

## Review Article

STAPHYLOCOCCUS AUREUS; A SUCCESSFUL PATHOGEN?

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### 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.

### 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 example; 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.

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*S. aureus* is gradually evolving in animals (Livestock-associated methicillinresistant *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

Comment [P.J.3]: Methicillin resistant

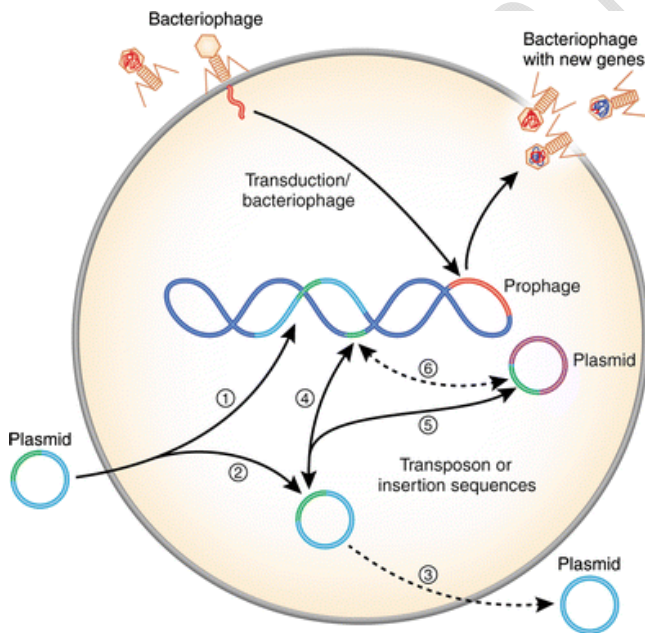
37 encodes different virulence factors such as toxins, enzymes which are mediated by horizontal  
38 gene transfer, and this, however, contributes to the emergence of antibiotic resistance to  
39 multiple classes of antimicrobial drugs.

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

43

#### 44 HORIZONTAL GENE TRANSFER IN *S. AUREUS*.

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



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54 **Fig.1:** The Acquisition of mobile genetic elements by *Staphylococcus aureus*: 1.  
55 Incorporation of plasmids into a bacterial DNA. 2. Plasmid integrated into the chromosome  
56 of a bacterium. 3. Plasmids as an independent circular DNA. 4. Transfer of a transposon

57 between plasmid and genomic DNA. 5. Transfer of transposons between plasmids. 6.  
58 Transfer of transposons from a genomic DNA to a plasmid. [3].

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## 62 **GENERALIZED TRANSDUCTION**

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

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

## 82 **CONJUGATION AND PLASMIDS**

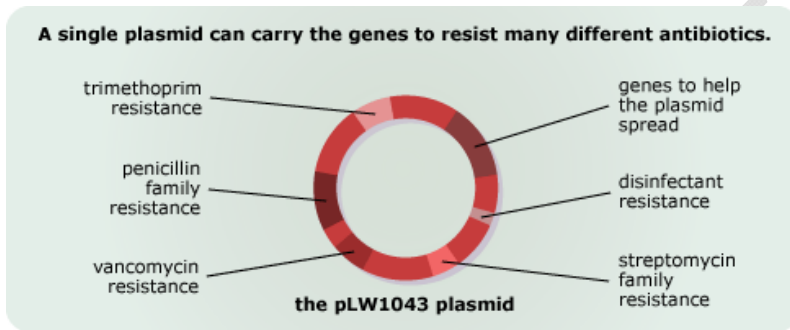
83 This is a mechanism whereby DNA is transferred from one cell to another through a pilus or  
84 a pore [6]. In *S. aureus*, it is assumed that the pores are made between cells that are in close  
85 contact with each other because the pili are not seen. A range of plasmids carrying resistance  
86 genes is transferred during the process of conjugation. as shown in Fig 2. (Adopted by  
87 evolution website). These conjugative plasmids are too large, and they carry an extensive  
88 range of antibiotic resistance genes and virulence factors which they transfer from one  
89 organism to another [7,8]. Most of the staphylococcal strains contain plasmids with 1-60kbp.  
90 *S. aureus* plasmids are made up of three classes. Class I is made up of tiny multi copies of  
91 plasmids per cell carrying resistance genes. The plasmids in this class do not have  
92 transposons nor prophages. Class II plasmids are known to be larger in size and they appear in

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93 lesser copy numbers. This class of plasmids includes the penicillinase, aminoglycosides  
94 resistance plasmids. Class III plasmids consist of bigger plasmids which carry conjugative  
95 transfer genes. The class III plasmids most often possess transposons including many copies  
96 of insertion sequences. Before these plasmids get integrated into the host chromosome, they  
97 are usually free DNA. They are known to code for some virulence factors such as exfoliative  
98 toxin and bacteriocin [9]. They also encode resistance to various organic and inorganic ions  
99 that are usually toxic to living cells and thermostable genes [10].

