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## The Emergence of Vancomycin Resistance Staphylococci in Egypt: A Review

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### Abstract

Staphylococcus aureus is a standout amongst the most pathogenic bacteria worldwide. The occurrence of methicillin-resistant Staphylococcus aureus (MRSA) made vancomycin as the only therapeutic choice. Due to excessive use of vancomycin, diminished susceptibility and increased resistance against this antibiotic are reported worldwide. The current study aimed at investigating the emergence and development of vancomycin-resistant Staphylococcus aureus (VRSA) in Egypt. This paper highlighted the genetic basis and virulence determinants of vancomycin resistance in *S. aureus*. It also focused on important considerations in the detection of vancomycin resistance and the development of this resistance all over the years. Finally, the expanding reports on the emergence of VRSA in Egypt mentioned in this study, revealed the necessity for new and effective drugs against Staphylococcus infection and more attention should be paid to the proper prescription of this antibiotic as the only choice for *S. aureus* infections.

**Keywords:** Vancomycin; Resistance; Staphylococci; Egypt

### Introduction

Over the past few decades, there has been a disturbing increment in the prevalence of antibiotic-resistant pathogens and strains in serious infections [1, 2]. The occurrence of bacterial infection had diminished with the disclosure of penicillin in 1940 until Staphylococcus aureus began producing  $\beta$ -lactamase, which destroys the penicillin  $\beta$ -lactam core ring [3, 4]. This increase in resistance towards penicillin drove the development of methicillin drugs, which are virtually resistant against many genetic variants of the  $\beta$ -lactamase enzyme. Infection by *S. aureus* was well controlled using methicillin until the seclusion of the first strain of methicillin-resistant *S. aureus* (MRSA) in 1961 [4,5]. Since then, MRSA has become endemic in hospitals and nursing homes around the world [1,6, 7].

Vancomycin has served as the cornerstone of therapy for serious (MRSA) infections for 50 years. Despite the fact that microbiological resistance to vancomycin in *S. aureus* remains very rare, recent years have seen a shift upwards in vancomycin MICs [8, 9].

Vancomycin, a glycopeptide antibiotic, acts against Gram-positive bacteria only, by inhibiting the synthesis of the long polymers of (NAM-NAG) that form the backbone strands (polypeptide into the growing peptidoglycan (PG) chain) of the bacterial cell wall. It inhibits this process by the formation of a hydrogen bond with D.Ala.-D.Ala. Of the stem pentapeptide of bacterial cell wall which consequently blocks the release of terminal D.Ala [10] as shown in (Figure 1).

An elevated mutation rate of Enterococcus species led to the rapid development of vancomycin resistance, indicating that a high mutation frequency could be one of the factors that favor the emergence and transformation of vancomycin resistance to *S. aureus* [11]. The glycopeptide antibiotic vancomycin has long been reserved for treatment of infections with methicillin-resistant *S. aureus* (MRSA). But, over the past decade, vancomycin intermediately resistant *S. aureus* strains have emerged in many countries [12]. The first strain of *S. aureus* with reduced susceptibility to vancomycin was reported in Japan in 1997 and had a

