https://doi.org/10.47278/book.vpph/2021.023

STAPHYLOCOCCUS AUREUS: COMMENSAL TO MUTATING PUBLIC HEALTH PATHOGEN

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INTRODUCTION

The word "*Staphylococcus*" is taken from two Greek words i.e., "staphyle" meaning a bunch of grapes and "kokkos" meaning berry, which indicates the microscopic appearance of organism following Gram staining. The word *Staphylococcus aureus* (*S. aureus*) means "Golden Cluster Seed" that's why named as "golden staph". *S. aureus* is coccus in shape, Gram-positive, non-spore forming, non-motile, opportunistic, catalase positive, coagulase positive, and oxidase negative bacterium. It gives different color colonies on different agar media, such as yellow on mannitol salt agar, greyish to greyish white or golden colonies on blood agar, and pink color colonies on chromogenic agar. Under microscopy, *S. aureus* appears as round, in the form of bunches, that shows the multiplication of *S. aureus* in different planes. This bacterium is categorized as an important pathogen that causes mild to life-threatening diseases. Its commensal and opportunistic capabilities make it able to colonize at different sites of animals, and humans. The *S. aureus* is a common inhabitant of skin, mucosa, GIT (gastrointestinal tract), urinary tract, and especially the respiratory tract in anterior nares (Cuny et al. 2010). It produces many kinds of different substances like proteins, enzymes, toxins etc. The proteins produced by *S. aureus* include protein A and fibronectin binding protein that help the bacterium to adhere and colonize on cell surfaces. The enzymes include coagulase, catalase, lipases, nucleases, proteases, collagenases, and beta-lactamase, while the toxins include exotoxin, endotoxins, enterotoxins, alpha, beta, gamma hemolysin and PVL (Panton-Valentine leukocidin). These all substances enhance the ability of *S. aureus* to infect healthy persons, which may lead to necrotizing and hemorrhagic fatal pneumonia (Gillet et al. 2002).

The infectivity of *S. aureus* has been aggravated by increasing resistance to antibiotics, and methicillin-resistant *S. aureus* (MRSA); it is usually encoded by a gene called *mecA* that encodes the penicillin-binding protein 2a (PBP 2a) that is linked with increased mortality and morbidity compared to methicillin-sensitive *S. aureus* (MSSA) (Katayama et al. 2000). Firstly, MRSA was mainly linked with hospital or health-care settings and its acquisition-related with known risk factors (Chambers 2001). But recently, it is propagated into the community known as Community-Associated MRSA (CA-MRSA) and has been concerned in reports of threatening infections in salubrious persons. These reports of infection in humans and companion animals have exhibited the animal potential to act as a source for the spread of MRSA (Cefai et al. 1994). Increasing interest about MRSA in the community has recommended for surveillance, including carriage rates in healthy cats, dogs and also in humans. Almost 25% of humans contain *S. aureus* in the nasal cavity, which acts as significant source for infection (Noskin et al. 2005). The pathogen holds zoonotic and humanotic transmission of MRSA from humans to animals and animals to humans that puts the community at a great risk. It causes light to severe life-threatening infections in humans and animals. An investigation in the USA showed the huge loss due to HA-MRSA, approximately seven million admissions in the hospital were due to *S. aureus* infections. The annual loss due to these infections is estimated to be $2.7 million, which is a huge loss that puts the country to an economic burden of somewhat $9.5 billion with 12,000 mortalities annually (Noskin et al. 2005). MRSA is exceptionally predominant at medical centers around the world. However, Higher MRSA prevalence (>50%) was observed at medical centers of North America, South America, Asia, Sri Lanka, South Korea, Vietnam, Taiwan, Thailand and Hong Kong. Conversely, lower number of reports are observed in India (22.6%) and Philippines (38.1%) (Song et al. 2011).

Staphylococcus aureus to methicillin-resistant *Staphylococcus aureus*

The bacterium derived from the puss was named as "*Staphylococcus aureus*" in 1881. In 19th century, a strong wave of mortality, reaching 90% of deaths from *S. aureus* infected people, remained prevalent until availability of penicillin, discovered in 1928 by Sir. Alexander Fleming.

Sooner, this bacterium developed resistance against penicillin, using beta-lactamase enzyme that hydrolyses beta-lactam ring of penicillin and makes the drug ineffective. A long way after this resistance, there was discovery of methicillin in 1950s, an antibiotic equally effective against *S. aureus*. To the dismay, the drug no longer remained effective due to strong resistance developed by the bacteria. The resistance was so strong that new strain has to be named as methicillin-resistant *Staphylococcus aureus* (MRSA). The resistance was due to penicillin-binding protein 2a (PBP 2a), and was equally resistant against all beta-lactam antimicrobials. Going into molecular analysis, it was found that resistance was due to *mec A,* which is present on large mobile genetic element called as Staphylococcal cassette mec (SCC mec) (Vengust et al. 2006). In 1961 in an experiment, 18 out of 50 Staphylococci were regarded as Celbenin (Methicillin) resistant Staphylococci. These isolates were found to have ability to retain both hemolytic and coagulase activities. Not to this only, the isolates even in the absence of methicillin, kept on retaining typical culture characteristics and resistance patterns. In initial years of emergence of MRSA, only three isolates out of 5444 of tested *S. aureus* could be identified (Barrett et al. 1968) due to complication in identification of MRSA because methicillin resistance in *S. aureus* varied in many isolates. Therefore, heterogeneous strains mainly consist of comparatively sensitive cells and extremely resistant cells that show phenotypical susceptibility to methicillin. However, phenotypic resistance expression can be increased by adding sodium chloride (NaCl) or sucrose to culture medium in the presence of β-lactam antibiotics (Datta et al. 2011).

