NARRATIVE REVIEW

Methicillin resistant *Staphylococcus aureus*: A brief review of virulence and resistance

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Abstract

Staphylococcus aureus is a common gram-positive human pathogen involved in both community-acquired and nosocomial infections ranging from localised superficial lesions to food poisoning and fatal systemic infections owing to its impressive array of virulence factors responsible for attaching, colonising, invading, and avoiding host immune system. The discovery of antibiotics effectively checked the once deadly infections. However, resistance started soon after their discovery and the first methicillin-resistant strain of staphylococcus aureus was reported in the early 1960s. The most important attribute of methicillin-resistant *staphylococcus aureus* is its acquisition of mecA gene coding for penicillin-binding protein-2a that blocks inhibitory action on peptidoglycan cross-linking. Methicillin-resistant *staphylococcus aureus* presents a serious global healthcare concern being responsible for prolonged hospital stays and increased mortality. The precise information of virulence factors and resistant traits of methicillin-resistant staphylococcus aureus and their interplay in a community is key to minimize the intermixing of resistant and susceptible pathogens in the community.

Keywords: Methicillin resistant *staphylococcus aureus*, Virulence, Resistance, mecA.

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Introduction

Staphylococcus (S.) aureus is a gram-positive spherical non-motile coccoid bacterium around 1µm in diameter and a well-known human pathogen in both community-acquired and nosocomial infections. It is often found on skin, skin glands, and mucous membranes, predominantly in the nose, of healthy individuals. The bacterium has an ability to asymptomatically colonise healthy individuals and it is believed that 30% of human population carries *S. aureus*.[1] The bacteria form medium-sized colonies that appear as golden coloured on rich medium and cause β-haemolysis on blood agar plates. Staphylococci are facultative anaerobes capable of generating energy by aerobic respiration and produce lactic acid by fermentation, catalase-positive and oxidase-negative. *S. aureus* and *S. intermedius* are coagulase-positive, while all other *staphylococci* are coagulase-negative.

S. aureus infections are mainly of three types; superficial lesions, toxinosis, and systemic and fatal conditions. Wound infections and food poisoning, toxic shock syndrome, and scaled skin syndrome belong to superficial lesions and toxinosis, respectively, whereas endocarditis, osteomyelitis and pneumonia present as some systematic and fatal diseases among many caused by the bacterium. *S. aureus* can survive inside host cells like nonprofessional phagocytes that include fibroblasts, osteoblasts, endothelial cells, epithelial cells and mesenchymal cells. Once inside the host cell, it can escape the host defence mechanisms and further disseminates by colonisation and tissue damage through specific pathogenic determinants.[2]

Methicillin-resistant *S. aureus* (MRSA) presents a serious global healthcare concern being responsible for prolonged hospital stays and increased mortality. The impact of this infection burden on meagre healthcare resources of resource-limited countries is believed to be much worse, considering the scarce data available on the issue. The World Health Organisation (WHO), therefore, proposed its global antimicrobial resistance surveillance system (GLASS) programme for member countries in 2015.[3] In response, several countries started reporting resistance data in a more synchronised manner that highlighted the severity of the issue. South Korea promulgated its Kor-GLASS programme and reported 54.3% MRSA among all *S. aureus* isolated from blood cultures during the first year (2016-17) of programme implementation.[4] Pakistan entered the GLASS programme in 2018 and is currently implementing its first phase. The WHO report on early implementation data for Pakistan indicated that around 62% *S. aureus* isolates were resistant to oxacillin when a selective testing approach was applied.[5]

There has been decreasing prevalence of MRSA in Europe over the years and MRSA ranges from as low as 1% in northern Europe to more than 50% of bacteraemia cases in southern Europe.[6] In the United States, there has been a

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trend of 17% decrease in MRSA bloodstream infection compared to that reported during 2005-16. Even though there had been substantial reduction trends, MRSA-associated morbidity and mortality was still alarming, as 119,247 infections and 19,832 deaths were reported.[7]

In resource-limited countries where proper documentation of MRSA is scarce, some studies have reported heterogeneous prevalence of MRSA ranging from 4% to 47%.[8] The situation is not different in Pakistan, where hospital-acquired MRSA is an important dominating factor forever increasing resistance-related mortality. Antibiotic resistance data from various hospitals and laboratories throughout Pakistan is available on the website of Pakistan Antibiotic Resistance Network (PARN).[9] The data from different hospitals indicates *S. aureus* resistance against penicillins, like oxacillin and cloxacillin, ranging from 43% to 55%. Another study reported that 66% of the total *S. aureus* isolated from pus and wounds samples of patients were resistant to methicillin.[10] A report from Rawalpindi district revealed 44% MRSA prevalence.[11] Such data not only provides a useful resource for implementing antibiotic stewardship programmes, but can also be utilised in devising infection control and screening strategies. The current narrative review was planned to summarise virulence factors, resistance traits and mechanisms of resistance development in MRSA.

