 ml of 40% KOH were added and shaken well. Observed for5 minutes.

Interpretation

A positive test is indicated by the development of red color due to acetoin. All staphylococcus aureus isolates were VP test positive.

Modified Hugh and Leifsons test (O/F test) [75]

Procedure

Duplicate tubes of semisolid OF medium containing carbohydrate with bromothymol blue as indicator are inoculated with bacterial growth from 18-24 hour culture by stabbing to a depth of 1cm. One tube was overlaid with sterile liquid paraffin,and both tubes were incubated at 37°C for up to 7 days.

Interpretation

Staphylococcus aureus produce acid by fermentation throughout the medium in both tubes indicated by yellow colour. Oxidising organism produce acid in the aerobic tube only.

Indole test[76]

This test is done to find the ability of organism to split Tryptophan to form the indole.

Procedure

Tryptophane broth was inoculated with one drop from a 24 hour brain heart infusion broth culture. Then it was incubated at 37ºC. After 48 hours 0.5 ml of Kovac's reagent was added and gently shaked.

Interpretation

Pink color ring appears if the test is positive.If the test is negative there is no color change. Indole test is negative in staphylococcus aureus isolates.

Urease test[77]

The organism produces the enzyme urease, which decomposes the urea in the medium by hydrolysis into ammonia and carbondioxide. This results in increase of pH, of the medium producing purple pink color.

Procedure

The colonies isolated from 18-24 hour culture plate was heavily inoculated over the Christensen's urease agar slope and incubated at 37ºC overnight.

Interpretation

All Staphylococcus aureus isolates were urease test positive.

Disc diffusion method[78]

Inoculum Preparation

Four to five colonies of the same morphology is selected from an agar culture plate. With a sterile bacteriological loop, the growth was inoculated into broth medium which was incubated for 3 to 5 hours to achieve a turbid suspension .This is compared with 0.5 McFarland standard.

0.5 McFarland Turbidity standard preparation

This is prepared by adding 0.05ml of 1% anhydrous BaCl2 to 9.95 ml of 1% H2SO4 in a test tube, which is sealed and kept in refrigerator.

Inoculation and incubation

The sensitivity to common antibiotics was done by Kirby Bauer disc diffusion method as recommended by CLSI. Control strains used are staphylococcal aureus ATCC -25923 and MRSA -43300.

A swab was submerged in bacterial suspension and was inoculated into, Mueller Hinton Agar plate. The surface of the plate is swabbed in three directions so that there is even and complete distribution of the inoculum. Within 15 minutes of inoculation antibiotic discs were applied using a sterile forceps.

The antimicrobial discs used were procured from Himedia. The drugs oxacillin(1μg), cefoxitin(30μg), penicillin(10u), linezolid(30 μg),

vancomycin(30µg) , doxycycline (30µg), amoxyclavulanic acid (30µg), cephelexin(30µg), cotrimoxazole(25µg), cefotaxime(30µg), amikacin(30µg), ciprofloxacin(5µg),were dispensed onto the surface of the inoculated agar plate using sterile forceps.

Each disc was pressed down to ensure complete contact with the agar surface. Then plates were inverted for incubation as accumulation of moisture leads to interference in test interpretation.

Incubation is at 37ºC for 24 hrs after which , the zone of inhibition was measured by using zone measuring scale and interpreted as per the CLSI standards. Transmitted light was used to examine the light growth of methicillin resistant isolates.

Interpretation of disc diffusion test

Disc diffusion test for detecting Methicillin resistance.

Oxacillin disc diffusion test[38]

Zone diameter of 13mm or more was taken as sensitive,11 to 12mm was taken as intermediate sensitive and 10 mm or less is considered as MRSA.

Cefoxitin disc diffusion test

Zone diameter of 22 mm or more was taken as sensitive and 21 mm or less was considered as resistant. These resistant isolates were considered as MRSA.

'D' test[3,55,56]

A 0.5 McFarland suspension of staphylococci was inoculated on Mueller Hinton agar plate. Clindamycin (2μg), and erythromycin (15μg), discs were placed at an edge-to-edge distance of 15 to 20mm, followed by overnight incubation at 37^{o}C.

Description of different types of phenotypes that were looked for:

Inducible Clindamycin resistance: (iMLS$_B$ resistance)

Staphylococcal isolates showing resistance to erythromycin (zone size ≤ 13mm) and a clear, D- shaped zone of inhibition round the clindamycin disc was designated as the inducible clindamycin resistance (D phenotype).

MS phenotype

In this phenotype Staphylococcal isolates were erythromycin resistant (zone size ≤ 13mm) .But sensitive to clindamycin (zone size ≥ 21mm) showing circular zone of inhibition around it.

Constitutive resistance (cMLS$_B$ resistance)

Staphylococcal isolates resistant to erythromycin (zone size ≤ 13mm) and resistant to clindamycin (zone size ≤ 14 mm) were brought under this phenotype.

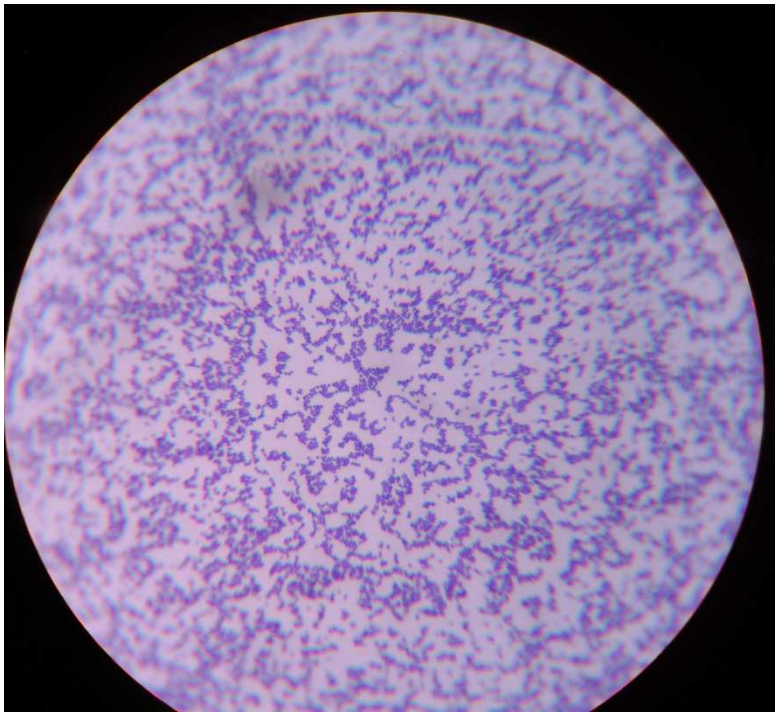
Susceptible phenotype(S phenotype)

Staphylococcal isolates sensitive to both erythromycin (zone size ≥ 23mm) and clindamycin (zone size ≥ 21mm) were categorized in this phenotype.

Antimicrobials with interpretation of zone size

Antimicrobial agent (µg)	Inhibition zone in mm		
	Resistant ≤	Intermediate	Sensitive ≥
Oxacillin 1 µg	10	11-12	13
Cefoxitin 30µg	21	-	22
Erythromycin 15µg	13	14-22	23
Clindamycin 2µg	14	15-20	21
Linezolid 30 µg	-	-	21
Vancomycin 30 µg	-	-	15
Amikacin 30 µg	14	15-16	17
Doxycycline 30 µg	12	13-15	16
Cotrimoxazole 25 µg	10	11-15	16
PenicillinG 10 units	28	-	29
Amoxyclavulanicacid 30µg	19	-	20
Cephelexin 30µg	14	15-17	18
Cefotaxime 30 µg	14	15-22	23
Ciprofloxacin 5 µg	15	16-20	21

