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METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS:* RESISTANCE GENES AND THEIR REGULATION

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ABSTRACT

Mobile genetic elements like transposons and plasmids, along with antibiotic resistant genes, play an important role in sensitising organisms to various antibiotics. More specifically, in methicillin resistant *Staphylococcus aureus*, resistance to ß-lactams is mediated by *mecA,* which encodes for penicillin binding proteins (PBP2a) and *blaZ* gene encoding for ß-lactamase. The regulators, *mecR-mecI* and *blaR-blaI* of *mec* and *bla* respectively, encodes for inducer-repressor combinations and co-regulates PBP2a and ß-lactamase production via MecR1 and BlaR1. In vancomycin intermediate resistant *Staphylococcus aureus*, the resistance is associated with thickened peptidoglycan layer which sequesters vancomycin and prevents it from reaching the site of cell division. High level vancomycin resistance is encoded by *van* operon which is regulated by *vanRS* genes via VanRS system. In clinical *S. aureus* isolates, methylation and dimethylation of ribosomal rRNA by methyltransferases is the most frequent mechanism of resistance to macrolids. Expression of *ermC* gene is induced by macrolide antibiotics, under the control of a 19-codon ORF, *ermCL.* In addition to the primary determinants, additional factors like temperature, pH and nutrients are also important for high-level antibiotic resistant phenotypes. In short, antibiotic resistance in *Staphylococcus aureus* is the combined effect of primary genetic determinants, additional genes and other environmental factors.

Keywords: Antibiotic resistance, Methicillin resistant *Staphylococcus aureus*, *mecA-blaZ*, *van* operon, Gene regulation

INTRODUCTION

Staphylococcus aureus, which was first isolated by Alexander Ogston in 1880s, is known to cause post-operative wound infections. The mortality rate of the individuals, due to *S. aureus* infections was around 80% before the introduction of penicillin[1].The first penicillin resistant *S. aureus* was isolated from clinical environment in 1942. The problem of penicillin resistance was later circumvented by the introduction of methicillin. In 1961, methicillin resistant *Staphylococcus aureus* (MRSA) made an appearance, probably due to the acquisition of the *mecA* gene, leaving vancomycin as the drug-of-last resort to treat it. Since resistance was not because of the antibiotic destruction by enzyme ß-lactamase, the resistance was termed as "intrinsic"[2]. Increased outbreaks had subsequently been reported from many countries after the emergence of MRSA as nosocomial pathogen in the early 1960s. There were reports of life-threatening sepsis, endocarditis, and osteomyelitis caused by this organism[3]. Dissemination of clones of various hospital-associated MRSA (HA-MRSA) has been found worldwide during last five decades. The clones of community associated MRSA (CA- MRSA) also increased worldwide, appearing both in the community and healthcare facilities. Introduction of vancomycin to combat MRSA proved ineffective, as strains resistant to this antibiotic emerged rapidly. The quick and high bacterial replication rate was conducive in spreading these "superbugs" everywhere.

The issue of pathogens, continuously developing resistance to various classes of antibiotics can be better understood and addressed at the genetic level. The remarkable observation is that the pathogen resistances, associated with wide varieties of bacterial toxins, especially under clinical settings, are encoded by a set of mobile genetic elements [4]. *S. aureus* DNA codons, for super antigen toxins, reside as mobile genetic elements in novel pathogenic islands in its genome[5]. The gene for enterotoxins D and A are encoded by plasmids and prophages respectively [6, 7]. In *S aureus,* scientists have identified mobile genetic elements of 15-20kb which are called staphylococcal pathogenicity islands (SAPIs)[8]. These are mobilized at high frequencies by certain staphylococcal phages. The prototype of this family is SAPII. Its genetic analysis was done by construction of a derivative, with *tetM* inserted into *tst,* which is the gene encoding for toxic shock syndrome toxin-1 (TSST-1).

Mode of action of ß-lactam antibiotics

Growth, cytoplasm content, continual synthesis, polymerization of peptidoglycan precursor, along with cell wall degradation and turnover is essential in order to maintain cell shape. The main role of most cell wall targeted antibiotics is to disrupt or block peptidoglycan biosynthesis[9]. In *S. aureus,* a mobile genomic island that contains a gene, *mecA,* is called staphylococcus cassette chromosome (*SCC mec*)[10]. This gene codes for penicillin-binding proteins (PBPs). The latter catalyzes the crosslinking of peptidoglycan in bacterial cell wall which is the last step of cell wall synthesis. ß-lactam antibiotics are structural analogs of peptidoglycans which inactivate the PBP's by covalently binding to their serine active sites. These antibiotics acylate the transpeptidase-active sites of PBPs, preventing them from acting on their peptidoglycan precursors, thus inhibiting cell wall synthesis [11].