Virulence factors of *S. aureus*

S. aureus virulence factors are responsible for diverse range of infections that allow it to attach to host surface, colonise, invade, and avoid immune system of host cells, causing harmful effects.

Cell surface factors: *S. aureus*'s attachment to the host cell surface is mediated by several adhesion molecules that initiate the colonisation process. Microbial surface component recognising adhesive matrix molecules (MSCRAMMs) is one of the major classes of adhesins. Proteins from this class anchor themselves covalently through threonine residue in C-terminus of cell peptidoglycan. These molecules identify prominent components of blood plasma, including fibrinogen, fibronectin and collagens. Typical members of the MSCRAMM family include staphylococcal protein A (SpA), fibronectin-binding proteins (FnbpA and FnbpB), collagen-binding protein, clumping factor proteins (ClfA and ClfB), and Serine rich proteins like SdrD and SraP whereas autolysin (Atl) also plays an important part in adhesion.[12]

***S. aureus* cytolytic toxins:** Almost all *S. aureus* strains produce exotoxins and enzymes, including nucleases, proteases, lipases, hyaluronidase and collagenase. These proteins can convert local host tissue into nutrients that are later used by bacteria for their growth.[13] Exotoxins of *S. aureus* possess cytolytic activity that causes leakage of the cell's content in surrounding environment and consequent lysis by forming β-barrel pores in the plasma membrane. The important exotoxins are α-haemolysin, β-haemolysin, γ-haemolysin, leukocidin, and panton-valentine leukocidin (PVL). *S. aureus* α-toxin (Hla) plays essential role in the pathogenesis of diseases by inserting itself into the eukaryotic cell membrane where it oligomerises into a β-barrel resulting in pore formation. This triggers alterations in ion gradients, activation of stress signalling pathways, membrane integrity loss which leads to osmotic cytolysis and eventually cell death. This toxin is particularly cytolytic to human platelets and monocytes.[14,15] Mutants of *S. aureus* lacking Hla showed reduced virulence.[16] β-Haemolysin, or β-Toxin, causes hydrolysis of plasmatic membrane sphingomyelin of monocytes, erythrocytes, neutrophils and lymphocytes, making them susceptible to other lytic agents, such as α-toxin and PVL.[14] Further, γ-Haemolysin is also a pore-forming toxin that induces lysis on erythrocytes and leukocytes. HlgAB and HlgCB are recognised as the bi-component proteins of the γ-Haemolysin that facilitate the bacterium in evading the phagocytic response of the host.[17] PVL is categorised as a bi-component cytolysin; LukF-PV and LukS-PV. It forms pore by inserting itself in plasma membrane followed by adopting heterooligomer conformation. PVL shows a high affinity toward leukocytes.[18]

***S. aureus* superantigens:** Exotoxins produced by *S. aureus* include the toxic shock syndrome toxin-1 (Tsst-1), staphylococcal enterotoxins, including Sea, Seb, Secn, Sed, See, Seg, Seh and Sei, and exfoliative toxins Eta and Etb. Tsst-1 and the staphylococcal enterotoxins belong to pyrogenic toxin superantigens (PTSAgs) family.[13] This family stimulates proliferation of T-lymphocytes, causing superantigenicity, and is responsible for toxic shock syndrome and food poisoning. The presence of Eta and Etb causes staphylococcal scalded skin syndrome (SSSS). The role of *S. aureus* enterptoxins in its pathogenesis needs to be understood (Table).

Table: Role of virulence factors in pathogenesis of *Staphylococcus (S.)* aureus.

***Staphylococcal* Enterotoxin**	**Feature**
Sea	Most common toxin responsible for staphylococcal food poisoning
Seb	biological weapon
Sec	Frequently isolated from animals
Sed	Food poisoning
See	Food poisoning
Sef	Associated with toxic shock syndrome
Seg	Little role in food poisoning
She	Food poisoning
Sei	Minor role in food poisoning

Antibiotic resistance

Resistance against antibiotics was first observed in hospitals soon after the start of their clinical use. Resistance against sulphonamide in streptococcus pyogenes appeared as early as in 1930s whereas penicillin-resistant *S. aureus* and streptomycin-resistant mycobacterium tuberculosis were reported soon after their clinical use. Multidrug resistance (MDR) in enteric bacteria, including *Escherichia coli, Shigella* and *Salmonella*, first appeared in the 1950s and 1960s.[19] Initially being considered as a small health concern, the emergence of resistance was later considered to be the major factor responsible for increasing mortality and cost of treatment. Currently MDR has been reported throughout the world.

