Genotypic and Phenotypic characterization of Methicillin Resistant *Staphylococcus aureus* from ocular isolates and its clinical correlation

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CERTIFICATE

Certified that this dissertation entitled **"GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF METHICILLIN RESISTANT *Staphylococcus aureus* FROM OCULAR ISOLATES AND ITS CLINICAL CORRELATION"** submitted for MS (Branch III) Ophthalmology, April 2014, is the bonafide work done by **DR.PRIYA.S** , under our supervision and guidance in the Ocular Microbiology Services of Aravind Eye Hospital and Post Graduate Institute of Ophthalmology, Madurai, during her residency period from May 2011 to April 2014.

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INTRODUCTION

Staphylococcus aureus is a bacterium that belongs to the family of *Staphylococcaceae.* The bacteria form part of the normal flora of the skin, intestine, upper respiratory tract and vagina. It can proliferate when there is appropriate pH, temperature and nutrition and can become pathogenic. The pathogenicity of *S. aureus* is determined by the production of several toxins. It has been documented that there is probably no other bacterium that produces as many cellular components, enzymes, extra cellular toxins and hemolysins as this organism [1]. It is known for its intrinsic virulence and multidrug resistance which poses a great challenge to the clinician.

Sir Alexander Ogstan first described the clinical picture in 1880s, *S. aureus* can affect almost every organ and tissue of human body [1]. It results in suppurative infections, wound and catheter related infections. It is reported to be associated with diseases that arise exclusively from staphylococcal toxins including food poisoning, toxic shock syndrome and Staphylococcal scalded skin syndrome [2].It can also cause severe ocular infections and its prevalence varies from 3% to 30% . Ocular infections range from blepharitis to sight threatening panophthalmitis [3.]

In early 1960s, treatment of *S. aureus* infections included semi-synthetic penicillin drugs, such as methicillin. However, soon methicillin-resistant *S. aureus* (MRSA) strains started appearing. In the early 1980's they became the major cause of nosocomial infections. The possibility of transmission of health-care associated MRSA - HA-MRSA) to the patient population was unavoidable. Since 1987, MRSA was also found in the community where there is no exposure to any known risk factors such as hospital admission. (community associated- methicillin-resistant *S. aureus* - CA-MRSA) Patients presented with severe skin and soft tissue infections and necrotizing pneumonia. HA-MRSA strains were genetically and phenotypically different than CA-MRSA strains. CA-MRSA were associated with a smaller composition, a higher incidence of virulence, and a lack of multidrug resistance.

Recent studies suggest that the prevalence of MRSA in the community is increasing [4]. Inspite of the increasing prevalence, not much has been reported from India regarding ocular MRSA infections. Proposed study on phenotypic and genotypic characterization of ocular MRSA will help us know the type of MRSA prevalent in south India that are responsible for ocular infections. Also this study will correlate the clinical manifestations with the morphological and genetic characteristics. The results will give a

better understanding of the bacterial virulence and clinical prognosis. Last but not the least, by finding the antibiotic susceptibility of the prevalent strain, appropriate and early antibiotic therapy can be started which will improve the prognosis and result in better outcome.

REVIEW OF LITERATURE

Staphylococcus aureus is a Gram positive, facultative anaerobic bacterium . They are non-motile, non- sporing and catalase positive organism. The cocci commonly form irregular clusters with a grape like appearance under the microscope (Figure 1) The individual coccus size is approximately 0.5 to 1.5 μm in diameter 5. Although more than 200 species of *Staphylococcus* are described, only *Staphylococcus aureus* and *Staphylococcus epidermidis* are significant in their interactions with humans.Summary of the classification of *Staphylococcus aureus* is given in table [1].

Classification of *Staphylococcus aureus*:

A) Based on coagulase production:

1. Coagulase positive: Eg- *S. aureus*
2. Coagulase negative: Eg- *S. epidermidis, S. saprophyticus*

B) Based on pathogenicity:

1. Common pathogen: Eg- *S. aureus*
2. Opportunistic pathogens: Eg- *S. epidermidis S. saprophyticus*
3. Non pathogen: Eg- *S. Homonis*

Table 1: Summary of the classification of *Staphylococcus aureus*

Domain	***Bacteria***
Kingdom	***Eubacteria***
Phylum	***Firmicutes***
Class	***Bacilli***
Order	***Bacillales***
Family	***Staphylococcaceae***
Genus	**Staphylococcus**
Species (cause of human disease)	***S. aureus*** ***S. epidermidis*** ***S. saprophyticus*** ***S. haemolyticus*** ***S. lugdunensis***

Culture Characteristics:

They can be grown in several media given below.

1. Non selective media:
    - Nutrient agar
    - Blood agar
    - Chocolate agar
2. Selective media:
    - Salt-milk agar
    - Ludlam's medium
    - MacConkey's agar.

On nutrient agar- The colonies are large, circular, convex, smooth, shiny, opaque and easily emulsifiable. Most strains produce golden yellow pigments. (Figure 2) On MacConkey's agar the colonies are small & pink in colour and on blood agar- Most strains produce β- haemolytic colonies.

Figure 1: Staphylococcus aureus - Gram positive cocci in clusters

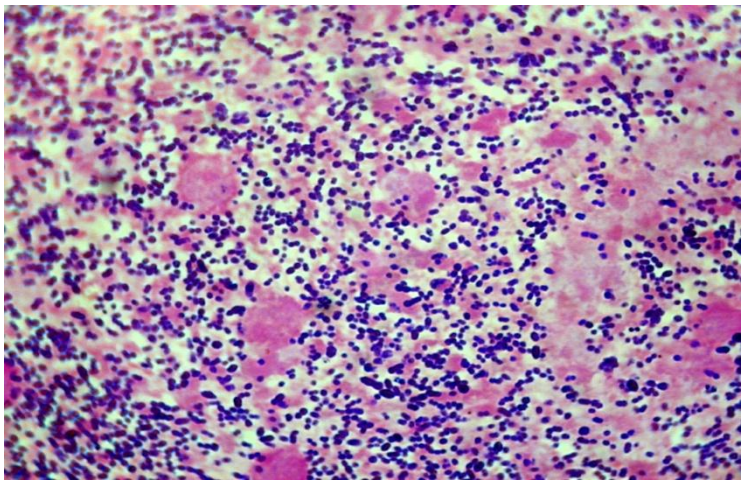
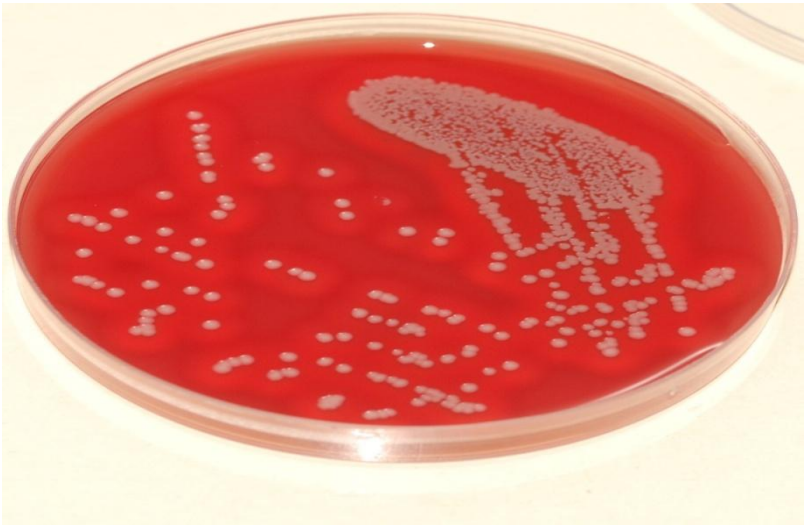


Figure 2: Staphylococcus aureus colony morphology in Blood agar plate



β – Haemolytic golden yellow colonies of S. aureus are seen in blood agar.

Important phenotypic characteristics of *Staphylococcus aureus :*

- Gram-positive, cluster-forming coccus (Figure 1)
- Nonmotile, nonsporeforming facultative anaerobe
- Fermentation of glucose produces mainly lactic acid
- Ferments mannitol (distinguishes from S. Epidermidis)
- Catalase positive (Figure 3)
- Coagulase positive(Figure 4)
- Reduces nitrate to nitrite.
- Urea hydrolysis test- Positive.
- Gelatin liquefaction test- Positive.
- Produces Lipase.
- Produces Phosphatase.
- Produces Thermostable nuclease

Figure 3:Catalase test

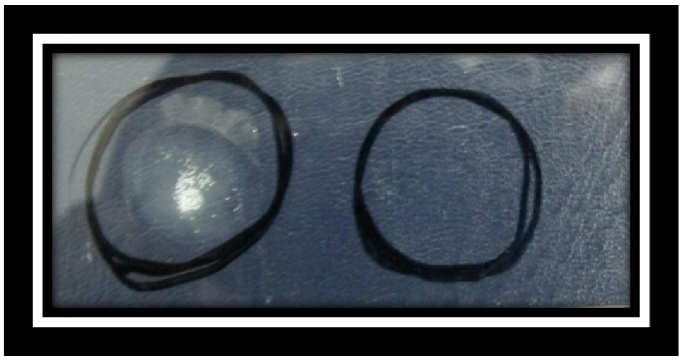


Figure on left shows MRSA with formation of air bubbles confirms positive catalase reaction Figure on right St.pneumoniae with no air bubble confirming a negative catalase eaction

Figure 4. Coagulase test

Figure on left shows MRSA Clot formation- a positive Coagulase reaction
Figure on right shows negative Coagulase reaction of S.epidermidis – absence of Clot Formation

Source of infection:

A) Exogenous: patients or carriers

B) Endogenous: From colonized site

Mode of transmission:

A) Contact: direct or indirect(through fomites)

B) Inhalation of air borne droplets

C) Hematogenous

Pathogenisis:

Staphylococcus aureus is a "tissue" invasive, pyogenic extracellular pathogens produce purulent lesions. They are normal skin flora. But can cause hospital-acquired infections from patient skin or skin of hospital personnel and nosocomial bacteremia. The high risk factors for infection include infections of prosthetic devices (artificial heart valves, CNS shunts, hip prostheses, other orthopedic devices), indwelling catheters, vascular grafts, peritoneal dialysis, and wounds. Infections caused by *S. aureus* can occur in two stages:

(i) *S. aureus* cells enter the body through damaged endovascular points of the host where platelet-fibrin-thrombi complex have formed and attach via microbial surface components that recognize adhesive matrix molecules (MSCRAMM) mediated mechanisms. (Figure 5)

(ii) Bacterial cells may attach to endothelial cells *via* adhesion receptor interactions or by bridging ligands, including serum components such as fibrinogen. Upon entry into the host tissue, immune cells phagocytose *S. aureus* cells, which promotes the production of proteolytic enzymes and toxins (table 2) that facilitate the spread to adjoining tissues and the release of the staphylococci into the bloodstream resulting in bacteraemia [1]. The infected endothelial cells produce tissue necrosis factor as part of the immune response to infection, which results in necrosis and abscess formation [1].

Figure 5 : Pathogenesis:

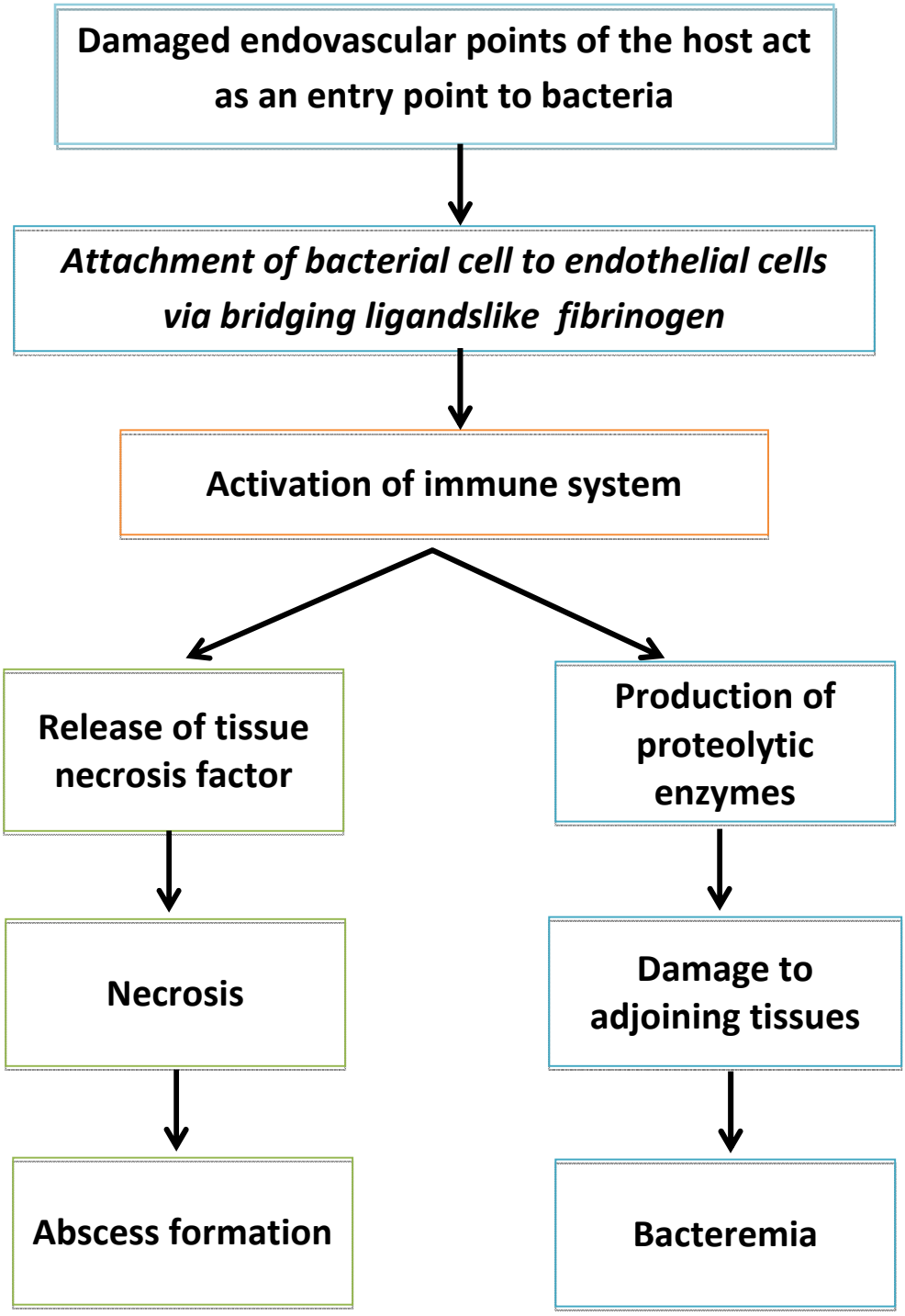


Table 2. Toxins and toxic components produced by *Staphylococcus aureus* (Timbury *et al*., 002)

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Toxin	Activity
Haemolysins α, β and δ	Cytolytic , lyse erythrocytes
Coagulase	Clots plasma
Fibrinolysin	Digets fibrin
Leukocidin	Kills leukocytes
Hyaluronidase	Breaks down hyaluronic acid
DNAse	Hydrolyses DNA
Protein A	Lypolytic (produces opacity in egg-yolk medium)
Capsule	Antiphagocytic
Epidermolytic toxins A and B	Epidermal splitting and exfoliation
Enterotoxin (s)	Food poisoning toxins that cause vomiting and diarrhoea
Toxic shock syndrome toxin-1	Shock, rash and desquamation

Virulence factors:

Different strains of *S. aureus* produce different virulence factor which result in their ability to multiply and spread across adjacent tissue [1]. The cell wall of *S. aureus* is composed of a thick peptidoglycan layer, which contributes to the virulence of the bacterium [6]. The peptidoglycan stimulates the production of cytokines by macrophages resulting in complement system activation and platelet aggregation [6].

Although numerous studies have contributed to the current knowledge of these components and products responsible for the development of infection, little information regarding the interactions of the bacteria with each other exists. The suppression of toxins is an important part in the treatment and management of *S. aureus* infections [7]. A thorough and complete understanding of the interaction of these*S. aureus* products and components is necessary to apply the correct treatment and to prevent infections [8].