Genetic elements of antibiotic resistance in *S. aureus*

The genome sequence of MRSA has revealed that it is composed of a complex mixture of genes. Most of the antibiotic resistance genes are carried either by plasmids or by mobile genetic elements which includes a unique resistance island. Pathogenicity islands identified in the genome of *S. aureus* belongs to three classes, *viz.,* exotoxin islands, toxic-shock-syndrome toxin islands and enterotoxin islands [12]**.** The length of the *mecA* gene, which is a mobile genomic island, is 2.1kb[10]. The genetic elements of this staphylococcus cassette chromosome (SCC *mec)* are of types I to VII and ranging from 20.9 to 66.9 kb (Fig. 1 & Table 1). The genes of cassette chromosome recombinases (*ccr*) are located on all types of *SCCmec* and encode for invertase/resolvase class of enzymes. These are involved in either integration of SCC*mec* into or excision of SCC*mec* from, *S. aureus* genome at the specific site called the SCC*mec* attachment site (*attBscc*). These processes occur at the 3' end of an open reading frame (*orf*X)[13]. The types of *mec* complex and *ccr* genes determine the class of SCC*mec*. The regions which are not part of the *mec* complex and *ccr* genes are called Junkyard (J) region. Thus, SCC*mec* element mainly consists of J3-*mec*-J2-*ccr*-J1 sequence[14, 15,16, 17, 18,19.]

In MRSA strains, near *pur-nov-his* gene cluster, an additional chromosomal DNA of approximately 30 to 50 kb of *mec* has been found[26, 27]. *mecI* and *mecR1* are regulatory elements controlling *mecA,* which is the structural gene encoding for a 76-kDa PBP 2a.

The *mecA* gene determines methicillin resistance in *S. aureus* and in susceptible strains, there is no *mecA* homolog. PBPs are membrane bound DD-peptidases that have evolved from serine proteases, and their biochemical activity is mechanistically similar to that of the serine proteases [28, 29].

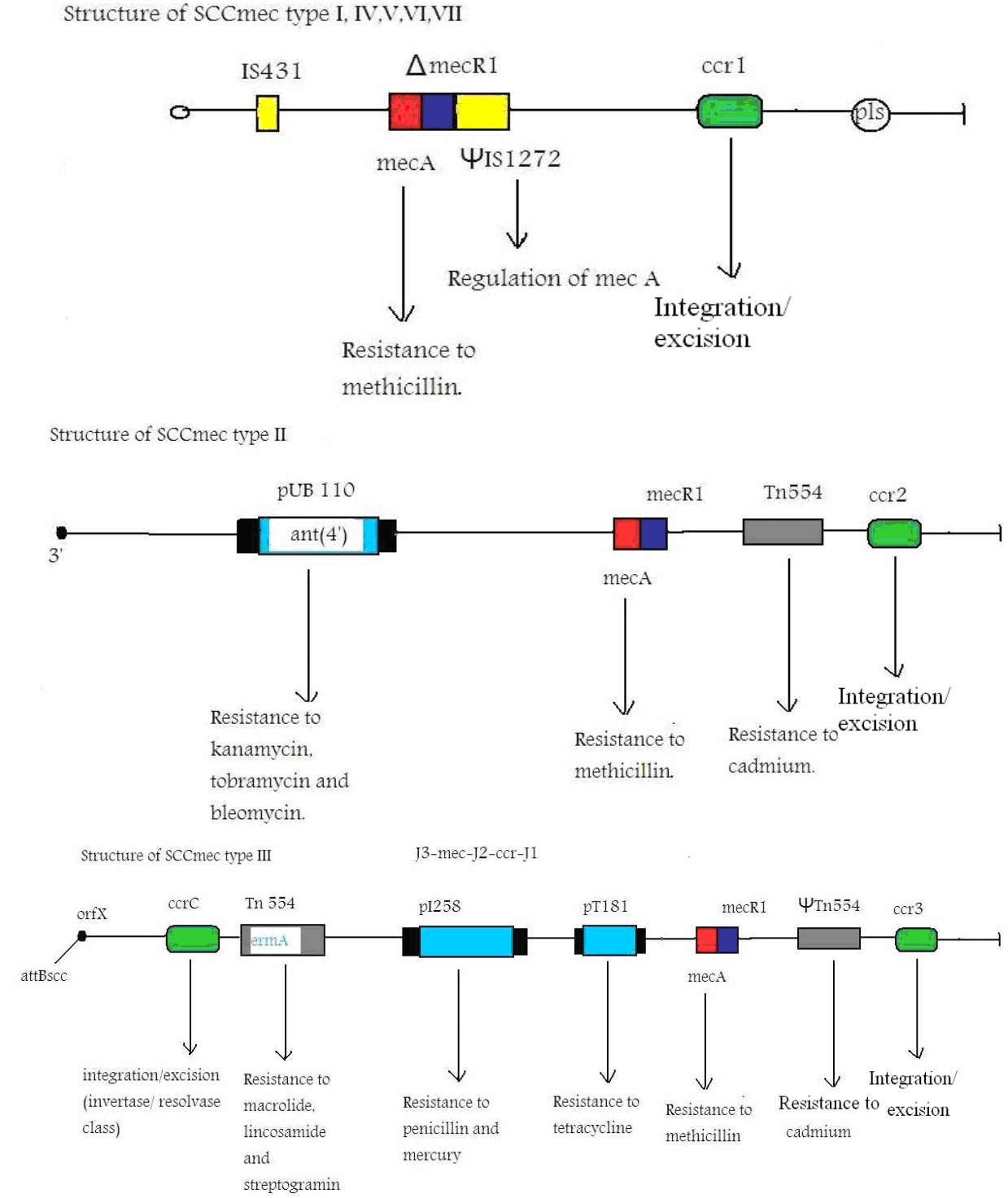

Fig. 1: A schematic representation of SCC*mec* types I-VII in MRSA [13 ,20,10,19,16,21, 22,23, 24,17, 14,18].

