Review Article

MICROBIAL GIMICS: STRATEGIES OF SUCCESFUL PATHOGENICITY BY *STAPHYLOCOCCUS AUREUS*

ABSTRACT

Staphylococcus aureus is an opportunistic pathogen responsible for several infections in humans which results in high mortality and morbidity rates. It is also known to be resistant to multiple classes of antibiotics which make treatment very difficult. The pathogenicity of *S. aureus* is greatly enhanced by its ability to produce various toxins and enzymes as well as transfer and acquire resistance genes from the environment. This review provides a summary of the different mechanisms that enhance its evolutionary nature, some virulence determinants and antibiotic resistance mechanisms in the organism. This would help in a better understanding of how its evolutionary nature can be artificially manipulated to control infections caused by the organism.

Keywords; antibiotic resistance, pathogenicity, infection.

SUMMARY

Staphylococcus aureus is a major human pathogen associated with a variety of clinical diseases. It is the leading cause of wound infections, skin infections, respiratory infections as well as device-related infections. This review comprehensively covers the virulence determinants of the organism and the different mechanisms of antibiotic resistance in the organism. Recently, *Staphylococcus aureus* has become a serious threat because of its ability to evolve which has led to challenges in the treatment of infections caused by the organism

INTRODUCTION

Staphylococcus aureus is a Gram-positive, non-motile, non-spore forming microorganism. It is present in the normal flora of the human nasopharynx and skin and makes up about 30% in a healthy human population [1]. It does not cause disease as a component of the normal flora but a break in the skin causes the bacterium to enter a wound and colonize it, thereby causing infections. However, *S. aureus* has the potential of being an opportunistic pathogen, producing a broad variety of diseases in humans, starting from a minor skin infection to a fatal form of pneumonia resulting in human mortality. *S. aureus* has a typical evolutionary nature which makes it a successful pathogen. It is associated with a variety of diseases Examples include; acute sepsis, respiratory infections, wound infections amongst others. It has also been implicated in different skin infections such as boils, impetigo, carbuncles, folliculitis etc. *S. aureus* is a major cause of bloodstream infections which occurs following a puncture on the mucosal membrane or on the surface of the skin following surgery, injury and the use of the catheter in hospital settings. Once inside the bloodstream, it has the capacity to infect numerous organs in the body and as well produces different pigments and molecules that help it to escape the host immunity and establish an infection such as protein A, staphyloxanthin etc. it, however, produces biofilms by producing different adhesins that enable it to adhere to host surfaces.

S. aureus is gradually evolving in animals (Livestock-associated Methicillin resistant *S. aureus*). This group of *S. aureus* heavily colonize pigs and calves in farms and because of this, the farmworkers and veterinarian are susceptible to infection by LA-MRSA [2]. It also encodes different virulence factors such as toxins, enzymes which are mediated by horizontal gene transfer, and this, however, contributes to the emergence of antibiotic resistance to multiple classes of antimicrobial drugs.

This review would be focused on the different mechanisms by which *S. aureus* acquires resistance to antibiotics (horizontal gene transfer), some virulence determinants that are mediated through this means and some antibiotic resistance in the organism.

HORIZONTAL GENE TRANSFER IN *S. AUREUS*.

This is a mechanism by which *S. aureus* can transfer DNA (mobile genetic elements) (MGE) from one bacterial cell to another. This mechanism enhances the circulation of (MGE) which encodes for virulence as well as antibiotic resistance. There are diverse ways by which the genetic information can be transferred or acquired from other cells or the environment as shown in Fig.1. [3]. They include: through conjugation, generalized transduction, plasmids, transposons, bacteriophages, genomic islands, staphylococcal cassette chromosome (SCC), transformation etc.