Antimicrobial resistance of *S. aureus* strains

Staphylococcus aureus causes the tissue destruction as a pathogen because of the intrinsic virulence and its ability to rapidly adjust to different environmental conditions[6]. The trend of multidrug resistance in *S. aureus* is particularly alarming because of the severity and diversity of diseases caused by this pathogen [9]. Despite the availability of novel drugs as an approach to staphylococcal therapy, the bacteria seem to be able to rapidly develop resistance to these drugs [10]. Perhaps the most commonly known resistance of *S. aureus*, is methicillin resistance, which has caused alarming reports with regard to the spread of *S. aureus* in hospitals and the community [11-14]

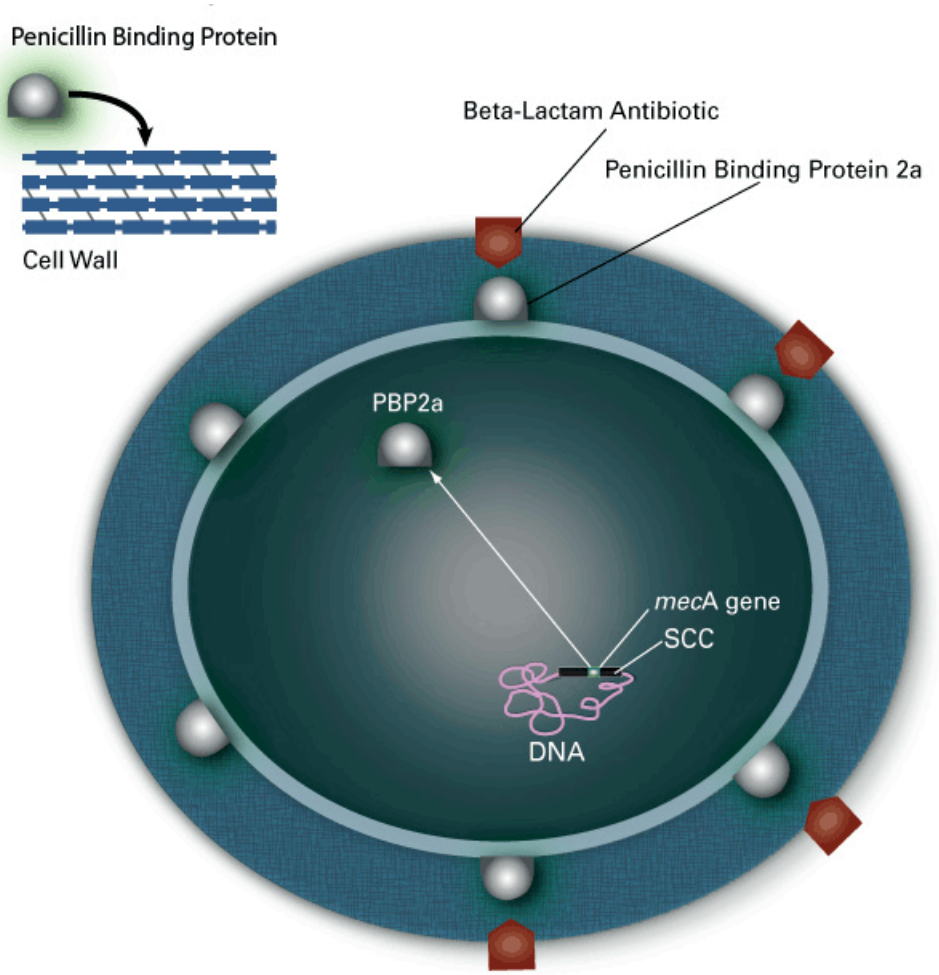
Penicillin Resistance:

The inactivation of penicillin in *S. aureus* strains was first demonstrated in 1944 by Kirby [15]. Resistance to penicillin is mediated by penicillinase which is a form of β-lactamase production, an enzyme that cleaves the β-lactam ring of the penicillin molecule, rendering it ineffective. The beta-lactamase enzyme is produced when the bacterial cells are exposed to beta-lactam antibiotics including penicillin and its derivatives.

Methicillin resistance in *S. aureus* strains:

Penicillinase-resistant β-lactam antibiotics, such as methicillin is able to resist degradation by staphylococcal penicillinase. However resistance to methicillin by *Staphylococcus* is mediated via the mec operon, part of the staphylococcal cassette chromosome mec (SCCmec). *MecA* gene, the structural determinant encoding PBP 2a, located on the chromosome of MRSA, is therefore considered a useful molecular marker of methicillin resistance in *S.aureus* . [16]While a few clinical isolates of MRSA express homogeneous oxacillin reistance, the majority of isolates have heterogeneous drug resistance due to interaction of PBP 2a and various gene products such as those encoded by *fem* (factor essential for methicillin resistance) genes that are involved in cell wall peptidoglycan synthesis. [17]

Figure 6: Mechanism of *S.aureus* resistance to methicillin



Staphylococcal methicillin resistance resulted from the due the mecA gene, which is part of a larger mobile genetic element known as the staphylococcal chromosomal cassette (SCCmec)--a genetic element that integrates into the staphylococcal chromosome. The mecA gene codes for an altered penicillin binding protein known as penicillin binding protein 2a (F**igure** 6).This has reduced affinity for methicillin binding and thus confers resistance to methicillin and other similar beta-lactam agents. Although methicillin is no longer used, the term methicillin-resistant *Staphylococcus aureus* (MRSA) has remained since methicillin was the most common anti-staphylococcal agent used when this resistance was discovered.

Emergence of MRSA

In the 1940s, medical treatment for S. aureus infections became routine and successful with the discovery of antibiotics, such as penicillin. However, use of antibiotics has aided natural bacterial evolution by helping the microbes become resistant to the drugs designed to fight them. In the late 1940s and throughout the 1950s, S aureus developed resistance to penicillin. [1]. Methicillin, a form of penicillin, was introduced to counter the increasing problem of penicillin-resistant S. aureus. Methicillin was one of most common types of antibiotics used to treat S. aureus infections; but, in 1961, British scientists identified the first strains of S. aureus bacteria that resisted methicillin. This was the so-called birth of MRSA.

The first reported human case of MRSA in the United States came in 1968. Subsequently, new strains of bacteria have developed that can now resist previously effective drugs, such as methicillin and most related antibiotics. MRSA is actually resistant to an entire class of penicillin-like antibiotics called beta-lactams. This class of antibiotics includes penicillin, amoxicillin, oxacillin, methicillin, and others. [1]

S. aureus is evolving even more and has begun to show resistance to additional antibiotics. In 2002, physicians in the United States documented

the first *S. aureus* strains resistant to vancomycin, which had been one of a handful of antibiotics of last resort for use against *S. aureus*. Though it is feared that this could quickly become a major issue in antibiotic resistance, vancomycin-resistant strains are still rare. [1]

Community acquired and Hospital acquired MRSA:

MRSA has been circulating in hospitals since the early 1960s but reports of infection in the community were relatively rare. However, in the early 1990s strains of highly virulent CA-MRSA were reported in Western Australia [18]. In recent years the incidence of CA-MRSA has been increasing with outbreaks occurring in various parts of the world [19,20]**.**

While one group believes that CA-MRSA isolates evolved from HA-MRSA[21] another introduces the differences in SCCmec complexes as evidence that CA-MRSA and HA-MRSA are in fact not related. HA-MRSA consists of SCC*mec* types I-III, while CA-MRSA consists of type IV and V [22,23] SCC *mec* type IV differs from the other types because of its small size and absence of non-beta-lactam genetic resistance determinants [24]. Therefore, SCC*mec* type IV is susceptible to a broader array of antibiotics [24]. SCC*mec* typing in community and health-care -associated methicillin-resistant *Staphylococcus aureus* is given in table 3 The clinical difference between

both types are given in table 4 . The guidelines in diagnosing both Community acquired and Hospital acquired MRSA and the differences are given in Table 5.

Table 3 : Major Difference Between Community – and Hospital-acquired Methicillin-resistant Staphylococcus Aureus

	Community Acquired	**Hospital Acquired**
Antibiotic Profile	More Susceptible to Beta lactams, clindamycin	Multiple drug resistance including clindamycin
Population affected	Young, otherwise healthy person	Predisposed patients
Area of infection	Skin, lungs	Varies
Genetic traits	Panton Valentine gene, Staphylococcal cassette chromosome IV	Various Staphylococcal cassette chromosome

Table 4 SCC*mec* typing in community and health-care -associated methicillin-resistant *Staphylococcus aureus*

Strain	**SCC*mec* type**	**Antibiotic resistance**	**Toxins**	**PVL genes**	**Infection spectrum**
HA-MRSA	Types I, II and III	Multi-drug resistant	Few	Rare	Bloodstream, respiratory tract and urinary tract infections
CA-MRSA	Type IV and V	Resistance is typically limited to betalactam betalactam and erythromycin although multidrug resistance can occur	usually PVL presence	Common	Skin and softtissue infections and necrotising pneumonia

CA-MRSA community-associated methicillin-resistant *Staphylococcus aureus*

HA-MRSA health-care associated methicillin-resistant *Staphylococcus aureus*

PVL Panton-Valentine leukocidin SCC*mec* staphylococcal cassette chromosome *mec*

Table-5 Community -Associated Methicillin – Resistant *Staphylococcus aureus*(CA-MSRA) Criteria

Diagnosis of CA MRSA made in the outpatient setting of within 48 hours of hospital admission.

- No medical history of MRSA infection or colonization.
- In the past year, no medical history of the following:
  - Hospitalization
  - Admission to a nursing home, skilled nursing facility, or hospice
  - Dialysis
  - Surgery
- No Permanent indwelling catheters or Medical devices that pass through the skin into the body

Health Care -Associated Methicillin – Resistant *Staphylococcus aureus*(HA-MSRA) Criteria - defined by the CDC

MRSA infection occurring in individuals who have been

- Hospitalized for more than 48 hours or
- Received surgery within the last year, or
- Have a permanent indwelling medical device, or
- Reside in a long-term care facility, or
- Who have recently received dialysis.

Global prevalence of Health Care associated MRSA :

Isolates of MRSA were initially recovered in hospitals; the first isolate was detected at a hospital in the United Kingdom. Within a few years, MRSA was found in other European countries, Japan, and Australia, and the first isolate in the United States was discovered at Boston Hospital. [25]

By the late 1980s, MRSA had become endemic in many hospitals, according to results from large surveillance studies such as the National Nosocomial Infections Surveillance conducted by the Centers for Disease Control and Prevention (CDC). In the United States hospitals, the proportion of *S aureus* isolated that was resistant to methicillin rose from 2.4% in 1975 to 29% in 1991 ; the proportion of MRSA continued to increase during the next decade and rose by approximately 3% per year in intensive care units between 1992 and 2003.

The mean was 59.5% in ICUs in 2003, reflecting an 11% overall increase, compared with the time period from 1998 through 2002. In a surveillance study conducted from 2000 through 2002, the proportion of

MRSA was also high, but variable, in ICUs in other industrialized countries, ranging from 21% in Ger many to 59% in Italy , but only 20% in Canada. [26]

Global prevalence of Community-acquired MRSA :

The most recent and alarming epidemiologic change in the community since late 1990s is the rapid emergence of MRSA and its increasing prevalence.Previous ly reported drug resistant organisms were first detected in the hospital prior to the community.In contrast, the strains causing community-acquired MRSA (CA-MRSA) infection seems to have arisen from non-healthcare sources, and show distinct characteristics that differentiate them from healthcare-associated (HA-MRSA) infections.

CA-MRSA strains have a pathogenic advantages due to their ability to produce a host of various virulence factors. Even though CA-MRSA infections were initially limited to selected populations as discussed earlier, the present scenario has changed.

CA-MRSA infections are becoming common in the general population. This necessitates changes in the therapeutic approach, such as culturing specimens from the lesions to determine the presence of MRSA . According to a survey in United Nations , it is estimated that out of 2 billion *S aureus* infection, 58 million were MRSA carriers. [24]

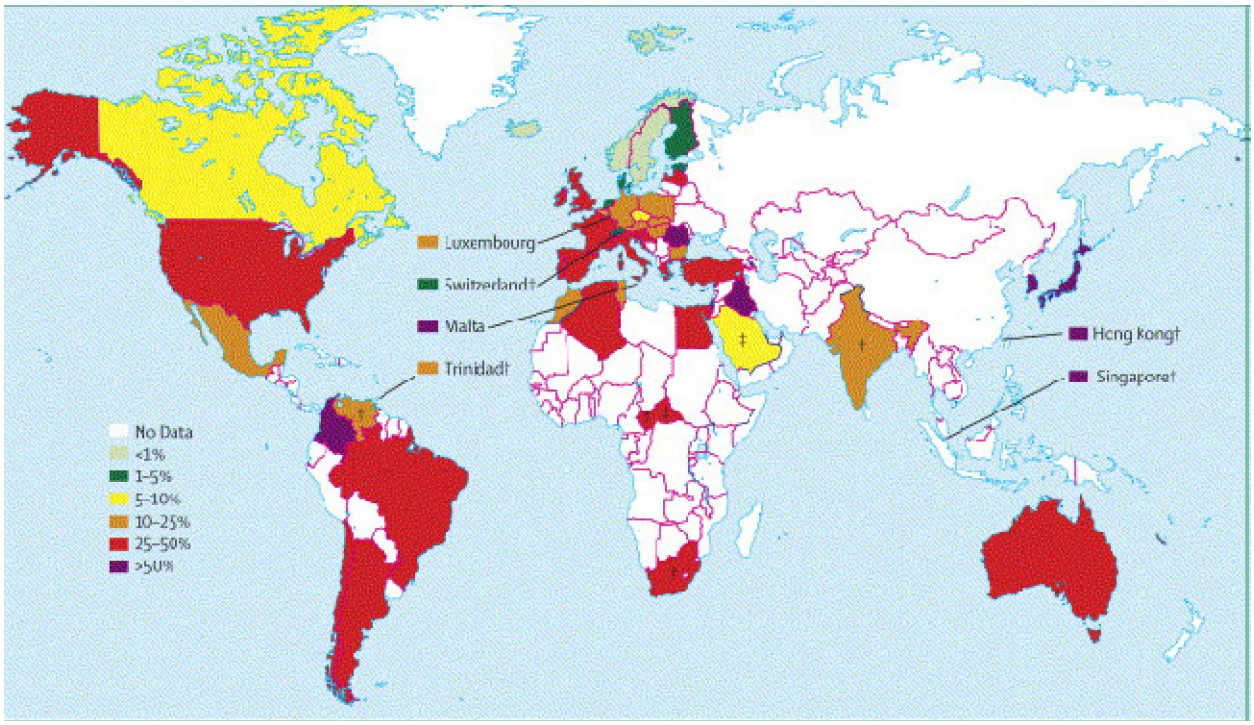
The carrier state of *S. aureus* among healthy individuals ranges between 15% to 35%. The risk of these individuals developing infection is 38%. A further risk of 3% is reported when colonised with methicillin-susceptible *S. aureus* (MSSA) [24,25] Certain groups of individuals have increased susceptibility to S. aureus colonisation compared to others including health-care personnels, nursing home inmates, prison inhabitants, military personnel and children [26.]

A review study was conducted by the University of the Witwatersrand and the University Hospital of Geneva in 2007 which showed that health-care workers accounted for 93% of personnel to patient transmission of infection [26]. In 1997, several outbreaks were reported in Taiwan that suggested MRSA transmission associated with health-care workers, including surgeons [26].

In countries such as Singapore (1993-1997), Japan (1999-2000) and Colombia (2001-2002) a prevalence of > 50% was reported in 2006 by Grundmann et al. Countries like South Africa (1993-1997), Brazil (2001), Australia (2003), Mexico and the United States showed a prevalence of 25-50%.. Norway,Sweden and Iceland (1993-1997) 26 had the lowest prevalence of <1%..Figure 6. Borg et al in 2007 reported that the prevalence rate was 50% in Cyprus, Jordan, Egypt, and Malta . This high rate of

prevalence was attributed to overcrowding ,poor sanitation and poor hand-hygiene facilities in the hospitals. [27]

Figure 7:**Global prevalence of MRSA (Grundmann et al., 2006)**



MRSA in India: Prevalence & susceptibility pattern:

According to a study conducted by Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group between 2008 and 2009, MRSA is endemic in India . This study was aimed to determine the prevalence pattern of MRSA and susceptibility of *S. aureus* isolates in India. [28]. It included 15 tertiary care centres during a two year period (2008 – 2009).