Table 1: Structural components of each SCC*mec* type

S. No.	SCCmec type	Size	Structure	Function
1.	I	34.3	*orfX-IS431-mecA-ΔmecR1-ΨIS1272-ccr1*	ß-lactam resistance
2.	II	53.0	*orfX-pUB110- mecA -ΔmecR1 -Tn554-ccr2*	Multidrug resistance
3.	III	66.9	*orfX-ccrC-Tn554-pI258-pI181-mecA-mecR1-Tn554-ccr3*	Multidrug resistance
4.	IV	20.9to 24.3	*orfX-IS431- mecA -ΔmecR1-ΨIS1272- ccr2*	ß-lactam resistance
5.	V	28.0	*orfX-IS431- mecA-ΔmecR1-ccrC*	ß-lactam resistance

6.	VI	20.9	*orfX-IS431- mecA -ΔmecR1-ΨIS1272-ccr4*	ß-lactam resistance
7.	VII	35.9	*orfX-ccrC1-mecA -ΔmecR1-ccrC2*	ß-lactam resistance

Source: [20, 19, 17, 16, 22, 25.]

Molecular basis of methicillin resistance

The major PBP types 1, 2, 3 and 4, with approximately 85, 81, 75, and 45 kDa molecular weights respectively are produced by both resistant and susceptible strains of *S. aureus*. PBPs types 1, 2 and 3 are essential for growth and survival of susceptible strains and they also show high affinity towards ß-lactams. Their binding to PBPs is lethal to the cell [30, 31, 32.] PBP2 functions as both transglycosylase and transpeptidase[33]. In MRSA, there are two mechanisms of resistance to ß-lactams. 95% of *S. aureus* resistant isolates produce an enzyme ß-lactamase (penicillinase) encoded by the *blaZ* gene, which hydrolytically cleaves ß-lactams of the penicillin class [34.] The second broader mechanism involves MRSA isolates containing *mecA* gene which encodes for PBP2a [35, 36]. In these isolates, there is an alteration in the active site of PBP2a due to which there is a decreased affinity for ß-lactams. As a result, the rate of their acylation is significantly reduced[37]. Hence PBP2a is able synthesize the bacterial cell wall, even in the presence of ß-lactam antibiotics, with the help of its ß-lactam-insensitive transglycosylase domain[33]. The distinctiveness of MRSA in their expression of heterogeneous antibiotic resistance is the generation of subpopulations among individual strains with different degrees of higher resistance.

Regulation of genes of ß-lactam antibiotics

Organisms respond to environmental changes through signaling pathways via receptor molecules present on cell envelopes and nuclear membranes. Different sensory transmembrane proteins that are present in bacterial cell envelopes establish interaction of the bacterial cells with the environmental signals. In case of bacterial antibiotic resistance strains like MRSA, VRSA, etc., resistance is directly and indirectly under the regulation of environmental signals, in which the main facilitator is a Two-Component System (TCS). The presence of antibiotics in the media (environmental signal) is detected by membrane-anchored sensor kinases (TCS), which activate transcriptional regulators[38]. In antibiotic resistant bacteria, *mecA, blaZ* genes and *van* operon are controlled by their respective cognate TCS like MecR1-MecI, BlaR1-BlaI and VanRS. These dedicated target loci, located adjacently to their inducer, are highly specific and active only in presence of single antibiotic class. They are switched off when antibiotics are absent in the media, i.e., under normal conditions.

Regulation of mec & bla genes

The structural genes *mecA* and *blaZ,* encoding for PBPs and ß-lactamase respectively are controlled by MecR1 and BlaR1 that are divergently transcribed from the structural genes, from an overlapping promoter/operator region. Both TCS contain ß-lactam sensor-transducers (MecR1/ BlaR1), but rather than acting as transcriptional activators, the second components act as repressors (MecI/BlaI). Because of the structural and functional similarity of MecR1 and BlaR1, both repressors bind as homodimers to *mecA/mecR1* and *blaZ/blaR1* operator regions. Hence blocking of the transcription of structural and regulatory genes occurs[39,40]. MecR1 and BlaR1 are multidomain membrane-spanning proteins, each composed of an extracellular C-terminal, N-terminal transmembrane domain made of four transmembrane α-helices and an intracellular metalloprotease domain[41].