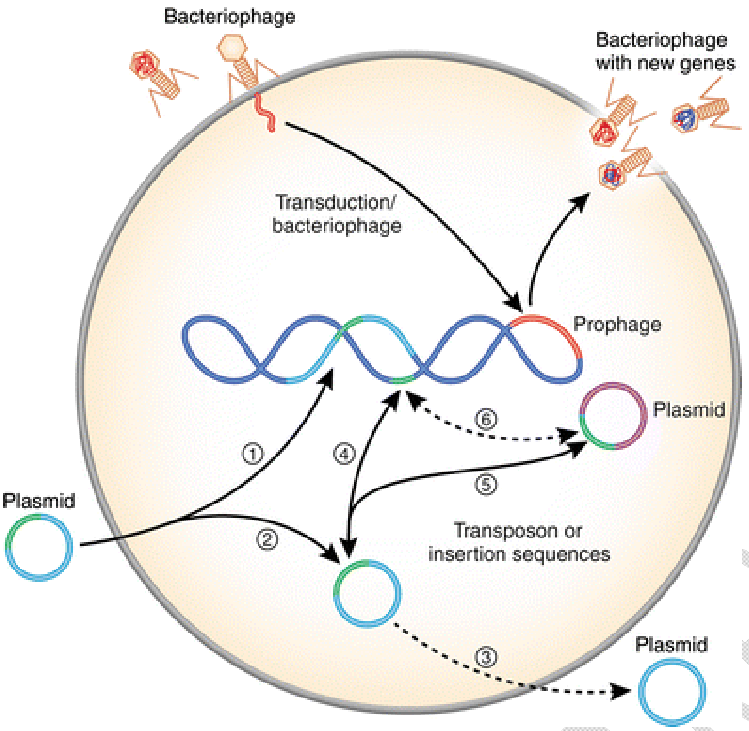

Fig.1: The Acquisition of mobile genetic elements by *Staphylococcus aureus*: 1. Incorporation of plasmids into a bacterial DNA. 2. Plasmid integrated into the chromosome of a bacterium. 3. Plasmids as an independent circular DNA. 4. Transfer of a transposon between plasmid and genomic DNA. 5. Transfer of transposons between plasmids. 6. Transfer of transposons from a genomic DNA to a plasmid. [3].

GENERALIZED TRANSDUCTION

Transduction is the transfer of DNA from one cell to another through a bacteriophage (Fig.1) During replication, the bacteriophage gets integrated into the chromosome and can be transferred to its daughter cells (Fig.1). A prophage can be instigated by stress, resulting in the cutting of the phage DNA, reproducibility of the prophage DNA, synthesis of novel prophage proteins etc. The size of the prophage is typically 45kb and they are known to code for virulence determinants like the Panton-Valentine Leucocidin(PVL), chemotaxis inhibitory protein amongst others. The phage particles can either kill the recipient host (lytic pathway) or get integrated into the recipient's chromosome as a prophage (lysogenic pathway). The lysogenic pathway is common in *S. aureus* where isolates carry between 1-4 different prophage types [1]. However, in generalized transduction, the new growing phage particles package the bacterial chromosomal DNA instead of the phage DNA. It has been shown that some bacteriophages do this while some others do not, but the mechanism is still not known.

However, this could be a natural mechanism of conserving its host DNA as well as transferring its genetic element to like or non-like bacterial cells. The phage particles that are released during lysis binds to the *Staphylococcus aureus* recipient's receptor and introduces its DNA into the cell [4,5]. Because the DNA is not a phage, it does not get integrated into the chromosome like a lysogenic phage will do nor does it kill the recipient cell as the lytic cell will do. However, some host DNA seems to be selectively packaged by the phage leading to an elevated level of transfer.

CONJUGATION AND PLASMIDS

This is a mechanism whereby DNA is transferred from one cell to another through a pilus or a pore [6]. In *S. aureus*, it is assumed that the pores are made between cells that are in close contact with each other because the pili are not seen. A range of plasmids carrying resistance genes is transferred during the process of conjugation. as shown in Fig 2. (Adopted by evolution website). These conjugative plasmids are too large, and they carry an extensive range of antibiotic resistance genes and virulence factors which they transfer from one organism to another [7,8]. Most of the staphylococcal strains contain plasmids with 1-60kbp. *S. aureus* plasmids are made up of three classes. Class I is made up of tiny multi copies of plasmids per cell carrying resistance genes. The plasmids in this class do not have transposons nor prophages. Class II plasmids are known to be larger in size and they appear in lesser ~~copy~~ numbers. This class of plasmids includes the penicillinase, aminoglycosides resistance plasmids. Class III plasmids consist of bigger plasmids which carry conjugative transfer genes. The class III plasmids most often possess transposons including many copies of insertion sequences. Before these plasmids get integrated into the host chromosome, they are usually free DNA. They are known to code for some virulence factors such as exfoliative toxin and bacteriocin [9]. They also encode resistance to various organic and inorganic ions that are usually toxic to living cells and thermostable genes [10].