Fig1: Gram stain showing Staphylococcus aureus



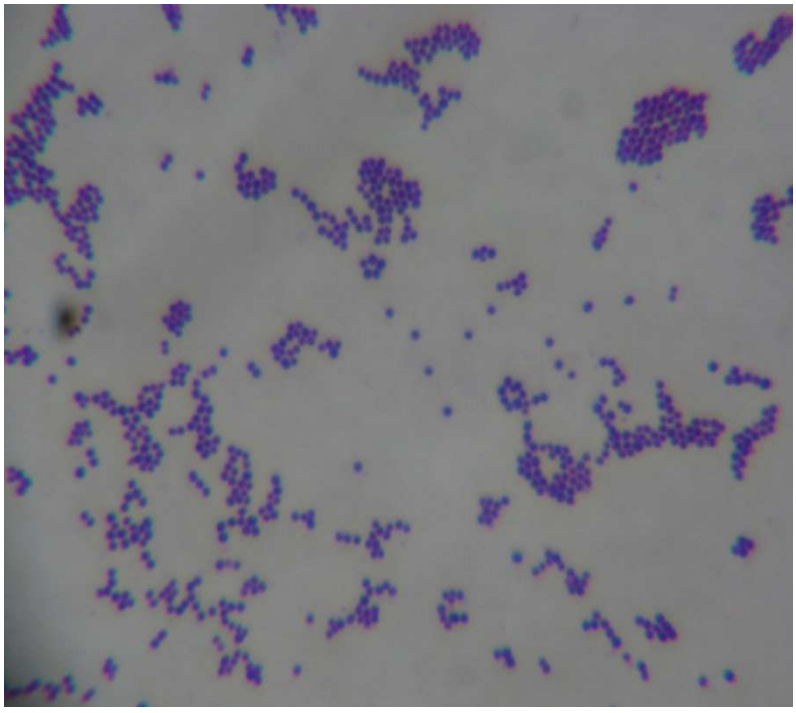


Fig2: Beta Haemolysis on Blood agar

Fig3: Staphylococcus aureus colonies on Mannitol salt agar

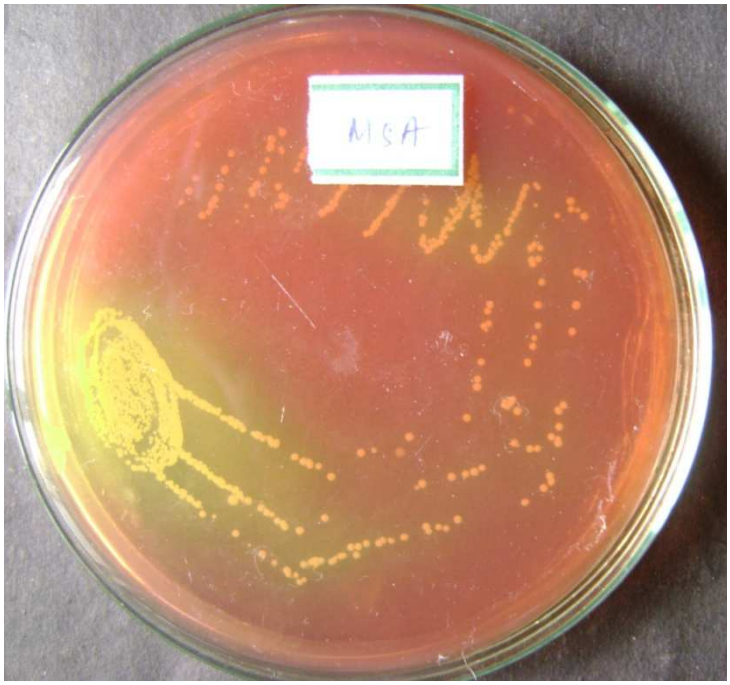


Fig4: Slide coagulase test

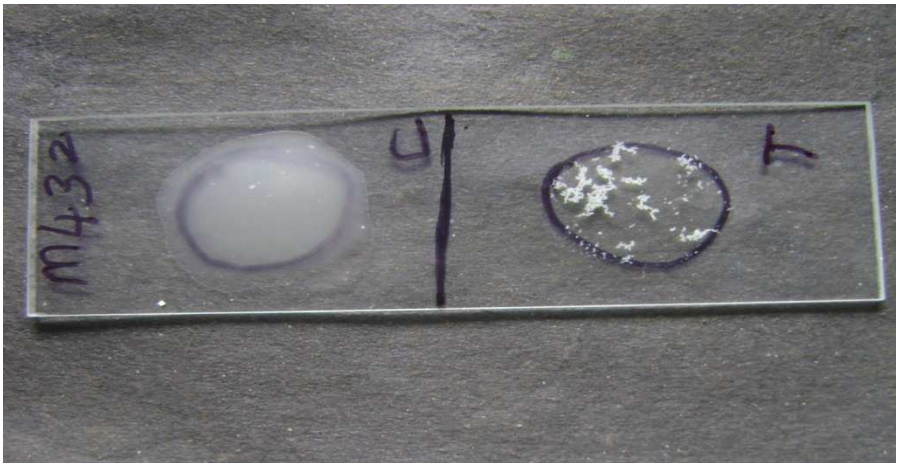


Fig5: Tube coagulase test

Fig6: Antibiogram of S.aureus isolates

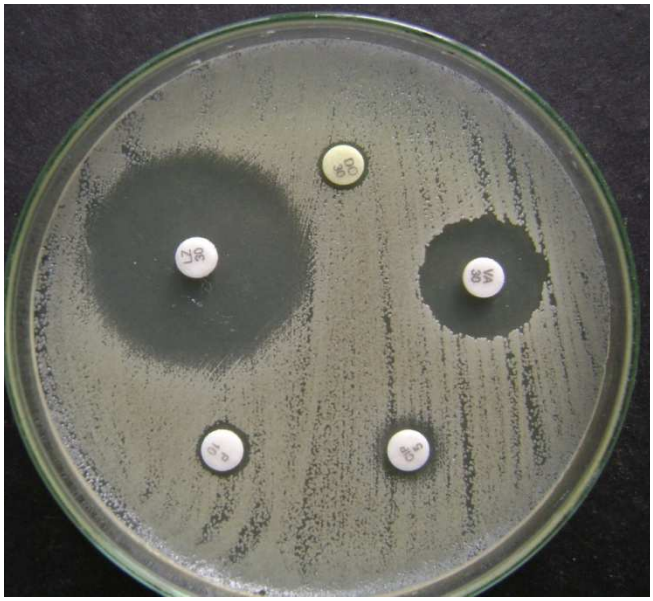


Fig7: MRSA detection using cefoxitin and oxacillin discs

Fig8: Constitutive Clindamycin resistance

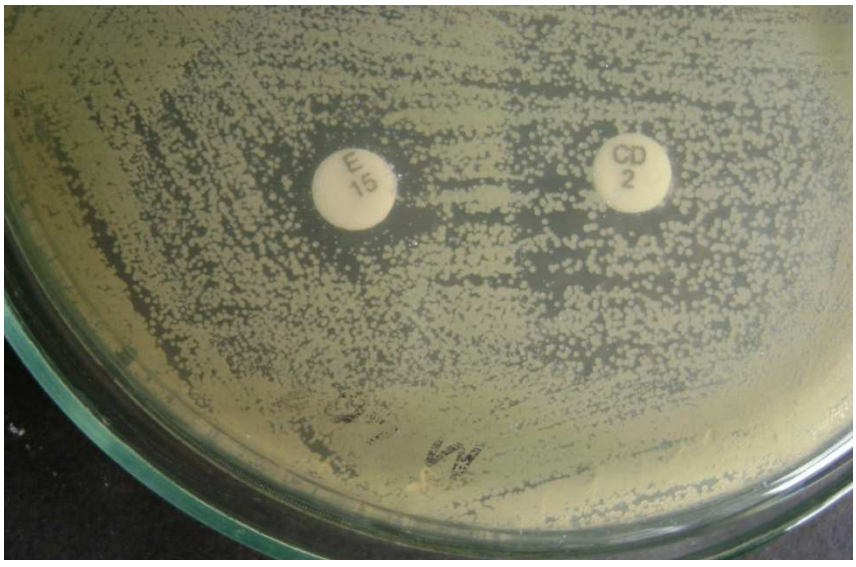


Fig9: Inducible clindamycin resistance

RESULTS

RESULTS

The study was performed during the period from September 2011 to August 2012 at department of microbiology, Coimbatore Medical College Hospital. This study was done to find the incidence of MRSA using oxacillin and cefoxitin disc diffusion methods and to compare inducible clindamycin resistance with constitutive resistance.

The study included 200 staphylococcus aureus isolates from samples like pus, blood, sputum,vaginal swab, urine and body fluids.

Among 200 staphylococcus aureus isolates the sample wise distribution was as follows. Pus constituted 175 (87.5%), urine 10 (5%), blood 6 (3%), sputum 4 (2%), vaginal swab 3 (1.5%) and synovial fluid 2 (1%), as given in Table1 and Chart1.

The above observation shows that staphylococcus aureus was isolated maximally from pus Samples (87.5%) and only few were isolated from urine, blood, sputum, vaginal swab and other body fluids.

The resistant and sensitivity pattern of staphylococcus aureus isolates to different antibiotic groups is given in Table2 and Chart 2.

Out of the 200 staphylococcus aureus isolates100% were sensitive to linezolid and 99% were sensitive to vancomycin.

77 % were sensitive to amikacin, 73%were sensitive to doxycycline, 69% were sensitive to cotrimoxazole, 68.5 % were sensitive

to cephalexin, 66.5 % were sensitive to amoxy clavulanicacid, 64 % were sensitive to cefotaxime, 59 % were sensitive to ciprofloxacin.

Staphylococcus aureus strains were highly sensitive to linezolid and vancomycin. Moderate level sensitivity was seen in amikacin, doxycycline, cotrimoxazole, cephalexin, amoxy clavulanicacid, cefotaxime and ciprofloxacin.

Table2 and Chart 2 lists the resistance pattern of staphylococcus aureus isolates. Out of the 200 isolates 100% were resistant to penicillin G, 33.5% were resistant to ciprofloxacin, 33.5 % were resistant to amoxy clavulanicacid, 27.5 % were resistant to cephalexin, 27.5% were resistant to cotrimoxazole, 26.5 % were resistant to cefotaxime, 24.5% were resistant to doxycycline, 20.5 % were resistant to amikacin and 1% were resistant to vancomycin.

Staphylococcus aureus isolates were 100% resistant to penicillin and 100% sensitive to linezolid. Moderate level of resistance were seen to amikacin, ciprofloxacin, doxycycline, co-trimoxazole, cephalexin, cefotaxime and amoxy clavulanicacid. Very minimal resistance was noted in vancomycin.

As evident from Table 3 and Chart 3 among 200 isolates of staphylococcus aureus, 26% were resistant and 74 % were sensitive to

cefoxitin whereas 24% were found to be resistant and 76% were sensitive to oxacillin as determined by disc diffusion method.Cefoxitin disc detected higher percentage of methicillin resistant staphylococcus aureus by disc diffusion method.

Among 200 staphylococcus aureus isolates74% MSSA and 26% MRSA were observed as given in Table 4 and Chart4.

Age wise distribution as given in Table 5 and Chart 5 shows out of 200 S.aureus isolates taken for study, 15.50% between 1-12 years, 9.50% between 13-20 years, 42.50% between 21-40 years, 19.50% between 41-60 years and 13% more than 60 years of age.

Out of 52 MRSA isolates 9.61% were between 1-12 years, 11.54% were between 13-20 years, 51.92% were between 21-40 years, 15.38% were between 41-60 years, and 11.54% were more than 60 years of age.

From this it is inferred that maximum staphylococcus aureus and MRSA isolates were from the age group between 21-40 years followed by 41-60 years age.

Among 52 MRSA isolates sex ratio was found to be 65.38 % Males and 34.61 % Females .This is given in Table 6 and Chart 6 indicating predominance of MRSA among males.

As listed in Table 7 and Chart 7 out of 200 Staphylococcal isolates 40 % were isolated from wound infection, 9 % from cutaneous ulcer, 8 %

from abscess, 7.5 % from cellulitis, 7.5 % from suppurative otitis media, 6 % from pyoderma, 5% from urinary tract infection 4% from osteomyelitis, 3% from burns, 3% from septicemia, 2% from pneumonia 1.5 % from gangrene, 1.5 % from vaginal infection 1% from necrotizing fascitis, and 1% from septic arthritis.

MRSA were isolated from 44.23 % of wound infection, 11.54 % of cutaneous ulcer, 9.62% of abscess, 7.69 % of cellulitis, 7.69 % of pyoderma , 5.77 % of osteomyelitis, and 3.85 % of urinary tract infection. Burns, septicemia, gangrene, necrotizing fascitis, and suppurative otitis media cases constituted 1.92% of MRSA each.

It is inferred from the above data that wound infections constituted higher percentage of MRSA.

Analysis of clindamycin Resistance in 52 MRSA isolates showed 42.30% of inducible clindamycin Resistance, 30.76 % of constitutive clindamycin Resistance, and 26.92% were sensitive to both erythromycin and clindamycin. MS phenotype was not observed as given in Table 8 and Chart 8.

Above observation shows that, inducible clindamycin resistance was reported in a higher percentage than constitutive clindamycin resistance.