The ß-lactams antibiotics binds to the extracellular domain of BlaR1/MecR1 proteins and acylates the active-site serine resulting in change of conformation of C-terminal penicillin-binding domain (Fig.2 and Fig.3)[42]. The activated sensor domain of BlaR1/MecR1 triggers activation of cytoplasmic zinc metalloprotease domain by inducing autocatalytic cleavage. Finally the active form of BlaR1 / MecR1, along with an unknown cofactor cleaves repressors BlaI / MecI, permitting the transcription of *mec* and *bla* genes[43]. This process not only allows the transcription of *blaZ* / *mecA*, but also results in transcription of *blaI* / *mecI* and *blaR1* / *mecR1*. Lactamase enzymes, which are expressed into the surroundings, hydrolyze the antibiotic (signal), thus bringing down the latter's levels. Consequently, the expression of lactamase enzymes is efficiently terminated[44]. Autoproteolytic cleavage of MecR1 / BlaR1 is an irreversible process. Hence in order to maintain its continuous replenishment in the presence of ß-lactam antibiotics, steady syntheses of the sensor-transducers is mainly required to proportionately lessen repressor binding. *blaR2,* an unknown genetic factor was shown to be involved in regulation of *blaZ* gene[45]. In strains of *S. aureus* harboring penicillinase plasmids, continuous-lactamse expression was observed from plasmid-borne *bla* divergeon (*i.e., blaZ*, *blaI*, and *blaR1*) [45]. *In vivo* it has been shown that regulatory genes of *mec* and *bla* are interchangeable. In *S. aureus,* BlaR1 and MecR1 share significant sequence identity with 34% of full-length proteins, 43% of sensor domains and 33% of protease domains[46, 47]. Induction of *mec* gene by the MecR1-regulated system is slower compared to the BlaR1-controlled system, taking hours instead of minutes[47, 48, 49]. Blocking the MecR1 regulatory pathway may be a novel strategy to combat MRSA infections.

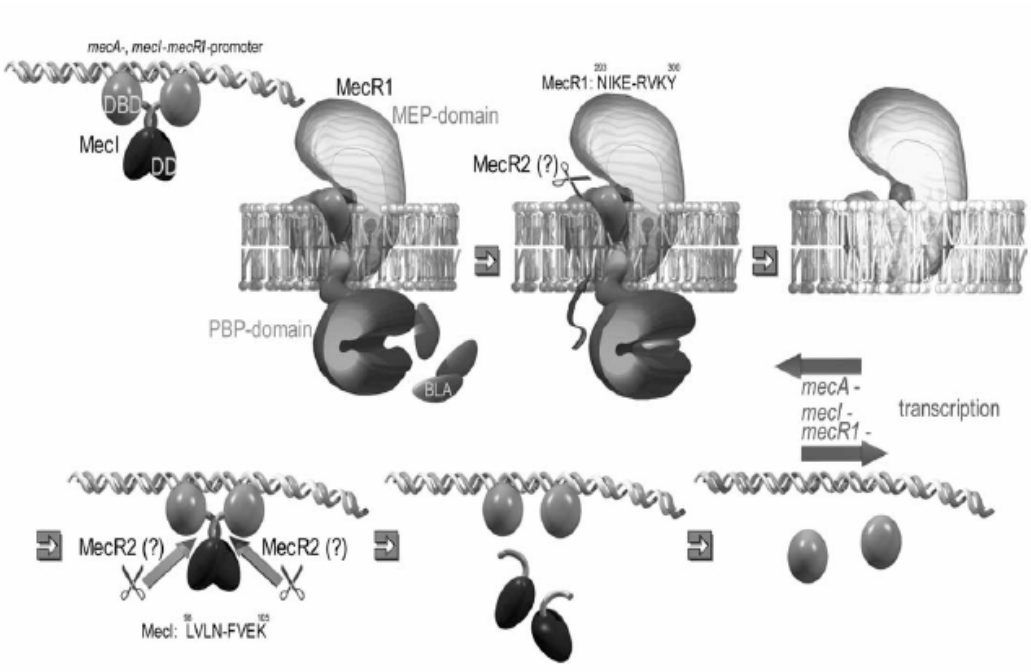

Fig. 2: Mechanism of regulation of MecA synthesis [50]

The signal-transduction system triggering MecA synthesis is activated in presence of ß-lactam antibiotics that binds to the sensor/transducer MecR1 which activates its cytoplasmic zinc metalloprotease domain by inducing autocatalytic cleavage. Activated MecR1 then cleaves MecI, along with an unknown cofactor (MacR2), permitting the transcription of *mecA* gene.

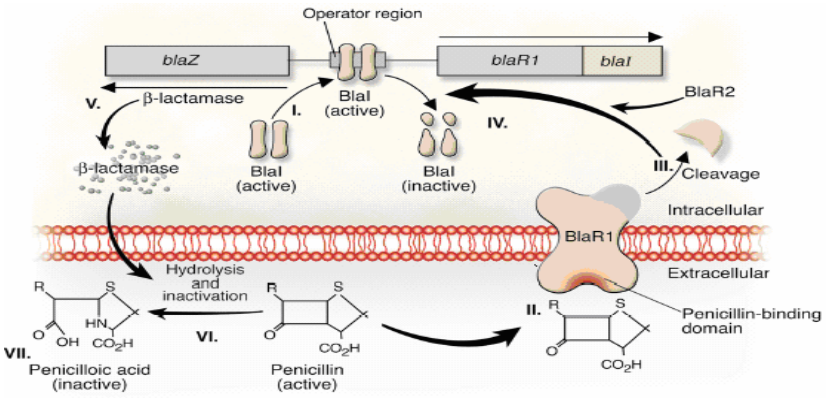

Fig. 3: The signal-transduction system triggering ß-lactamase synthesis

I. BlaI preventing transcription of *blaZ-blaR1-blaI* by binding to the operator region in the absence of ß-lactam antibiotic (penicillin) thus expressing lactamase at low levels

II. In presence of penicillin, the transmembrane sensor-transducer BlaR1 stimulates autocatalytic activation of BlaR1

III-IV. Active BlaR1 (via BlaR2) cleaves BlaI into inactive fragments, resulting in transcription of both *blaZ-blaR1-blaI.*

V-VII. ß-lactam ring of penicillin (VI) is hydrolyzed by an extracellular enzyme ß-Lactamase encoded by *blaZ* (V), thereby rendering it inactive

In addition to the above primary genetic determinants, many other different chromosomal loci, like expression of alternate sigma factors SigB (global regulators of virulence gene), the SarA protein family, the quorum-sensing agr system and other transcription factors have shown to be involved in modulation of antibiotic resistant phenotypes. The other phenotypic characters like virulence, resistance, metabolism and interconnecting fitness are also influenced by these regulators. Some of these regulators are given in Table 2.