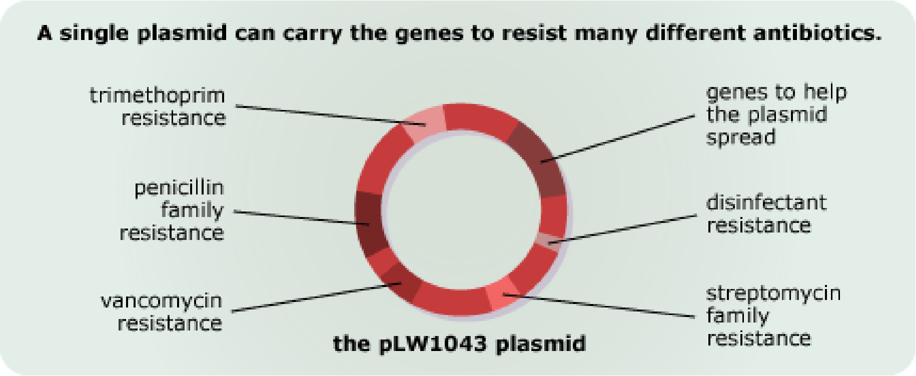

Fig 2: An illustration of a single plasmid (pLW1043) which carries resistance genes to be conferred on different antibiotics.

TRANSFORMATION

This is a horizontal gene transfer mechanism that involves the uptake/intake of free DNA from the environment by a competent bacterium. Some bacteria are readily competent such as *Bacillus subtilis*, *Streptococcus pneumonia* whereas some are not readily competent such as *E. coli*. For bacteria that are not readily competent, competence can be induced chemically (addition of calcium ions) or through electroporation. However, previous studies show that *S. aureus* has low transfer efficiency in taking up free DNA from the environment. This transfer requires phage proteins (tail proteins) and is dependent on the presence of a lytic phage. The phage proteins bind to the cell when DNA is present, and this facilitates the transfer of the DNA into the cell. It has now been shown that *S. aureus* can engage in natural transformation through a bacterial encoded protein [11]. In this case, its ability to take up DNA is being controlled by sigma H factor which is needed for the maintenance of the lysogenic phage. Interestingly, the *S. aureus* sigma H gene does not switch on competence until it is able to duplicate itself and change its promoter region. This impulsive chromosomal arrangement happens at low frequencies so that a tiny proportion of the population will finally express the sigma factor. The expression of the sig H gene also requires specific nutritional requirements, and this was known using a lysogenic bacterium which carries the sig H on a plasmid and was able to take up the plasmid demonstrating that the process is phage independent.

TRANSPOSONS

S. aureus genome is also made up of transposons, insertion sequences, and transposon-like elements. These mobile genetic elements contribute to the evolutionary nature of the bacterium and can be found in the chromosome or in close contact with other mobile genetic element either as single or multiple copies [3]. Insertion sequences are involved in carrying genetic information that is needed for transposition. They don’t encode for resistance but oversee the recombining and upkeep of these resistance genes. Because of this, they are vital in the development of *S. aureus* genome by promoting alterations in the bacterial gene expression. Insertion sequences are also capable of inactivating numerous genes through direct insertion or through a polar effect on close gene transcription [12]. Insertion elements are mostly in a combination form e.g. Insertion sequence 256 and 1nsertion sequence 257 are moderated by Transposons 4001 and 4003 forming a pair which mediates resistance to some antibiotics like gentamicin and kanamycin. The insertion of Insertion 256 and 257 into *S. aureus* chromosome function in the rearrangement of its genome. *Staphylococcus aureus* transposons are little genetic elements which code genes that are resistant to a wide range of antibiotics such as erythromycin, macrolide-lincosamide, spectinomycin, methicillin amongst others. Various copies are found being integrated into plasmids or Staphylococcal cassette chromosome [2].