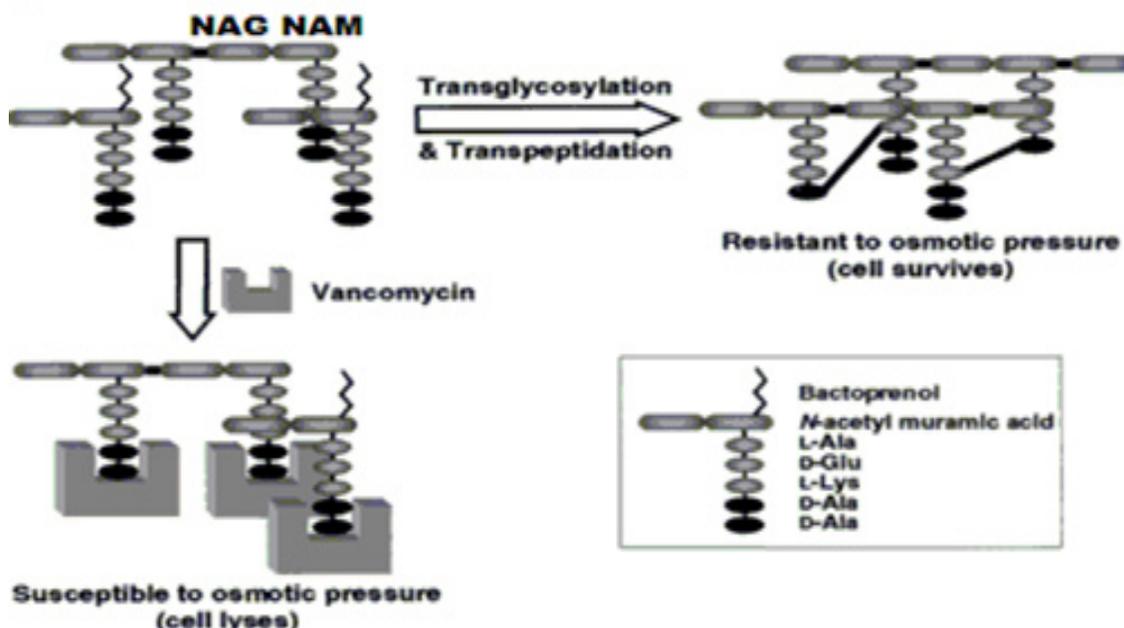


Figure 1: Mechanism of vancomycin action.

vancomycin MIC in the intermediate susceptibility range [13]. Presently, VRSA has been isolated in different countries; hence, the burden has become a global phenomenon. There have been no sufficient literature explaining the emergence of VRSA strains in Egypt as yet. Therefore, the current paper focuses on the problem of evolution vancomycin acquired resistance of gram-positive cocci in Egypt.

### Testing of Vancomycin Resistance in Vitro

MIC tests should be performed to determine the susceptibility of staphylococci isolates to vancomycin. The disc diffusion test doesn't differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, intermediate and resistant isolates of coagulase-negative staphylococci, all of which will give similar size zones of inhibition [14]. *S. aureus* isolates with vancomycin MICs between 4 and 8 µg/ml are classified as vancomycin-intermediate and those with MIC ≥16 µg/ml as vancomycin resistant. These MIC cutoffs are different from those for coagulase-negative staphylococci and enterococci where isolates with vancomycin MIC ≥32 µg/ml are classified as vancomycin resistant.

Apart from vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA), there is one more entity, heterogenous VISA (hVISA), which has a vancomycin MIC ≤2 µg/ml by routine testing methods, but has a great population of cells with reduced vancomycin susceptibility (in the disc diffusion method) [15].

### Genomic Organization of Vancomycin Resistance

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### Genes

The first documented cause of vancomycin resistance in *S. aureus* due to the acquisition of the van genes occurred in Michigan in 2002 [16]. The second case of VRSA was isolated from a morbidly obese 70-year-old male in Pennsylvania [17]. The third case of VRSA was isolated from the urine of a long-term care facility patient [18]. The van genes were first found in 1988 in an *Enterococcus faecium* isolated from France [19]. Transfer of vancomycin resistance from enterococci to staphylococci was detected and light-cycler PCR results illustrated that enterococci and staphylococci are known to exchange their genetic information (plasmid and related element) [20-22].

Six sorts of vancomycin resistance genes have been described on both a phenotypic and a genotypic premise in enterococci. Five of these sorts (van A, B, D, E, and G) correspond to acquired resistance and one sort (vanC) is an intrinsic property of *Enterococcus gallinarum* and *Enterococcus flavescens*. Classification of glycopeptide resistance is currently based on the primary sequence of the structural genes for the resistance ligases rather than on the levels of resistance to glycopeptides because the minimum inhibitory concentration (MIC) ranges of vancomycin and against the various types overlap. VanA-type strains display high levels of inducible resistance to both vancomycin and teicoplanin, whereas vanB-type strains have variable levels of inducible resistance to vancomycin only [23], [24]. VanD-type strains are characterized by constitutive resistance to moderate levels of the 2 glycopeptides [25] while vanC, vanE and vanG-type strains are resistant to low levels of