TABLE NO.1

FREQUENCY OF STAPHYLOCOCCUS AUREUS

ISOLATES IN DIFFERENT SPECIMENS

n-200

Samples	Total no of S.aureus isolates	Percentage
Pus	175	87.5%
Urine	10	5%
Blood	6	3%
Sputum	4	2%
Vaginal swab	3	1.5%
Synovial fluid	2	1%

TABLE 2

ANTIBIOTIC SENSITIVITY PATTERN OF

STAPHYLOCOCCUS AUREUS

n=200

Drugs	Sensitive	Intermediate sensitive	Resistant
Linezolid	200 (100%)	-	-
Vancomycin	198 (99%)	-	2 (1%)
Amikacin	154 (77 %)	5 (2.5 %)	41 (20.5%)
Doxycycline	146 (73%)	5 (2.5%)	49 (24.5%)
Cotrimoxazole	138 (69%)	7 (3.5%)	55 (27.5%)
Cephalexin	137 (68.5%)	8 (4%)	55 (27.5%)
Amoxy clavulanic acid	133 (66.5%)	-	67 (33.5%)
Cefotaxime	128 (64%)	19 (9.5%)	53 (26.5%)
Ciprofloxacin	118 (59%)	15 (7.5%)	67 (33.5%)
penicillin G	-	-	200 (100 %)

TABLE-3

DETECTION OF METHICILLIN RESISTANCE BY DISC DIFFUSION TEST USING OXACILLIN AND CEFOXITIN DISCS

n=200

Discdiffusion test	Cefoxitin(30µg) disc	Oxacillin (1µg) disc
Resistant	52 (26 %)	48 (24 %)
Sensitive	148 (74 %)	152 (76%)

TABLE 4

PREVALENCE OF MRSA AMONG STAPHYLOCOCCUS AUREUS ISOLATES

Total isolates	MRSA	MSSA
200	52 (26%)	148 (74%)

TABLE -5

AGE WISE DISTRIBUTION OF MRSA

Age in years	Total no of S.aureus isolates(200)	MRSA(52)
1-12	31 (15.50%)	5 (9.61%)
13-20	19 (9.50%)	6 (11.54%)
21-40	85 (42.50%)	27 (51.92%)
41-60	39 (19.50%)	8 (15.38%)
> 60	26 (13.%)	6 (11.54%)

TABLE.6

GENDER DISTRIBUTION OF MRSA

Sex	Total (200)	MRSA (52)
Male	120	34 (65.38 %)
Female	80	18 (34.61%)

TABLE-7

DISTRIBUTION OF MRSA AMONG VARIOUS INFECTIONS

Diseases	Total (200)	MRSA (52)
Wound infection	80 (40 %)	23 (44.23 %)
Cutaneous ulcer	18 (9 %)	6 (11.54 %)
Abscess	16 (8 %)	5 (9.62%)
Cellulitis	15 (7.5 %)	4 (7.69 %)
Pyoderma	12 (6 %)	4 (7.69 %)
Osteomyelitis	8 (4%)	3 (5.77 %)
Urinary tract infection	10 (5%)	2 (3.85 %)
Suppurative otitis media	15 (7.5 %)	1 (1.92%)
Burns	6 (3%)	1 (1.92%)
Septicemia	6 (3%)	1 (1.92%)
Gangrene	3 (1.5 %)	1 (1.92%)
Necrotizing fascitis	2 (1%)	1 (1.92%)
Pneumonia	4 (2%)	0 (0%)
Vaginal infection	3 (1.5%)	0 (0%)
Septic arthritis	2 (1%)	0 (0%)

Table.8: Clindamycin Resistant phenotypes of MRSA by D-test

Susceptibility pattern (phenotype)	MRSA(52)	Percentage (26%)
ERY R, CLI-S (D -Test positive; $iMLS_B$)	22	42.30%
ERY-R, CLI-R ($cMLS_B$)	16	30.76%
ERY-S, CLI-S (S - Phenotype)	14	26.92%
ERYR, CLI-S (D –Test negative;MS Phenotype)	Nil	0 %

ERY- R: Erythromycin resistant.

CLI-R : Clindamycin resistant

CLI-S: Clindamycin sensitive

ERY-S: Erythromycin sensitive.

$iMLS_B$- Inducible Clindamycin resistance

$cMLS_B$- Constitutive Clindamycin resistance

S – Phenotype: Susceptible phenotype

MS phenotype- Macrolide Streptogramin (type B) resistance.