STAPHYLOCOCCAL CASSETTE CHROMOSOME (SCC)

This is another mobile genetic element of *S. aureus* family. The SCC elements can insert into the 'orfX' gene in the *staphylococcus* chromosome and are responsible for methicillin resistance in *S. aureus*. Its integration requires a specific attachment site (attBscc) in the orfX region. They are classified into two groups; the mec-staphylococcal chromosome and the non -mec staphylococcal chromosome.

Mec- Staphylococcal cassette Chromosome

All MRSA strains contain the SCC mec element. One of the genes it encodes is the mecA gene. The 'mecA' genes confer resistance to all beta-lactam antibiotics most notably the methicillin [13]. *S. aureus* can resist the methicillin antibiotic because of the production of a modified penicillin-binding protein (PBP2a) which has a low affinity for beta-lactams thereby rendering them clinically ineffective. There are various types of SCC mec ranging from type 1 to type XI and they all encode resistance genes (Table 1). About six different classes have been shown about their arrangement and associated genes [14,15].

Non-mec staphylococcal cassette chromosome

These are SCC elements that are not limited to encoding for only methicillin resistance. They also contain virulence or survival determinants and have been identified in *S. aureus*. They share some characteristics with the major mec sec such as the integration into the staphylococcal chromosome, the presence of flanked repeated sequences. Regarding the nomenclature of these elements, it was proposed to include a suffix that describes the gene functions. Examples include SCCcap1 which is a type 1 capsule gene cluster, SCCfur which harbours the resistance for fusidic acid) and SCChg which carriers an operon for mercury resistance [15].

Table 1:

Scc mec types	Mec gene complex	Structure of the mec gene complex	Reference
I	Class B	IS1272 Δ mec RI-mec A IS431	[16]
II	Class A	mec I-mec RI-mec A-IS431	[16]
III	Class A	mec I-mecRI-mec A-1S431	[16]
IV	Class B	IS431-mec A-Δmec RI- IS1272	[17]
V	Class C2	1S431-mecA-Δmec RI-IS431	[18]
VI	Class B	ISI 272-Δmec RI-mec A- IS431	[19]
		IS431-mecA-ΔmecRI IS431	
VII	Class CI	mec- mecRI-mec A-IS431	[20]

VIII	Class A	IS431-mecA Δ mec RI- IS431	[20]
IX	Class C2	IS431-mecA-Δmec RI- IS431	[21]
			[21]
X	Class CI		
XI	Class E	bla Z-mec A-mec RI-mecI	[22]

GENOMIC ISLANDS

They are mobile genetic elements that are present among the core genes of a bacterium either in the chromosome or in a plasmid and they are usually acquired by horizontal gene transfer. [23,24]. Among the *S. aureus* strains that have been sequenced, three families or groups of genomic islands are present [1,25, 26) known as the VSAa, VSAb, and VSAy. The VSAa family carry a lipoprotein gene and a staphylococcal enterotoxin gene (SEI) [27]. The VSAb family encodes for bacteriocin, enterotoxins, hyaluronate lyase in addition to a serine protease gene group [26, 28,29]. The VSAy family comprises of genes coding Beta type phenol soluble modulins (PSM) and a group of staphylococcal enterotoxin gene (SEI). [25]. These islands are usually flanked by 16-20 base pair direct repeats. These repeats are as a result of the integration of the island into a specific site for it to exert its enzymatic function. The genomic island's stability is enhanced by an upstream and downstream flanking of DNA segments. However, most of the islands are not seen to be mobile since they have to degenerate before they can be transferred.