The cause of resistance is associated with two main components; the antibiotic which kills the susceptible microbes and sparing the resistant population, and the genetic characteristics of the microorganism. The resistance against the antibiotic develops when both of these components play their role, either in a bacterial host or in general environment. The resistant organism thus evolved continues to propagate, and transfers its resistance trait to similar and related organisms over time. Most of the antibiotics classes discovered so far act by interfering with physiological and metabolic functions, causing resistance development in their target bacteria. Antibiotics have been and are being used extensively in humans, animals and in agriculture, mainly for treatment and prevention of diseases, thus selecting the resistant strains and driving towards newer resistance mechanisms and strains. The mechanisms of resistance development involve the transfer of genetic elements responsible for mediating resistance, between different bacteria, since most are encoded on mobile genetic elements or deoxyribonucleic acid (DNA) and also by bacteriophages. In addition to the acquisition of resistance by mobile elements, spontaneous resistance or elevation in resistance level from low to high resistance may occur by mutations in the bacterial genome.

Mechanisms of antibiotic resistance in biofilms: Like nearly all human pathogens, *S. aureus* also has the ability to establish complex biofilms for successfully thriving in the host for a chronic infection.[20] When microorganisms bind to a surface, they produce extracellular polymeric substance (EPS) and form biofilm. Biofilm is posing a great problem for public health due to its resistant nature to antibiotics and disease associated with indwelling medical devices. Mechanisms of antibiotics resistance in bacterial biofilms include inactivation of active molecule, altering sensitivity to target, reduction of drug concentration before reaching the target site and efflux systems. However, resistance levels in biofilms may vary among different settings. Intrinsic mechanisms have been attributed to resistance development in both adhered bacteria and biofilms. However, primary mechanisms that are main attributes to resistance development in the growth of adherent cells seem unable to describe the high resistance to antibacterial agents related to biofilms. Several mechanisms have been attributed to this high resistance characteristic of biofilms, including limited diffusion, enzyme-based neutralisations, slow growth rate, presence of persistent non-dividing cells, efflux pumps and membrane alteration.[21]

Low penetration of antibiotics: Diffusion of antibiotics can take place through the matrix of biofilm. Penetration of antibiotics to deeper layers of biofilm is affected by exopolysaccharide acting as a physical barrier. When molecules directly interact with this matrix, their transport to the interior is altered, resulting in resistance to these antibiotics. In liquid culture, bacterial cells are readily exposed to antibiotics compared to compact-structure biofilm. Bacteria escape from biofilm that do not produce polysaccharide and are easily attacked by immune system cells. Inactivation of antibiotic takes place when it binds to biofilm matrix. Pseudomonas aeruginosa possess alginate exopolysaccharide that causes slow penetration of fluoroquinolones and aminoglycosides.[22]

Neutralisation by enzymes: Presence of neutralising enzymes which degrade or inactivate antibiotics may also confer resistance. Enzyme accumulation is stimulated by antibiotics in the glycocalyx, and their activity increases due to slow penetration of antibiotics and subsequent degradation in the biofilm. Overproduction of cephalosporinase AmpC (Amp C beta lactamase) enzyme together with an efflux transporter has been reported to cause resistance in P. aeruginosa against third-generation cephalosporins and carbenams in cystic fibrosis patients.[23]

Heterogeneous nature of biofilms: Biofilms are heterogeneous both structurally and metabolically as both aerobic and anaerobic processes occur simultaneously. This fact has been explained by many studies performed to determine the microbial growth in biofilms across its different areas.[24] The response against antibiotics may be different in different areas of the biofilms. On the surface of the biofilm there is a high level of activity of antibiotics compared to the inside of the biofilms where slow or absent growth reduces the sensitivity of the cells to antimicrobials. The action of aminoglycosides is affected by limitation of oxygen and anaerobic growth of microorganisms, which is affected by the presence of oxygen and potential of hydrogen (pH) gradients.[25]