Pathogenesis of *Staphylococcus aureus*

S. aureus is a commensal, as well as pathogenic, organism. It is normally present in the anterior nares of humans and animals. Some other sites of its colonization may include the axillae, groin, and gastrointestinal tract. Colonization increases chances for bacterial infection when host defense is broken, either due to shaving, aspiration, insertion of a catheter or surgery (Wertheim et al. 2005). Virulence factors for *S. aureus* may include numerous surface proteins, called "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs), which are responsible for attack on host tissues by binding with collagen, fibronectin, and fibrinogen, and produce endovascular, bone, joint, and prosthetic-device infections (Menzies 2003). *S. aureus* can make biofilms (slime) on host and prosthetic surfaces, permitting it to stick on them by evasion of host immune system and antimicrobials. *S. aureus* can also make small-colony variants (SCVs), leading to tenacious and recurring infection. Chief protection of *S. aureus* is by making an anti-phagocytic microcapsule (type 5 or 8). The zwitterionic capsule can also make abscess. The MSCRAMM protein A can inhibit opsonization by binding with Fc portion of immunoglobulin (Gordon and Lowy 2008). *S. aureus* is responsible for neutrophil extravasation and chemotaxis to the site of infection due to secretion of chemotaxis inhibitory protein or extracellular adherence protein. During infection, *S. aureus* infection can metastasize to other sites by producing several enzymes like proteases, lipases, and elastases (Fig. 1). Septic shock develops in case of *S. aureus* due to its ability to activate immune system and coagulation pathways by peptidoglycan, lipoteichoic acid, and toxins production. In addition to this, some *S. aureus* strains also produce super antigens, responsible for toxicosis, like food poisoning and toxic shock syndrome (Dinges et al. 2000).

Pathogenesis of HA-MRSA

Methicillin-resistant *S. aureus* (MRSA) is a major pathogen in comparison to methicillin-sensitive *S. aureus* due to higher chances of disease occurrence and death rate. However, particular mechanism of pathogenicity is not known. Though, it is considered that protein related b-lactam antibiotic resistance, penicillin-binding protein 2A (encoded by *mecA* gene), directly causes immunopathology during MRSA infection. PBP2A is responsible for poor peptidoglycan cross-linking, which causes increased degradation and detection by phagocytes, and it leads to vigorous IL-1b production. Peptidoglycan separated from b-lactam confronted MRSA powerfully stimulates the NLRP3 inflammasome in macrophages, however these effects disappear due to peptidoglycan solubilization (Turner et al. 2019). Transmuted MRSA containing decreased peptidoglycan cross-links produce high IL-1b levels *in vitro* and cause severe diseases *in vivo*. Treatment of MRSA skin infection by b-lactam aggravates IL-1 related immunopathology. So, antibiotic provoked appearance of *mecA* during MRSA skin infection is responsible for immunopathology due to change in peptidoglycan structure (Madzgalla et al. 2016).

Pathogenesis of CA-MRSA

Virulence of CA-MRSA strains is increased due to increased fitness, enhanced evasion of the host immune system, and exclusive toxin production by *S. aureus*. Some researchers have suggested that PVL protein present in *S. aureus* has leukocyte lytic and dermonecrotic activity, leading to CA-MRSA infection (Chini et al. 2006). However, other studies proposed that the linkage of PVL with higher *S. aureus* virulence is multifaceted, so it needs additional research. Additionally, recent studies have revealed that phenol-soluble modulins are up-regulated in CA-MRSA strains, in comparison to the level in HA-MRSA strains; so, it damages neutrophils and causes inflammation in mouse and bacteremia models. Additionally, enterotoxins may also play important role in these infections (Wang et al. 2007; Lee et al. 2018).

Pathogenesis of LA-MRSA

Livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) is a comparatively new classification in veterinary medicine. Initially, it was caused by a single