BACTERIOPHAGES

Phages also play a key role in *S. aureus* adaptation and evolution, and they are transferred through horizontal gene transfer. They are also involved in the induction, packaging, and transfer of genomic islands. *S. aureus* phage is classified into three families known as Siphoviridae, Myoviridae, and Podoviridae. The Podoviridae family contains the lytic and chronic phages, and they habour the smallest set of genomes compared to the genomes present in the other families. The Myoviridae also contains the lytic and chronic phages but the Sophoviridae family contains all the temperate phages and they are capable of living for a very long time in the host. The virulent phages present in Myoviridae and Podoviridae are used as a phage therapy in humans against *S. aureus* infections and for food preservation as well. These phages also encode different virulence factors such as staphylokinase, enterotoxins amongst others and these genes are located close to the attachment site in the host chromosome [30]. In *S. aureus* pathogenicity islands (SaPI), helper phages are needed for its mobilization and the helper phages that can perform this function include the temperate phages which belong to the Sophoviridae family [31]. They help to increase the mobility of *S. aureus* pathogenicity island to other staphylococci [32]. The SaPI are not mobile on their own therefore they depend on a helper phage for its replication between different *S. aureus* isolates [30,33]. For example, the Panton Valentine leucocidin is transferred through a helper phage from a PVL-positive to a PVL- negative *S. aureus* strain. It is also of importance to note that only certain helper phages can increase the mobility of certain SaPI.

EXPRESSION OF VIRULENCE OR SURVIVAL DETERMINANTS IN *S. AUREUS*.

S. aureus produces a wide range of virulence factors which helps it to establish infections in humans either by adhering to surfaces or tissues, by invading the immune system and by causing lethal toxic effects to the host. As we have seen from above that some of these virulence factors are encoded by the horizontal gene transfer mechanisms.

PANTON VALENTINE LEUKOCIDIN (PVL)

PVL is encoded by bacteriophages which enables them to be transferred from one organism to another. It is classified as a cytotoxin, one of the beta forming toxins. It has been reported to be present in community-associated methicillin-resistant *S. aureus* (CA-MRSA), a major cause of necrotizing pneumonia. It lyses neutrophils, leading to the release of enzymes that damages the surrounding tissues [34].

ENTEROTOXINS

S. aureus enterotoxins belong to a family of pyrogenic toxin superantigens (SAG). These superantigens bind to the MHC Class II molecules in host animals, therefore, forming a complex with the T cell receptor. The formation of the complex activates the T cell to proliferate in a non- specific manner resulting in host immune suppression [34-35]. The superantigen genes are the major cause of acute clinical syndromes such as toxic shock syndrome, food poisoning etc. The superantigens have been classified into two groups: classical and new enterotoxins (Argudin *et al*., 2010, Hennekinne *et al*., 2012, Wilson *et al*., 2011). However, about 23 types of *S. aureus* enterotoxins have been reported and they are all encoded on horizontal gene transfer mechanisms [10,36,37]. Moreover, they have also contributed to the evolution of *S. aureus* as a pathogen. Some of these enterotoxins are components of the enterotoxin gene cluster which is found on genomic islands.

TOXIC SHOCK SYNDROME TOXIN (TSST)

TSST is a superantigen that is produced by a small percentage of *Staphylococcus aureus* isolates. Once these toxins are released into the bloodstream, they cause the over stimulation of the immune system which subsequently leads to symptoms of toxic shock syndrome (TSST). They are however known to live in the vagina of women that are infected which is highly encouraged using a tampoon [38]. They are also present in other sites of the body. It has been reported that children, men, and non-menstruating women also have the potential of developing TSST. TSST also has the capacity to stimulate the release of cytokines enhancing the leakage of endothelial cells in low concentrations thereby producing a cytotoxic effect at high concentrations. It also causes systemic infection by penetrating mucosal barriers even though the infection is localized in the vagina or at any other location in the body.

STAPHYLOKINASE

This is another virulence factor of *S. aureus* which is encoded by lysogenic bacteriophage. It is present in the DNA of some bacteriophage and can be transferred from one organism to another. Staphylokinase interacts with plasminogen and α-defensins which enhances *S. aureus* invasion into the host tissues. It has been shown that *S. aureus* that carries the staphylokinase- plasminogen complex on their surface can lyse extracellular matrix by activating the metalloproteinases present in the host. Staphylokinases also encourages bacterial resistance in *S. aureus* especially to phagocytosis which is mediated by the interaction of HNPs (Human neutrophil peptides), an important part of the innate immunity. Most importantly, the production of staphylokinase enables *S. aureus* to persist longer on the host skin and mucosa [38].