CHART: 1

FREQUENCY OF STAPHYLOCOCCUS AUREUS ISOLATES IN DIFFERENT SPECIMENS

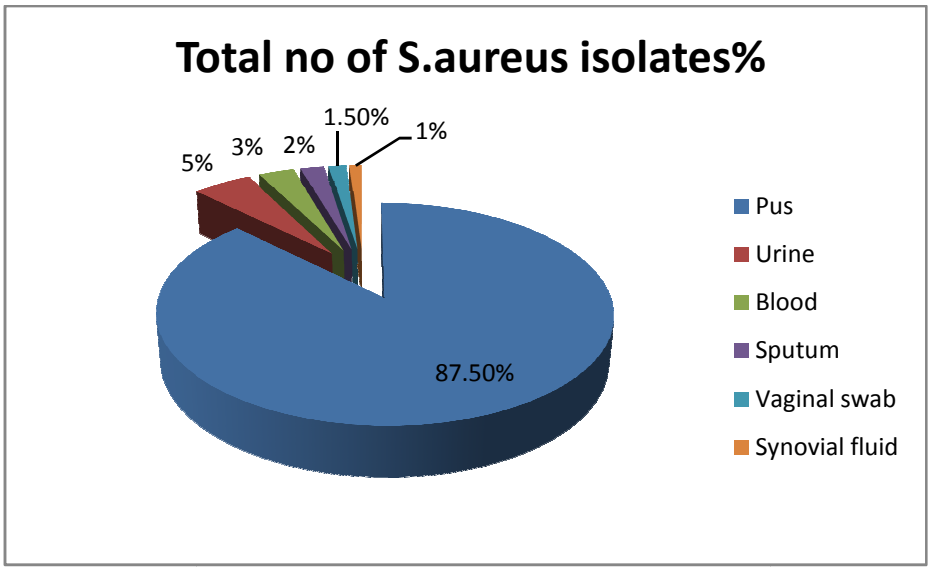


CHART: 2

ANTIBIOTIC RESISTANT PATTERN OF STAPHYLOCOCCUS AUREUS

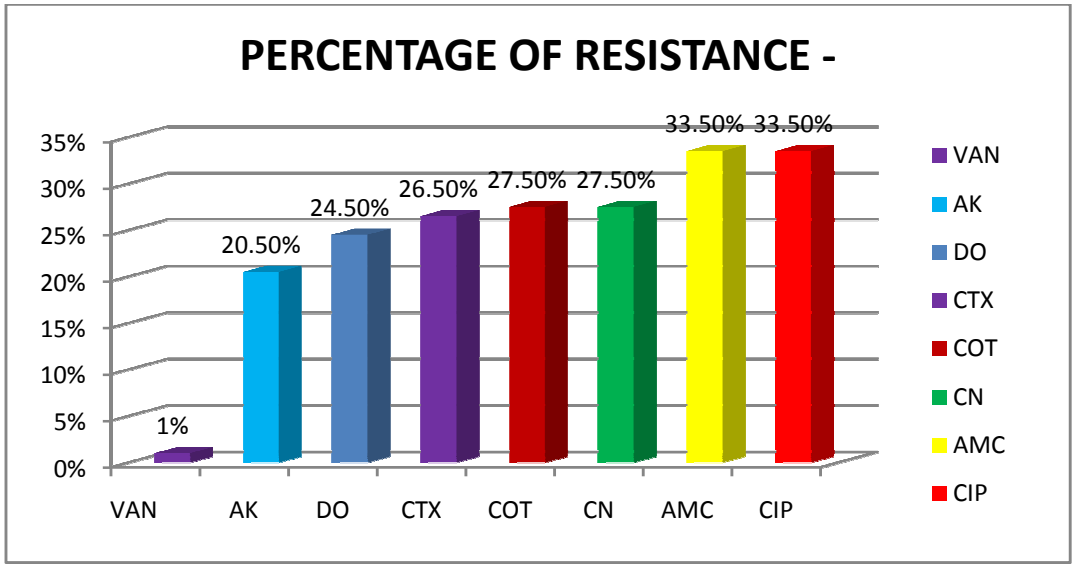


CHART: 3

DETECTION OF METHICILLIN RESISTANCE BY DISC DIFFUSION TEST USING OXACILLIN AND CEFOXITIN DISCS

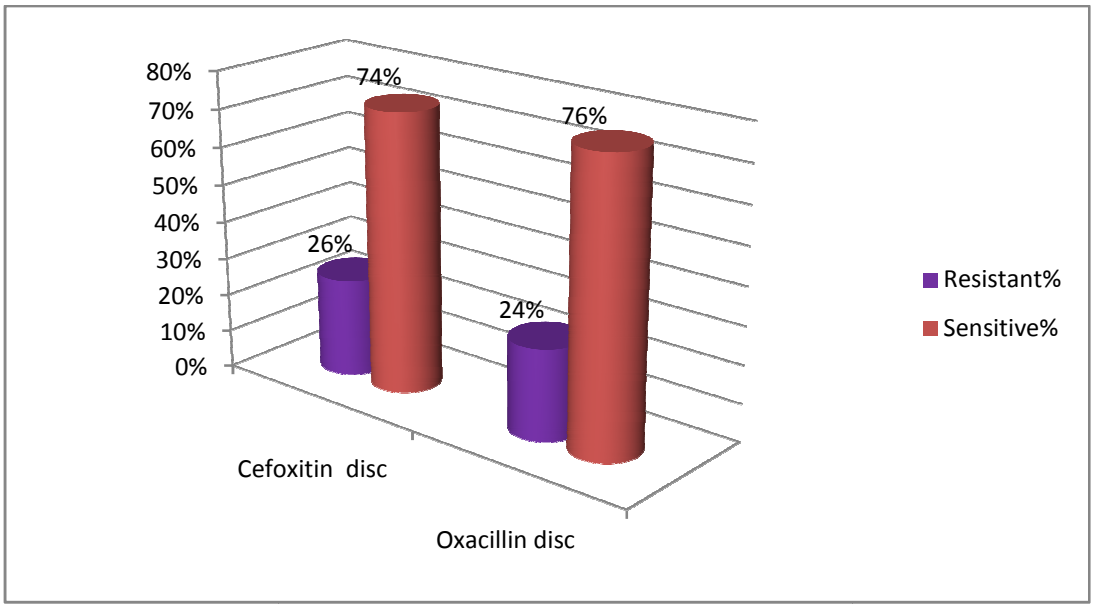


CHART: 4

PREVALENCE OF MRSA AMONG STAPHYLOCOCCUS AUREUS ISOLATES

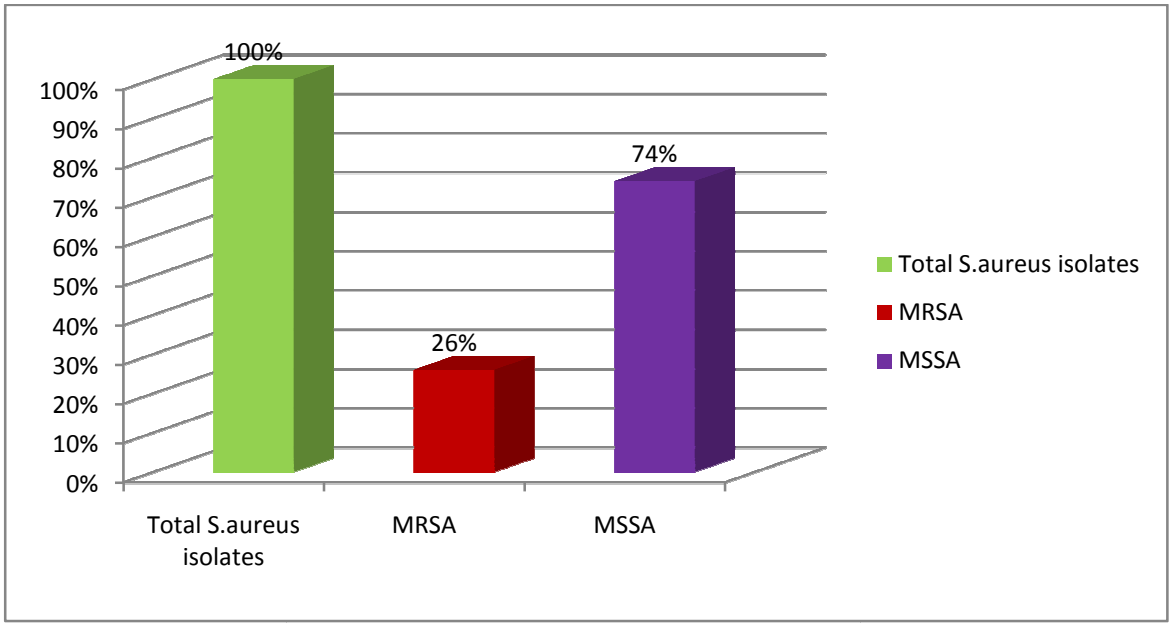


CHART: 5

AGE WISE DISTRIBUTION OF MRSA

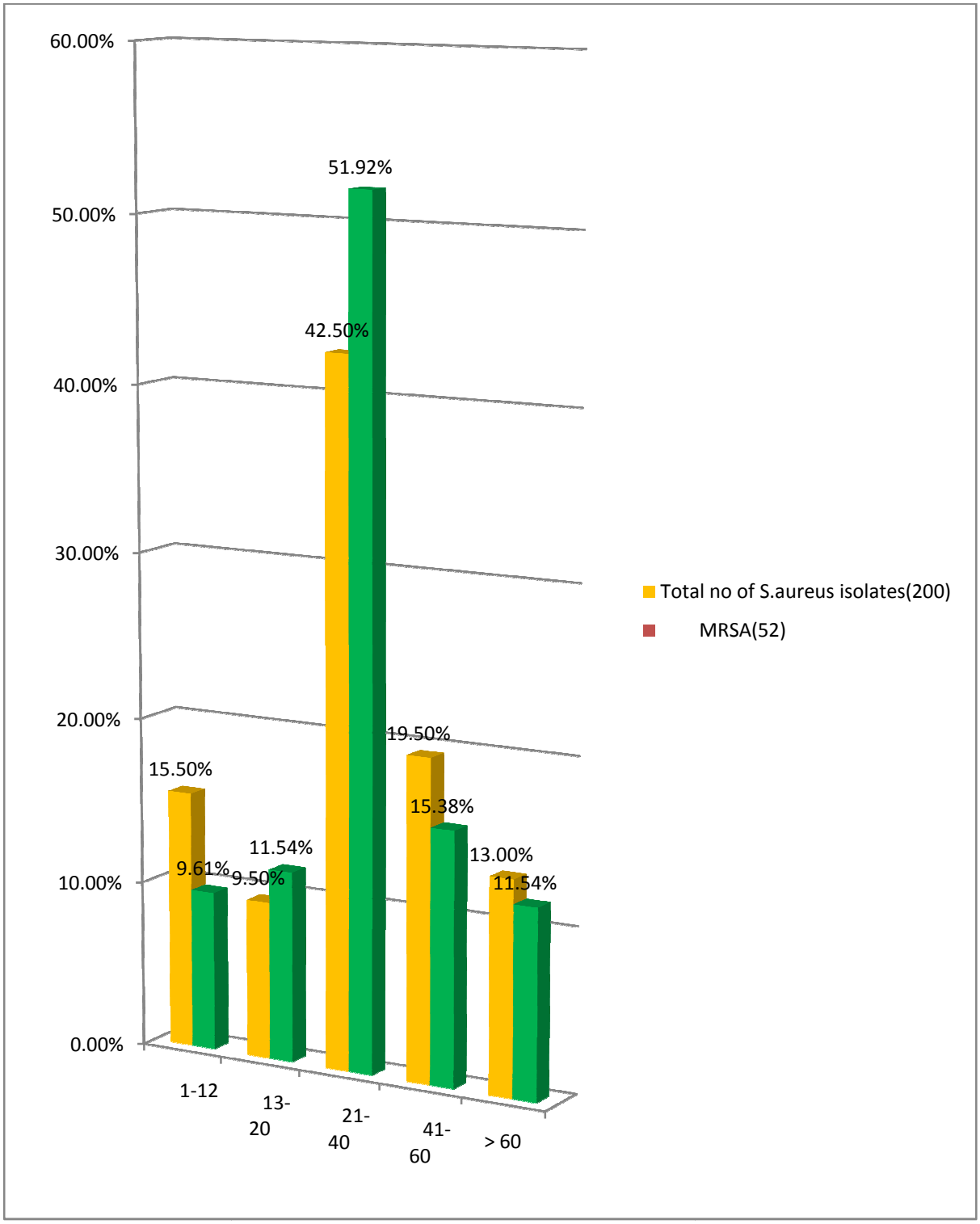


CHART: 6

GENDER DISTRIBUTION OF MRSA

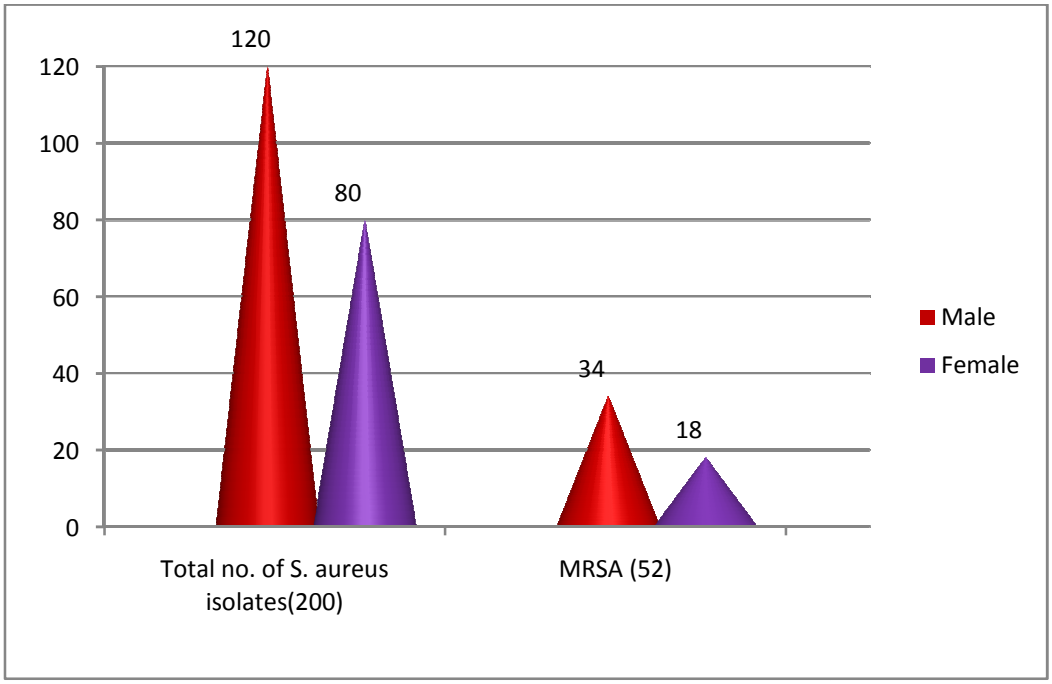


CHART: 7

DISTRIBUTION OF MRSA AMONG VARIOUS INFECTIONS

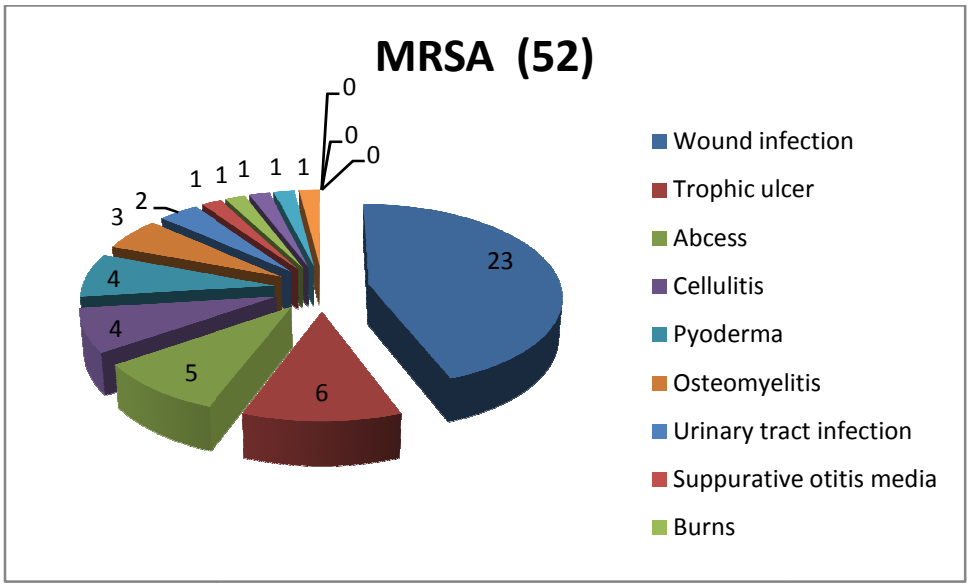
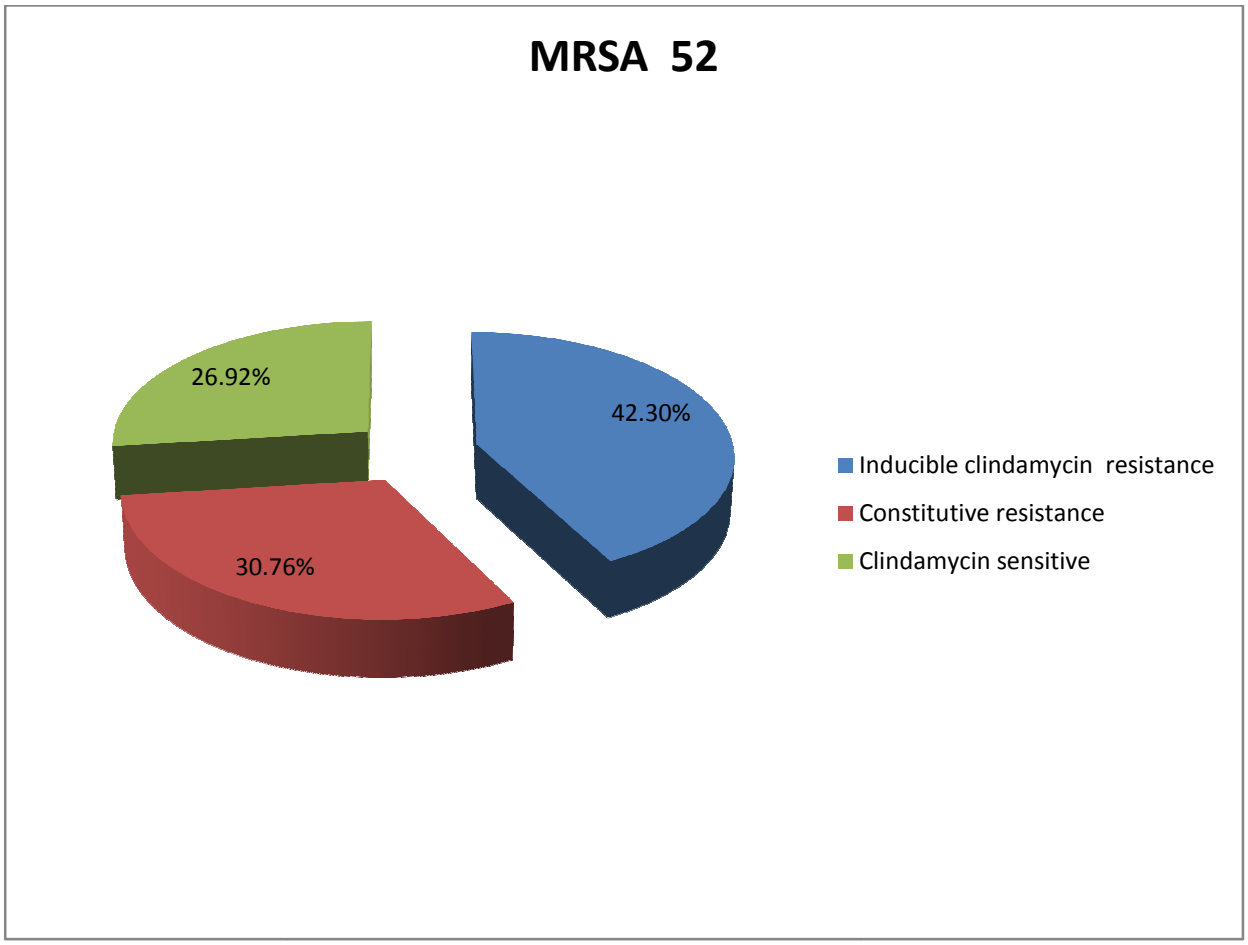