This study included a total of 26000 isolates . The prevalence of methicillin resistance during the study period was 41 per cent. Isolation rates for MRSA from ICU ,ward inpatients and outpatients were 42, 43 and 28 per cent, in 2008 and 49 , 47 and 28 per cent in 2009 respectively. A study from Chennai reported the prevalence of MRSA as 40-50 per cent. [29]. Majority of the isolates were obtained from skin and soft tissue infections.This was followed by blood stream and respiratory infections. As demonstrated by this study,the prevalence of MRSA varies between regions and between hospitals . Sensitivity to ciprofloxacin was low in both MSSA (53%) and MRSA (21%). MSSA isolates showed a higher sensitivity to gentamicin, co-trimoxazole, erythromycin and clindamycin in comparison to MRSA isolates. None of the isolates were found resistant to vancomycin or linezolid.

CA-MRSA infections are now being increasingly reported from India. In a study by D' Souza et al,28he studied 412 confirmed cases of MRSA and found that 54 per cent were proven CA-MRSA.They possessed the SCCmec IV and SCC mec V genes. These were mainly isolated from SSTIs. These strains also showed variable resistance to ciprofloxacin, erythromycin, clindamycin and tetracycline. Chatterjee et al found the overall prevalence of S. aureus nasal colonization was 52.3 per cent and that of MRSA was 3.89 per cent in the community. [29,30]

MRSA is a challenging problem in India. Multidrug resistance is seen more among MRSA strains as compared with MSSA isolates. Vancomycin and linezolid continue to remain the mainstay for treatment for MRSA infections.

Vancomycin-resistance in *S. aureus* strains

The increased prevalence of MRSA strains in the community resulted in the increased usage of the glycopeptide, vancomycin . However, the increased usage of vancomycin to treat MRSA infections lead to the emergence of vancomycin-resistant staphylococci. The first case of vancomycin resistance among staphylococci was reported in 1987 and was identified in a *Staphylococcus haemolyticus* strain . In 1997, the first report of a vancomycin-intermediate resistant *S. aureus* (VISA) strain was reported from Japan, with reports subsequently following from other countries including France ,Scotland and two isolates in South Africa. These VISA isolates were all MRSA strains[7]. Complete resistance to vancomycin was reported in Michigan in the United States in 2002 and subsequently in Pennsylvania two months later.

Identification of two forms of vancomycin resistance have been demonstrated. The first form involves the VISA strains with a minimum inhibitory concentration of 8 to 16 μg/ml. The reduced susceptibility to vancomycin by *S. aureus* is hypothesised to be a result of changes in peptidoglycan synthesis .

There is a visible irregularly shaped and thickened cell wall in these VISA strains due to increased amounts of peptidoglycan. Evidently, there is a decrease in crosslinking of the peptidoglycan strands resulting in the exposure of more D-alanyl-D-alanine residues (Figure 8).

Mechanism of S.aureus Resistance to Vancomycin

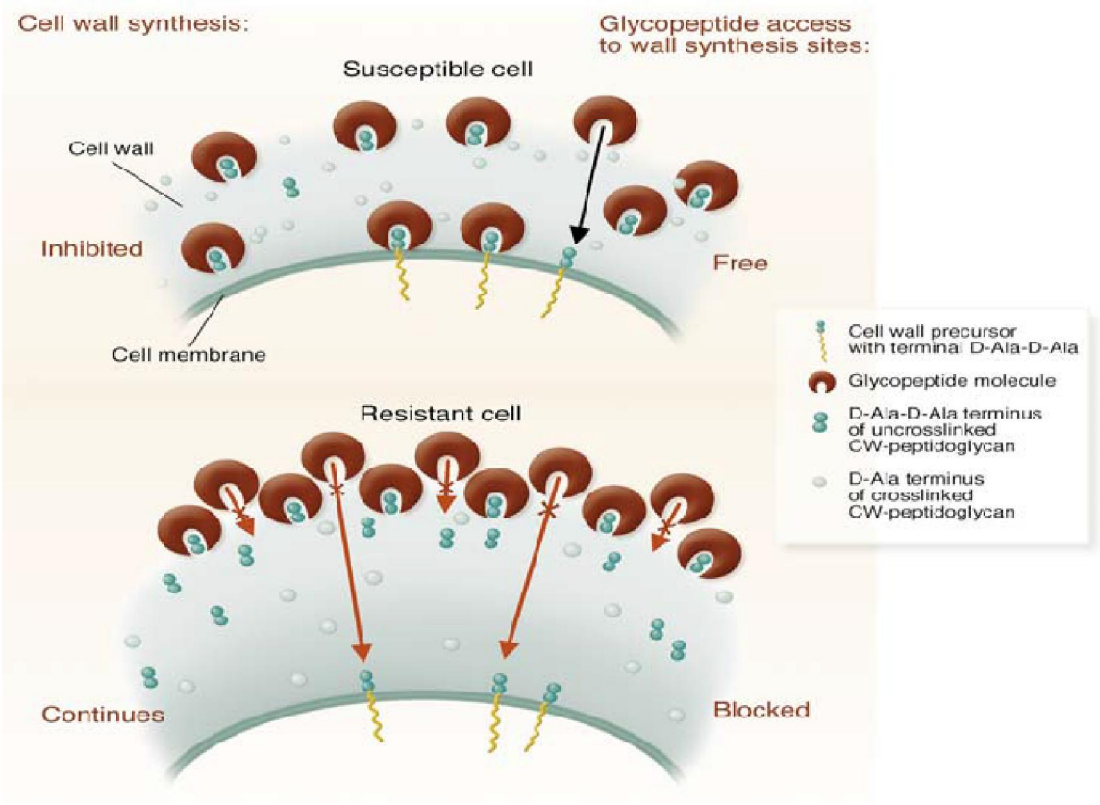


Figure 8 : Schematic representation of the mechanisms of *S. aureus* intermediate resistance to vancomycin (Lowy, 1998). The vancomycin-intermediate *S. aureus* strains synthesise additional quantities of peptidoglycan with increased numbers of D-Ala-D-Ala residues that bind vancomycin, thus preventing the molecule to bind to its bacterial target (cell wall) (Lowy, 1998).

The second form of vancomycin resistance involves vancomycin-resistant *S. aureus* (VRSA) with a minimum inhibitory concentration (MIC) of ≥128 μg/ml. The mechanism is hypothesised to be due to conjugation with vancomycin resistant *Enterococcus faecalis* (VRE). The process of conjugation results in the transfer of the *vanA* operon of the *E. faecalis* bacterium to the MRSA strain. The *vanA* gene together with its regulator genes, *vanSR*, from VRE is carried by a transposon, *Tn1546*, which is integrated into the plasmid (pLW1043) and conjugatively transferred into *S. aureus*. Vancomycin-resistant *S. aureus* is therefore, an MRSA with a pLW1043 carrying the *vanA* gene. The pLW1043 also carries other resistance mediating genes against gentamycin, penicillin and trimethoprim[7].

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Fluoroquinolone resistance in *S. aureus* strains

Fluoroquinolones are broad spectrum and bacteriocidal antibiotics. The fluoroquinolone drugs kill bacteria by inhibiting bacterial DNA synthesis. Important examples of the fluoroquinolone group include ciprofloxacin, ofloxacin and norfloxacin. Introduced in the 1980s, fluoroquinolones were initially developed for the treatment of Gram-negative bacteria, such as *Pseudomonas* species with limited activity against Gram-positive bacteria. Over the years, new fluoroquinolones with increased activity against Gram-positive cocci were developed including grepafloxacin, levofloxacin, moxifloxacin, sparfloxacin and trovafloxacin. However, the use of these drugs have been highly regulated because of increased development of resistance by bacteria to this group of drugs.

Fluoroquinolone resistance of *S. aureus* emerged rapidly in US hospitals in 1988 after the introduction of ciprofloxacin with 80% of the infections identified as MRSA[11]. Ciprofloxacin was initially developed for the treatment of Gram negative and Gram-positive bacteria other than *S. aureus*, thus exposure of *S. aureus* to fluoroquinolones was minimal. *Staphylococcus aureus* resistance to fluoroquinolones is suggested to be as a result of exposure of the bacteria to fluoroquinolones in the mucosal and cutaneous surfaces in the nasal cavity. In 2005, MacDougall and colleagues reported a 38% resistance in 616 *S. aureus* strains from 17 US hospitals isolated in 2000 [11].Recently, a study reported a 85% fluoroquinolone-resistance in 846 MRSA strains isolated from Kuwaiti hospitals between March and October 2005.

Diseases caused by *S. aureus:*

Staphylococcal diseases are usually a result of the production of a toxin or through the invasion and destruction of tissue. Diseases that arise from exclusively staphylococcal toxins include staphylococcal scalded skin syndrome (SSSS), taphylococcal food poisoning and toxic shock syndrome (TSS). Other staphylococcal diseases include suppurative infections, wound infections and catheter related infections.

Systemic Manifestations:

S. aureus causes a wide variety of suppurative diseases in humans(figure 8a and 8b). Most infections are minor and superficial.

Minor infections:

1. Impetigo

2.Cellulitis

3.Folliculitis

4.Carbuncles

5.Scalded skin syndrome and

6.Abscesses

7.Serious infections occur in association with a predisposing conditions like newborns, persons with traumatic or operative wounds, burn victims or other serious skin lesions, chronic debilitating disorders (diabetes mellitus, cancer, cystic fibrosis) and in IV drug abusers.

Major infections:

1. Pneumonia,

2.Meningitis,

3.Osteomyelitis,

4.Endocarditis,

5.Toxic shock syndrome,

6.Bacteremia and

7.Sepsis.

S.aureus is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections.

Bacteraemia

Staphylococcus aureus remains a common cause of community onset bloodstream infections. Staphylococcal bacteraemia mortality rate was approximately 20% to 50% between 1992 and 1998 in Belgium. The increased risk in staphylococcal bacteraemia is mostly attributed to catheterisation and patients with a high nasal carriage (85%) of *S. aureus* in hospital. It is estimated that more than 50% of *S. aureus* associated bacteraemia are acquired in the hospital after surgical operation or resulting from constant use of contaminated intravascular catheters . Other risk factors for HA-MRSA bacteremia include immunosuppressive diseases, such as cancer; diabetes; human immunodeficiency virus (HIV) and the extensive use

of corticosteroids and foreign bodies, which include prosthetic heart valves as well as central and peripheral venous catheters

Endocarditis

Staphylococcus aureus related endocarditis has accounted for 25% to 35% of cases worldwide between 1985 to 1993. The infection is abundant in elderly patients, prosthetic valve patients, intravenous drug users and hospitalized patients. Infective endocarditis is a complication often arising from *S. aureus* associated bacteraemia with a 12% incidence in infants and children in North Carolina,USA, between 1998 and 2001. Echocardiography is one way of exploring the heart valves thus diagnosing endocarditis. Prognosis of *S. aureus* related endocarditis is worsened in patients with HIV infection, as it usually presents as an advanced infective endocarditis.

Toxic shock syndrome

Toxic shock syndrome was first described by Todd and his collaborators (1978) in Denver, USA, in children aged 8 to 17 years . The disease is characterised by diarrhoea, erythroderma, high fever, hypotension, mental confusion and renal failure. Female cases have been associated with caesarean section surgeries,tampoon use and long-term diaphragm use . Hypovolemic shock develops due to loss of colloids and fluids . A sunburn-like rash develops within a few hours with the involvement of conjuctival inflammation[10] .

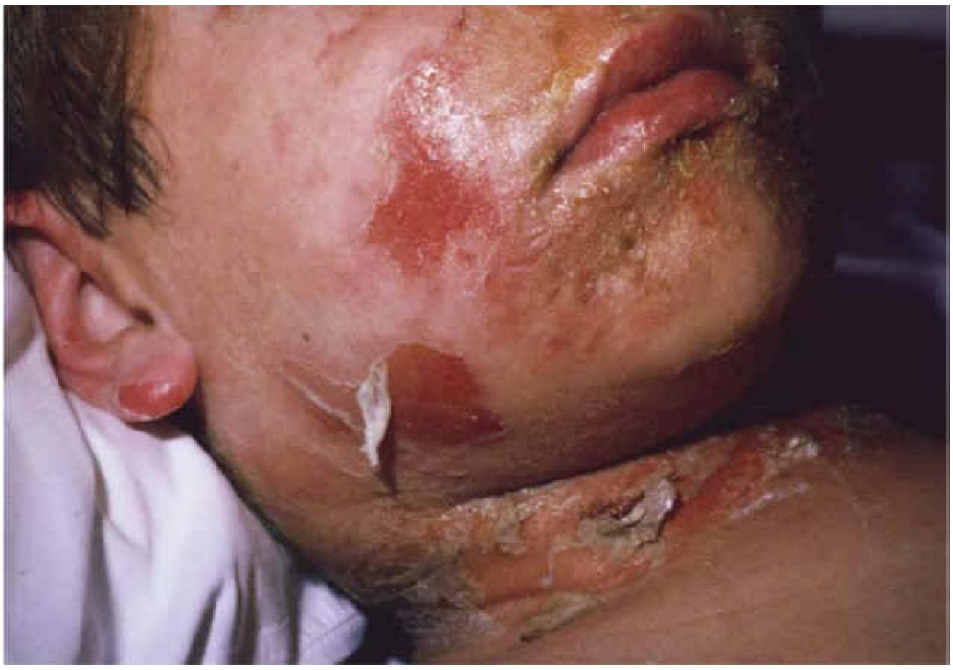
Food poisoning

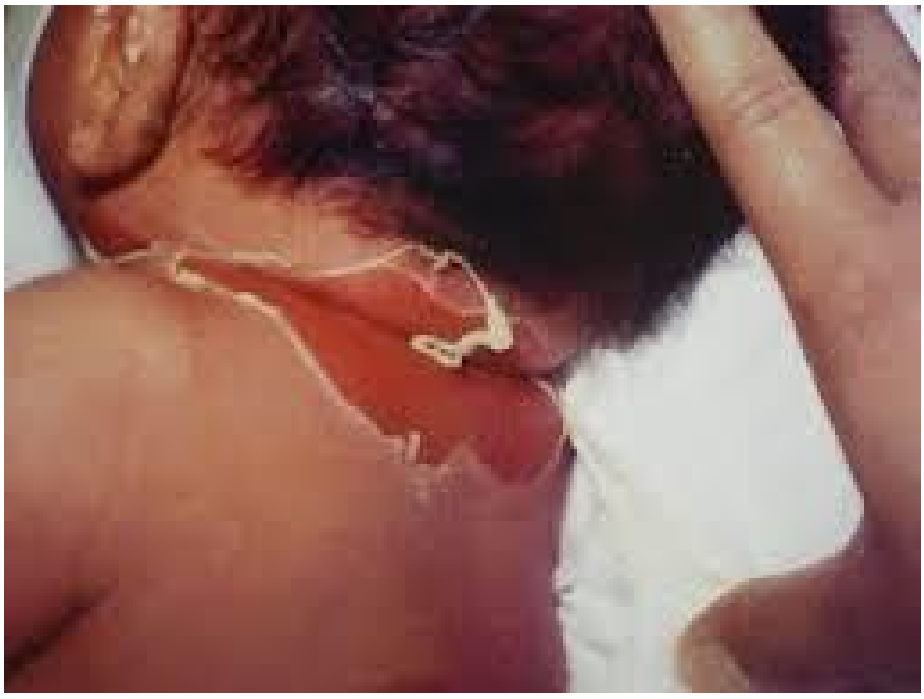
Staphylococccus aureus is the leading cause of gastroenteritis resulting from the consumption of contaminated food. *Staphylococcus aureus* food poisoning is due to the release of toxins in the food during its growth, causing symptoms ranging from abdominal pain to nausea, vomiting and sometimes diarrhoea but never diarrhoea alone. The onset of *S. aureus* food poisoning is rapid, ranging from 30 min to 8 h after ingestion, with spontaneous remission after 24 hrs.