However, there are several other virulence factors produced by *S. aureus* which makes it a versatile pathogen, having the ability to induce a wide range of infections. (Table 2).

Table 2: Other virulence factors of *S. aureus* [38].

Virulence factors	Biological effects
Structural components	
Capsule	Inhibits chemotaxis and phagocytosis; inhibits proliferation of mononuclear cells
Slime layer	Facilitates adherence to foreign bodies; inhibits phagocytosis
Teichoic acid	Binds to fibronectin.
Protein A	Inhibits antibody-mediated clearance by binding to IgG
Toxins	
Exfoliative toxins	Serine proteases that split the intercellular bridges in the stratum granulosum epidermis

Cytotoxins	Toxic for many cells including erythromycin, fibroblasts, leucocytes, macrophages and platelets.
Enzymes	
Coagulase	Converts fibrinogen to fibrin
Hyaluronidase	Hydrolyses hyaluronic acids in connective tissues, promoting the spread of staphylococci in tissues
fibrinolysin	Dissolves fibrin clots
Lipases	Hydrolyses lipids
Nucleases	Hydrolyses DNA

ANTIBIOTIC RESISTANCE IN *STAPHYLOCOCCUS AUREUS*

Several antimicrobial resistance genes are also carried on the mobile genetics' elements discussed such as transposons and plasmids. The resistance genes confer resistance to a wide range of antibiotics such as penicillin, macrolides, aminoglycosides, tetracyclines, chloramphenicol, linezolid etc. The capacity of *S. aureus* to easily acquire these resistance genes is one of the characteristics that make it successful in establishing infection, thereby making the control of infection more difficult and complicated. *S. aureus* has been shown to develop resistance to β lactam antibiotics such as penicillin, methicillin and glycopeptide such as vancomycin amongst others.