clonal complex CC398, but now it is caused by many varieties of clonal complexes. Most common clonal complex is CC398 in Europe, while CC9 is common in Asia. LA-MRSA contains SCCmec cassettes limited mostly to SCCmec IVa and SCCmec V, but non-typeable cassettes and SCCmec type XI, containing *mecC*, also have been found. Livestock associated MRSA (LA-MRSA) was first identified in 2005 due to CC398 (Voss et al. 2005). Numerous studies have shown that CC398 MSSA of human origin misplaced human related factors such as Panton Valentine Leukocidin (PVL)-associated phages, toxic shock syndrome toxin I and exfoliative toxins (Ballhausen et al. 2017) and developed antibiotic resistance genes e.g., *mecA* and *tetM* related to livestock. Moreover, it is reported that chances of LA-MRSA infection are higher in individuals who are in contact with livestock (Goerge et al. 2017). Nonetheless, colonization depends upon frequency, strength and period of animal contact, as livestock are supposed to be momentarily colonized (Bangerter et al. 2016). Though CC398 is the chief MRSA strain separated from livestock, some clonal complexes, and sequence types other than CC398, have also been found in livestock and animal products. Amount of staphylococcal protein A gene (spa) types within CC398 is presently increasing (Peeters et al. 2015). Moreover, other *S. aureus* strains of animals have developed methicillin resistance. Now-a-days, methicillin resistance is being reported more commonly in pet animals compared to livestock, and resistance is also increasing. Though, the epidemiology in pets is totally altered and is restricted to some Staphylococcus pseudo intermedius and human related clones in addition to methicillin-susceptible *S. aureus* (MSSA) of CC398 (Gómez-Sanz et al. 2013; Lee et al. 2018).

Microbiological and Molecular Techniques for diagnosis of MRSA

MRSA is detected by using conservative approaches involving oxacillin disc diffusion, MIC and oxacillin screen agar methods. But in recent times, the Clinical and Laboratory Standards Institute (CLSI) suggested the use of cefoxitin disc diffusion technique for MRSA identification. Cefoxitin is a cephamycin type antibiotic that acts as an inducer of the PBP2a-encoding *mecA* gene (Velasco et al. 2005). Other method to identify MRSA is the latex agglutination assay, which is specific monoclonal Abs against PBP2a antigen. Additionally, CHROM agar is a new method to detect MRSA by using chromogenic medium (Diederen et al. 2005).

Agar plate methods

Cefoxitin and Oxacillin Disc Diffusion Test

S. aureus suspension equal to 0.5 McFarland standards was made for all isolates and tested with cefoxitin (30 µg) and oxacillin (1 µg) disc, on Muller Hinton agar. Incubation was done at 35°C for 24 hours. Zones of inhibition were then measured and compared with guidelines of CLSI. CLSI approves use of cefoxitin rather than oxacillin in disk diffusion method to monitor resistance against methicillin for *S. aureus*. Cefoxitin disk diffusion test gives better results with greater sensitivity as compare to oxacillin. Cefoxitin disk diffusion test has 97.3% sensitivity and 100% specificity, in contrast to the oxacillin disk. This higher sensitivity of cefoxitin is due to its higher ability to activate *mecA* gene to express PBP 2 compared to oxacillin (Broekema et al. 2009).

Test with Oxacillin Resistant Screening Agar (ORSA)

The 0.5 McFarland standards *S. aureus* suspension is inoculated on ORSA medium at 35°C for 48 hours incubation. ORSA comprises oxacillin (2 µl), 5.5% NaCl to prevent non-*staphylococcal* growth and aniline blue dye to identify mannitol fermentation by *S. aureus*. Development of blue colonies specifies the existence of MRSA. The ORSA is used to identify MRSA in laboratories, because it has ability to recognize mannitol fermenting bacteria. To confirm better sensitivity of MRSA, an enrichment broth is required and incubation time of 48h is given to primary culture on ORSA. Although ORSA is inexpensive and can be easily performed, but its chief disadvantage is delay in getting results. So, cefoxitin can be a better substitute indicator to detect MRSA. However, an additional E-test along with cefoxitin disc diffusion can be used to identify *S. aureus* strains, which show 20-22 mm inhibition zone diameter (Panda et al. 2016).

MIC by E-Test

Oxacillin MICs is examined by E-test on Muller Hinton agar with 2% NaCl. Incubation of plates is done at 35°C for 24 hours. The MIC value >4 µg/ml is deliberated as MRSA (CLSI 2012). E-test for MIC gives better results in comparison to other tests, because it is easy to perform as disc diffusion test and gives quite accurate results in specific test conditions under the support of PCR for *mec-A* gene (Ercis et al. 2008).

Genomic analyses

S. aureus genome was first sequenced by Kuroda et al. (2001) and until now eighteen genomes have been sequenced. Numerous other incomplete sequences have been kept in gene bank. Examination of this tremendous measure of information indicates that the genome structure has three chief segments, a spine of core genes, Mobile Genetic Elements (MGEs), and extensive discrete parts of DNA that encode activation ability, appearance of continuous exchange and (less normally) recombination. The *mecA* gene is placed on a versatile hereditary component, named staphylococcal cassette chromosome mec (SCCmec) embedded in the *Staphylococcus* chromosome up stream to the orf X (Ali et al. 2018). Four contrastingly composed SCC-mec components have been described. But, three kinds of SCC-mec components are ordinarily found in HA-MRSA strains, namely type I, type

II, and type III, present in various countries (Katayama et al. 2003). Chromogenic agar is the best medium to identify 92.9% MRSA isolates. PCR result was found to be positive in all isolates showing resistance to cefoxitin discs. Generally used marked genes to confirm *S. aureus* are *femA*, *orfX*, *Sa442*, and the *nuc* quality genes. The *femA* gene of *S. aureus* has a few areas of similarity with CoNS, and requires much greater attention during primer development (Kobayashi et al. 1994). *Sa442*-particular PCR has appeared to be valuable after DNA extraction from positive blood culture isolates, yet few disease-causing *S. aureus* strains may not have this particular locus. Other valuable targets incorporate staphylococcal chromosomal cassette (SCCmec)- related loci form the stable DNA nuclease gene (nuc).