Glycopeptides resistance and regulation of van operon

Clinical isolates of *Staphylococcus haemolyticus* were the first reported strains to be resistant to vancomycin [51]. In 1997, vancomycin intermediate-resistant *S. aureus* (VISA) was reported from Japan. Subsequently several cases of vancomycin resistance were reported from different parts of the world [52] [53]. The first clinical isolate of *Staphylococcus aureus,* fully resistant to vancomycin (VRSA), was isolated in the USA[51]. Vancomycin and teicoplanin belong to glycopeptide class of antibiotics and mainly inhibit cell wall biosynthesis. These cell wall antibiotics have high affinity for D-ala-D-ala terminus of nascent uncrosslinked peptidoglycan and extracellular precursor. Binding of vancomycin to D-ala-D-ala terminus of extracellular peptidoglycan precursor sterically obstructs the penicillin binding protein reactions of cell wall synthesis (Fig. 4)[54].

Two mechanisms of vancomycin resistance have evolved in *S. aureus* isolates. In vancomycin/glycopeptide intermediate resistant *Staphylococcus aureus* (VISA/GISA) with MIC of 8–16 mg/l[18], the cell wall is composed of thickened and inadequately cross-linked peptidoglycan. Glycopeptides are lethal to the cell when it interacts

with the target site of the cell division. But in this kind of cell wall structure, glycopeptides are confiscated and prevented from reaching the site of cell division. Due to increased number of free D-ala-D-ala dipeptide, glycopeptide molecules bind within the outer layer of the cell wall[55]. As a result, their rate of diffusion decreases. This indicates the importance of this pathway for glycopeptides to reach the site of synthesis at the septal tip and at the growth stage of the cell[56]. Although heterogeneous GISA (hGISA) are not highly vancomycin resistant, they are considered to be the precursors of GISA because they can evolve as a subpopulation of higher resistance in the presence of glycopeptides[52].

A higher level of vancomycin resistance in *S. aureus* is attained by conjugal transfer of *vanA* operon from a vancomycin-resistant *E. faecalis*[57]. The *van* operon contains Tn1546-like *vanRSHAXYZ* gene complexes, in which *vanH* and *vanA* genes are encoded for an altered peptide. This has 1000 fold lower affinity towards glycopeptides than D-ala- D-ala but still can used as a substrate for PBPs (Fig. 4)[58]. The D, D-peptidases, acting on precursors ending in D-ala-D-ala, are encoded by *vanX* and *vanY* genes and they also contribute to glycopeptide resistance by the elimination of susceptible stem peptide termini from cell wall of the resistant strains[59]. The exact function of *vanZ* gene is unknown and it may encode for a low-level teicoplanin resistance phenotype[60].

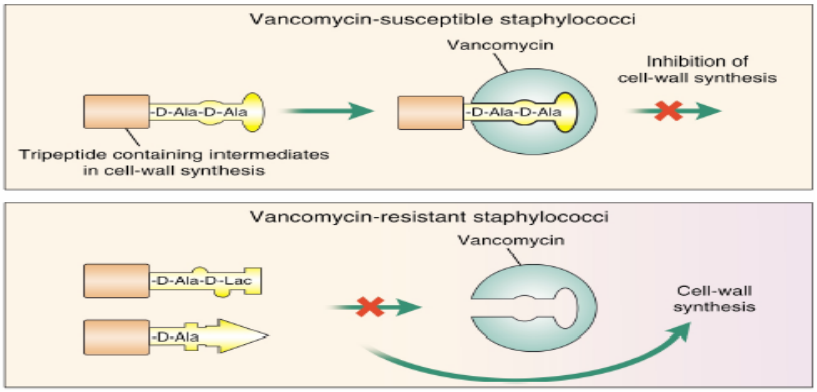

Fig. 4: Mechanism of vancomycin resistance in VRSA strains

Vancomycin resistance in VRSA is due to the transfer of *vanA* operon from an *Enterococcus.* The above Figure explains the inhibition of cell wall synthesis by vancomycin that binds to cell wall precursors, D-Ala-D-Ala in susceptible strains. It also explains the synthesis of modified precursors D-Ala-D-Lac by resistant organisms that cannot bind to vancomycin, thereby allowing continued peptidoglycan assembly [61].