BETA-LACTAM RESISTANCE

S. aureus resistance to beta-lactam antibiotics was first seen in penicillin which was mediated by the production of penicillinase (a beta-lactamase) which hydrolyses the beta-lactam ring present in penicillin. Thereby rendering it ineffective. However, methicillin was introduced to subdue penicillin resistance, but it was not possible because *S. aureus* has a way of evolving and adapting to new or nearly or classes of antibiotics which were used to treat it. Therefore, Methicillin-resistant *S. aureus* (MRSA) strains evolved and this has been shown to be mediated by the mecA gene. The mecA gene is present on the mobile genetic element which is known as staphylococcal cassette chromosome (SCCmec) [39]. The methicillin resistance is not acquired during infection as it has not been observed. However, studies have shown the horizontal transfer of the staphylococcal cassette chromosome at the time of infection giving rise to the emergence of methicillin-resistant *S. aureus* strains [40]. The MRSA strains become resistant to beta-lactam antibiotics by producing a modified penicillin-binding protein (PBP2a) which has a low affinity for beta-lactam antibiotics thereby rendering them clinically ineffective. MRSA has been identified in hospitals; Hospital-associated MRSA (HA-MRSA). Several clones accounted for most of the HA-MRSA include ST22, ST36, ST239, and ST5. These clones successfully evolve and establish themselves mostly due to the intensive use of antibiotics, mutations and poorly registered regimens [41]. MRSA has also been identified in communities; Community associated MRSA (CA-MRSA). Previously, CA-MRSA greatly affects immunocompromised individuals with predisposing factors and those with health care exposure. However, in recent times, it affects healthy hosts particularly children and middle-aged adults. This could be attributed to increased transmission of infection, activation of more virulence genes and an increased pathogenicity during infection [40]. Interestingly, MRSA has now been identified in animals: Livestock-associated MRSA (LA-MRSA) as a cause of infection in humans. Infections due to LA-MRSA occur in persons who have close access to farm animals such as pigs, poultry, dogs, cats etc. it affects mostly the farmers and veterinarians. LA-MRSA was identified in a cow in 1972. In 2005, CC398 MRSA lineage was reported in pigs in Europe showing that the livestock was a good reservoir for MRSA. The main reservoir for CC398 is in pigs but it has also been found in veal calves, poultry, horses, dogs, cats and to an extent, in cows. There has been a general agreement that CC398 is increasing worldwide although information on prevalence rate has been difficult to obtain. Other complex MRSA lineages in livestock that have been found include the CC9, CC1, CC5, CC97, CC121, CC130, and ST 425 [42]. It is of interest that a human CA-MRSA type descended from bovine MSSA after bovine-host adaptation [43-44]. Risk factors for its transmission are not fully understood although one of the important risk factors is the trade of pigs that are MRSA positive. However, some farmers have been found positive even without buying new animals before the MRSA CC398 was detected. In these exceptional cases, it could be that they become MRSA positive from MRSA-positive humans like veterinarians. The use of antibiotics amongst farmers most notably the beta-lactams and tetracyclines also induce selective pressure on the clones [45]. The most crucial risk factor for LA-MRSA in humans is the close occupational access with animals which are MRSA positive which depends on the contact time and intensity. In a study at Denmark in 2013, most of the new cases, (about, 70%) that were reported had to do with direct contact with pigs, (17%) were linked with members of the house who had close access to pigs while the remaining 13% where those who had no contact with pigs but lived in places that had high pig density indicating that transmission takes place probably from the people working at the farms or through access with farm surroundings itself [46]. However, the comparative contribution of transmission whether through the surroundings of the farm or through humans hasn't been elucidated. Although, from the knowledge of *S. aureus* transmission in other settings, human-human contact is predominant [47]. It has also been shown that MRSA has been found on meat which raises the likelihood of MRSA being acquired through the food chain. From the epidemiology of LA-MRSA, it clearly indicates that meat is not one of the routes of transmission [46]. The increasing rate of LA-MRSA in pigs including humans who have close access with pigs has resulted in an increase in cases in the communities, especially in the immunocompromised persons. Therefore, it is possible that increasing numbers of infections caused by LA-MRSA will be seen unless the epidemic is monitored. Furthermore, if the human carriage of LA-MRSA clone is increased, then it would lead to a greater chance of these clones undergoing adaptation which will enhance human-human transmissibility. Measures to reduce the increasing reservoir in pigs is highly needed.

GLYCOPEPTIDE RESISTANCE

MRSA strains have also developed resistance to glycopeptide antibiotics such as vancomycin. Vancomycin acts by binding to the D-ala D-ala residues of the peptidoglycan thereby inhibiting cell wall synthesis. It is used in the treatment of infections caused by MRSA such as osteomyelitis, endocarditis, bacteraemia [48]. Two mechanisms of vancomycin resistance have evolved in *Staphylococcus aureus*. The first resistance to evolve were *S. aureus* isolates which had decreased susceptibility to vancomycin known as vancomycin- intermediate resistant *S. aureus* (VISA) strain. (with a MIC of 8µg/ml). These strains have an excess binding site which can 'confine' the antibiotic [49]. They also show characteristics of a decreased autolysis, attenuation of virulence and thickened cell wall [50]. The thickness of the VISA cell wall was first reported in a 4-month-old infant who had a heart surgery; it showed that the VISA strain known as 'Mu50' which was isolated from the discharge at the surgery site had a cell wall that was two times thick as the control strains seen under the microscope. [49] demonstrated this and showed that the thickness of the cell wall was a common characteristic of the VISA isolates. Due to the thickened cell wall, present, it makes these strains more resistant because the antibiotic is being 'confined' by the free D-ala residues in the cell wall [49]. Furthermore, VISA strains also show decreased autolytic activity. It has been proved when cell assays were carried out in the VISA strain, 'Mu50' [51]. The reduced autolysis has been suggested to may have contributed to the thickened cell wall thereby preventing the antibiotic from getting into its site of action. However, the acquisition of resistance to antibiotics among VISA strains could be a disadvantage towards its virulence [52]. Animal~~s~~ models have been used to ascertain the extent of VISA pathogenesis; in an insect model, it was shown that the clinical VISA isolates had decreased virulence [53,50]. Also, in a rat model, the VISA isolate was shown to have a decreased virulence likewise in a mouse sepsis model, the VISA isolates had reduced infectivity and there was no capacity to cause liver abscesses. The VISA strains tend not to cause acute infections because of its attenuated virulence, however; this may be a 'sneaky' strategy to evade host immune responses [54]. In addition, multiple mutations in different loci with VISA have also emerged and has been shown to contribute to its level of resistance to antibiotics. To identify these mutations, whole genome sequencing of the isolates has been carried out and it showed the presence of several mutations which were associated with resistance to other antibiotics such as β lactams, rifampicin including vancomycin [55]. Recently, a second-high level vancomycin-resistant *S. aureus* (VRSA) emerged. The first case of VRSA was seen in a patient who was diabetic and had a co-infection of *Staphylococcus aureus* and *Enterococcus faecalis* [56]. Evidence has shown that resistance in MRSA strain was mediated by the acquisition of the Tn1546 transposon which encodes for vancomycin resistance factor (van A) in the *Enterococcus feacalis* strain. However, there hasn't been a person-person spread, therefore, the importance of van-mediated resistance hasn't been fully elucidated [57].