Staphylococcal scalded skin syndrome

Staphylococcal scalded skin syndrome was first described in 1878 by Ritter von Rittershain as a disease manifested by a bullous exfoliative dermatitis in infants less than 1 month old. The skin looks and feels as though it had been scalded by hot water (Figure 9). The disease presents occasionally with an onset of general localised erythema and spreads to the entire body in less than two days The symptoms are usually followed by an upper respiratory infection or a purulent conjunctivitis. The disease has been attributed to the production of an exotoxin known as epidermolytic toxin (ET).

Figure 9a and 9b:Staphylococcal scalded skin syndrome (skin looks scalded by hot water)





Ocular manifestations [31](figures 9-12)

Minor infections:

1.Staphylococcal blepharitis

2. Phlyctenular conjunctivitis

Major infections

1. Preseptal and orbital cellulitis

2.Lid abscess

3.Corneal ulcers

4.Endophthalmitis

5.Blebitis

6.Scleral abscess

7.Panophthalmitis

MRSA is known to cause a wide spectrum of ocular diseases ranging from conjunctivitis to panophthalmitis. The commonest manifestation is conjunctivitis[31].It is commonly associated with patients in long term care facilities, particularly in those with immuno compromised state. Keratitis due to MRSA is usually chronic in onset and slowly progressive usually not responding to treatment. Patients with obstruction of nasolacrimal duct are at an increased risk for infection. Scleritis and scleral abscess due to MRSA can lead to extensive tissue destruction resulting in panophthalmitis.

MRSA causes lid and orbital infections more commonly than methicillin sensitive strains. Closed space infections like abscesses usually respond well to surgical drainage. A delay in surgical intervention in such cases can promote development of resistance. Of special concern to ophthalmic surgeons is the increasing reports of post operative endophthalmitis. Refractive surgeries have also not escaped MRSA infections. Cases have been reported following laser insitu keratomileus and penetrating keratoplasty. Socket infections due to MRSA have also been encountered following exentration and evisceration surgeries[31].

Fig 9: 2 year old Child infected with MRSA causing Cellulitis

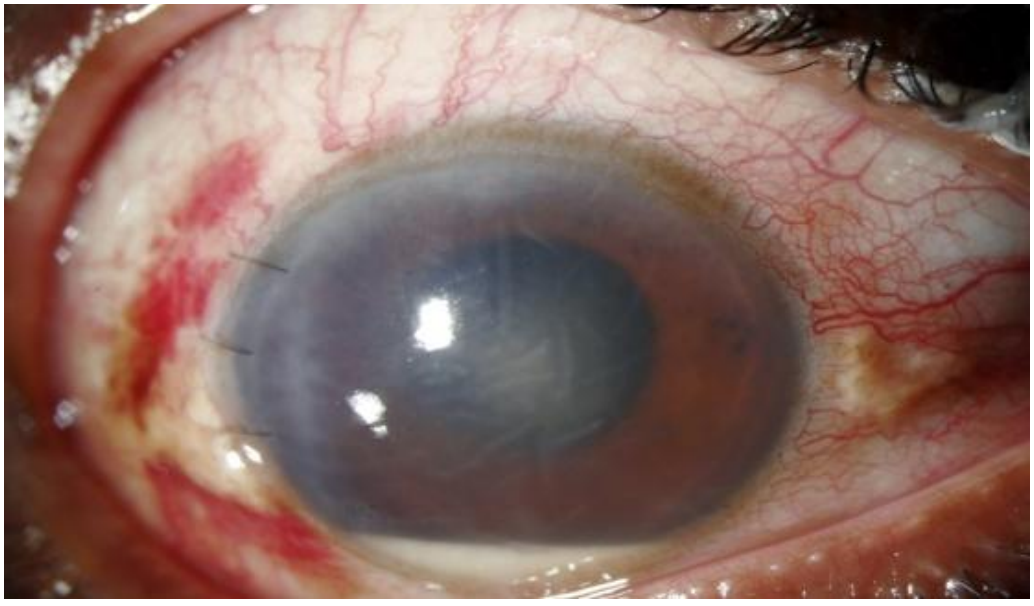


Fig 10: Endophthalmitis with Hypopyon

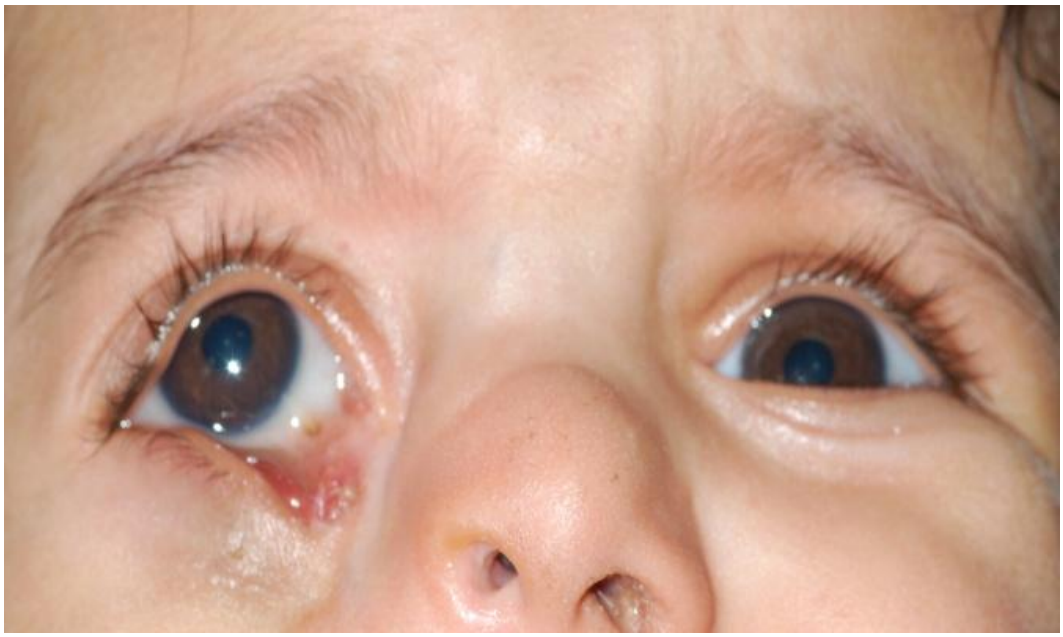


Fig 11 : Lower lid coloboma

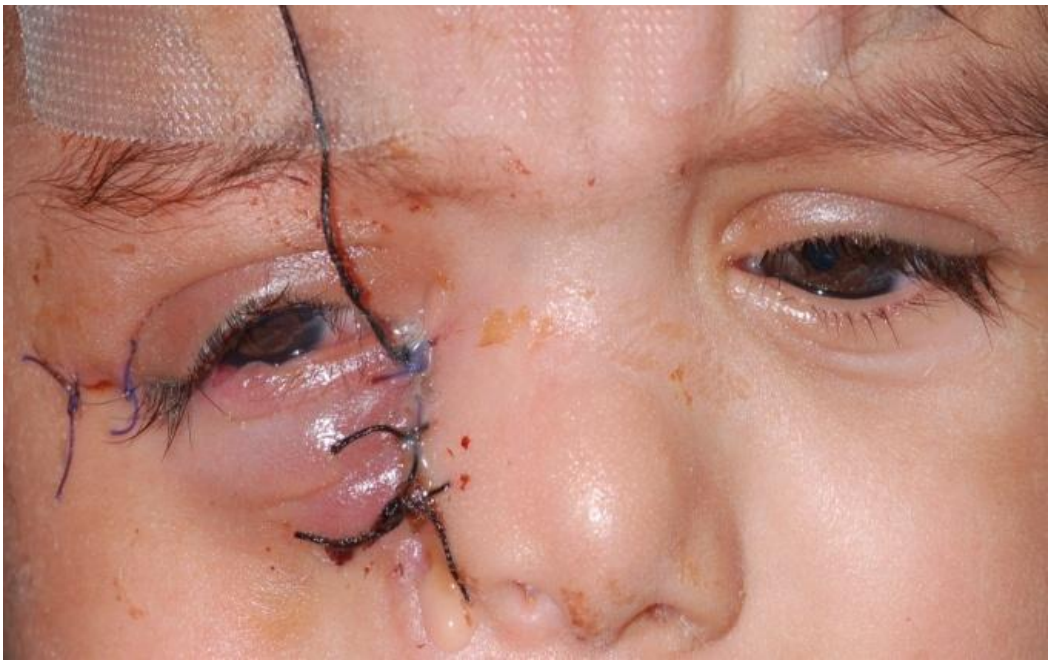


Fig 12 : MRSA suture infection seen post lid repair surgery

Diagnosis of MRSA:

Early diagnosis and determination of antimicrobial susceptibility is not only necessary for the optimal antimicrobial therapy but also to monitor the of the spread of MRSA strains or resistance genes throughout the hospital and the community [32]

Phenotypic identification of MRSA strains:

Upon identifying *S. aureus* by Gram-staining (Gram-positive cocci), catalase (positive), fermentation tests (oxidase positive) and tube coagulase (positive) or DNase (positive), the sample is grown on mannitol salt agar or blood agar at 37^{o}C for 18 to 24 hrs [33]. The colonies appear yellow on mannitol salt agar and creamy white on blood agar [33]. *Staphylococcus aureus* colonies are subjected to antimicrobial susceptibility testing by the disk diffusion methods. The Kirby-Bauer disk diffusion method is the most routinely used detection method for methicillin resistance in *S. aureus* in clinical laboratories despite the increasing development of commercial methods and automated systems [34]. The Kirby Bauer disk diffusion method is a standardised antimicrobial susceptibility test, which is recommended by the Clinical and Laboratory Standards Institute (CLSI) [35].

Antimicrobial susceptibility testing

Staphylococcus aureus colonies grown on Mueller-Hinton agar plates in the presence of thin disks containing relevant antibiotics at standardized concentrations . Susceptibility of *S. aureus* is demonstrated by a clear zone around the disk known as the zone of Inhibition.(figure 13)

Commercially available susceptibility testing methods are also used in addition to Kirby Bauer disk diffusion method.

Figure 13. Kirby Bauer disk diffusion method

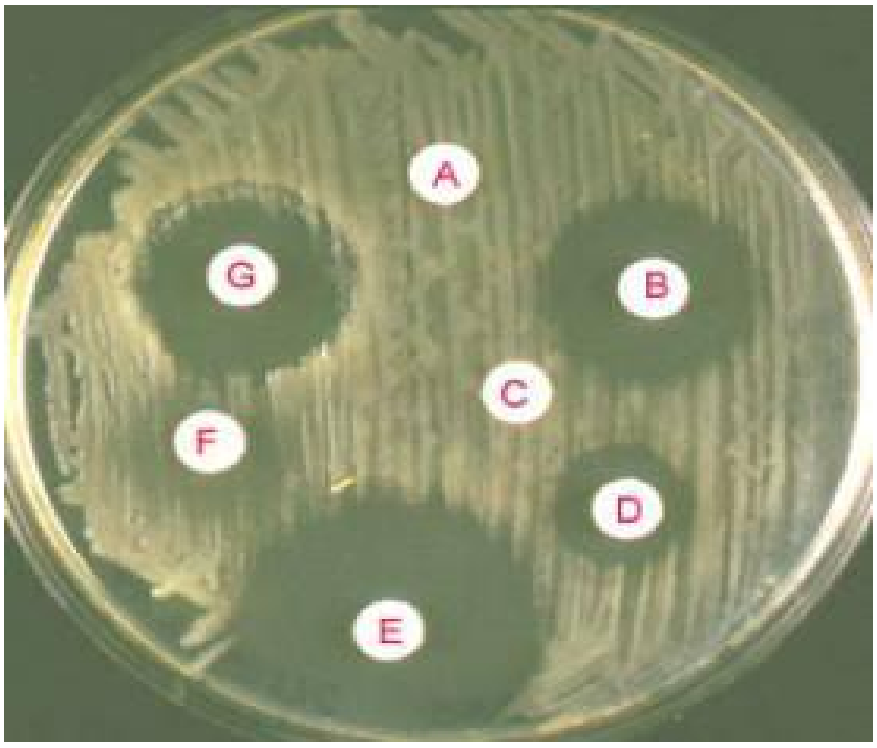


Figure 13 shows Mueller-Hinton agar plate -antibiotic disks A-G. Disks B, E and G have clear zones indicating susceptibility to these antibiotics. Discs A, C, D and F show the resistance of the bacterium to these antibiotics

Molecular identification of MRSA strains

Since conventional identification and antibiotic resistance detection often take more than 48 hr, molecular based detection techniques, including conventional PCR and real-time PCR, have been developed for the rapid and accurate identification and characterization of MRSA isolates [32,36]. Molecular techniques are often applied for the routine diagnostic MRSA detection along with antimicrobial susceptibility testing methods, partly because susceptibility testing alone is not enough to confirm MRSA presence due to the sensitivity of the test conditions [37]. The identification of MRSA was simplified by the polymerase chain reaction (PCR) technique [38](figure 14).

Multiplex PCR typing methods of MRSA have been previously described [40]. The M-PCR typing method is based on the characterization of MRSA's specific *ccr* gene complex, which encodes for site-specific recombinases responsible for the mobility of SCC*mec* [39]. The *ccr* gene complex together with *mec* complexes which are classified into class A, class B, class C and class D 39 can type MRSA isolates into the different SCC*mec* types thus enabling researcher to distinguish between HA-MRSA and CA-MRSA [41].

Recently, another M-PCR assay was developed for the subtyping of the SCC*mec* type IV into eight subtypes [42]. The "SCC*mec* IV" M-PCR is important to trace clones of CA-MRSA characterized by SCC*mec* type IV to understand the mechanism of SCC*mec* assembly and acquisition in these clones [42]. The M-PCR assays can be useful in infection control strategies and be implemented for epidemiological studies to determine clonal relatedness during outbreaks in clinical settings [43].

Typing assays of MRSA strains

Following the development of PCR, various techniques became available for the typing of MRSA and MSSA strains including random amplified polymorphic DNA (RAPD) and variable number tandem repeat (VNTR) typing techniques. [43]. However, prior to the development of PCR, several molecular techniques were used for identification and typing of *S. aureus* and MRSA strains. The section below discusses the different non-PCR based and PCR based techniques used in the genotyping of MRSA.

2.10.1 Non-PCR based typing techniques of MRSA strain typing

Before the development of PCR, several efficient typing methods were used for *S. aureus* strain typing. These methods including

1)Bacteriophage typing (1952)

2)Capsular typing (1984)

3)PFGE(1984) and

4) Zymotyping

have been applied for discriminating between *S. aureus* and MRSA strains [44,45]. Amongst these methods, PFGE is the most extensively used method to date for the typing of MRSA strains as it is the "gold standard". Most novel MRSA typing studies couple PFGE as a reference method for MRSA strain typing as it is the most sensitive and specific MRSA strain typing method to date. [46]

PCR-based typing methods for MRSA typing

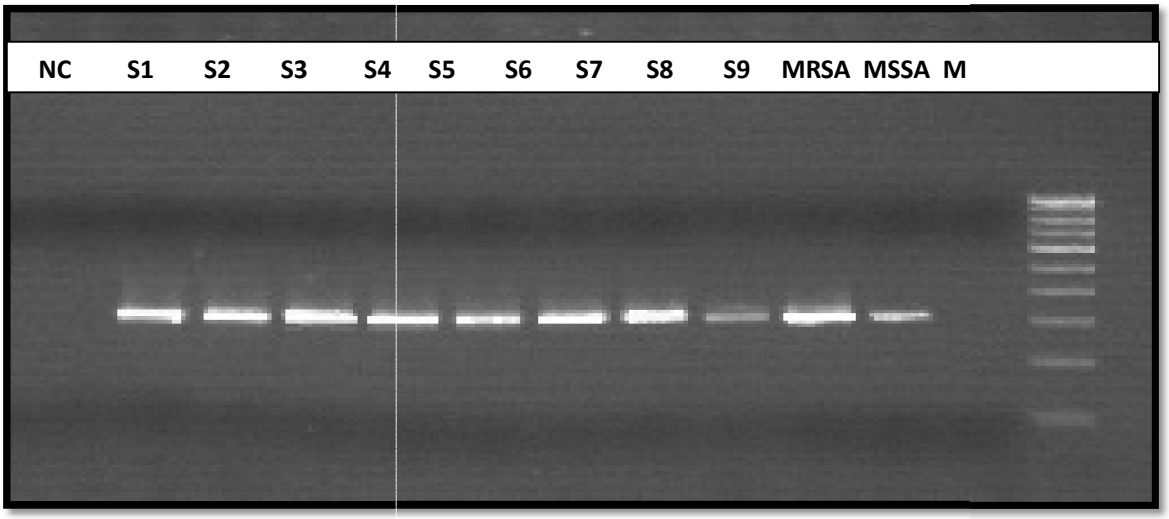
Following the development of PCR, typing of MRSA strains evolved to the detection of polymorphic regions of the MRSA genome. Polymerase chain reaction based typing techniques have increased the understanding of MRSA strains by identifying the different genotypes and related MRSA strains [37].