VanRS two-component system (TCS) regulates the expression of *van* operon in response to the presence of glycopeptides (Fig. 5)[62]. This is a membrane-bound VanS sensor/transducer protein with cytoplasmic C-terminal kinase domain consisting of two helices, and a sensor domain of N-terminal[63]. In the presence of glycopeptide antibiotic, autophoshorylation of membrane bound VanS occurs by an unidentified inducing signal, which activates cytoplasmic VanR by phosphorylation[63].*vanA* operon is activated subsequently by phosphorylated VanR, which binds to upstream promoter region of *vanRS* with the structural *vanHAX* genes permitting the transcription. When glycopeptides are absent, the VanS enzyme acts as a phosphatase, deactivating the activated VanR resulting in the repression of *van* operon transcription.

The sensor-transducer VanS plays a dual role as an activator (kinase) and deactivator (phosphatase). When glycopeptides are present in the medium, phosphorylation of VanR by VanS activates transcription of *vanRSHAX* genes. Lack of glycopeptides in medium phosphorylates VanR by cellular acetyl phosphate and its deactivation also by VanS- kinase activity, blocking *van* gene transcription [64, 38].

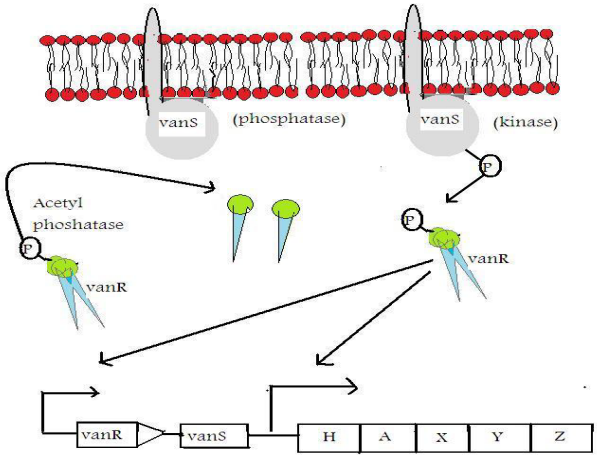

Fig. 5: VanRS two-component system regulation of *van* operon in vancomycin resistant *S. aurues*

Macrolides, Lincosamides, Straptogramins$_B$ (MLS$_B$) and Ketolides: Mode of action, resistance and regulation

These classes of antibiotics mainly act on the protein synthesizing machinery of the cell that binds to 50S subunit of ribosome resulting in prevention of 50S subunit assembly (Fig. 6)[65]. The MLS$_B$K antibiotics blocks the formation of peptide bond, and also the translation, by occupying similar, overlapping binding sites, in the domainV of peptidyl transferase centre which is close 23S rRNA[66]. Gram-positive organisms are more sensitive to ketolides in which they bind to an adenine nucleotide in 23S rRNA domain II.

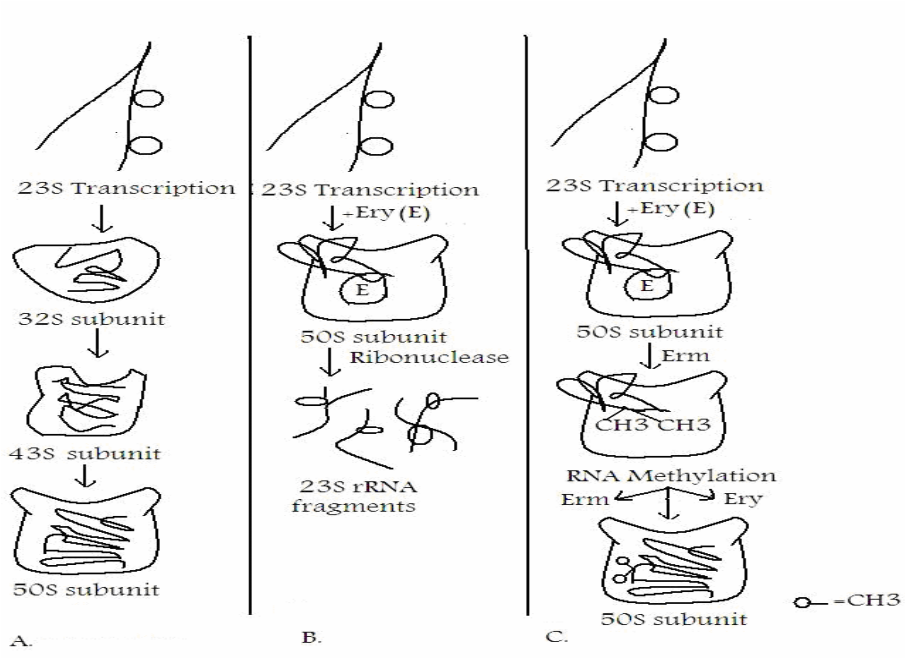

Fig. 6: Model for 50S subunit formation in *S. aureus* cells

(A) Formation of 50S subunit in normal control cells showing the 32S and 43S intermediate particles. (B) Inhibition of 50S formation by erythromycin in antibiotic-inhibited cells. (C) Prevention of erythromycin (E) inhibition of subunit formation by Erm methyltransferase in antibiotic-induced cells.

Methylation and dimethylation of ribosomal rRNA by methyltransferases is a common resistance mechanism to MLS_B antibiotics in most clinical isolates [67, 68]. There are two types of methyltransferases; Erm methyltransferase alters the adenine residue of domain V A2058 of the 23S rRNA. In *E.coli*, S-adenosyl methionine and Cfr methyltransferase, also methylate the 23S rRNA at A2503 residue. In addition, even broader resistance to lincosamides, phenicols, pleuromutilins streptograminA and oxazolidinones is conferred by Methyltransferase[69, 70]. Dimethylation influences MLS_B resistant phenotype, expanding it to resistance to ketolides. Methylation enhances the cross-resistance to macrolides, streptograminB and lincosamides[71].