Mechanism of resistance of *S. aureus* to other antibiotics are also common and have been summarised in (Table 3). It is also important to note that resistance to new drugs like linezolid and daptomycin has been shown amongst MRSA in clinical settings.

Table 3: Mechanisms of *S. aureus* resistance to other antimicrobials [58, 3]

Antibiotic	Resistance genes	Mechanism of resistance	Location
Quinolones	par C, (a component of topoisomerase IV), gyrA,gyrB(a component of gyrase).	mutations in the QRDR region	Chromosome
Aminoglycosides	Modifying enzymes (acetyltra transferase,	Acetylating or phosphorylating	Plasmids

	phosphotrans ferase)	enzymes	
Trimethoprim- Sulfamethoxazole	Sulfonamide: dihydropteroate synthase, TMP; dihydrofolate reductase	Acetylating or phosphorylating enzymes overproduction of para amino benzoic acid decreased affinity for hydrofolate reductase.	Plasmids
Tetracyclines	Tetracyclines tetracycline, doxycycline and minocycline, TetM	Binding to the ribosome and removing the drug from its binding site.	Plasmids; Transposons
		. .	
Erythromycin	msrA (efflux protein), erm (ribosomal methylase)	efflux pump and alteration of 23Srna Transposons	Plasmids
Linezolid	Cfr	methylation of the 23S rRna that interferes with Ribosomal binding.	Plasmid

Daptomycin	mprF	increasing synthesis of total LPG translocation and positive net charges on the cell membrane	Chromosomal

The resistance of *Staphylococcus aureus* to beta lactam antibiotics as well as other antibiotics such as Tetracyclines, Lincosamides and Gentamicin has led to the development of newer drugs which are now exploited for the treatment of infections caused by the organism. However, some of which are still undergoing clinical trials. Some of the promising molecules such as Triclosan etc have been designed to target fatty acid biosynthesis, cell division protein, the Clp P protease activator and the Lipid A moiety of lipid II.[59].

CONCLUSION

S. aureus is a successful pathogen due to its versatility and evolutionary nature and this has contributed to its success in invading the human immune system thereby establishing an infection. This has been seen from its ability to cause a wide range of mild infections and life-threatening diseases in humans. There is a close relationship between the horizontal gene transfer mechanisms and its virulence factors. These mechanisms not only encode for resistance but also encodes for virulence determinants which are responsible for causing infections in humans. This is important for our knowledge of how *Staphylococcus aureus* is being shaped by selective pressures. This also allows us to understand the versatility of S. *aureus* and discover ways by which its evolutionary nature can be genetically manipulated to control infection and reduce its level of resistance to multiple antibiotics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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