PCR-based methods include

1)Random amplified polymorphic DNA (RAPD) ,

2) Variable-numbers of tandem repeat **(**VNTR)- based typing techniques including *coa* , *spa* and hypervariable region typing

Figure 14.PCR for screening *mecA* gene:



NC - Negative control
S1 – S9 - Clinical Isolates
MRSA - Methicillin Resistant Staphylococcus aureus
MSSA - Methicillin Susceptible Staphylococcus aureus

The rate of mutations and genetic rearrangements of strains control the consistency of the various PCR based typing techniques [43]. Using these typing techniques in combination will provide better results when compared to using one technique. The sensitivities and specificities can thus be compared when more than one technique is used. Differentiation between HA-MRSA and CA-MRSA is primarily based on the harbored SCC*mec* element [22]. Several M-PCR assays have been proposed to distinguish between these two types of MRSA [40,41]

Methicillin-resistant *S. aureus* classification and sub typing is important for recognising MRSA outbreaks, determining the source of outbreak and recognizing virulent strains that might be circulating in the clinical setting [40]. The monitoring of multi-drug resistant MRSA strains (HA-MRSA) and virulent strains (CA-MRSA) is essential in enforcing the correct and adequate control measures and adjusting guidelines for antimicrobial chemotherapy in different hospital settings [47].

Treatment options

Appropriate surgical drainage is the definitive management of many soft tissue infections due to MRSA and it acts as an important adjunct to antibiotic therapy in deep, closed-space infections. Data from the surgical literature suggest that adequate surgical drainage allows many CA-MRSA infections to resolve regardless of whether the isolate is susceptible in vitro to the antibiotic chosen [48] **.**Vancomycin is the empirical drug of choice for the treatment of MRSA [35] . With MRSA isolates being widespread, it is imperative that treating physicians de-escalate to β-lactams once the culture sensitivity results reveal a MSSA isolate. Preservation of glycopeptides and linezolid for use only in MRSA cases should be encouraged.

Fluoroquinolones, cephalosporins, lincosamide, tetracycline, chloramphenicol and trimethoprim-sulfamethoxazole can also be used in sensitive cases. But they are of limited value due to rapid development of resistance during therapy. [7]. Adult and pediatric dosages of agents that may be used for the treatment of CA-MRSA are listed in **Table 6**.

Table 6 : Antimicrobial Dosing Recommendations for MRSA

Drug	**Adult IV Dosage**	**Adult oral Dosage**	**Peadiatric IV Dosage**
Clindamycin	1,200 – 2,700mg/day divided every six to eight hours↑	300 – 450mg every six hours↑	Age >1 month 20-40mg/kg/day divided every eight hours↑
Doxycycline/ Minocycline	100mg every 12 hours↑	100mg every 12 hours	Age < 8 years: contraindicated
Linezolid	600mg every 12 hours 400mg every 12 hours	600mg every 12 hours 400mg every 12 hours	Age <12 years:10mg/kg every eight to 12 hours:
Rifampin	600mg every 12 hours□	600mg every 12 hours	15-20mg/kg/day divided in one to two doses: maximum 600mg/dose↑
Trimethoprim-Sulphamethazole	15-20mg/kg/day divided every six hours(TMP component)↑	One to two double strength tablets every 12 hours↑	Age > 2 months: 15-20mg/kg/day divided every six hours(TMP component)↑
Vancomycin	15-20mg/kg/dose every 12 hours: then dosage and interval adjusted to trough levels	N/A	15mg/kg/dose every eight hours: then dosage and interval adjusted to trough levels

Adult and pediatric dosages of agents that may be used for the treatment of CA-MRSA

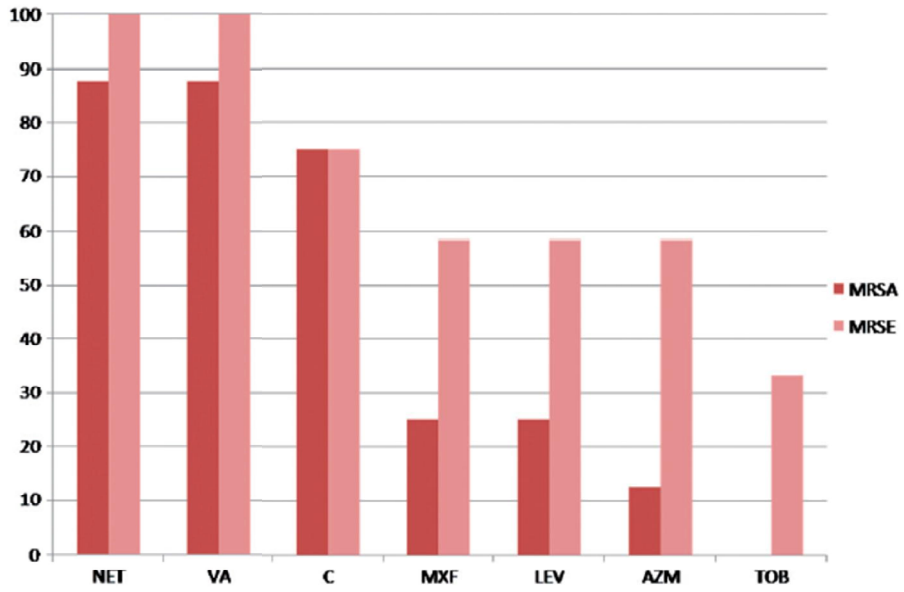
Treatment of ocular infection :

Incision and drainage of pus material is very important followed by treatment based on antibiotic susceptibility pattern . Anna Rita Blanco found vancomycin, netilmicin and chloramphenicol to be effective in ocular isolates. (Chart 1).

Eye infections are frequently treated with multiple topical antibiotics, the chance of antibiotic-resistance development among microorganisms is high. Indiscriminate use of antibiotics in the community allows resistant strains to colonize eyes in the community population.

Study by Anna Rita Blanco1 et al, Italy 2013

CHART 1



Authors found Vancomycin, Netilmicin and chlormpenicol to be effective in ocular isolates

AIM

1) To find the prevalent MRSA strains causing ocular infection in South India.
2) To correlate the ocular manifestations of MRSA with its phenotypic and genotypic characteristics.
3) To find the antibiotic susceptibility pattern of the prevalent MRSA strains

Design:- Prospective study.

Participants:-

Patients with culture proven MRSA ocular infection seen between January 2012 to December 2012

Setting :

University affiliated teaching centre attached to a community based eye hospital offering primary to tertiary care.

Centre :

Aravind Eye Hospital ,Madurai .

Department :

Microbiology, Aravind Eye Hospital ,Madurai

Methodology:- The study was approved by the research committee and the Institutional Review Board of Aravind eye hospital. A waiver of consent was granted as the study is observation of standard practice of care.

Sample and data collection:

Clinical isolates were collected by culturing pus, corneal scraping, vitreous fluid, aqueous humor or conjunctival swab of infected patients. Patient who had a positive culture for MRSA between January 1, 2012, and December 31, 2012 were included in this study. Isolates were identified and confirmed as MRSA on the basis of drug resistance and Polymerized chain reaction(PCR) test the patient was included in the study. Comprehensive systemic and ophthalmologic histories were obtained from each patient**.** A complete ocular examination was performed at every visit, including best-corrected visual acuity, adnexal examination, slit-lamp biomicroscopy and fundus examination. Demographic and clinical details were recorded in the proforma (Appendix).

Data collected included age at time of culture, gender, laterality, clinical manifestation, pre existing risk factors, diagnosis, treatment and final visual outcome. Possible risk factors investigated included hospitalization in the past year, recent stay at a long-term care facility, diabetes, intravenous drug use, immune compromised state systemic or ocular corticosteroid use, or use of other ocular medications.

Data collected also included the following: antibiotics initially prescribed, whether antibiotics were begun empirically prior to culture results, if antibiotics were changed after culture results were known and any procedures performed, including incision and drainage. Sensitivity (or resistance) of isolates of MRSA to antibiotics tested was also reviewed. Visual acuity at discharge and subsequent follow ups were recorded. Cause for defective vision, if any were also noted.

Identification of MRSA:-

Ocular specimens were inoculated on blood agar for 24 – 48 hours at 37° C. Typical staphylococcal colonies were examined under microscopy by gram staining and routine biochemical tests like catalase, coagulase, and mannitol fermentation test.

All the confirmed *S.aureus* strains were subsequently tested for methicillin resistance based on Kirby- Bauer disk diffusion method using oxacillin(1µg) and cefoxitin (30µg) discs (Himedia, Mumbai, India).Oxacillin was used instead of methicillin as it is more stable invitro. The isolates were considered to be methicillin resistant, if the zone of inhibition was 13mm or less and 17mm or less for oxacillin and cefoxitin respectively according to CLSI standards (2013).

Confirmation of MRSA:

Molecular methods were used for confirmation of MRSA. It included:

1. Detection of *MecA* gene by uniplex PCR.
2. Staphylococcal cassette chromosome (SCC*mec*) typing by multiple PCR.

Antibiotic susceptibility pattern:-

The antibiotic susceptibility pattern of various antimicrobial agents such as levofloxacin,gatifloxacin,moxifloxacin,cefotaxime,gentamycin,tobramycin,ciprofloxacin,ofloxacin,chloramphenicol,cefazolin,vancomycin and tetracycline against MRSA was determined by the modified Kirby- Bauer disc diffusion on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials (Figure 13) according to CLSI standard.

Clinical correlation:-

Based on the structures involved, ocular infections were grouped into one of seven diagnoses: conjunctivitis, keratitis, lid disorder, lacrimal system disorder, wound infection, endophthalmitis and others (e.g., blebitis, buckle or implant infection and scleritis). If the chart showed more than one diagnosis, the primary pathology or the more severe diagnosis was chosen.

If the patients had either of the following they were considered to have health care exposure.

1) A MRSA infection identified after 48 hours of admission to a hospital

2) A history of hospitalization, surgery, dialysis, or residence in a long-term health care facility within one year of the MRSA culture date

3) A permanent percutaneous medical device present at the time of culture

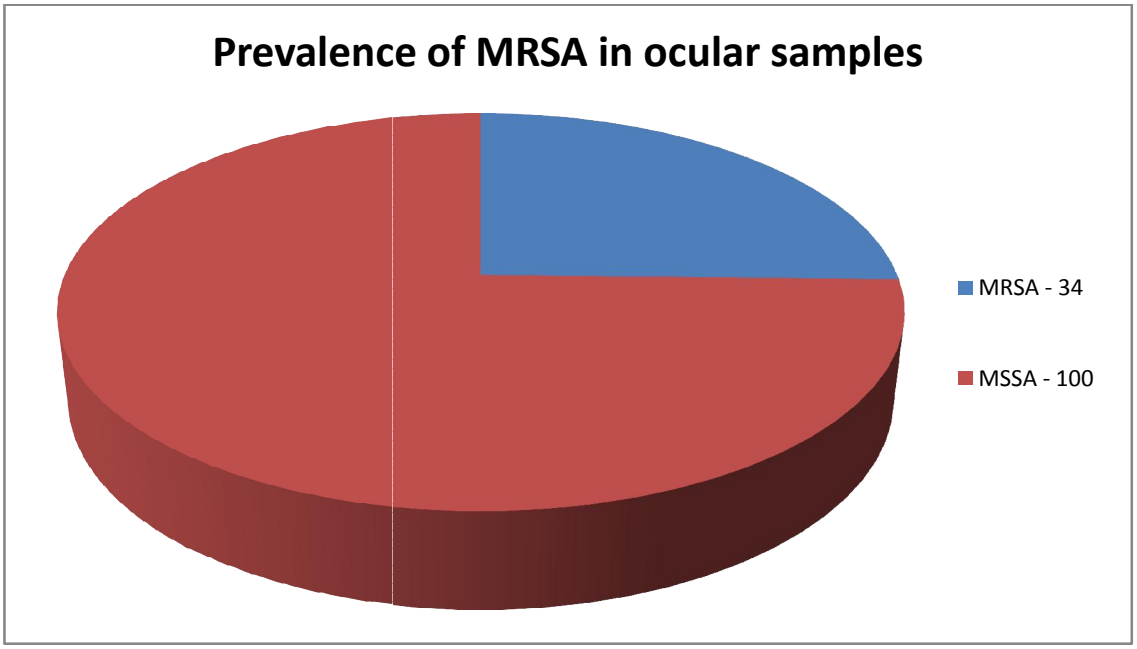
4) A known positive culture for MRSA prior to the study period.

Patients were followed up for control of infection for 3 to 6 months. Details of treatment and treatment response were noted in the data sheet.

RESULTS

During the study period of one year, from Jan 2012 to December 2012, S. aureus was isolated from 134 patients. Of these, 34(25.3%) were MRSA and 100 (74.6%) were Methicillin-sensitive S. aureus. (figure 16).

Fig:16 Prevalence of MRSA in ocular samples



Of 34 patients who were MRSA positive, nineteen were males and 15 were females (figure 17) . The mean age was 31.8 years, range, one month to 78 years .