The 16S rRNA amplification of gene sequencing (479 bp) is the most ordinarily used strategy for distinguishing the bacteria, including Staphylococci. Transferring binding protein (Tbp), having size of 42-kDa, encodes gene situated inside the cell wall of the *Staphylococcus* and also contains an enzyme known as Glyceraldehyde 3-Phosphate Dehydrogenase (housekeeping gene) (Ghebremedhin et al. 2008). PCR identification of the nuc gene (270 bp) is also recommended for the identification of *S. aureus*. Different mechanisms of resistance in MRSA include presence of resistant genes TcaR, TcaA, TcaB, TetR, TetM, PBP2a (*mecA*), or secretion of enzymes like DNA gyrase (A), DNA gyrase (B), Topoisomerase IV (A), Topoisomerase IV (B) and Beta-lactamase repressor); these are responsible for the use of efflux pump mechanism (Li et al. 2014). In short, typical phenotype of MRSA is due to the existence of mecA encoding penicillin-binding protein (PBP2a), having less efficiency for b-lactams. The *mecA* is entrenched in a large heterologous chromosomal cassette (SCCmec) element. Several MRSA strains bring upstream to the mecA gene (mecI-mecR1) coding for a repressor and an inducer of the *mecA* expression, correspondingly, as shown in Fig. 2 (Oliveira et al. 2011).

Proteomic analyses

Proteomics is defined as the study of structure and function of proteins in living organisms to understand the multifaceted nature of the organism. A few studies have used this method to clarify the efficacy of natural product as antibiotic agents (Khairon et al. 2016). Regulation system associated with the multidrug protection, pathogenesis and transmission of MRSA is basic system for the improvement of new antimicrobial agents developed for the treat MRSA infections and to monitor their anti-microbial activity for the anticipation of this superbug. The Bla and mec frameworks predict a basic region that protects from β-lactam drugs in MRSA. The regulation mechanism associated with the outflow of methicillin protection has been uncovered (Wilke et al. 2004). As mobile genetic element (MGE), MRSA contains the Staphylococcal cassette chromosome mec (SCC mec) region that develops the multidrug resistance. Resistant from beta-lactam anti-toxins, *S. aureus* is fundamental because of 1-lactamase, which is a chemical activated by β-lactam anti-toxins. The 1-lactamase gene (*blaZ*) is kept on a plasmid with two firmly connected loci firmly under the regulation of P-lactamase generation. After the confirmation of blaZ gene, two new genes have been recognized and sequenced named as blaI and blaR1. In spite of the fact that the elements of the blaI and blaRl genes have not been built up, their structural parts have been supposed similar to genes that control 1-lactamase generation in *Bacillus licheniformis*. In that framework, the chromosomally found structural genes blaI and blaRi are likewise encoded upstream of the 3-lactamase genes (blaP). *S. aureus* has also been equipped with *mecA* gene that encodes one kind of trans-peptidase, called penicillin binding protein 2a (PBP-2a). PBP2a opposes hindrance to β-lactam group of anti-microbials.

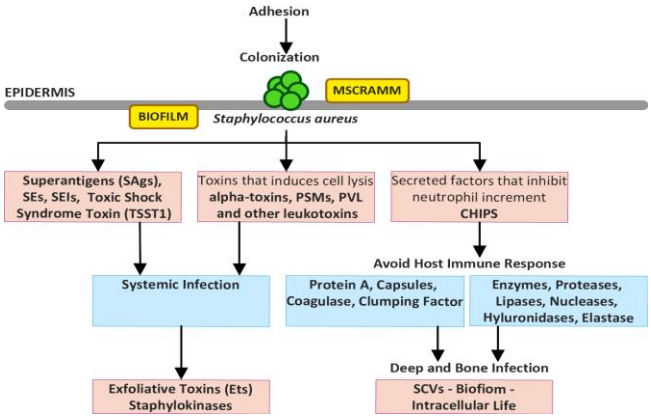

Fig. 1: Pathogenesis of *Staphylococcus aureus*.

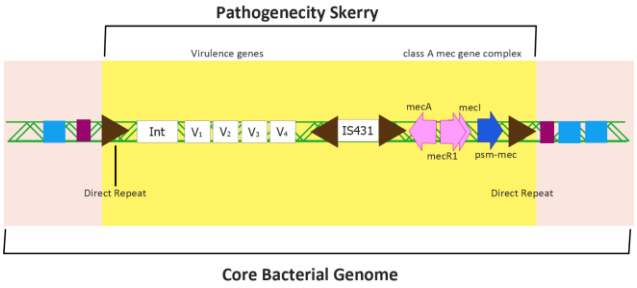

Fig. 2: Genome structure of MRSA.