CHART: 8:

RESISTANT PHENOTYPES OF MRSA BY D-TEST



DISCUSSION

DISCUSSION

MRSA is a major cause of hospital and community acquired infections. Clindamycin is an excellent drug to treat not only serious infections like sepsis, endocarditis, osteomyelitis, pneumonia, and staphylococcal scalded skin syndrome caused by MRSA but also MSSA. It is less expensive compared to newer antibiotics.

As it can be given orally it can be used in outpatient therapy. Drugs like tetracyclines and fluroquinolones are not advised for treating children and pregnant women due to side effects. But clindamycin is a treatment of option in children and it can also be used in penicillin allergic individual[1,62].

It is very necessary to distinguish between staphylococci having inducible clindamycin resistance from those with MS Phenotype. Because MS Phenotype in staphylococcal strains does not result in failure of therapy, whereas it occurs in inducible clindamycin resistance[1].

D test is a simple, reliable and significant test. Sensitivity of D test performed at 15mm disk spacing is 100% corelated with detection of erm genes by polymerase chain reaction[63].

In the present study 200 samples were processed and results were analysed.

In this study majority of the staphylococcus aureus isolates ,(87.5%) were from pus samples while 5%, were from urine, 2% were from sputum, 3%, were from blood, 1.5% were from vaginal swab and 1% were from synovial fluid. This is supported by the study of **Vidyapai et al 2011** who has isolated 181(76.3%) of staphylococcus aureus in pus samples followed by 28(11.81%) from urine, 17(7.17%) from respiratory specimen, 9(3.79%) from blood and 2(0.84%) from body fluids[65]. This also correlates with the study conducted by **Anupurba et al 2003,** in which, they have reported 381(69.39%) of staphylococcus aureus in pus samples followed by 59 (10.74%) from urine, 25(4.55%) from high vaginal swab, 27(4.91%) from body fluids, and sputum 23(4.18%)[79]. **Lakari Saikia et al 2009** has reported 46.67% of staphylococcus aureus from pus and 42.86% from sputum[24].

The present study showed multidrug resistant pattern of staphylococcus aureus as amikacin 20.5%, ciprofloxacin 33.5%, doxycycline 24.5%, cotrimoxazole 27.5%, cephelexin 27.5 % cefotaxime 26.5 %, penicillin G 100%, vancomycin 1% and amoxy clavulanic acid 33.5 %.The present study showed 100% sensitivity to linezolid. In accordance with present study, **Shilpa Arora et al 2010** has reported antimicrobial resistance of staphylococcus aureus as amikacin(22%), ciprofloxacin (52.8%) , cephelexin (56.8%) and penicillin(78.4%).

Staphylococcus aureus was 99.2% sensitive to linezolid and 100% sensitive to vancomycin[49]. **Adebayo O Shittu et al** 2006 in his study of 227 staphylococcus aureus isolates has reported that 70 isolates (30.8%) were resistance to cotrimoxazole, 68 isolates (30%) were resistant to tetracycline[54]. **Vidhani S**. **et al 2001** has documented 87% of staphylococcus aureus isolates resistant to amoxy clavulanic acid, 100% resistant to penicillin and 78.5% resistant to cefotaxime[80].

The present study showed 26% of MRSA among 200 staphylococcus aureus isolates. The above data correlates with the result of **Vidyapai et al 2011** who has documented 29.1% MRSA[65]. This is in accordance with study of **Gupta V et al 2009** who has documented 25% of MRSA among 200 staphylococcus aureus isolates[66]. **Pal N 2010** has documented 31.60% of MRSA[56] , **Oommen S.K 2010** has reported 28.7% of MRSA[81]. **Jadhav Savita Vivek et al 2011** has reported 32.5% of MRSA[82].In contrary **Anupurba S et al 2003** has reported 54.8% of MRSA in their study[79].

Presence of predisposing factors such as prolonged hospital stay and antibiotic intake as evidenced by **Mathanraj etal** may be the reason for high MRSA report among inpatients[25]. Invasive procedures and use of resistant antibiotics results in bacteremia by MRSA[7].

By disc diffusion method the present study showed 26% of MRSA using cefoxitin disc,and 24% of MRSA by oxacillin disc. Similarly **Shilpa Arora et al** 2010 have detected 46% of MRSA by cefoxitin disc diffusion method and 40.4% of MRSA by oxacillin disc diffusion method .This shows that cefoxitin is superior to oxacillin in detecting MRSA [49].

Maximum number of MRSA isolates in this study were among 21-40 years (51.92%) followed by 41-60 years (15.38%). Similarly **Gayathri Naik et al 2011** studies report maximum number of patients belong to the to age group of 21- 30 years. the males being 25.9% and females 22.2%[83].

In the Present study among MRSA isolates 65.38 % were males and 34.61% were females. In the study by **Shaileshkumar et al 2011,**59 % of males and 41 % of females infected with MRSA has been reported [61]. **Waness A in 2010** has mentioned that MRSA infections have male predilection[7]. This may be attributed to the increased mobility of the male population.

In this study highest MRSA is reported in wound infections (44.23%). Orthopaedic patients operated for open fractures showed highest incidence of wound infection followed by surgery and obstetrics

and gynaecology**. Shilpa Arora et al** 2010isolated 54.8% of MRSA from surgical units, and 27.8% from orthopaedic wards [49] . **Shaileshkumar et al** isolated MRSA, commonly from surgical site infection, orthopaedic infection and bone fractures [61].

Mathanraj et al isolated highest number of MRSA in orthopaedic ward and dermatology ward. Patients with extensive skin lesions are heavy shedders of MRSA .So the rate was high in orthopaedic ward and dermatology ward. It was due to big surface area of denuded skin with large inoculam of organism that can easily be transmitted to other patients via hands of health care workers[25].

The common complications following all operative procedure is surgical site infections. Pre operative care, the theatre sterility, postoperative care, overcrowding, and the type of surgery are some of the factors which determine the surgical site infections. Contamination from the external environment is the most probable reason for the wound infection (**Gayathri Naik et al 2011)** [83]. In a study at AIMS New Delhi **Arti Tyagi et al 2008** has reported high intensity of MRSA in ICU and surgical units due to greatest antibiotic usage [84]. The increased incidence of MRSA in wound infection is due to the production of PVL by MRSA which is associated with tissue necrosis, leucocyte destruction[20].

Clumping factor, coagulase and hyaluronidase helps in invasion and existence in tissues[20]. Higher rate of MRSA carriage has been reported in the surgical, orthopaedic, obstetric and gynecological wards [25,61,84]. This might be attributed to prolonged hospital stay due to fractures and operative procedures. **J B Sarma, G.U.Ahmed in 2010** reported that surgery is a risk factor as prophylactically used antibiotics is irrationally continued for several days which may account for the acquisition of MRSA [85].

Present study showed higher rate (42.30%), of inducible clindamycin resistance than constitutive clindamycin resistance (30.76%), among MRSA. Similarly **Ciraj AM et al 2009** has reported 38% of inducible clindamycin resistance and 15.3% of constitutive clindamycin resistance[86]. Likewise **Bidya Shrestha et al 2009** has reported higher percentage (44.4%) of inducible clindamycin resistance than constitutive clindamycin resistance (39.7%)[87]. **Angel MR et al 2008,** [60] and **Sureerat Chelae 2009**[88] have reported 37%, 35.9% of inducible clindamycin resistance respectively. **Vasanthi R et al** has reported17.3% of inducible clindamycin resistance and 9.6% of constitutive clindamycin resistance [63]. **Deotale et al 2010** have reported 14.5% of inducible clindamycin resistance and 3.6% of constitutive clindamycin resistance[55]. **Jadhav Savita Vivek et al 2011** have reported 24.8% of inducible clindamycin

resistance and 8.2% of constitutive clindamycin resistance[82]. **Kavitha prabhu et al 2011** have documented 20% of inducible clindamycin resistance and 16.6% of constitutive clindamycin resistance [5].

Ajanta GS et al have documented high rate (74%) of inducible clindamycin resistance[57]. **Amruthkishan Upadhya et al 2011** have documented high rate (61.08%) of inducible clindamycin resistance in their study [89]. **Shailesh kumar et al 2012** reported 75% MRSA isolates found to be $iMLS_{B.}$ The low constitutive resistance was that the drug is not commonly used and so there is less selection of resistant strains [61] . Contrasting results were published by **Gupta V et al** 2009 who has documented high rate of constitutive clindamycin resistance (46%) than inducible clindamycin resistance (20%)[66]. **Hwan Sublim 2006** has reported high rate of 78% in constitutive clindamycin resistance and 19% inducible clindamycin resistance (20%)[62]. Mohamad 2007 has reported 47.6% constitutive clindamycin resistance and 22.6% inducible clindamycin resistance[16]. **Angel MR et al 2008** has not reported any constitutive clindamycin resistance in their study[60].

The present study haven't found out any MS Phenotype.Likewise **Sureerat Chelae 2009** has reported only 1.1 % of MS Phenotype [88]. In contrary 7.97% of MS Phenotype has been reported by **Amruthkishan**

Upadhya et al 2011 [89]. Clindamycin susceptible rates are higher than erythromycin regardless of methicillin susceptibility. The difference in percentage of $iMLS_B$ resistance and constitutive resistance is explained by the difference in bacterial susceptibility in various geographical areas and varied antibiotic prescription by the clinicians[64].

Staphylococcus aureus has emerged as a major cause of nosocomial infections for quite some time. Clindamycin is a very useful drug in treating skin and soft tissue infections. It can be used in penicillin allergic individual. It is a promising therapeutic option in the era of drug resistance. The costly antibiotics like vancomycin can be reserved for severe illness.The erythromycin resistant Staphylococcal isolates will be misidentified as clindamycin sensitive if D test is not performed. To avoid prescribing clindamycin to those who exhibit inducible clindamycin resistance, D test must be done routinely.