Fig:17 Gender distribution

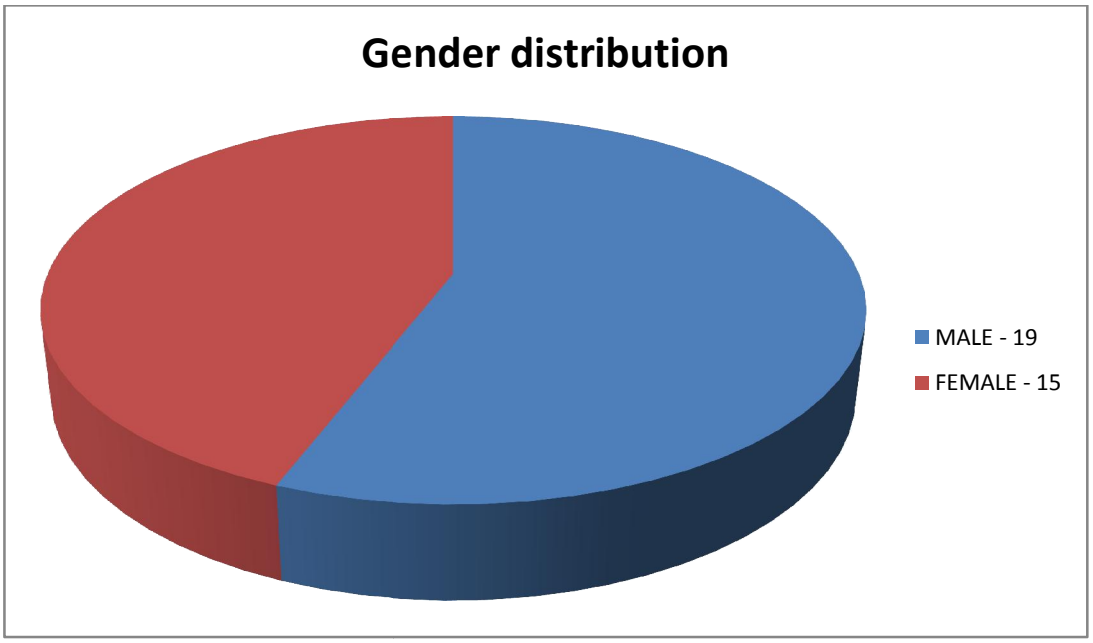


Table 7 : Clinical signs of patients with MRSA infection:

	N	%
Lids		
Lid Edema	22	65%
Blepharitis	1	3%
Trichiasis	2	6%
Lagopthalmas	2	6%
Conjunctiva		
Congestion	27	79%
Chemosis	10	29%
Dryeye	1	3%
Discharge	8	24%
Cornea		
Edema	8	24%
Cornea ulcer	3	9%
Infiltrate	9	26%
Pannus	2	6%
Vascularization	2	6%
Marginal keratitis	2	6%
Satellite lesion	1	3%
Exposure keratitis	1	3%
Anterior chamber		
Evidence iritis	7	21%
Hypopyon	3	9%
Anterior synechiae	1	3%
Lens		
Clear lens	24	71%
Cataract	2	6%
Aphakia	4	12%
Posterior synechiae	1	3%
Posterior segment		
Vitreous cells	1	3%
Exudates	1	3%
Fundus Normal	20	60%
No view	10	30%
Macular edema	1	3%
Exudatine retinal detachment	1	3%

Previous treatment taken outside		
If yes,		
Antibiotic	7	3%
Steroids	4	72%
Native treatment	1	3%
Prior hospitalization	10	31%
Predisposing risk factors local		
Trauma	5	17%
Eye surgery	8	25%
Ocular surface disorders	2	7%
Lid anomalies	5	17%
Naso lacrimel duct patency		
Free with clear fluid	10	31%
Not free with clear fluid	1	3%
Not free with clear pus	1	3%
Systemic risk factors		
Immuno compromised state	2	7%
DM	8	26%
Hemodialysis	1	3%
Steroid use	1	3%

Table 8: Clinical diagnosis of patients with MRSA infection

Clinical Details	No	%
1.Orbit		
Dermis fat graft infection post evisceration	1	2.9%
Suture Infection post DCR	1	2.9%
Orbital cellulitis and corneal inflitration	1	2.9%
Sling infection	1	2.9%
Socket infection post exentration	1	2.9%
Lacrimal Abscess	4	11.7%
Acute dacryocystitis	1	2.9%
2.Lid		
Preseptal Cellulitis	1	2.9%
Lid abscess	9	26.4%
Blepharities	1	2.9%
3.Sclera		
Scleral Abscess	1	2.9%
Necrotizing scleritis	1	2.9%
Infectious Nodular Scleritis	1	2.9%
4.Cornea		
Corneal inflitration	1	2.9%
Suture infection	1	2.9%
Exposure keratitis due to lagophthalmos	1	2.9%
Neurotropic keratitis	1	2.9%
Graft infection	1	2.9%
Corneal ulcer	2	5.8%
Moorens ulcer	1	2.9%
Corneal graft	1	2.9%
5.Endophthalmitis	1	2.9%
Total	34	100%

The commonest clinical sign encountered was lid edema (65%) followed by congestion,chemosis and corneal edema.Majority of the infections were limited to anterior segment while 2 patients had posterior segment involvement.10(31%) had prior history of hospitalization.8 out of 10 had undergone prior ocular surgery within the past year like cataract extraction, exentration, evisceration, lid repair, keratoplasty, dacryocystorhinostomy and sling surgery. The commonest systemic risk factor seen in our patients was diabetes(26%).

Lid abscess and lacrimal abscess were the most commonly encountered diagnosis (38%).This was followed by corneal infections (26%) and sclera involvement (8.7%).Other infections like cellulitis, blepharitis, conjunctivitis, orbital cavity infection, dacryocystitis and suture infection were also encountered each contributing around 3%.Endophthalmitis was seen in 1 patient (2.9%).

Table 9 : Phenotypic and genotypic characterisation of MRSA in ocular samples

S.No	Lab No	Infection	Sample	Phenotypic Characters		Identification of MRSA	Confirmation and typing	
				Grams stain	Catalase& Coagulase	Oxacillin Cefoxitin disc diffusion	PCR for MecA	SCCmec Type
1	14519	Sclera – Abscess	Pus	+	+	Resistant	+	V
2	16923	Pre septal cellulitis	Pus	+	+	Resistant	+	V
3	16614	Dermis graft Infection	Pus	+	+	Resistant	+	V
4	17816	Suture infection Lacrimal abscess	Pus	+	+	Resistant	+	V
5	16538	Orbital cellulitis corneal infilteration	Pus	+	+	Resistant	+	III
6	18373	Lid abscess	Pus	+	+	Resistant	+	V
7	20485	Acco corneal infilteration	Conj Swab	+	+	Resistant	+	V
8	14400	Infected sling	Pus	+	+	Resistant	+	V
9	18314	Lid abscess	Pus	+	+	Resistant	+	V
10	18148	Post excentration cavity infection	Pus	+	+	Resistant	+	V
11	14632	Lacrimal abscess	Pus	+	+	Resistant	+	IV
12	16625	Suture Infection	Corneal scraping	+	+	Resistant	+	IV
13	15115	Lid abscess	Pus	+	+	Resistant	+	IV
14	19983	Neurotrophic ulcer	Corneal scraping	+	+	Resistant	+	IV
15	12966	Suture infection Lid abscess	Pus	+	+	Resistant	+	Non typeable
16	16825	Lid abscess	Pus	+	+	Resistant	+	Non typeable
17	20302	Acute dacryocystitis	Pus	+	+	Resistant	+	IV

18	24355	Neurotrophic ulcer	Corneal scraping	+	+	Resistant	+	III
19	23003	L acrymal abscess	Pus	+	+	Resistant	+	IV
20	23892	Necrotising Scleritis	Conj Swab	+	+	Resistant	+	IV
21	19881	Lid abscess	Pus	+	+	Resistant	+	IV
22	23052	Graft infection	Corneal scraping	+	+	Resistant	+	IV
23	21352	Corneal abscess	Corneal scraping	+	+	Resistant	+	IV
24	13159	Lacrimal abscess	Pus	+	+	Resistant	+	V
25	20242	Blepharitis	Pus	+	+	Resistant	+	V
26	22084	Lid abscess	Pus	+	+	Resistant	+	V
27	13220	Moorens Ulcer	Conj Swab	+	+	Resistant	+	V
28	16938	graft Infection	Corneal scraping	+	+	Resistant	+	V
29	17676	Corneal Ulcer	Corneal scraping	+	+	Resistant	+	V
30	19084	Lacrimal abscess	Pus	+	+	Resistant	+	V
31	21039	Sclera – Abscess Endophthalmitis	Pus	+	+	Resistant	+	V
32	19148	Scleral Abscess	Conj Swab	+	+	Resistant	+	V
33	20167	Lid abscess-preseptal	Pus	+	+	Resistant	+	V
34	20418	Lacrimal abscess	Pus	+	+	Resistant	+	V

Type I to III - Hospital acquired Type IV and V- Community acquired

All isolates were gram positive and showed positive results to catalase and coagulase tests . All isolates showed resistance to oxacillin and cefoxitin in disc diffusion method.Confirmation of MRSA was done by PCR which showed all isolates to be positive for MecA gene.SCCmec typing showed majority of the strains (88.2%) to be community acquired (type 4 and 5). Two were not typeable and two isolates were hospital acquired(type 3).Type 1 and 2 were not encountered in our study. Orbital infections were caused by both types 4 and 5 while corneal and scleral infections were mainly caused by type 5.

Systemic co - morbidities and and treatment details are given in **table 10**.

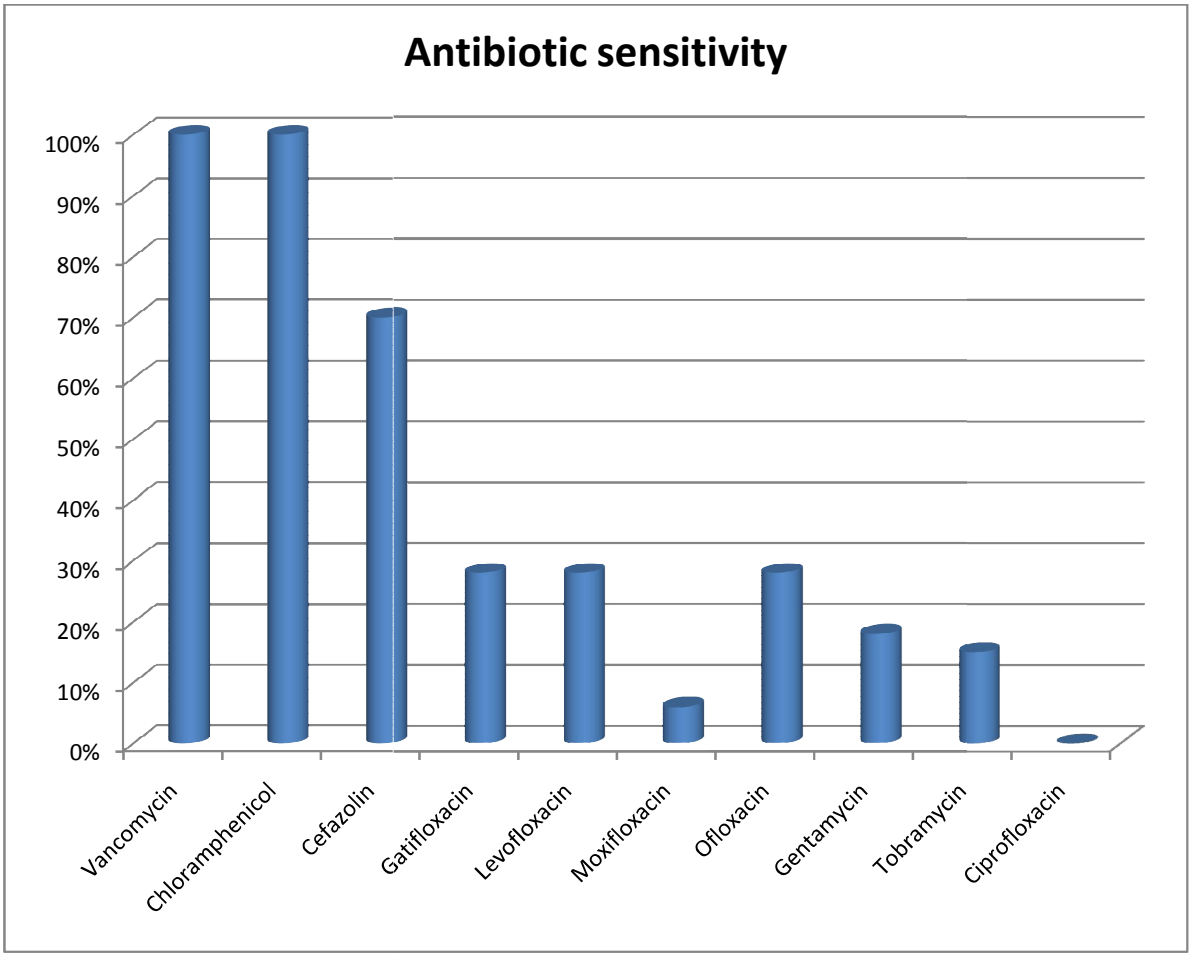
S. No	Lab No	Age/Gender	Comorbidities	CA/HA CDC guideline	Previous hospitalisation	Diagnosis	Treatment	
							Empirical	Surgical
1	14519	61/M	Diabetes Mellitus	CA	Nil	Scleral Abscess	Gatifloxacin,inj.amikacin	Incision & Driange
2	16923	1/F	Nil	CA	Nil	Preseptal Cellulitis	Ofloxacin,inj.ceftriaxone	Incision & Driange
3	16614	25/F	Trauma Eye Surgery	HA	Yes	Dermis fat graft infection post evisceration	Gatifloxacin inj.gentamycin, oral amoxycillin	graft removal
4	17816	78/F	Diabetes Mellitus	HA	YES	Suture Infection post DCR	Gatifloxacin,tab.ofloxacin	Incision & Driange
5	16538	1 Month /F	Trauma	HA	Yes IV and oral antibiotic	Orbital cellulitis and corneal inflitration	Gatifloxacin ,inj.ceftriaxone ,syp.metrogyll	Incision & Driange
6	18373	9/M	Nil	CA	Nil	Lid abscess	Moxifloxacin,tab.cefipime	Incision & Driange
7	20485	68/M	Diabetes Mellitus	CA	Nil	Corneal inflitration	Ofloxacin,chloramphenicol	Nil

8	14400	9/ M	Eye Surgery and lid anomalies	HA	Yes	Sling infection	Ofloxacin,cap. amoxycillin	Sling removal
9	18314	28/ M	Nil	CA	Nil	Lid abscess	Ofloxacin,tab.cefipime	Incision & Driange
10	18148	41/ M	Eye surgery	HA	YES	Socket infection post exentration	Ofloxacincap.amoxycillin	Nil
11	14632	58/ F	Diabetes Mellitus	CA		Lacrimal Abscess	Tobramycin,tab.amoxyclav	Incision & Driange
12	16625	61/ F	Nil	HA	Yes	Suture infection	Ofloxacin	Nil
13	15115	37/ M	Nil	CA	Nil	Lid abscess	chlorompenicol	Incision & Driange
14	19983	33/ M	Immunio suppression	CA	Nil	Exposure keratitis due to lagophthalmos	chlorompenicol	Tarsorrhaphy
15	12966	1/F	Eye Surgery and lid anomalies	HA	Yes	Suture Infection lid abscess	Tobramycin,inj Ceftriaxone	Suture removal and Incision & Driange
16	16825	36/ M	Nil	CA	Nil	Lid abscess	chlorompenicol ,tab.cefixime	Incision & Driange
17	20302	1 Month /F	Nil	CA	Nil	Acute dacryocystitis	tobramycinl and sac massage	Nil

18	24355	38/M	Trauma lid anomaly	HA	Yes	Neurotropic keratitis	Moxifloxacin,chloramphenicol	Tarsorrhaphy
19	23003	21/F	Ocular surface disorder	CA	Nil	Lacrimal Abscess	gatifloxacin,tab.cefixime	Incision & Driange
20	23892	30/M	Nil	CA	Nil	Necrotizing scleritis	Moxifloxacin,inj.amikacin	Nil
21	19881	8/F	Eye surgery	CA	Nil	Lid abscess	Chlorpenicol ,tab.amoxyclav	Nil
22	23052	70/F	Eye surgery	HA	YES	Graft infection	Gatifloxacin,fortified 10%cefazolin	Nil
23	21352	17/F	Trauma and steriod use	CA	Nil	Corneal ulcer	chlorompenicol ,moxifloxacin, natamycin	Therepeutic keratoplasty
24	13159	69/M	Diabetes Mellitus	CA	Nil	Lacrimal Abscess	Ofloxacin,tab.ciprofloxacin	Incision & Driange
25	P2809831	21/M	Nil	CA	Nil	Blepharities	Azithromycin	Nil
26	22084	21/F	Skin furunculosis	CA	Nil	Lid abscess	Ofloxacin,chloramphenicol	Incision & Driange
27	13220	71/M	Nil	CA	Nil	Moorens ulcer	Ofloxacin,tab.levofloxacin	Nil
28	16938	70/F	Corneal sutures	HA	YES	Corneal graft infection	Gatifloxacin,10%ceftazidime,natamycin	Suture removal

29	17676	65/F	Nil	CA	Nil	Corneal ulcer	Ofloxacin	Nil
30	19084	29/F	Diabetes Mellitus	CA	Nil	Lid abscess	chlorompenicol,	Incision & Driange
31	21039	60/M	Eye Surgery and Diabetes Mellitus	HA	Yes	Endophthalmitis	moxifloxacin,inj.amikacin,intravitreal vancomycin,ceftazidime	Scleral debridement, Ac wash
32	19148	53/M	Diabetes Mellitus	CA	Nil	Infectious Nodular Scleritis	Gatifloxacin,inj.amikacin	Nil
33	20167	7 months/F	Lid anamoly	CA	Nil	Lid abscess and preseptalcellulitis	Gatifloxacin	Incision & Driange
34	20418	40/F	Nil	CA	Nil	Lacrimal Abscess	Tobramycin	Incision & Driange and Dacryocystorhinostomy