Therapeutics and preventive measures

S. aureus is the leading cause of mortality in patients having developed MRSA infections. Almost 50% of the population may face invasive infections that lead to bacteremia and may cause death within 90 days (Nickerson et al. 2009). However, the incidence rate of MRSA is declining as a result of prevention strategies and use of a new combination of antibiotics available in the market. The reason behind the high mortality rate may be the lack of proper treatment at the initial stages of infections (Simor et al. 2016). Moreover, various virulence factors are associated with the mortality e.g., accessory gene regulator (agr) of Group I is an intrinsic virulence factor that is detected among MRSA isolates. In recent years, vancomycin has been considered as a drug of choice after methicillin resistance for aggressive MRSA

infections. But now the trend has been changed after the introduction of various new antibiotics in the market. Furthermore, researchers now suggest the treatment shift toward the combined therapy for MRSA infections.

Bloodstream infections and their management with combination therapy

A condition known to be caused by MRSA, called bacteremia, is more severe as compared to that caused by MSSA infection, and a long period of bacteremia will result with a more serious outcome. A study conducted in Australia has highlighted that 17% of MRSA cultures are found to be resistant to cephalosporin and ceftaroline. Many types of new agents are licensed in the market but they are not showing results superior to vancomycin (Abbott et al. 2015). A combination therapy recommended by the Spanish Society of Clinical Microbiology and Infectious Diseases includes a glycopeptide daptomycin along with B-lactam antibiotics against the MRSA infections (Gudiol et al. 2015). The phenomenon of action of daptomycin is also very important to know; it crosses the plasma membrane like calcium influx and potassium efflux, leading to apoptosis. Daptomycin reduces the expression of *mecA* gene by blocking both fem and aux factors. Daptomycin has an ability of reducing the attachment of PBP-2a to its peptidoglycan moieties before the synthesis of peptidoglycan in its early stages in the presence of oxacillin (Fig. 3). The benefit of using the daptomycin with B-lactam antibiotics is to enhance the binding of daptomycin (Dhand et al. 2011). During the previous decades, vancomycin has been considered as the most effective drug for treating the severe infections caused by MRSA. Collecting confirmation of aggregate resistance, unattainable pharmacokinetic/pharmacodynamic (PK/PD) targets and lesser consequences encounter the appropriateness of the primary place of vancomycin. The glycopeptides are used extensively for the treatment of VISA (vancomycin intermediate *S. aureus*) and hetero resistant VISA (hVISA). So, resistance also started developing against them, reducing the sensitivity to glycopeptides that resulted in the development of VRSA (vancomycin resistant *S. aureus*). Such isolates are more sensitive to other class of antibiotics, especially β-lactam antibiotics, even in the presence of *mecA* gene. This 'seesaw effect' describes sensitization of MRSA isolates to anti-staphylococcal β-lactam antibiotics by using higher minimum inhibitory concentrations (MICs) (Fig. 4) to vancomycin and daptomycin. The promising combined effects of vancomycin and b-lactam antibiotics are quite encouraging (Werth et al. 2013).

Fifth generation cephalosporin's role against MRSA infections

Out of five generations of cephalosporin, fifth generation is the most active against MRSA infections. Among the cephalosporin, the Ceftaroline and Ceftobiprole are the most commonly available drugs used at clinics. Ceftaroline is the approved drug against community-acquired pneumonia and acute bacterial skin and skin structure infections (Purrello et al. 2016). In 2006-2007, multicenter clinical trials were conducted among the hospitalized patients infected with community acquired pneumonia (CAP) to compare the ceftobiprole with ceftriaxone and linezolid. From 2008-2009, a multicenter FOCUS-1 trial was conducted by a scientist to check the efficacy of Ceftaroline against CAP. Almost 168 sites were selected across the world. Clinical curative rates of Ceftaroline were found to be high 86.6 and 83.3% in CE (clinical evaluable) population and mITT (modified intention-to-treat) population, respectively, while cure rates of Ceftriaxone were 78.2 and 77.7% in CE and mITT, respectively (File et al. 2011).

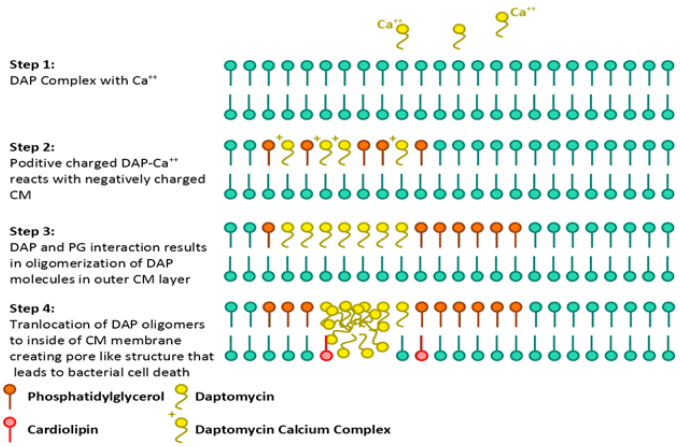

Fig. 1: Mode of action of Daptomycin against MRSA.