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

## 106 TRANSFORMATION

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

121 sigma factor. The expression of the sig H gene also requires specific nutritional requirements,  
122 and this was known using a lysogenic bacterium which carries the sig H on a plasmid and  
123 was able to take up the plasmid demonstrating that the process is phage independent.

## 124 TRANSPOSONS

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

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## 143 STAPHYLOCOCCAL CASSETTE CHROMOSOME (SCC)

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

### 149 Mec- Staphylococcal cassette Chromosome

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

**Comment [P.J.5]:** Please provide a diagram showing the attachment site in the orfX region

**Comment [P.J.6]:** SCC mec

157 **Non-mec staphylococcal cassette chromosome**

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

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167

168 **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-IS431	[16]
IV	Class B	IS431-mec A-Δmec RI- IS1272	[17]
V	Class C2	IS431-mecA-Δmec RI-IS431	[18]
		ISI 272-Δmec RI-	

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		mec A- IS431	
VI	Class B		[19]
		IS431-mecA- ΔmecRI IS431	
VII	Class CI		[20]
		mec- mecRI-mec A-IS431	
VIII	Class A		[20]
		IS431-mecA Δ mec RI- IS431	
IX	Class C2		[21]
		IS431-mecA- Δmec RI- IS431	
			[21]
X	Class CI		
XI	Class E	bla Z-mec A-mec RI-mecI	[22]

172 **GENOMIC ISLANDS**

173 They are mobile genetic elements that are present among the core genes of a bacterium either  
174 in the chromosome or in a plasmid and they are usually acquired by horizontal gene transfer.  
175 [23,24]. Among the *S. aureus* strains that have been sequenced, three families or groups of  
176 genomic islands are present [1,25, 26) known as the VSAA, VSAb, and VSAY. The VSAA  
177 family carry a lipoprotein gene and a staphylococcal enterotoxin gene (SEI) [27]. The VSAb  
178 family encodes for bacteriocin, enterotoxins, hyaluronate lyase in addition to a serine  
179 protease gene group [26, 28,29]. The VSAY family comprises of genes coding Beta type  
180 phenol soluble modulins (PSM) and a group of staphylococcal enterotoxin gene (SEI). [25].  
181 These islands are usually flanked by 16-20 base pair direct repeats. These repeats are as a  
182 result of the integration of the island into a specific site for it to exert its enzymatic function.  
183 The genomic island's stability is enhanced by an upstream and downstream flanking of DNA  
184 segments. However, most of the islands are not seen to be mobile since they have to  
185 degenerate before they can be transferred.

186 **BACTERIOPHAGES**

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

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

208 *S. aureus* produces a wide range of virulence factors which helps it to establish an infection  
209 in humans either by adhering to surfaces or tissues, by invading the immune system and by

Comment [P.J.7]: Establish infections

210 causing lethal toxic effects to the host. As we have seen from above that some of these  
211 virulence factors are encoded by the horizontal gene transfer mechanisms.

#### 212 **PANTON VALENTINE LEUKOCIDIN (PVL)**

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

#### 218 **ENTEROTOXINS**

219 *S. aureus* enterotoxins belong to a family of pyrogenic toxin superantigens (SAG). These  
220 superantigens bind to the MHC Class II molecules in host animals, therefore, forming a  
221 complex with the T cell receptor. The formation of the complex activates the T cell to  
222 proliferate in a non-specific manner resulting in host immune suppression [34-35]. The  
223 superantigen genes are the major cause of acute clinical syndromes such as toxic shock  
224 syndrome, food poisoning etc. The superantigens have been classified into two groups:  
225 classical and new enterotoxins (Argudin *et al.*, 2010, Hennekinne *et al.*, 2012, Wilson *et al.*,  
226 2011). However, about 23 types of *S. aureus* enterotoxins have been reported and they are all  
227 encoded on horizontal gene transfer mechanisms [10,36,37]. Moreover, they have also  
228 contributed to the evolution of *S. aureus* as a pathogen. Some of these enterotoxins are  
229 components of the enterotoxin gene cluster which is found on genomic islands.

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#### 230 **TOXIC SHOCK SYNDROME TOXIN (TSST)**

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

#### 241 **STAPHYLOKINASE**

242 This is another virulence factor of *S. aureus* which is encoded by lysogenic bacteriophage. It  
243 is present in the DNA of some bacteriophage and can be transferred from one organism to  
244 another. Staphylokinase interacts with plasminogen and  $\alpha$ -defensins which enhances *S.*  
245 *aureus* invasion into the host tissues. It has been shown that *S. aureus* that carries the

246 staphylokinase- plasminogen complex on their surface can lyse extracellular matrix by  
247 activating the metalloproteinases present in the host. Staphylokinases also encourages  
248 bacterial resistance in *S. aureus* especially to phagocytosis which is mediated by the  
249 interaction of HNPs (Human neutrophil peptides), an important part of the innate immunity.  
250 Most importantly, the production of staphylokinase enables *S. aureus* to persist longer on the  
251 host skin and mucosa [38].