Antibiotic sensitivity (Bar diagram) Chart 4

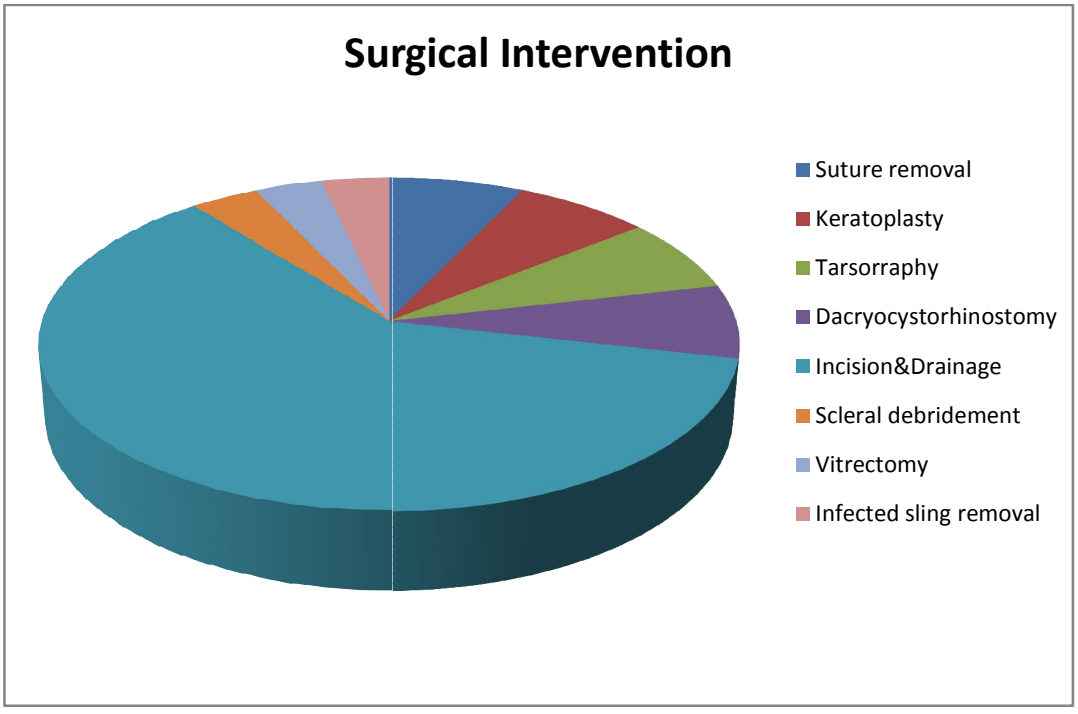


All isolates were 100% sensitive to vancomycin and chloramphenicol , 70% were sensitive to Cefazolin, 28% to gatifloxacin,ofloxacin and levofloxacin ,6% to Moxifloxacin, 17% to gentamycin, 14% to tobramycin , none of the isolates were sensitive to ciprofloxacin.Antibiotic sensitivity is given in **chart 4.**

Table 11: Surgical intervention for patients who had MRSA infection

S.No	*Surgical Intervention*	n	%
1	Suture removal	2	6.67
2	Keratoplasty	2	6.67
3	Tarsorraphy	2	6.67
4	Dacryocystorhinostomy	2	6.67
5	Incision&Drainage	17	77.27
6	Scleral debridement	1	3.45
7	Vitrectomy	1	3.45
8	Infected sling removal	1	3.45

Chart :5 **Surgical Intervention**



28 out of 34(82.5%) patients needed surgical intervention.The most commonly done procedure was incision and drainage.

Inflammatory and infection control was achieved in 30 patients except in patient no: 15,18,27 and 28 among which patient no 15 was not typeable and patient no 18 was hospital acquired.

Visual recovery was recorded in 23 patients who completed 6 months follow up . Of them 10 patients had 6/6 vision and they maintained their initial good vision. Five patients improved their vision in the study eye. Seven patients did not have improvement in vision but maintained the same initial vision. One patient worsened inspite of treatment.

Pre treatment and post treatment vision are given in **Chart 6**.Of the 7 patients who did not have improvement in vision, 5 had successful infection control and the reason for poor vision was not MRSA. It was due to pre-existing conditions the details of which are given in table 12 .

Chart – 6

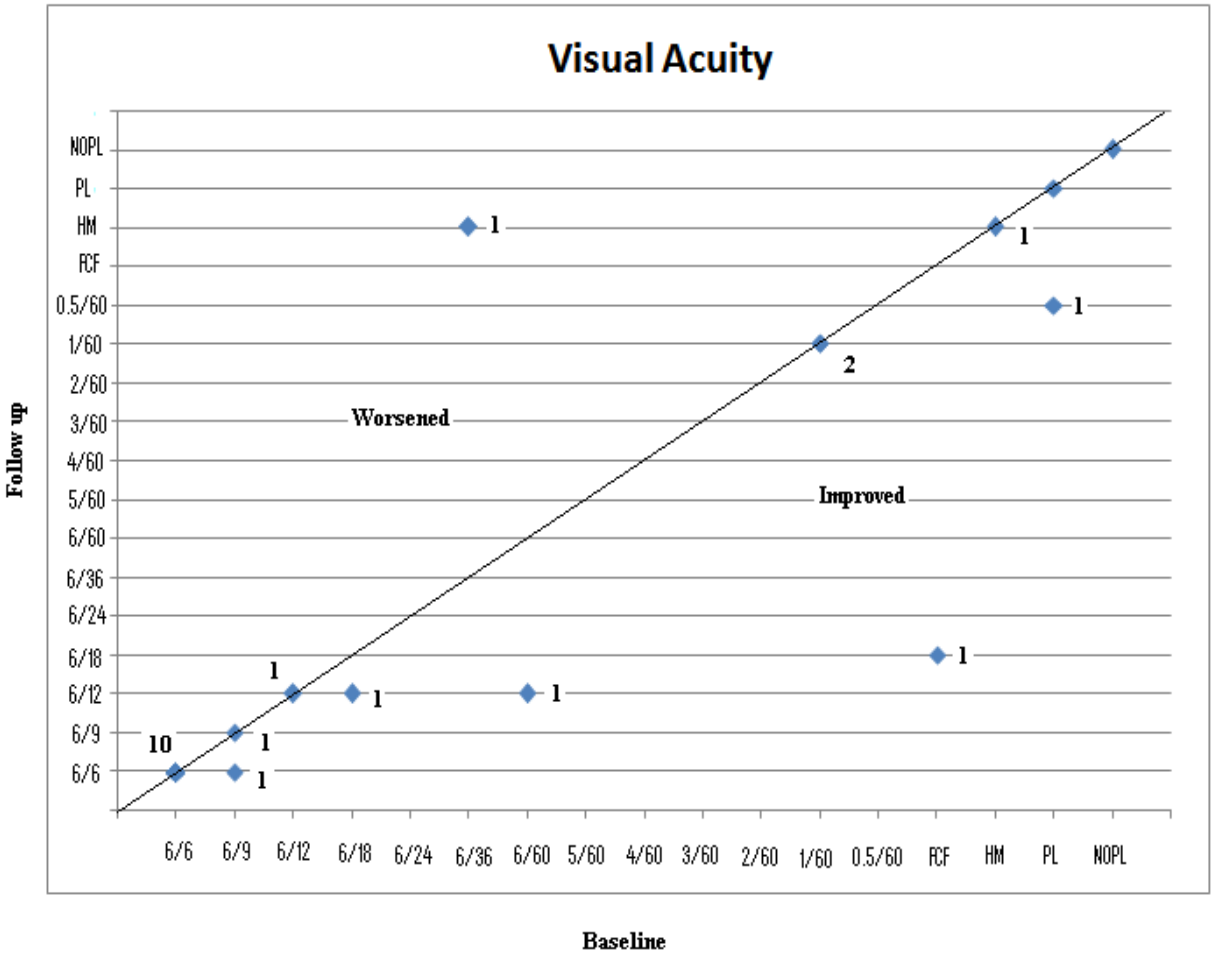

Visual Acuity
Follow up
Baseline
NOPL
PL
HM
FCF
0.5/60
1/60
2/60
3/60
4/60
5/60
6/60
6/36
6/24
6/18
6/12
6/9
6/6
Worsened
Improved
10
1
1
1
1
1
1
1
1
1
1
2
1
1
1
6/6
6/9
6/12
6/18
6/24
6/36
6/60
5/60
4/60
3/60
2/60
1/60
0.5/60
FCF
HM
PL
NOPL

Table 12: Other coexsisting causes for defective vision in MRSA infected patients.

S.No	Cause for defective vision	n
1	Anophthalmos	1
2	Central retinal vein occlusion	1
3	Graft failure	1
4	Media opacity	1
5	Traumatic optic neuropathy	1

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* has been a common nosocomial pathogen in health care settings for more than four decades. However reports of MRSA from within the community began to emerge in early 1980s and in the early 1990s, Patients of community-associated MRSA emerged sporadically in various populations without any hospital exposure. Development of these outbreaks has become an ever-increasing concern among health care professionals. In addition the epidemic of CA-MRSA is being experienced in hospital exposed patients as well[20.] The exact incidence of MRSA in systemic infection is documented meticulously from many parts of the world and outbreaks are investigated with regard to the source based on the clonal relatedness. In India, the few studies suggest that the prevalence of MRSA in hospitals is rising, and nationally MRSA is speculated to account for about 30% of *S. aureus* infections in hospital [22] . Here in this study we analyse the prevalece of MRSA, including HA – MRSA and CA_ MRSA in ocular infection over one year period.

Community-associated MRSA is an important pathogen of ocular infections[49] CA- MRSA strains carry the SCC*mec* type IV or V element, which is shorter than other SCC*mec* elements [22] The short size of the SCC*mec* type IV element makes it easier to transmit to other MSSA strains [22]. Most outbreaks of MRSA

involves CA-MRSA rather than HA-MRSA[22]. Of 34 samples of our study, 11 had prior hospital exposure . According to CDC criteria they are clinically grouped under HA – MRSA, however only 2 had molecular evidence to be grouped under HA-MRSA. In our study also majority of our patients had CA- MRSA infections. Type IV and V were the predominant strains, two were type III strains . SCC*mec* type I , and II were not detected in the MRSA isolates analyzed in this study. Several studies found community-associated MRSA to play an important role in MRSA ocular infections probably because most ophthalmologic patients are seen and treated as outpatients instead of inpatients [49]. In our study also the sources of ocular infections are most likely from the community rather than hospital.

Life-threatening bloodstream infections such as pneumonia and surgical site infections in the health care settings can be caused by MRSA. It is believed to cause a more severe disease than MSSA in systemic infections however in ocular infections results did not show that MRSA caused more severe ocular diseases than MSSA as seen from Taiwan study [49]. Similarly we had milder infection with MRSA. Being an ophthalmic hospital none of our patients had any life threatening bloodstream infection. They were healthy immuno competent patients.

There has been several reports of ocular MRSA infections from across the globe ranging from conjunctivitis to endophthalmitis**.** A 10 year retrospective study in a tertiary care hospital in Taiwan by Chuang et al. raised the clinician's attention to

the increasing prevalence of MRSA . Keratitis was most predominant in this study [49].A report from Korea recommended to rule out MRSA infection in corneal ulcers with mild superficial infiltration that have a slow progression and resistant to β-lactam antibiotics.

Corneal infections:

In our study ten patients had corneal involvement. They were slow to progress and responded to treatment. Patient no 5 and 7 had corneal infiltration. Both did not respond to initial empirical therapy. Patient no 5 improved with antibiotics based on sensitivity report. Patient no 7 was lost for follow up. Three patients, (Patient no:12,22 and 28) had corneal graft infection. Selective suture removal and appropriate antibiotic therapy resulted in control in one patient. The other two needed change of antibiotic from empirical treatment to10%ceftazidime for the inflammatory control and infection clearance. The last patient suffered from graft failure and needed regrafting for which patient was not willing.

Three patients (no: 23,27 and 29) had corneal ulcer and two underwent therapeutic keratoplasty. In patient no 23 and 29, infections were controlled and grafts were clear after changing the empirical antibiotic to fortified cefazoline and chlorampohenicol. However one of them did not have visual improvement due to pre exsisting central retinal artery occlusion. Patient no 27 had Moorens ulcer, inspite of

using appropriate antibiotic after sensitivity results patient. Patient no:14 developed exposure keratitis due to post radiation lagophthalmos after nasopharyngeal carcinoma in whom infection was controlled with topical chloramphenicol and tarsorrhaphy. Empirical therapy correlated with sensitivity report. However vision remained poor because of corneal scaring.One of the two patients who had HA-MRSA in this study, Patient no:18 had neurotropic keratitis.SCC mec typing showed a HA strain. Inspite of tarsorraphy and appropriate antibiotics(moxifloxacin and chloramphenicol based on sensitivity), infection persisted. His vision was light perception due to traumatic optic neuropathy. In summary majority of corneal MRSA infection cleared well with appropriate antibiotic and surgical treatment because they were mainly due to CA- MRSA. The one HA-MRSA that was encountered did not respond to treatment.

Conjunctivitis is the most commonly reported manifestation and has been associated with long-term care units, especially in patients with neurologic impairment[31] . However our patients were from the community, they were ambulatory, healthy individuals and only one patient presented with conjunctivitis. In the present study orbital, lid , corneal, and intraocular infections were the common manifestations.

Lid infections:

Lid and lacrimal abscess were more common than any other infection. Most of the patients responded very well after incision and drainage. Inflammatory control was achieved.

Eight patients had lid abscess. 6 out of 8 improved with surgical intervention and empirical antibiotics. Patient no: 24 had to receive a change in antibiotics based on sensitivity report after which he improved. Patient no:16 had a recurrence following incision and drainage.SCC mec typing showed a non typeable strain. Patient no:15 was an one year old female baby who developed suture infection and lid abscess following surgery for coloboma correction. Suture removal and appropriate antibiotic therapy based on sensitivity report (inj Ceftriaxone and topical tobramycin) did not control the infection.SCC mec typing showed a non typeable strain.Infection persisted even after a period of one year. Patient no: 2 and 33 had preseptal cellulitis.Both improved after incision and drainage.

Community-acquired MRSA conjunctivitis that progressed to palpebral conjunctival ulceration and destruction of postseptal soft tissue with invasion of extraconal fat has been previously reported (Brown Archieve). The patient had an invasive infection that arose from the ocular surface. Of 34, one of our patients also had conjunctivitis but that resolved with antibiotic eye drops without any tissue destruction.

Socket infections with MRSA strains have been reported after enucleation and exenteration. [31] In our study post orbital surgery as well as post retinal surgery MRSA infection was seen. Our patient no : 3 had post evisceration dermis fat graft infection. Graft was removed. Based on the sensitivity report, patient was given inj.cefataxime, inj.gentamycin and gatifloxacin . Complete clearing of infection was achieved and there was no reinfection or chronic inflammation because of use of appropriate antibiotics. In addition, patient no 10 had post exentration orbital cavity infection. He was empirically treated with oral Amoxycillin, systemic Gentamycin and ofloxocin eye drops which were replaced with inj.cefotaxime and gatifloxacin eye drops on the basis of sensitivity report. Patient started showing improvement in three days. Osawa and Shanmuganathan reported patients of MRSA scleral buckle infection after retinal detachment repair who presented with exudative retinal detachment without endophthalmitis after surgery. Resolution occurred after buckle removal and antibiotic administration**.** Patient no;8 had sling infection. Sling removal resulted in infection control. Though SCC mec typing showed it to be a HA infection, patient had a good prognosis because of the prompt removal of the source of infection.

Scleral infections:

Patient no:1 had scleral abscess who responded to incision and drainage. Patient 20 and 31 had necrotizing scleritis. Both did not respond to emperical therapy. However both the patients improved rapidly after receiving vancomycin.