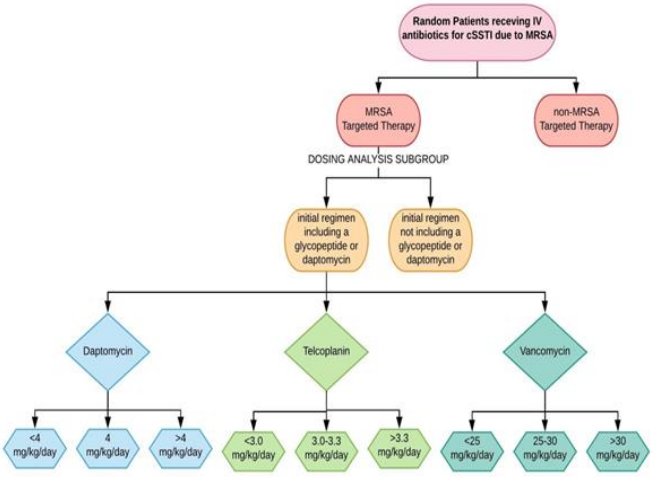

Fig. 4: Minimum inhibitory concentration (MIC) of various antibiotics against MRSA.

Novel antimicrobial agents for the intervention of MRSA infections

Several licensed and new agents have shown efficacy against MRSA infections, though defining their exact use needs further investigations. In this section, some of the newer antimicrobials active against MRSA have been discussed.

Oxazolidinones

Oxazolidinone is a new group of antibiotics, which are effective against a variety of Gram-positive bacteria, including methicillin-resistant and vancomycin-resistant Staphylococci. Oxazolidinone binds to P-site of 50S ribosome subunit and inhibits protein synthesis. Its activity is not affected by resistance to other inhibitors of protein synthesis though development of oxazolidinone resistance with 23S rRNA. Its high infiltration and accumulation in the tissue including bone, lungs, hematoma and cerebrospinal fluid permit its use for surgical infections (Bozdogan and Appelbaum 2004). Among the oxazolidinones, a new drug with the name of tedizolid is licensed for the treatment of skin and soft tissues infections for a standard course of 6 days. Tedizolid is a more effective drug and gives more advantages as compared to Linezolid. Among various benefits of tedizolid, the salient one is its efficacy against those isolates which are resistant to chloramphenicol-florfenicol resistant (cfr) methyltransferase gene (Flanagan et al. 2015). Cadazolid is a new oxazolidinone agent and it is quite effective against *Clostridium difficile* (Gerding et al. 2016), while radezolid is effective against the *S. aureus* isolates that are resistant to linezolid (Lemaire et al. 2010).

Tetracycline

Tetracycline antibiotics are inhibitors of protein synthesis. They inhibit translation initiation by binding to the 30S-ribosomal subunit and inhibit the binding of aminoacyl-tRNA to the translational mRNA complex. Several studies have shown that Tetracycline can bind to 16S and 23S rRNA (Chukwudi 2016). A new synthetic fluorocycline drug, Eravacycline, is effective against both Gram negative and Gram-positive pathogens including MRSA. Ribosomal hydrolysis and efflux pumps resistance is due to a mechanism called fluorination. Eravacycline is two to four times more active drug than tigecycline for Gram-positive organisms (Zhanel et al. 2016). Among aminomethylcyclines, omadacycline is more effective against MRSA infections, in addition to CAP and ABSSSI (Pfaller et al. 2017).

Fluoroquinolones

Quinolones are one of the most widely used antibacterial drugs in the world for the treatment of various bacterial infections in humans and animals. Structurally, these drugs contain a quinoline ring system due to which these are called quinolones. Quinolones and fluoroquinolones inhibit the replication of bacteria by blocking their path of DNA replication. Quinolones act by converting the target, gyrase and topoisomerase IV into toxic enzymes that break down the bacterial chromosome (Aldred et al. 2014). An investigational fluoroquinolone drug, delafloxacin, is active against Gram-negative and Gram-positive organisms. Due to its specific electro chemical properties includes uncharged at acidic pH and anion at physiological pH. A study was conducted in USA in 2011 to monitor the efficacy of Delafloxacin in comparison with vancomycin and Linezolid. The highest cure rates were observed with delafloxacin, followed by linezolid, while vancomycin showed the lowest cure rates (Kingsley et al. 2016).

Lipoglycopeptides

Lipoglycopeptides are a class of antibiotics with lipophilic side chains attached to glycopeptides that exhibit concentration-dependent bactericidal activity. They inhibit cell wall synthesis and disrupt the barrier function of the bacterial cell membrane. So, glycopeptide core binds to the terminal acyl-d-alanyl-d. The alanine chain of the cell wall has high affinity through hydrogen binding and interaction with hydrophobic filling. This prevents polymerization and crosslinking of the precursors of the cell wall (Damodaran and Madhan 2011). Among the lipoglycopeptides, three new agents are licensed and available in the market. Dalbavancin is a lipoglycopeptide licensed by FDA in 2014 and also by EMA (European medicine agency) after one year in 2015 for the treatment of ABSSSI (Bambeke 2015). Dalbavancin is semisynthetic lipoglycopeptide with long half-life derived from an actinomycete "*Nonomuria*" (Chen et al. 2007). Because of its long half-life of 10 days, it shows long activity for 7 days against MRSA with a single dose of 500 mg. Dalbavancin is especially used for the treatment of outpatient having complicated infections (Juul et al. 2016). Dalbavancin was also compared with vancomycin in a multicentre trial in 2011-2012 for ABSSSI. Excellent results were seen by dalbavancin with fewer adverse effects as compared to vancomycin (Boucher et al. 2014). Oritavancin is a second lipoglycopeptide approved by FDA and EMA in 2014 and 2015 respectively. It is a long-acting lipoglycopeptide for the treatment of ABSSSI (Takahashi and Igarashi 2018). It is dual in nature that suppresses transglycosylase and transpeptidase. This ability enhances its bactericidal property, and it shows the broad-spectrum activity against Gram-positive organisms including VRSA, VISA and vancomycin-resistant enterococci (VRE) (Bambeke 2014). Telavancin showed very effective response against HAP (hospital acquired pneumonia) caused by Gram-positive pathogens including MRSA (Sandrock and Shorr 2015).