Giving false report that patient is infected with MRSA will lead to fatal consequences due to inadequate therapy, whereas wrongly labelling the patient infected with MSSA as MRSA will lead to unwanted usage of costly drugs like vancomycin.

SUMMARY

SUMMARY

- Majority of staphylococcus aureus were isolated from pus samples (87.5%).
- Staphylococcus aureus was highly sensitive to linezolid (100%) and vancomycin (99%).
- Antimicrobial sensitivity results showed that staphylococcus aureus were 100% resistant to penicillin.
- Moderate level of antimicrobial resistance were seen to amikacin (20.5%), ciprofloxacin (33.5%), doxycycline (24.5%), cotrimoxazole (27.5%) , cephelexin(27.5 %), cefotaxime (26.5 %), and amoxy clavulanic acid (33.5 %).
- Cefoxitin disc detected higher percentage (26%) of MRSA by disc diffusion method on comparison to oxacillin disc diffusion method which detected (24%) of MRSA.
- Sex distribution revealed predominance of males (65.38%) over females (34.61%) among the 200 staphylococcus aureus isolates.
- Among 52 MRSA maximum number of isolates were from the age group between 21-40 years (51.92%) followed by 41-60 years (15.38%).
- Wound infections constituted higher percentage (44.23%) of MRSA followed by cutaneous ulcer (11.54%) and abscess(9.62%).

- MS Phenotype was not reported in the present study.Erythromycin and clindamycin sensitivity was noted in 26.92 %.Higher percentage of inducible clindamycin resistance (42.30%)was reported in MRSA than constitutive clindamycin resistance(30.76%).

CONCLUSION

CONCLUSION

Among the 200 staphylococcus aureus isolates 26% were methicillin resistant. Analysis of clindamycin resistance in 52 MRSA isolates showed 42.30% of inducible clindamycin resistance. These isolates are seemed to be susceptible to clindamycin in vitro but, treatment failure occurs when this drug is instituted as invivo therapy. MRSA infection in surgical site is commonly noted. Multidrug resistance to commonly used drugs like ciprofloxacin, amikacin, doxycycline and cotrimoxazole are to be noted with concern.

Staphylococcus aureus is a leading cause of hospital acquired infections including pneumonia, endocarditis, bacteremia, and surgical wound infections. The problem is exacerbated by the ability of the MRSA to colonize the individuals years together and infect them frequently.

The increase in staphylococcus aureus infections is a outcome of organism's ability to adapt to a changing environment and its capability to spread. MRSA is a threat not only to immunocompromised individuals, but also to general public. Moreover emergence of drug-resistance among MRSA is now a major concern.

So detection of methicillin resistance in staphylococcus aureus is very important for treating patients and to prevent its spread.

Drugs like clindamycin are needed to stem the severe consequences of MRSA. Use of clindamycin avoids costly, intravenous glycopeptides for treating MRSA. Clindamycin is a treatment of option in children. It can be used in penicillin allergic individual. It has good oral bioavailability. So it can be used by clinicians as outpatient therapy as well as to switchover after intravenous antibiotics in hospitalized patients.

The pattern of clindamycin resistance to MRSA varies in different regions. When clindamycin is considered for therapy, the kind of resistance (inducible or constitutive clindamycin resistance) which exists to be detected.

'D test' is absolutely necessary in microbiology laboratories. This is because it avoids misinterpretation of clindamycin resistance by clearly delineating inducible clindamycin resistance from constitutive clindamycin resistance. Moreover it is simple, cost effective, and reliable.

So 'D' test is suggested along with routine antibiotic susceptibility testing to detect inducible clindamycin resistance and thus avoid treatment failure. Hence this study was done.

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ANNEXURES

PROFORMA

Name**:** Date:

Age**:** IP /OP No:

Sex**:** Ward:

Address**:**

Diagnosis of the patient**:**

Specimen**:**

Laboratory analysis:

Microscopy:

Culture:

Biochemical reactions:

Antibiomicrobial sensitivity:

Interpretation:

Details of Investigator:

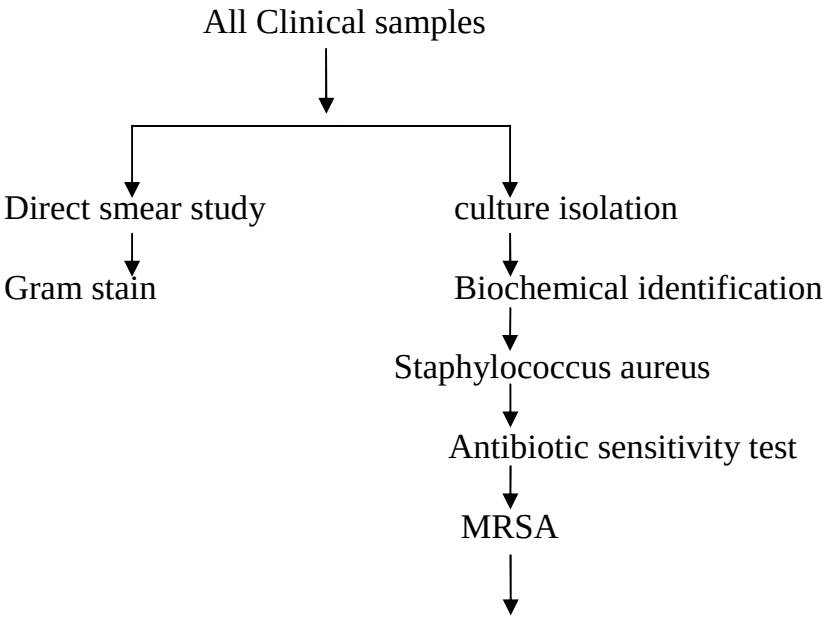
Name: Signature

PROTOCOL

Study Design – Prospective Cohort study

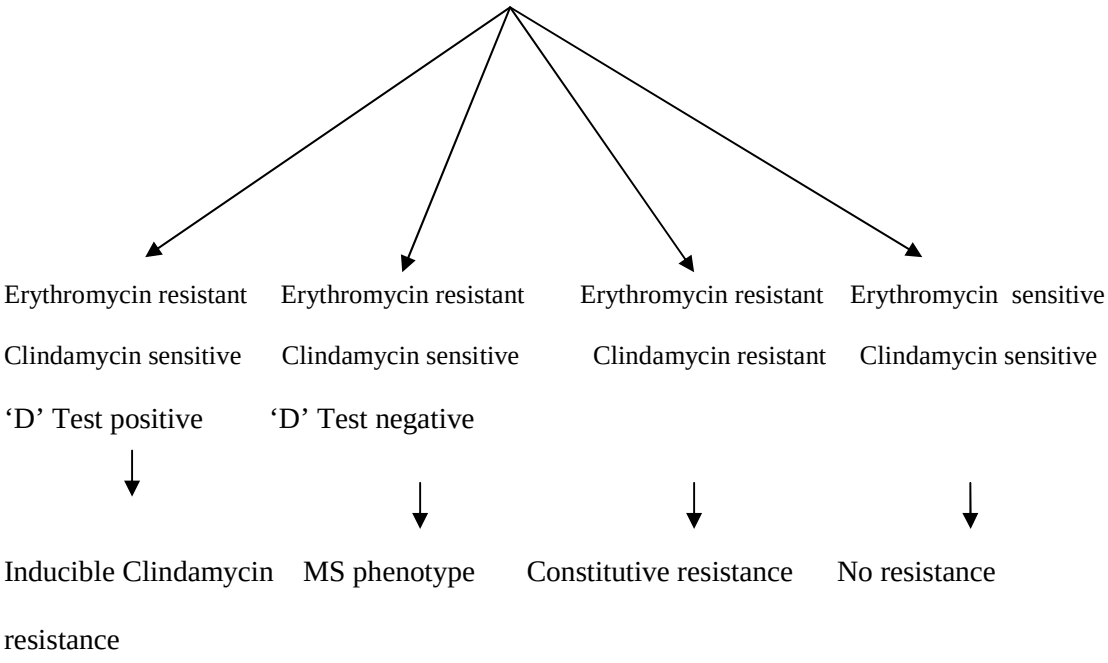
Study Materials – 200 Staphylococcus aureus isolates

Methodology-



'D' Test

(Disc approximation test -Erythromycin,clindamycin disc)



MASTER CHART

S.NO	AGE	SEX	WARD	DIAGNOSIS	SAMPLE	Type of SA	CD Susceptibility in MRSA			DRUG -S	DRUG-IS	DRUG -R
							IR	CR	E,CD-S			
1	26	F	OG	WOUND INFECTION	PUS	MRSA	-	CR	-	LZ,VAN,AK,CIP,DO, COT	-	P, AMC, CN,CTX,
2	3	M/CH	SURGERY	BURNS	PUS	MSSA	-	-	-	AMC,CTX, AK LZ,VAN,DO,COT	-	P, CN,CIP
3	61	M	SURGERY	FOURNIERS GANGRENE	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
4	14	M	SURGERY	ABSCESS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
5	38	M	ORTHO	WOUND INFECTION	PUS	MRSA	-	-	E,CD-S	LZ,VAN,AK, DO,COT ,CN, CTX,	-	P,AMC, CIP
6	34	F	OG	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, CN,CTX,AMC	-	P,COT
7	27	M	MEDICINE	FURUNCLE	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
8	34	M	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
9	25	M	SURGERY	ABSCESS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
10	5	M/CH	ENT	ASOM	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
11	50	M	ORTH0	WOUND INFECTION	PUS	MRSA	IR	-	-	LZ,VAN	COT	P, AMC,AK,CIP, CN,CTX,DO
12	23	F	OG	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
13	44	F	SURGERY	CELLULITIS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P

14	29	M	SURGERY	NECROTIZING FASCITIS	PUS	MRSA	IR	-	-	LZ,VAN	-	P, AMC,AK,CIP, CN,CTX,DO,CO T
15	37	F	OG	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
16	35	M	ORTH0	WOUND INFECTION	PUS	MRSA	IR	-	-	LZ,VAN	-	P, AMC, AK,CIP, CN,CTX,DO,CO T
17	25	M	ORTH0	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,CIP,COT,CN	AK	P, AMC DO,CTX
18	45	M	SURGERY	CELLULITIS	PUS	MRSA	-	-	E,CD-S	LZ,VAN,AK,CIP,DO, CN,COT	-	P,AMC,CTX
19	35	M	SURGERY	WOUND INFECTION	PUS	MRSA	IR	-	-	LZ,VAN,AK	DO, CTX,	P,AMC,COT, CIP,CN ,
20	23	F	SURGERY	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
21	62	M	SURGERY	ABSCESS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
22	58	M	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	AK, DO, CN,CTX, VAN,LZ	,CIP	P,AMC,COT
23	20	F	ORTHO	OSTEOMYELITI S	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
24	4	M/CH	SURGERY	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,DO,COT CN,CTX,AMC	CIP	P,
25	45	F	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,DO,COT CN,CTX,AMC	CIP	P
26	36	F	SURGERY	CELLULITIS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC,	-	P
27	80	M	ORTHO	WOUND INFECTION	PUS	MRSA	-	CR	-	LZ,VAN,AK,CIP AMC, CTX	-	P, COT, DO,CN,