Slow growth rate of cells: Slow growth of microorganisms

occurs due to limited availability of nutrients as there exists a gradient of nutrients within the biofilm environment, resulting in metabolically active surface layer and peripheries, and inactive interior part. It has been postulated that antibiotics, like penicillin and ampicillin, attack the bacterial cells in growing phase. However, other antibiotics can attack the cells in stationary phase as well, like aminoglycosides, cephalosporin and fluoro-quinolones.[26]

Existence of persistent cells: Even after extensive antibiotics treatment, small number of bacterial cells may still remain viable in the biofilm. There exists a biphasic dimension in biofilms, indicating that large number of cells population is attacked sparing some resistant population even with substantial antibiotics usage. Secondly, bacteriostatic antibiotics contribute to the growth of persistent cells and biofilm preservation by inhibiting growth of sensitive cells. These persistent cells may or may not give this resistance to their progeny once the pressure is removed. The persistent cells stop their replication for small duration to survive. Their adaptive mechanism is not related to the mechanism followed by the cells during stress (environmental damage). When cell density reaches the highest number in the stationary phase, persistent cells increase in number.[27] This reshapes the biofilm character into original form when the antibiotics therapy is withdrawn.

Biofilm phenotype: During the formation of biofilm, bacteria produce secondary metabolites, such as antibiotics, pigments and other diffusible molecules, functioning as signalling molecules for biofilm formation. Biofilm phenotype is community of cells conferring resistance to antibiotics, indicating the presence of specific genes regulating such processes. Bacillus subtilis biofilms indicated a considerable difference of 6% in gene expression compared to planktonic culture when subjected to DNA microarray-based studies.[28] This differential gene expression, however, is not conclusive in describing this mechanism. It has been reported that, like biofilm, their planktonic cells in stationary phase rapidly regained antibiotic susceptibility when diluted in fresh medium that provides nutrients and might also dilute the protective cell-cell signalling. This reversibility established that biofilm tolerance represents a phenotype rather than the product of genetic alterations.

Efflux pumps: Efflux pumps are protein structures that transport compounds to the exterior of bacterial surface. Their substrates may include, but not limited to, those involved in MDR. Efflux pumps in the periplasmic area of resistant bacteria are involved in the accumulation of antibiotics, including macrolides, tetracycline, fluoro-quinolones and β-lactam, thus reducing these antibiotics concentration to sub-toxic levels. Five families of efflux transporters have been identified in prokaryotes, and over-expression of the efflux pumps has been considered to be responsible for antibiotic resistance in *P. aeruginosa* biofilms.[24]

Membrane proteins alterations: Outer membrane channel proteins (porins) present in gram-negative bacteria play a key role in transporting hydrophilic molecules from external environment to the periplasmic space. Mutation of porins-encoding genes can result in production of non-functional or altered proteins having low permeability for hydrophobic molecules. The differential expression of porins-coding genes in biofilm may lead to antibiotic resistance as increased expression of ompC (Outer membrane porin C) and other osmotically-regulated genes has been reported during biofilm growth of bacteria.[29]

Phase variation: The presence of diverse phenotypes inside the biofilms may also play an important role in resistance. Antibiotic resistance and phase reversal have been reported to be related in Staphylococcus and Pseudomonas genera and some species of *Enterobacteriaceae*.[26] Biofilms can develop bacterial subpopulation that switch to the dormant state, known as small colony variants (SCVs), having less susceptibility to growth phase dependent antibiotics. Detectable morphological changes in biofilms due to SCVs might lead to increased adherence, auto-aggregation, increased hydrophobicity and low-level motility. Such attributes help these to withstand wide range of harsh environmental stress conditions, a major survival mechanism for biofilms.

Target-site alteration: Alterations in drug targets interfere with the bacteriostatic/bactericidal effects of antibiotics, limiting their effectiveness and causing resistance. In macrolide antibiotics, ribosomal target-site is modified as one of the adenine residue is methylated by a family of transposon-encoded N-methyltransferases in 23S ribosomal ribonucleic acid (rRNA). Resistance to methicillin is usually due to production of a low-affinity penicillin-binding protein-2a (PBP2a), which results in resistance to almost all b-lactams.[30] Vancomycin resistance is caused by synthesis of abnormal pentapeptide precursors, resulting in the alteration of target-site (D-ala–D-lac or D-ala–D-ser instead of D-ala–D-ala termini of the UDP (Uridine diphosphate)-N-acetylmuramyl-pentapeptide precursor of peptidoglycan). This alteration confers reduced affinity for vancomycin, catalysed by products of the van genes.[31] Similarly, resistance to fluoroquinolones is caused by mutations in the fluoroquinolone targets, DNA gyrase (gyrA) and DNA topoisomerase (parC), whereas resistance to aminoglycosides is caused by ribosomal mutations that

alter its target-site.