Clinical management of MRSA by antibiotic combination

For clinical management of MRSA, many new combinations of drugs are being used, for example vancomycin and daptomycin are considered effective in bacteremia, while in hospital-acquired pneumonia (HAP) vancomycin or linezolid show more effective results. In case of acute bacterial skin and skin structure infections (ABSSSIs), any of both combinations can be used to treat MRSA. Other antimicrobial agents, such as doxycycline, clindamycin and trimethoprim/ sulfamethoxazole, are also effective in cases of ABSSSIs, depending upon the severity of infection (Liu et al. 2011). The use of these

agents also has adverse effects or drawbacks, for example, vancomycin is available only for parenteral use, minimum inhibitory concentration (MIC) creep, difficulties in the achievement of curative levels and emergence of vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA) and heteroresistant VISA (hVISA). The drawbacks associated with daptomycin are that it is not indicated in case of pneumonia, and is available only for parenteral use. Myelosuppressive, bacteriostatic and significant drug interaction type of drawbacks are associated with linezolid (Dhand et al. 2011).

Future drifts in the control of MRSA Infections

An act was passed in 2012 by US Congress with the name "US FDA Safety and Innovation Act" to facilitate the pharmaceutical industry by providing incentives about generating new antibiotics for the market. This act also provides fast-tracked approval of antibiotics with additional patent protection of five years for qualified products (Tillotson and Tillotson 2015). After the approval of this act, many antibiotics such as Oritavancin and dalbavancin, belonging to group lipoglycopeptides, and tedizolid belonging to group oxazolidinones, were approved by FDA. However, after their approval from FDA, several clinical trials were required, which are still in progress to explore the advantages of combination therapy of antibiotics for their clinical use.

Drug Modulation of MRSA strains

Outstanding amongst other techniques to control bacterial resistance and expand the life of existing antibiotics, is to connect them with modulators of drug resistance. For example, numerous β-lactam antibiotics mixed with potassium clavulanate proved to be best against MRSA. Coumarins involve a class of characteristic phenolic compounds described by solitary benzene intertwined to a α-pyrone ring. They are emerging with great organic potential, as exhibited in a few examinations; these are compounds with antifungal and antibacterial properties, and modulators of anti-toxin resistance (Bazzaz et al. 2010). The mending properties of some restorative plants against irresistible infections are outstanding and recorded through the human development. The dynamic auxiliary metabolites created from plants are for the most part responsible for these remedial properties. Various examinations have also shown the antibacterial properties of numerous plants extracts against MRSA. Although not all plants discovered dynamic against MRSA are enrolled in this group, however it is expected that the value of therapeutic plants as an elective hotspot for antibacterial specialists against MRSA would be demonstrated. Strangely, some restorative plants, when joined with a few anti-microbial agents, could upgrade the ability of the anti-toxins against MRSA pathogens (Babra et al. 2013). This upgrade of the anti-microbial action can be ascribed to Phyto-mixtures, which may obstruct the efflux pumps of microscopic organisms and enable the anti-microbial to cooperate and wreck the bacterial cell. This procedure is called "Synergistic multi target impact" (Coutinho et al. 2009). Numerous plants indicated synergistic impacts on safe pathogens. A combined effect of ethanol removed from *Turnera ulmifolia* leaves with antibiotics includes gentamicin and kanamycin that may improve anti-toxin activity against MRSA strains. The oil extracted from grapefruit was found to be effective against MRSA as potential efflux pump modulator (Abulrob et al. 2004). In a fascinating investigation, a Korean customary natural plan, known as Sami Hyanglyum-Hwan comprises of four herbs (Arecae semen, Coptidis rhizome, Aucklandiae radix and Rhei rhizome), has restabilized the adequacy of the anti-microbial ciprofloxacin after it demonstrated no impact when tried alone against some MRSA strains (Choi et al. 2015).

Antibacterial activity of non-steroidal anti-inflammatory drugs (NSAIDs)

Numerous studies have reported that non-steroidal anti-inflammatory drugs (NSAIDs) show antimicrobial properties but the mechanism of action is not clear. It has been reported that ibuprofen, diclofenac and aspirin show antimicrobial action against certain Gram-positive bacteria at 5 mg/mL, except mefenamic acid. Because Gram-negative bacteria have lipopolysaccharide layer which restricts the diffusion of most drugs due to its hydrophilic nature, so just aspirin has efficacy against Gram-negative bacteria. Conversely, Gram-posative bacteria lack this lipopolysaccharide layer, hence allowing easy infiltration of the antimicrobial agents into the cells (Zhong et al. 2015). NSAIDs show antimicrobial activity at much less concentration compared to normal therapeutic dose used for inflammation, pain or fever. In contrast to diclofenac, both aspirin and ibuprofen expressed bacteriostatic and bactericidal ability against tested MRSA, so they can be used as antibiotic adjuvant to treat MRSA infections (Chan et al. 2017).