28	28	M	SURGERY	ULCER LEG	PUS	MSSA	-	-	E,CD-S	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
29	30	F	NEPHROLOGY	ULCER RIGHT ARM	PUS	MRSA	-	CR	-	LZ,	-	P,AMC,CN,AK CIP, VAN,CTX, DO, COT
30	2	FCH	PAEDIATRICS	ASOM	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
31	45	F	SURGERY	GANGRENE	PUS	MSSA	-	-	-	LZ,VAN,AK,DO,COT CN, AMC	,CIP,CTX	P
32	30	F	MEDICINE	URINARY TRACT INFECTION	URINE	MRSA	-	CR	-	LZ,VAN,CN,	CTX	P,AMC, AK,CIP DO,COT
33	32	F	MEDICINE	URINARY TRACT INFECTION	URINE	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC	-	P
34	29	F	OG	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,DO,COT CN,CTX,AMC	-	P,CIP
35	25	F	MEDICINE	URINARY TRACT INFECTION	URINE	MSSA	-	-	-	LZ,VAN,AK,DO,CN, CTX,AMC	-	P,CIP,COT
36	10 days	FCH	PAEDIATRICS	TRANSIENT NEONATAL PUSTULOSIS	PUS	MRSA	-	CR	-	LZ,VAN,AK,CIP,DO,	CTX,CN	P, AMC, COT,
37	45	F	ORTHO	WOUND INFECTION	PUS	MRSA	-	-	E,CD-S	LZ, AK, DO, COT, CN	CIP, CTX	P, AMC VAN,
38	15 days	FCH	SURGERY	ABSCESS	PUS	MRSA	-	-	E,CD-S	LZ,VAN,AK,DO, COT	CTX	P, AMC, CN, CIP
39	33	M	SURGERY	WOUND INFECTION	PUS	MRSA CX-R OX-S	IR	-	-	LZ,VAN	-	P,AMC,CN,CTX, AK CIP, DO,COT
40	80	M	SURGERY	ULCER FOOT	PUS	MRSA	-	CR	-	CTX, LZ ,VAN,	-	P,AMC,CN,AK, DO, CIP, COT
41	40	M	SURGERY	ORAL CARCINOMA WITH ULCER	PUS	MRSA	-	CR	-	LZ,VAN,AK,CIP,COT, CTX,CN	-	P, AMC, DO,

42	25	M	ENT	ASOM	PUS	MSSA	-	-	-	LZ,VAN,AK,DO,COT CN,CTX,AMC	-	P ,CIP
43	52	M	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,DO CN,CTX,AMC	CIP	P , COT
44	11	FCH	SURGERY	ULCER SCALP	PUS	MSSA	-	-	-	COT, LZ, VAN		CTX, CN,DO, AK, CIP,P,AMC
45	50	M	SURGERY	WOUND INFECTION	PUS	MSSA	-	-	-	VAN,DO LZ		CTX,CN,COT, P, AMC,CIP,AK,
46	35	M	SURGERY	ULCER LEFT LEG	PUS	MSSA	-	-	-	LZ,VAN,COT,CN,CTX	AK,CIP,DO	P,AMC,
47	87	M	ENT	SUPPURATIVE OTITIS EXTERNA	PUS	MSSA	-	-	-	LZ,VAN, CIP, COT CN,AMC	AK,DO	P,CTX
48	62	M	ORTHO	OSTEOMYELITIS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
49	80	F	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,AMC	CTX	P, DO, COT, CIP, CN
50	21	F	OG	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK ,COT	CTX, CN	P,AMC,CIP, DO
51	50	M	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK	COT	P, AMC, CN,CTX CIP, DO
52	50	M	ORTHO	WOUND INFECTION	PUS	MRSA		CR	-	LZ,VAN, DO	CIP, COT,CTX	P, AMC, CN,AK
53	25	F	ORTHO	WOUND INFECTION	PUS	MRSA	IR	-	-	LZ,VAN		P,CN,CTX,AMC, AK,CIP,COT ,DO
54	15	M	MEDICINE	URINARY TRACT INFECTION	URINE	MRSA	IR	-	-	LZ,VAN, DO,COT		P, AMC ,CN,CTX, AK,CIP
55	23	M	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, CN,AMC	CTX,COT	P
56	28/365	FCH	PAEDIATRICS	SEPTICEMIA	BLOOD	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT ,CN,CTX,AMC		P

57	50	F	SURGERY	BURNS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT ,CN,CTX,AMC		P
58	32	M	MEDICINE	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT ,CN,CTX,AMC		P
59	29	F	MEDICINE	URINARY TRACT INFECTION	URINE	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT ,CN,CTX,AMC		P
60	25	M	SURGERY	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT ,CN,CTX,AMC		P
61	34	F	SURGERY	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT ,CN,CTX,AMC		P
62	50	M	MEDICINE	CELLULITIS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT ,CN,CTX,AMC		P
63	20	F	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,DO CN,CTX,AMC	COT,CIP	P
64	35	F	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT ,CN,CTX,AMC		P
65	20	M	ORTHO	PELVIC ABSCESS	PUS	MRSA	IR	-	-	LZ,VAN		P, AMC,CN,CTX, AK,CIP ,DO,COT
66	9	MCH	SURGERY	URINARY TRACT INFECTION	URINE	MSSA	-	-	-	LZ,VAN, DO,COT,AK	CTX,CIP CN	P, AMC
67	18	M	ORTHO	WOUND INFECTION	PUS	MRSA	IR	-	-	LZ,VAN		P, AMC ,CN,CTX, AK,CIP,DO,COT
68	19	M	SURGERY	ULCER	PUS	MRSA	-	CR	-	LZ,VAN		P, AMC CN,CTX, AK,CIP,DO,COT
69	51	M	SURGERY	CELLULITIS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
70	38	M	ORTHO	WOUND INFECTION	PUS	MRSA	-	CR	-	LZ,VAN,AK,	CTX ,CN	P, AMC, CIP,DO,COT,
71	31	M	ORTHO	WOUND INFECTION	PUS	MRSA	-	CR	-	LZ,VAN		P, AMC,CN,CTX, AK ,CIP, DO,COT

72	22	F	OG	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,DO,AMC	CTX, CN	P,CIP,AK,COT,
73	63	M	MEDICINE	FURUNCLE	PUS	MSSA	-	-	-	LZ,VAN,CN,CTX,AMC	AK , DO ,COT,CIP	P
74	9	FCH	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
75	25	F	OG	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK, CN,AMC	DO COT,CIP,C TX,	P,
76	13	F	SURGERY	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
77	40	M	SURGERY	CELLULITIS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
78	4YR	MCH	SURGERY	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
79	15	F	ORTHO	SEPTIC ARTHRITIS	SYNOVIA L FLUID	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
80	8	MCH	ENT	TONSILLAR ABSCESS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
81	46	M	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
82	22	F	OG	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
83	20	F	ORTHO	STUMP ULCER WITH WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT ,CN,CTX,AMC		P
84	75	F	SURGERY	ABSCESS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,CO T ,CN,CTX,AMC		P
85	21	F	ENT	CSOM	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,CO T,CN,CTX,AMC		P
86	12	F	SURGERY	BURNS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,CO T, CN,CTX,AMC		P

87	40	F	SURGERY	CELLULITIS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
88	24	F	SKIN	FURUNCLE	PUS	MRSA CX-R OX-S	IR	-	-	LZ,VAN		P, AMC, CN,CTX AK,CIP,DO,COT
89	50	M	SURGERY	ABSCESS LEFT THIGH	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
90	15	F	MEDICINE	VAGINITIS	VAGINAL SWAB	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
91	64	M	SURGERY	ULCER FOOT		MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
92	47	M	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
93	9	FCH	SURGERY	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
94	70	M	MEDICINE	PNEUMONIA	SPUTUM	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
95	2	FCH	PAEDIATRICS	IMPETIGO	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
96	24	F	ORTHO	WOUND INFECTION	PUS	MRSA	IR	-	-	LZ,VAN		P,CN,CTX,AMC, AK,CIP ,DO,COT
97	55	F	OG	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
98	43	M	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
99	17	M	SURGERY	GLUTEAL ABSCESS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
100	39	M	MEDICINE	PNEUMONIA	SPUTUM	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P

101	45	F	SKIN	FURUNCLE	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		p
102	1	MCH	SURGERY	MILIARY PUSTULOSIS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		p
103	12	M	ENT	CSOM	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		p
104	25	F	OG	VAGINITIS	HIGH VAGINAL SWAB	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		p
105	65	M	SURGERY	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,CIP,DO,COT, CN,CTX,AMC		P,AK
106	12	MCH	PLASTIC SURGERY	BURNS	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		p
107	32	M	SKIN	TROPHIC ULCER	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		p
108	13	M	ENT	ASOM	AURAL SWAB	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		p
109	28	F	ORTHO	OSTEOMYELITIS BOTH CALCANEUM	PUS	MRSA	IR	-	-	LZ,VAN,AK	COT	P,AMC, CN, CTX CIP,DO,
110	35	F	SURGERY	CELLULITIS	PUS	MRSA	-	-	E,CD-S	LZ,VAN,AK,CIP,DO,COT,		P,AMC, CN, CTX
111	27	M	ORTHO	WOUND INFECTION	PUS	MRSA	IR	-	-	LZ,VAN CN,	CTX,	P,AMC, DO , COT AK, CIP,
112	19	M	NEUROSURGERY	BEDSORES	PUS	MSSA	-	-	-	LZ,VAN,AK,DO,COT, CN,CTX,AMC		P,CIP
113	33	M	ORTHO	WOUND INFECTION	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
114	60	F	SURGERY	DIABETIC ULCER	PUS	MRSA	-	CR	-	LZ,VAN,AK,COT	CTX	P,AMC,CIP,DO, CN,
115	8	FCH	PAEDIATRICS	SLE WITH SKIN ULCERS	PUS	MSSA	-	-	-	LZ,VAN,DO,COT, CN,CTX,AMC	CIP	P, ,AK