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

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

255 <u>Virulence factors</u>	<u>Biological effects</u>
<b>Structural components</b>	
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
<b>Toxins</b>	
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

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### **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

268 develop resistance to  $\beta$  lactam antibiotics such as penicillin, methicillin and glycopeptide  
269 such as vancomycin amongst others.  
270

## 271 **BETA-LACTAM RESISTANCE**

272 *S. aureus* resistance to beta-lactam antibiotics was first seen in penicillin which was mediated  
273 by the production of penicillinase (a beta-lactamase) which hydrolyses the beta-lactam ring  
274 present in penicillin. Thereby rendering it ineffective. However, methicillin was introduced to  
275 subdue penicillin resistance, but it was not possible because *S. aureus* has a way of evolving  
276 and adapting to new or nearly or classes of antibiotics which were used to treat it. Therefore,  
277 Methicillin-resistant *S. aureus* (MRSA) strains evolved and this has been shown to be  
278 mediated by the *mecA* gene. The *mecA* gene is present on the mobile genetic element which  
279 is known as staphylococcal cassette chromosome (SCCmec) [39]. The methicillin resistance  
280 is not acquired during infection as it has not been observed. However, studies have shown the  
281 horizontal transfer of the staphylococcal cassette chromosome at the time of infection giving  
282 rise to the emergence of methicillin-resistant *S. aureus* strains [40]. The MRSA strains  
283 become resistant to beta-lactam antibiotics by producing a modified penicillin-binding  
284 protein (PBP2a) which has a low affinity for beta-lactam antibiotics thereby rendering them  
285 clinically ineffective. MRSA has been identified in hospitals; Hospital-associated MRSA  
286 (HA-MRSA). Several clones accounted for most of the HA-MRSA include ST22, ST36,  
287 ST239, and ST5. These clones successfully evolve and establish themselves mostly due to the  
288 intensive use of antibiotics, mutations and poorly registered regimens [41]. Interestingly,  
289 MRSA has now been identified in animals: Livestock-associated MRSA (LA-MRSA) as a  
290 cause of infection in humans. Infections due to LA-MRSA occur in persons who have close  
291 access to farm animals such as pigs, poultry, dogs, cats etc. it affects mostly the farmers and  
292 veterinarians. LA-MRSA was identified in a cow in 1972. In 2005, CC398 MRSA lineage  
293 was reported in pigs in Europe showing that the livestock was a good reservoir for MRSA.  
294 The main reservoir for CC398 is in pigs but it has also been found in veal calves, poultry,  
295 horses, dogs, cats and to an extent, in cows. There has been a general agreement that CC398  
296 is increasing worldwide although information on prevalence rate has been difficult to obtain.  
297 Other complex MRSA lineages in livestock that have been found include the CC9, CC1,  
298 CC5, CC97, CC121, CC130, and ST 425 [42]. It is of interest that a human CA-MRSA type  
299 descended from bovine MSSA after bovine-host adaptation [43-44]. Risk factors for its  
300 transmission are not fully understood although one of the important risk factors is the trade of  
301 pigs that are MRSA positive. However, some farmers have been found positive even without  
302 buying new animals before the MRSA CC398 was detected. In these exceptional cases, it  
303 could be that they become MRSA positive from MRSA-positive humans like veterinarians.  
304 The use of antibiotics amongst farmers most notably the beta-lactams and tetracyclines also  
305 induce selective pressure on the clones [45]. The most crucial risk factor for LA-MRSA in  
306 humans is the close occupational access with animals which are MRSA positive which  
307 depends on the contact time and intensity. In a study at Denmark in 2013, most of the new

308 cases, (about, 70%) that were reported had to do with direct contact with pigs, (17%) were  
309 linked with members of the house who had close access to pigs while the remaining 13%  
310 where those who had no contact with pigs but lived in places that had high pig density  
311 indicating that transmission takes place probably from the people working at the farms or  
312 through access with farm surroundings itself [46]. However, the comparative contribution of  
313 transmission whether through the surroundings of the farm or through humans hasn't been  
314 elucidated. Although, from the knowledge of *S. aureus* transmission in other settings, human-  
315 human contact is predominant [47]. It has also been shown that MRSA has been found on  
316 meat which raises the likelihood of MRSA being acquired through the food chain. From the  
317 epidemiology of LA-MRSA, it clearly indicates that meat is not one of the routes of  
318 transmission [46]. The increasing rate of LA-MRSA in pigs including humans who have  
319 close access with pigs has resulted in an increase in cases in the communities, especially in  
320 the immunocompromised persons. Therefore, it is possible that increasing numbers of  
321 infections caused by LA-MRSA will be seen unless the epidemic is monitored. Furthermore,  
322 if the human carriage of LA-MRSA clone is increased, then it would lead to a greater chance  
323 of these clones undergoing adaptation which will enhance human-human transmissibility.  
324 Measures to reduce the increasing reservoir in pigs is highly needed.