Treatment of MRSA by combination of antibiotics and NSAIDs

NSAIDs, along with antibiotics, are used to treat community-acquired MRSA. Cefuroxime and chloramphenicol alone are used to treat MRSA, but these drugs do not show effective results. Since aspirin and ibuprofen show bacteriostatic and bactericidal effects against the MRSA strains, so the collective effects of both NSAIDs with cefuroxime and chloramphenicol were examined. It was observed that Ibuprofen/aspirin in combination with chloramphenicol/cefuroxime might act on various target sites of the bacteria, and ultimately produce either an additive or a synergistic effect. Practical implication of antibiotic-adjuvant method is seen in Augmentin (amoxicillin/clavulanate potassium), in which a β-lactam is combined with β-lactamase (resistance enzyme inhibitor) and it explains the use of amoxicillin to treat infections of β-lactam resistant bacteria (Yin et al.

2014). This NSAID–antibiotic combination can be prepared to treat MDR bacterial infections.

Vaccine development against MRSA

The ongoing reports by both the World Health Organization and Centers for Disease Control have featured the issue confronting us because of antimicrobial resistance with methicillin sensitive *S. aureus* (MRSA). MRSA contaminations have expanded levels of mortality, doctor's facility stays, septic shock and ensuing diseases. The contamination by this pathogen has risen to greater than 94,000 cases, with 18,000 deaths every year in the United States. It also causes billions of dollars losses in the United States and in many other countries (Klevens 2007). Given its significance, the improvement of an immunization protocol and development of new antimicrobials to control *S. aureus* is highly important. At present, there is no well-established antibody against MRSA. Efforts in the past have depended on single antigen arrangements, while current endeavors weighted towards different antigens. An immunization ought to be planned in the light of lethal factors communicated in various periods of disease, so that it can effectively fight against an expansive range of disorders induced by the microorganism (Broughan et al. 2011). *S. aureus* has distinctive sorts of destructive virulence factors; so, endeavors to build up a compelling immunization against this pathogen have been basically unsuccessful. In order to produce viable antibodies against various *S. aureus* strains, more than one antigen ought to be chosen. Also, to upgrade the host resistant reactions, the immunization must be joined by a suitable adjuvant.

In such manner, three antigenic determinants, including clustering factor A (ClfA), alpha-enolase (Eno1), and iron surface determinant B (IsdB), were assessed by accessible bioinformatics instruments for outlining an effective multi-epitope subunit immunization for the enlistment of safe reactions against Staphylococcal contaminations. Eno1 is a cell divider and multiple functional protein that is limited on the outer covering of multiple prokaryotic and eukaryotic cells. This protein also holds the ability to reside in the cytoplasm of a cell. It is available in all tested strains of *S. aureus* and has an exceedingly saved succession. This protein also helps in the adhesion process and plays a significant role in the spreading of this pathogen. ClfA is also a cell divider secured protein, which is present on the surface of *S. aureus* and helps its adhesion to host fibrinogen γ-chain. Past examinations have demonstrated that ClfA assumes a significant part in the acceptance of Staphylococcal diseases (Garcı-Laura and Foster 2009). Thus, this harmful factor, as an immunization segment, gives potential focus to the enlistment of a hearty dynamic and aloof insusceptible reaction to *S. aureus*. IsdB, the third antigenic determinant, is also a protein that helps in anchoring to the cell surface. It is uncovered on the exterior of cell. This protein is saved among different strains of *S. aureus*, and is communicated just under restricting iron shape. Immunoglobulins speak to the primary immunotherapy approach utilized as a part of people. For Staphylococcal infections, diverse arrangements, generally polyclonal, focusing on amassing factor or capsular polysaccharide, have been tried with clashing outcomes regardless of persuading animal models, particularly in pneumonia (Liu et al. 2011). These days, immunoglobulins are infrequently utilized for *S. aureus,* except for some particular signs where a few specialists will think about them. Isolation of *S. aureus* lethal factors is a gigantic test for immunization advancement. Without dependable biomarkers for *S. aureus* and surrogate insurance, trial disappointment can't be profoundly examined. Since *S. aureus* infections are conceivably heterogeneous, contrasted with Pneumococcus in pneumonia, it is additionally hard to develop an immunization agent focusing on a particular infection (Proctor 2012).

Conclusion

MRSA is not restricted to humans and animals but now it is transferred from humans to animals, animals to humans and from environment to both humans and animals. So, it has become a threat to one health and needs immediate attention. MRSA new strains are emerging continuously through the development of resistance to antibiotics and causing light to severe infections in both humans and animals. So, it is recommended to use a combination of drugs and alternative medicines to avoid this resistance. World health authorities have also suggested the need for effective control strategies to control MRSA infections by limiting the excessive use of antibiotics and by urging the health workers to prescribe the drugs in combination after correct diagnosis.

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