Translation attenuation based regulation of ermC expression

Binding of streptogramin$_B$, lincosamide and macrolide to the ribosome, that mainly modifies A2058 in 23S Rrna, is prevented by Erm-type methyltransferases, thereby conferring MLS_B resistance[72]. In *S. aureus* and other bacteria, the *ermC* gene expression, induced by macrolides, is under the control of a 19-codon ORF, *ermCL* (suffix 'L' for leader), and presents 60 bps upstream of *ermC* (Fig. 7)[73, 74].

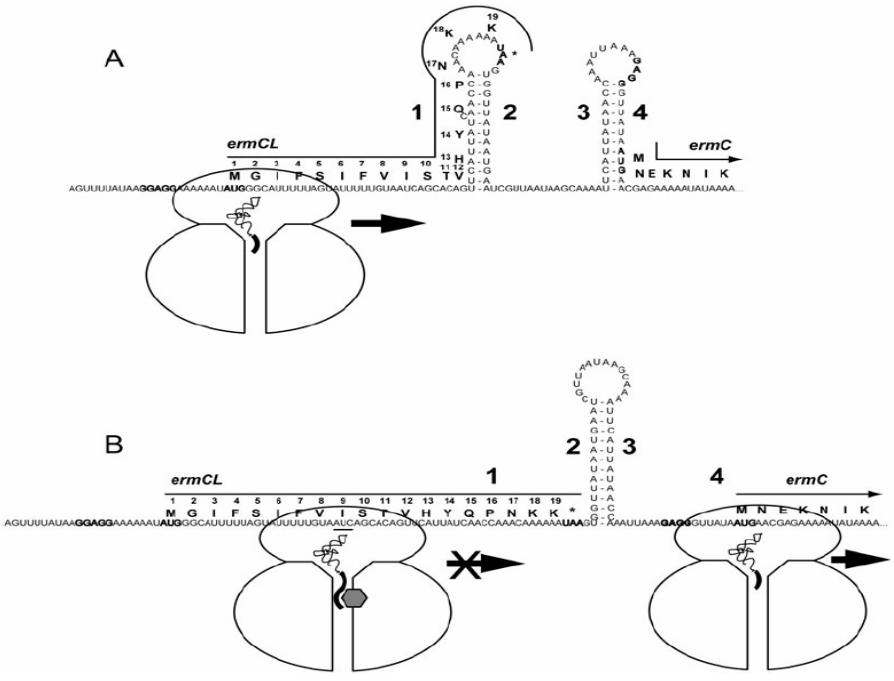

Fig. 7: Regulation of expression of *ermC* gene by translation attenuation *in* erythromycin resistance organisms.

In the absence of antibiotics, *ermCL* is constitutively translated and translation of *ermC* is attenuated because its RBS (shown in bold) is sequestered in mRNA secondary structure (A). After induction, in presence of an erythromycin (grey hexagon), ribosome stalls at the 9th codon of *ermCL* leading to a change in the mRNA conformation, allowing translation of *ermC*. The bold numbers (1–4) marked are mRNA segments involved in the conformational switch.

In absence of antibiotic, the leader segment *i.e., ermCL* of ermC gene is translated constitutively, but *ermC* gene translation is attenuated because of seizing of its RBS in a stem-loop structure (Fig. 7A). In order to activate expression of *ermC*, there must be rearrangement in the regulatory region of mRNA structure into 'induced' conformation form, which releases the translation initiation region of *ermC* gene. This is possible only when strand 1 of the first hairpin sequesters in the ribosome, and allows strand 2 to repair the strand 3, so that strand 4, which contains *ermC* RBS can be liberated and translated (Fig. 7 B).

Other factors contributing to antibiotic resistance

In addition to MecA, which is the primary determinant of intrinsic methicillin resistance, additional genes and other environmental factors are required for a high-level resistance phenotype [75, 76, 77, 78, 79]. These genes are mainly involved in cell wall synthesis and turnover that are native to genome of *S. aureus.* These chromosomal loci are global regulators of virulent gene expression *i.e.,* the SarA protein family, the quorum-sensing *agr* system, the alternate sigma factor SigB, and some transcription factors, which modulate phenotypes of antibiotic resistance. Cell virulence factor production, metabolism, resistance, virulence and interconnecting fitness are influenced by many of these regulators. Some examples of these regulators are summarized in Table 2.

Table 2: Genes involved in the high level antibiotic resistance

Gene(s)	Influence on methicillin resistance	Reference
lytH	Inactivation of this homologous lytic enzymeincreases methicillin resistance.	80
hmrA	Over expression putative aminohydrolase increasesmethicillin resistance.	81
hmrB	Over expression increases methicillin resistance; is ahomologue of acyl carrier protein.	81
dlt operon	It mainly transfer D-alanine into teichoic acids and disruptionof this gene increases methicillin resistance.	82
fmtA	Is a membrane protein; its inactivation decreasescross-linking and amidation of peptidoglycan, and reduction in methicillin resistance. .	83
fmtB (*mrp*)	Cell surface protein encodes substituted monomer of the cellwall fraction; inactivation reduces pentaglycyne whileincreasing the amount of unsubstituted pentapeptide and reduces methicillin resistance.	8485

Studies show that in addition to the genomic differences, resistance to methicillin in MRSA also depends on growth medium like Mueller-Hinton Agar[86]. The factors like anaerobiosis, temperature, pH, chelating agents, osmolality, light and metal ions, and prior exposure to β-lactam antibiotics influences the heterogeneity of the methicillin resistance in MRSA [87].