Orbital infections:

4 patients had lacrimal abscess. All 4 improved after incision and drainage. An one month old baby had acute dacryocystitis. Infection was controlled by empirical tobramycin. Empirical therapy correlated with sensitivity report. Post DCR suture infection and abscess at the site of incision revealed MRSA infection. Suture removal, incision and drainage resulted in infection control. Similarly rapid clearing of infection was noted in one another patient with orbital cellulitis (Case no;5) following incision and drainage and an appropriate antibiotic based on the sensitivity report.

MRSA infections are reported to be increasing following ocular surgery . Fukuda and associates found that 13 of 978 eyes (1.3%) swabbed preoperatively grew MRSA, and patients with nasolacrimal duct obstruction had a higher incidence of harboring MRSA. They also found that 6.6% of 1,000 asymptomatic eyes swabbed grew MRSA. In addition, they found that elderly conjunctival MRSA carriers were more likely to have anemia, cancer, liver dysfunction, or dementia; to be status post surgery; or to be chronically bedridden. [50].

Insler et al. in his report, described MRSA keratitis after recent uncomplicated phacoemulsification procedure with posterior chamber intraocular lens insertion [51]. More recently, MRSA wound infections have been reported with clear corneal phacoemulsification wounds. [52] One of two patients with MRSA clear

corneal wound infections described by Cosar and associates also had scleral extension of infection and endophthalmitis. Two other patients of postoperative MRSA endophthalmitis following cataract surgery have also been reported. [53] .In a recently reported patient, the patient had received moxifloxacin, a fourth-generation fluoroquinolone, for prophylaxis both before and after surgery inspite of which infection occured[53]. Fukuda and associates also reported a patient of MRSA endophthalmitis following vitrectomy in a patient with atopic dermatitis. [50]

Endophthalmitis:

In our series, patient no:32 had post operative endophthalmitis following cataract surgery.SCC mec typing showed a CA strain. Patient improved after sclera debridement, anterior chamber wash and vitrectomy. Intravitreal vancomycin, ceftazidine and dexamethasone were given along with topical moxifloxacin and I.V. amikacin. Infection clearance and inflammatory control were achieved.

Ching-Hsi Hsiao,reported that both CA-MRSA and HA-MRSA ocular infections to be multi-resistant in Taiwan. [49]. Similarly in our experience also both the strains were resistant to many antibiotics. All isolates in our study were resistant to penicillin and oxacillin A total of 94%,72%, 72%,72% and 30% of the isolates were resistant to moxifloxacin levofloxacin gatifloxacin ofloxacin and cefazolin respectively. However all isolates were sensitive to vancomycin and chrompenicol.

CONCLUSION

Methicillin Resistant *Staphylococcus aureus* (MRSA) infection is a challenge for clinicians to treat due to its multi-drug resistance and rapid progression. The emergence of community-associated MRSA is not limited to the community anymore but these strains are progressively introduced into the hospital setting. This study highlighted the prevalence of the resistance profiles and clinical presentations of MRSA strains circulating in the patients with ocular infection. Although 11 of our patients had history of hospital admission or previous surgery, only two had HA MRSA as per SCC mec typing (typeIII) . Majority of the isolates were type V (58.8%) and type IV (29.4%) which are community acquired.

Wider and empirical usage of newer antibiotics promotes resistance in bacteria such as *S. aureus* due to selective pressure.[54] The correct susceptibility pattern of the MRSA isolates needs to be determined rapidly for accurate treatment of MRSA infections. Lid abscess and lacrimal abscess were the most commonly encountered diagnosis (38%) . Appropriate and timely surgical intervention resulted in a better outcome . Commonly used fluoroquinalone are not very effective against these organisms . Chloramphenicol and vancomycin remain the drug of choice .

BIBLIOGRAPHY

1.Timbury, MC, McCartney, A and Thakker, B (2002) *Notes on Medical Microbiology*. Churchill Livingstone, Elsevier Limited, New York, USA:31-34

2.Murray, PR, Rosenthal, KS and Pfalter, MA (2005) *Staphylococcus* and related organisms. *In*: Medical Microbiolog. (5th ed). Elsevier Mosby, Edinburgh, United Kingdom: 221-236

3.Noble WC, Valkenburg HA, Wolters CHL. Carriage of *Staphylococcus aureus* in random samples of a normal population. *J Hyg (Lond)* 1967;65:567-573.

4.Casewell MW, Hill RLR. The carrier state: methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 1986;18(Suppl A):1-12.

5.Zetola N, Francis JS, Nuermberger EL, et al. Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. *Lancet Infect Dis* 2005;5:275-286.

6.Wilkinson, BJ (1983) Staphylococcal capsules and slime. *In*: Easmon, CSF and Adlam, C (ed) *Staphylococci and Staphylococcal Infections*. Academic Press, New York, USA:481-523

7.Lowy, FD (1998) *Staphylococcus aureus* infections. *New England Journal of Medicine* 339: 520-532

8.Viera-da-Motta, O, Ribeiro, PD and Dias da Silva, W (2001) RNAIII inhibiting peptide (RIP) inhibits *agr*-regulated toxin production. *Peptides* 22:1621-1627

9.Marrack, P and Koppler, J (1990) The staphylococcal enterotoxins and their relatives. *Science* 248:705-711

10.Waldvogel, FA (2000) *Staphylococcus aureus* (including toxic shock syndrome). *In*: Mandell, Douglas and Bennet's Principles and Practice of Infectious Diseases. (5th ed). Churchill Living Stone, New York, USA:2069-2092

11.Diekema, DJ, BootsMiller, BJ and Vaughn, TE (2004) Antimicrobial resistance trends and outbreak frequency in United States hospitals. *Clinical Infectious Diseases* 38:78-85

12.Kowalski, RP, Karenchak, LM and Romanowski, EG (2003) Infectious disease: changing antibiotic susceptibility. *Ophthalmology Clinics of North America* 16:1-9

13.Carleton, HA, Diep, BA and Charlebois, ED (2004) Community-adapted methicillin-resistant *Staphylococcus aureus* (MRSA): population dynamics of an expanding community reservoir. *Journal of Infectious Diseases* 190:1730-1738

14.Cepeda, JA, Whitehouse, T and Cooper, B (2008) Isolation of patients in single rooms or cohorts to reduce spread of MRSA in intensive-care units: prospective two-centre study. *Lancet* 365:295-304

15.Tattevin, P, Diep, BA and Jula, M (2008) What happened after the introduction of USA300 in correctional facilities? A long term follow-up of methicillin-resistant *Staphylococcus aureus* (MRSA) molecular epidemiology in San Francisco jails. *Journal of Clinical Microbiology* 46:4056-4057

16.Gaze, W, O'Neill, C, Wellington, E (2008) Antibiotic resistance in the enviroment, with particular reference to MRSA. *Advances in Applied Microbiology* 63:249-270

17.Sakoulas G, Eliopoulos GM, Moellering RC, Jr., Novick RP, Venkataraman L, Wennersten C, et al. Staphylococcus aureus accessory gene regulator (agr) group II: is there a relationship to the development of intermediate-level glycopeptide resistance? J.Infect.Dis. 2003;187:929-38.

18 . Udo, E. E., J. W. Pearman, and W. B. Grubb. 1993. Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. J Hosp Infect 25:97-108.

19 . Francis, J. S., M. C. Doherty, U. Lopatin, C. P. Johnston, G. Sinha, T. Ross, M. Cai, N. N. Hansel, T.Perl, J. R. Ticehurst, K. Carroll, D. L.Thomas, E. Nuermberger, and J. G. Bartlett. 2005. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. Clin Infect Dis 40:100-7.

20.Mulvey, M. R., L. MacDougall, B. Cholin, G. Horsman, M. Fidyk, and S. Woods. 2005. Community-associated methicillin-resistant *Staphylococcus aureus*, Canada. Emerg Infect Dis 11:844-50.

21. Aires de Sousa, M., and H. de Lencastre. 2003. Evolution of sporadic isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. J Clin Microbiol 41:3806-15.

22.Deresinski, S (2005) Review of MRSA. *Clinical Infectious Diseases* 40: 562-573

23.Popovich, KJ and Weinstein, RA (2009) The graying of methicillin-resistant *Staphylococcus aureus*. *Infection Control and Hospital Epidemiology* 30: 9-12

24.File, TM (2008) Methicillin-resistant *Staphylococcus aureus* (MRSA): focus on communityassociated MRSA. *Southern African Journal of Epidemiology and Infection* 23: 13-15

25.Heiman FL Wertheim a , Henri A Verbrugh a Global prevalence of meticillin-resistant Staphylococcus aureus The Lancet, Volume 368, Issue 9550, Page 1866, 25 November 2006

26.Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet 2006; 368: 874-885.

27.Borg, MA, De Kraker, M and Scicluna, E (2007) Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in invasive isolates from southern and eastern Mediterranean countries. Journal of Antimicrobial Chemotherapy 60:1310-1315

28.Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India. Methicillin resistant Staphylococcus aureus (MRSA) in India: prevalence & susceptibility pattern. Indian J Med Res. 2013 Feb;137(2):363–9.

29.Gopalakrishnan R, Sureshkumar D. Changing trends in 3. antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. *J Assoc Physicians India* 2010; 58 (Suppl): 25-31.

30.Chatterjee SS, Ray P, Aggarwal A, Das A,Sharma M. A 11. community based study on nasal carriage of *Staphylococcus aureus*. *Indian J Med Res* 2009; *130*:742-8.

31. Blomquist PH. MRSA infections of the eye and orbit. *Trans Am Opthalmol Soc* 2006; *104* : 322-45.

32.Fluit, AD, Visser, MR and Schmitz, FJ (2001) Molecular detection of antimicrobial resistance.*Clinical Microbiology Reviews* 14:836-871

33.Brown, DF, Edwards, DI and Hawkey, PM (2005) Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). *Journal of Antimicrobial Chemotherapy* 56:1000-1018

34.Jureen, R, Bottolfsen, KL and Grewal H (2001) Comparative evaluation of a commercial test for rapid identification of methicillin-resistant *Staphylococcus aureus*. *Acta Pathologica, Microbiologica et Immonulogica Scandinavica* 109:787-790

35.Shoeb, H (2008) Antimicrobial susceptibility testing (Kirby Bauer) animation. Microbelibrary.org

36.Huletsky, A, Giroux, R and Rossbach, V (2004) New real-time PCR assay for rapid detection ofmethicillin-resistant Staphylococcus aureus directly from specimens

containing a mixture ofStaphylococci. Journal of Clinical Microbiology 42:1875-1884

37.Trindade, PA, McCulloch, JA and Oliveira, GA (2003) Molecular techniques for MRSA typing:current issues and perspectives. The Brazil Journal of Infectious Diseases 7:32-43

38.Van Hal, SJ, Jennings, Z and Stark, D (2009) MRSA detection: comparison of two molecularmethods (BD GeneOhm® PCR and Easy-Plex) with two selective MRSA agars (MRSA-ID andOxoid MRSA) for nasal swabs. Clinical Microbiology and Infectious Diseases 28:47-53

39.Ito, T, Ma, XX and Takeuchi, F (2004) Novel type V staphylococcal cassette chromosome mecdriven by a novel cassette chromosome recombinase, ccrC. Antimicrobial Agents Chemotherap 48:2637-2651

40.Oliviera, DC, Milheirico, C and de Lencastre, H (2006) Redefining a structural variant ofstaphylococcal cassette chromosome mec, SCCmec type VI. Antimicrobial Agents and Chemotherapy 50: 3457-3459

41.Zhang, K, McClure, J and Elsayed, S (2005) Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillinresistant *Staphylococcus aureus. Journal of Clinical Microbiology* 43: 5026-5033

42.Chongtrakool, P, Ito, T and Ma, XX (2006) Staphylococcal cassette chromosome mec (SCCmec) typing of methicillin-resistant Staphylococcus aureus isolated in 11 Asian countries: a proposal for a new nomenclature for SCCmec elements. Antimicrobial Agents and Chemotherapy 50:1001-1012

43.Stranden, AM, Frei, R and Adler, H (2008) Emergence of SCCmec type IV as the most commontype of methicillin-resistant Staphylococcus aureus in a University Hospital. Infection 30

44.Schlichting, C, Branger, C and Fournier, JM (1993) Typing of Staphylococcus aureus by pulsedfield gel electrophoresis, zymotyping, capsular typing, and phage typing: resolution of clonal relationships. Journal of Clinical Microbiology 31:227-232

45.Basim, E (2001) Pulsed-field gel electrophoresis (PFGE) technique and its use in molecular biology. Turkey Journal of Biology 25:405-418

46.Ibrahem, S, Salmenlinna, S and Virolainen, A (2009) Carriage of methicillin-resistant staphylococci and their SCCmec types in a long-term-care facility. Journal of Clinical Microbiology 47:32-37

47.Baba, T, Takeuchi, F and Kuroda, M (2002) Genome and virulence determinants of high virulence community-acquired MRSA. Lancet 359:1819-1827

48.Young DM, Harris HW, Charlebois ED, et al. An epidemic of methicillin-resistant Staphylococcus aureus soft tissues infectionsamong medically underserved patients. Arch Surg2004;139:947-953

49.Chang et al. *Staphylococcus aureus* Ocular Infection: Methicillin-Resistance, Clinical Features, and Antibiotic Susceptibilities journal of ophthalmology 2012:119-522-527.

50.Fukuda M, Ohashi H, Matsumoto C, et al. Methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulasenegative *Staphulococcus* ocular surface infection: efficacy of chloramphenicol eye drops. *Cornea* 2002;21(7 suppl):S86-S89.

51. Insler MS, Fish LA, Silbernagel J, et al. Successful treatment of methicillin-resistant *Staphylococcus aureus* keratitis with topical ciprofloxacin. *Ophthalmology* 1991;98:1690-1692.

52. Cosar CB, Cohen EJ, Rapuano CJ, et al. Clear corneal wound infection after phacoemulsification. *Arch Ophthalmol* 2001;119:1755-1759.

53. Moshirfar M, Marx DP, Mirzaian G. Endophthalmitis in patients using fourth-generation fluoroquinolones following phacoemulsification. *J Cataract Refract Surg* 2005;31:1669-1670.

LIST OF ABBREVIATIONS

CA-MRSA	-	Community-associated methicillin-resistant *Staphylococcus aureus*
CDC	-	Center for Disease Control and Prevention
CLSI	-	Clinical and Laboratory Standards Institute
DNA	-	Deoxyribose nucleic acid
HA-MRSA	-	Health-care associated methicillin-resistant *Staphylococcus aureus*
HIV	-	Human immunodeficiency virus
HVR	-	Hyper-variable region (*S. aureus* specific)
MH	-	Mueller-Hinton medium
MIC	-	Minimum inhibitory concentration
µl	-	Microlitre
M-PCR	-	Multiplex polymerase chain reaction
MSCRAMM	-	Microbial surface components recognising the adhesive matrix molecules
MSSA	-	Methicillin-susceptible *Staphylococcus aureus*
MRSA	-	Methicillin-resistant *Staphylococcus aureus*
PBP2a	-	Penicillin-binding protein 2a
PCR	-	Polymerase chain reaction
PFGE	-	Pulsed-field gel electrophoresis

RAPD	-	Random amplified polymorphic DNA
SCC	-	Staphylococcal cassette chromosome
Spa	-	Staphylococcal Protein A
SSSS	-	Staphylococcal scalded skin syndrome
TNF-α	-	Tumor necrosis factor alpha
TSS	-	Toxic shock syndrome
VISA	-	Vancomycin-intermediate resistant *Staphylococcus aureus*
VRSA	-	Vancomycin-resistant *Staphylococcus aureus*

KEY TO MASTER CHART

Route of drug admission

1	-	Topical
2	-	Oral
3	-	Intra venous

Status on follow up

1	-	Improved
2	-	Same
3	-	Worsened

Other causes of defective vision

1	-	Central retinal artery occlusion
2	-	Anophthalmos
3	-	Media opacity
4	-	Traumatic optic neuropathy
5	-	Graft failure

Surgery

1	-	Penetrating keratoplasty
2	-	Therapeutic keratoplasty
3	-	Tarsorrhaphy
4	-	Vitrectomy

5	-	Incision and Drainage
6	-	Infected sling removal
7	-	Dermis fat graft removal and regrafting
8	-	Scleral debridement + AC wash + Vitreous tab
9	-	Dacryocystorhinostomy