Membrane permeability: Most antibiotics must access their intracellular targets to exert bactericidal or bacteriostatic actions. In gram-negative bacteria, the outer membrane acts as a major barrier. The substantial permeability acts as a necessary factor for antibiotics action against bacteria. The role of the outer membrane acting as a barrier resulting in resistance development has also been emphasised by the observations that chemical changes in membrane structure enhanced antimicrobial susceptibility.[32]

Ecology of antibiotic resistance: After the treatment of a microbial population by an antibiotic, the susceptible strains are out-competed by the resistant ones, which thrive and acquire dominant position in the ecological niche, resulting in microbial populations with pool of antibiotic resistant genes. The widespread usage of antibiotics is attributed to selective resistance strains as trends in resistance rates correspond to the total use of antibiotics by the community and not at individual levels. Another example is the treatment of acne by antibiotics that not only produces MDR skin flora in the patients, but also in other members of the same household.[33]

The acquisition of mecA gene in MRSA: The strains of MRSA get resistant to methicillin and other similar antibiotics through the acquisition of mecA gene. The gene product of mecA is PBP2a.[30] This gene is a part of part of 21-60-kb Staphylococcal cassette chromosome (SCCmec) which is a mobile genetic element.[35] During mecA gene-acquisition in MRSA, SCCmec type IV is incorporated into the *S. aureus* genome. After incorporation, the bacterium starts producing PBP2a which is incorporated in bacterial cell wall and is less likely to bind to beta-lactam antibiotics, compromising their inhibitory action on peptidoglycan cross-linking, eventually leading to penicillin resistance.

Management and control of resistance

The indiscriminate use of antibiotics and consequent global emergence of resistance has resulted in failure of even so-called last-line antibiotics,[35] not only threatening human health, but also endangering global economies by increasing health expenditure, thereby putting global health security at risk.[36] It has been estimated that antibiotic-resistant infections not only double the hospital stay time, but also increase mortality rates manifold.[37] The most important aspects in checking the emergence of bacterial resistance is devising and implementing antibiotic stewardship programmes in hospitals, limiting over-the-counter (OTC) availability of antibiotics, restricting antibiotics use in livestock and agriculture, ensuring proper disposal and effective surveillance in communities. This can be achieved by various strategies involving effective resistance tracking, isolating resistant patients in hospitals and finding new therapeutic interventions.

Commensal organisms are common reservoirs of antibiotic resistant genes and spread the same among their populations. For example, *S. epidermidis* disseminates resistance to pathogenic *S. aureus*. Similarly, vancomycin resistance, initially observed in enterococci, appeared in other commensal bacteria before it emerged in *S. aureus*. It is, therefore, important to track the frequency of resistance in various bacterial populations to predict the trends and possible threats of resistance dissemination to clinically important pathogens. The intermixing of resistant and susceptible pathogens in clinical and hospital settings results in dissemination of resistant traits across bacterial populations. It is, therefore, important to isolate the patients harbouring resistant bacteria to lower the risk of hospital-based transmission of MRSA and MDR to previously negative patients or hospital staff.

Rational and prudent use of antibiotics is one of the most important measures to reduce the emergence of resistance. The pace of emergence of resistance and development of new drugs is unmatched, and it is, therefore, necessary to strictly regulate the use of current drugs at global level to buy time for the scientific community to find effective antibiotics. In addition to conventional culture-based antibiotic therapy, novel therapeutic interventions are being increasingly proposed.[38] Research focus on developing novel antibiotics against newer targets is urgently warranted. Early and rapid diagnosis of infections, and vaccinations, can help limit the spread of infections. Alternative therapies, including the use of bacteriophages, also promise an infection control option that needs to be extensively explored.

Conclusions

Since their discovery in the 1940s, antibiotics have been widely used clinically for controlling bacterial infections, thus reducing morbidity and mortality. Widespread antibiotic resistance is now threatening global health security. The strategies being adapted in the developed world for checking this unprecedented spread of resistance need to be implemented in resource-limited countries as well. There is a dire need of monitoring resistance and virulence traits of indigenous pathogens through nationwide studies and establishing national registries. Devising effective strategies for identification, control and prevention of new resistance traits in community and hospital settings through promulgating necessary regulations and setting up of infection prevention and control measures in hospitals is warranted.

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