Tn551-mediated insertional inactivation of part of the normal set of genes present in susceptible and resistant strains of *S. aureus* indicate the factors essential for methicillin resistance (fem) or auxiliary factors (*aux*), necessary for expression of resistance to methicillin in MRSA [88]. Different types of *fem* factors *fem A, B, C, D, F, R* and *X* have been identified, which encode for cell wall synthesizing enzymes. FemA and FemB proteins encoded by *fem AB* operon play an important role in formation of gly2-gly3 and gly4-gly5 of pentylglycine side chain of the peptidoglycan precursor [89]. Addition of gly1 occurs by *femX* for which *fmhB* may be an essential gene[90]. Tn551 insertional studies in *femC* shows a glacial effect on transcription *of glnA*, resulting in reduction of GS activity and causes decrease in methicillin resistance[91]. The mutant form of *glmM,* termed as *femD* and *R* encodes for phosphoglucosamine mutase, which converts glucosamine-6-phosphate into glucosamine-1-phosphate, which is one of the peptidoglycan precursor[88, 92]. Decrease in methicillin resistance was noticed with disrupted *glmM* [93].

Inactivation of *femF,* encoding for UDP-N-acetylmuramyltripeptide synthetase, results in accumulation of UDP-linked muramyldipeptide, indicating that this gene is important for methicillin resistance in MRSA, Mutation of gene *llm* (lipophilic membrane protein) with Tn918 transposon indicates its importance on the rate of bacterial lysis and also on high-level resistance in MRSA[94]. Here, the product of *mecA* gene *i.e.,* PBP2a and PBP2 are important for the expression of optimal methicillin resistance [95]. The studies of Tn551 insertion mutant indicates that MRSA strains show reduced resistance to oxacillin in presence of 0.02% Triton X-100. Tn551 mutation in *fmt* gene shows its possible involvement in cell wall synthesis[96].

Density-sensing system, utilizing an octapeptide, acts as an environmental signal to regulate the secretion of virulence factors from pathogenic bacteria. Octapeptide, produced by pathogenic bacteria, is the main activator of expression of virulence response, including regulation of surface proteins, *agr* locus and secreted virulence factors genes. Degree of methicillin resistance in hMRSA is affected by global regulators Sar and Agr[97]. Decrease in the number of cells in the subpopulation expressing high methicillin resistance was observed in inactivated *sar* and / or *agr* and *pbps* MRSA[98]. The other factors that affect the methicillin resistance are modulating murein hydrolase expression and / or activity in the regulatory genes, *agr* and *sar* [99].

CONCLUSION

Since the time of its discovery, *Staphylococcus aureus* has been exposed to different environments that persuaded it to undergo genetic modifications like mutation, or acquiesce genes from the resistance organisms. Circumstances, like the use of antibiotics as preservatives, frequent practice of prescribing the same antibiotic to treat the diseases and also incomplete medication are some of major grounds for developing antibiotic resistance. Mortality rate due to MRSA is drastically increasing in contrast to that of MSSA [100]. In MRSA, the gene *mecA* encodes for penicillin binding proteins 2a (PBP2a) which confers methicillin resistance and *blaZ* gene encodes for an enzyme which confers resistance to penicillin. MecR1 and BlaR1 are two transmembrane proteins (receptors) which are activated after binding with methicillin and penicillin respectively. The intracellular domain of these activated transmembrane proteins regulates the expression of *mecA/blaZ* gene directly or indirectly. Vancomycin resistance in MRSA is associated with cell wall thickness and *van* operon *i.e., vanHAXYZ* is regulated by the transmembrane protein VanRS. Resistance to MLS_B is mediated through translational attenuation regulation. In brief, the antibiotic resistance in *staphylococcus aureus* is not only determined by a single genetic factor but is a combined effect of different genetic, biochemical, nutritional and physical factors.

Approaches are made in order to restrict infections of MRSA. Countries like Denmark, Netherlands and USA have launched strict isolation policies and MRSA infections control programs to control disseminations of MRSA. Novel preventive agents like rapid diagnostic tools, vaccines and usage of lysostaphin and bacteriophage lytic enzymes against MRSA have been under consideration[101, 102, 103]. Minocycline and vancomycin in a rabbit model, and also Oxazolidinones are effective against methicillin resistance *Staphylococcus aureus*[104, 105]. The newer antimicrobial agents, Quinupristin-dalfopristin and linezolid are active against drug-resistant staphylococci VISA and VRSA strains *in vitro*. The present review helps to understand the methicillin, vancomycin and other type of resistance mechanisms at molecular level. Understanding the regulation pathways may pave the way for development of new drugs that inhibit the routes of antibiotic resistance, by targeting some of their key stages.

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