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#### 326 **GLYCOPEPTIDE RESISTANCE**

327 MRSA strains have also developed resistance to glycopeptide antibiotics such as  
328 vancomycin. Vancomycin acts by binding to the D-ala D-ala residues of the peptidoglycan  
329 thereby inhibiting cell wall synthesis. It is used in the treatment of infections caused by  
330 MRSA such as osteomyelitis, endocarditis, bacteraemia [48]. Two mechanisms of  
331 vancomycin resistance have evolved in *Staphylococcus aureus*. The first resistance to evolve  
332 were *S. aureus* isolates which had decreased susceptibility to vancomycin known as  
333 vancomycin- intermediate resistant *S. aureus* (VISA) strain. (with a MIC of 8µg/ml). These  
334 strains have an excess binding site which can 'confine' the antibiotic [49]. They also show  
335 characteristics of a decreased autolysis, attenuation of virulence and thickened cell wall [50].  
336 The thickness of the VISA cell wall was first reported in a 4-month-old infant who had a  
337 heart surgery; it showed that the VISA strain known as 'Mu50' which was isolated from the  
338 discharge at the surgery site had a cell wall that was two times thick as the control strains  
339 seen under the microscope. [49] demonstrated this and showed that the thickness of the cell  
340 wall was a common characteristic of the VISA isolates. Due to the thickened cell wall,  
341 present, it makes these strains more resistant because the antibiotic is being 'confined' by the  
342 free D-ala residues in the cell wall [49]. Furthermore, VISA strains also show decreased  
343 autolytic activity. It has been proved when cell assays were carried out in the VISA strain,  
344 'Mu50' [51]. The reduced autolysis has been suggested to may have contributed to the  
345 thickened cell wall thereby preventing the antibiotic from getting into its site of action.  
346 However, the acquisition of resistance to antibiotics among VISA strains could be a  
347 disadvantage towards its virulence [52]. **Animals** models have been used to ascertain the

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348 extent of VISA pathogenesis; in an insect model, it was shown that the clinical VISA isolates  
 349 had decreased virulence [53,50]. Also, in a rat model, the VISA isolate was shown to have a  
 350 decreased virulence likewise in a mouse sepsis model, the VISA isolates had reduced  
 351 infectivity and there was no capacity to cause liver abscesses. The VISA strains tend not to  
 352 cause acute infections because of its attenuated virulence, however; this may be a ‘sneaky’  
 353 strategy to evade host immune responses [54]. In addition, multiple mutations in different  
 354 loci with VISA have also emerged and has been shown to contribute to its level of resistance  
 355 to antibiotics. To identify these mutations, whole genome sequencing of the isolates has been  
 356 carried out and it showed the presence of several mutations which were associated with  
 357 resistance to other antibiotics such as  $\beta$  lactams, rifampicin including vancomycin [55].  
 358 Recently, a second-high level vancomycin-resistant *S. aureus* (VRSA) emerged. The first  
 359 case of VRSA was seen in a patient who was diabetic and had a co-infection of  
 360 *Staphylococcus aureus* and *Enterococcus faecalis* [56]. Evidence has shown that resistance in  
 361 MRSA strain was mediated by the acquisition of the Tn1546 transposon which encodes for  
 362 vancomycin resistance factor (van A) in the *Enterococcus faecalis* strain. However, there  
 363 hasn’t been a person-person spread, therefore, the importance of van-mediated resistance  
 364 hasn’t been fully elucidated [57].

Comment [P.J.10]: strains

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

368 **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 (acetyltransferase, phosphotransferase)	Acetylating or phosphorylating enzymes	Plasmids

Trimethoprim- Sulfamethoxazole	Sulfonamide: dihydropteroate synthase, dihydrofolate reductase	TMP; 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 23S rna Transposons	Plasmids
Linezolid	Cfr	methylation of the 23S rRNA that interferes with Ribosomal binding.	Plasmid
Daptomycin	mprF	increasing synthesis of total LPG	Chromosomal

translocation and  
positive net  
charges on the  
cell membrane

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## 370 CONCLUSION

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

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## 382 CONFLICT OF INTEREST

383 The authors declare no conflict of interest.

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