116	45	M	SURGERY	WOUND GAPING	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO, COT		P, ,AMC,CN, CTX
117	20	F	MEDICINE	FURUNCLE	PUS	MRSA CX-R OX-S	-	-	E,CD-S	LZ,VAN,AK,CIP,DO,CO T	CTX	P,AMC,CN,
118	32	M	ORTHO	OSTEOMYELITI S	PUS	MSSA	-	-	-	LZ,VAN,AK,DO, CN,CTX,AMC		P,CIP,COT
119	21day s	MCH	SURGERY	WOUND INFECTION	PUS	MRSA	-	CR	-	LZ,VAN,DO,	CIP	P,AMC,AK,COT, CN,CTX,
120	38	M	ORTHO	OSTEOMYELITI S	PUS	MRSA	-	CR	-	VAN,LZ		P,AMC,AK,COT, CN,CTX,CIP, DO,
121	31	M	ORTHO	OSTEOMYELITI S	PUS	MRSA	IR		-	VAN,LZ		P,AMC,AK, CIP , DO,COT, CN,CTX,
122	37	M	ORTHO	PERITONITIS,A BSCESS	PUS	MRSA	IR		-	AK, VAN,LZ		P,AMC,COT,CN, CTX,CIP,DO,
123	71	M	ORTHO	SEPTIC ARTHRITIS	SYNOVIA L FLUID	MSSA	-	-	-	AMC,AK, DO, CN, VAN,LZ		P,CIP,COT,CTX
124	3mon ths	FCH	PAEDIATR ICS	ABSCESS	PUS	MRSA	IR	-	-	LZ, CIP,DO VAN	CN,CTX	P,AMC,AK,COT, ,
125	62	M	SKIN	PYODERMA	PUS	MRSA	IR	-	-	LZ ,VAN,	AK	P,AMC, ,CIP,COT, DO, CN,CTX,
126	21	M	ORTHO	WOUND INFECTION	PUS	MRSA	IR	-	-	LZ,VAN,COT		P,AMC,AK, CIP, DO CN,CTX,
127	39	M	ORTHO	OSTEOMYELITI S	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,CO T, CN, AMC		P,CTX
128	61	M	SURGERY	ULCER FOOT	PUS	MSSA	-	-	-	LZ,VAN,AK,CIP,DO,CO T, CN,CTX,AMC		P
129	24	F	OG	WOUND INFECTION	PUS	MSSA	IR			LZ,VAN,AK,CIP, DO, CN, CTX,AMC		P,COT
130	24	F	OG	WOUND INFECTION	PUS	MSSA				LZ,VAN,CIP,COT, CN,CTX,AMC		P,AK,DO

131	56	M	MEDICINE	PNEUMONIA	SPUTUM	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
132	38	M	SURGERY	GLUTEAL ABSCESS	PUS	MSSA				LZ,VAN,AK,COT, CN,CTX,AMC		P,DO,CIP
133	14	F	ORTHO	POST CELLULITIS ULCER	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
134	68	M	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK, DO,CN,CTX, COT,		P, AMC ,CIP,
135	13	M	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,DO,COT, AMC		P,CN,CTX,CIP
136	52	M	SURGERY	CELLULITIS	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
137	27	F	OG	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK, CN,CTX,AMC		P ,DO,CIP,COT
138	40	M	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
139	50	F	MEDICINE	BEDSORE	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
140	53	M	ENT	CSOM	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
141	50	F	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO		P, AMC , CN,CTX, COT,
142	58	M	SKIN	PYODERMA	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
143	55	M	ORTHO	OSTEOMYELITIS	PUS	MSSA				LZ,VAN,AK,COT, CN,CTX,AMC		P, DO,CIP
144	60	F	MEDICINE	ULCER FOOT	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
145	23	F	MEDICINE	PYODERMA	PUS	MSSA				LZ,VAN,CIP, CN,CTX,AMC		P,AK,DO ,COT
146	36	M	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P

147	27	M	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
148	37	M	SURGERY	ABSCESS	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
149	40	F	OG	WOUND INFECTION	PUS	MRSA OX-S, CX-R	IR			LZ,VAN,AK,DO,	CIP	P,AMC,CN,CTX COT,
150	23	F	ENT	CSOM	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT	CTX	P, AMC, CN,
151	22	F	OG	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
152	45	M	SKIN	CELLULITIS	PUS	MSSA				LZ,VAN, DO, AK CN,CTX,		P,AMC, COT,CIP
153	42	F	SURGERY	ULCER FOOT	PUS	MSSA				LZ,VAN,AK,CIP,DO, , CN,CTX,AMC		P ,COT
154	2	FCH	PAEDIATRICS	SEPTICAEMIA	BLOOD	MSSA				LZ,VAN,DO, COT, CN,CTX,AMC		P,AK,CIP
155	5dayS	M	PAEDIATRICS	SEPTICAEMIA	BLOOD	MRSA			ECDS	LZ,VAN,AK		P, AMC CN,CTX,COT,DO,CIP
156	70	M	MEDICINE	SEPTICAEMIA	BLOOD	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
157	60	M	MEDICINE	SEPTICAEMIA	BLOOD	MSSA				LZ,VAN,AK,COT, CN,CTX,AMC		P, DO,CIP,
158	24 days	M	NICU	URINARY TRACT INFECTION	URINE	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
159	77	M	MEDICINE	URINARY TRACT INFECTION	URINE	MSSA				LZ,VAN,AK, CN,CTX,AMC		P,DO,CIP,COT
160	24	F	OG	URINARY TRACT INFECTION	URINE	MSSA				LZ,VAN,AK,CIP,DO, COT, AMC		P, CTX ,CN
161	5	MCH	PAEDIATRICS	SEPTICAEMIA	BLOOD	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P

162	21	F	ENT	CSOM	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
163	28	F	ENT	CSOM WITH DNS	PUS	MSSA				LZ,VAN,AK, DO, CN,CTX,AMC		P,CIP,COT
164	40	M	SURGERY	CELLULITIS	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P,
165	24	F	SURGERY	BREAST ABSCESS	PUS	MSSA				LZ,VAN,AK, DO,COT, AMC		P, CTX,CN ,CIP
166	64	M	MEDICINE	FURUNCLE	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
167	23	M	ENT	ASOM	PUS	MSSA				LZ,VAN,AK,CIP,DO, CN,CTX		P,AMC,COT
168	30	M	SURGERY	UTI	URINE	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
169	5	MCH	SURGERY	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
170	5	MCH	SURGERY	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,AMC		P,CTX
171	30	M	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN, DO,COT, CN,CTX,AMC		P,AK,CIP
172	64	M	SURGERY	CELLULITIS	PUS	MRSA			E,CD-S	LZ,VAN, DO,COT		P, AMC, CIP,AK CN,CTX
173	65	M	ORTHO	WOUND INFECTION	PUS	MRSA	IR			LZ,VAN,AK		P,AMC,CIP, COT,DO ,CN,CTX
174	28	F	OG	WOUND INFECTION	PUS	MRSA			E,CD-S	LZ,VAN, DO,COT		P, AMC, AK, CIP CN,CTX
175	23	F	OG	WOUND INFECTION	PUS	MRSA			E,CD-S	LZ,VAN, AK		P, AMC ,CN, CTX, COT,DO,CIP
176	35	M	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN		P,AMC ,CTX
177	21	M	SURGERY	GANGRENE FOOT	PUS	MRSA	IR			LZ,VAN, AK		P, AMC, COT,DO CN,CTX, CIP

178	23	F	ENT	CSOM	PUS	MRSA		CR		LZ,VAN		P, AMC ,CN, CTX, COT,DO,CIP,AK
179	65	F	SURGERY	BURNS	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, AMC		P,CN,CTX
180	21	M	ORTHO	WOUND INFECTION	PUS	MRSA			ECD-S	LZ,VAN		P, AMC,COT , DO,CIP,AK CN,CTX,
181	2	FCH	SURGERY	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
182	44	M	SKIN	TROPHIC ULCER	PUS	MRSA			E,CD-S	LZ,VAN		P,AMC,COT,DO, CN, CTX , CIP,AK
183	25	M	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN, AK,CIP DO,COT, CN,CTX,AMC		P,
184	64	M	SURGERY	CELLULITIS	PUS	MRSA		CR		LZ,VAN		P,AMC,COT,DO, CIP,AK, CN,CTX
185	63	M	SURGERY	CELLULITIS	PUS	MSSA				LZ,VAN,AK,COT, CN,CTX,AMC		P, DO,CIP
186	44	M	SURGERY	ABSCESS	PUS	MRSA			E,CD-S	LZ,VAN, CIP		P, CN, CTX COT,DO,AK,AMC
187	74	M	MEDICINE	PNEUMONIA	SPUTUM	MSSA				LZ,VAN, ,DO, COT, CN, CTX,AMC		P,AK ,CIP
188	35	F	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
189	23	F	OG	VAGINITIS	VAGINAL SWAB	MSSA				LZ,VAN,AK,CIP,DO, CN,CTX ,AMC COT		P,
190	61	M	ENT	CSOM	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
191	51	M	MEDICINE	VENOUS ULCER,PUO	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P
192	48	F	ENT	CSOM	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT, CN,CTX,AMC		P

193	20	M	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO,COT,		CN,CTX,AMC,P
194	45	M	SURGERY	ABSCESS	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
195	40	M	SURGERY	NECROTISING FASCITIS	PUS	MSSA				LZ,VAN,AK,COT, CN,CTX,AMC		P, DO,CIP
196	10months	FCH	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P
197	12	MCH	SURGERY	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,DO CN,CTX,AMC		P,CIP,COT
198	18	M	ORTHO	WOUND INFECTION	PUS	MRSA	IR			LZ,VAN,CIP		P,AMC,COT,DO, AK CN,CTX
199	51	M	SURGERY	BURNS	PUS	MRSA			E,CD-S	LZ,VAN, DO,COT		P, AMC , CIP,AK CN,CTX
200	4	MCH	ORTHO	WOUND INFECTION	PUS	MSSA				LZ,VAN,AK,CIP,DO, COT, CN,CTX,AMC		P

Comparative study of inducible and constitutive clindamycin resistance among methicillin resistant staphylococcus aureus isolates

ABSTRACT

INTRODUCTION: Staphylococcus aureus is the commonly encountered pathogen isolated from clinical specimens.Methicillin Resistant Staphylococcus aureus (MRSA)causes variety of human infections resulting in high rate of mortality and morbidity.Clindamycin, lincosamide antibiotic is a good option for clinicians to treat MRSA infections.**AIMS AND OBJECTIVES:**The aim of the study was to screen for MRSA by disc diffusion method with cefoxitin and oxacillin discs and to determine the prevalence of inducible clindamycin resistance and constitutive clindamycin resistance in MRSA and compare them.**MATERIALS AND METHODS**:200 staphylococcus aureus were isolated from samples like pus, blood, sputum, vaginal swab, urine and body fluids received in microbiology department of Coimbatore Medical College Hospital.They were confirmed by microscopy,culture and biochemical reaction.Then MRSA were detected by disc diffusion test using Cefoxitin (30μg) and Oxacillin(1μg) discs. Clindamycin resistance were detected by performing D-test by placing erythromycin 15μg and clindamycin 2μg discs at 15-20mm interdisc distance. **RESULTS:**Majority of staphylococcus aureus were isolated from pus samples (87.5%). Staphylococcus aureus was highly sensitive to linezolid (100%) and vancomycin (99%) and 100% resistant to penicillin. Cefoxitin disc detected higher percentage (26%) of MRSA than oxacillin disc(24%). Analysis of clindamycin resistance in 52 (26%) MRSA isolates showed 42.30% of inducible clindamycin resistance, 30.76 % of constitutive clindamycin resistance and 26.92% were sensitive to both erythromycin and clindamycin. **CONCLUSION:**Detection of MRSA is very

important for treating patients and to prevent its spread. MRSA isolates exhibiting inducible clindamycin resistance are seemed to be susceptible to clindamycin in vitro but resistant invivo resulting in treatment failure. So‘D’test is suggested along with routine antibiotic susceptibility testing to detect inducible clindamycin resistance.

Key words: Inducible clindamycin resistance, constitutive resistance