vancomycin [26]. The *vanA* and *vanB* operons are situated on plasmids or in the chromosome [27], whereas the *vanD* [25], *vanC* [28], *vanE* [29] and *vanG* [30] operons have been discovered only in the chromosome. Along these lines, the incidence of *van* genes on *Staphylococcus* species responsible for the resistance to vancomycin was acquired by the action of *vanA* and *vanB*, where the enterococcus plasmid apparently behaved as a suicide conveyance vector to the plasmid in MRSA [31].

The *vanA*, *vanR*, *vanS*, *vanH* and *vanX* genes are basic for the articulation of the *vanA* phenotype (*vanA* cluster) [32]. The transposon encodes a dehydrogenase (*vanH*), which decreases pyruvate to d-Lac and the *vanA* ligase (*vanA*), which catalyzes the formation of an ester bond between d-Ala and d-Lac. The resulting d-Ala-d-Lac depsipeptide replaces the d-Ala-d-Ala dipeptide in peptidoglycan synthesis, a substitution that decreases the affinity of the molecule for glycopeptides considerably. *VanX*, a D, D-dipeptidase that splits the d-Ala-d-Ala dipeptide synthesized by the host. Expression of the *vanA* gene cluster is regulated by the two-component regulatory system *vanR-vanS*, which is responsible for recognition of the presence of glycopeptides in the culture medium and transcriptional activation of the resistance and regulatory genes [10]. As in *vanA*-type strains, acquired *vanB*-type resistance is due to synthesis of peptidoglycan precursors ending in the depsipeptide d-Ala-d-Lac instead of the dipeptide d-Ala-d-Ala [23, 33].

### Virulence Determinants In VRSA

The alternative transcription factor sigma B ( $\sigma$ B) is responsible for transcription of *Staphylococcus aureus* during the stress response. Many virulence-associated genes are directly or indirectly regulated by  $\sigma$ B. Expression of  $\sigma$ B-regulated virulence genes, including *hla* and *fnbA*, was related with the vancomycin-induced  $\sigma$ B activity in VRSA strains and the raise in the cytotoxicity upon vancomycin treatment. The sub-minimum inhibitory concentration (sub-MIC) levels of vancomycin act as ecological stressors and activate the stress response sigma factor,  $\sigma$ B [34].

### The Consideration Of Genetic Basis And Regulation For Vancomycin Resistance In Egypt

Following a systematic literature search using the keywords vancomycin-resistant *S. aureus*, few reports were retrieved. A systematic review of all available resources suggested an increased rate of Vancomycin Intermediate *S. aureus* (VISA) and VRSA emergence in Egypt initiated since 2008. From the first report until 2010, 19 VISA and 2 VRSA from several intensive cares were detected [35, 36] at which Medhat et al. was the first one who identified two

VRSA isolates from Mansoura University Hospital, children department and still susceptible to some antibiotics, which were not used widely such as, daptomycin, quinipristin/dalfopristin and tigecycline, also detected hVISA and VISA had an antimicrobial susceptibility pattern similar to VRSA isolates.

Then, the highly resistant vancomycin-resistant *S. aureus* strains were obtained from clinical samples of patients at the critical care unit, Cairo Hospital, University. Furthermore, *vanA* and *vanB* resistant genes were detected for the first time by using real-time PCR with percentages of 27% and 36%, respectively [37]. Subsequently, 10 VRSA isolates were detected from outpatient clinics as well as inpatient departments mainly from the intensive care unit and orthopedic department at Zagazig University hospitals and five out of them harbored *vanA* gene [9]. Hassanein et al. [38] obtained three VISA and one VRSA. The *vanA* gene was detected in two isolates only (one VISA and one VRSA). Also, the tested VRSA strain was characterized by the production of virulence factors hemolysin, lecithinase, and protease.

In 2014, profound cases of VRSA infection were detected in many intensive cares all over Egypt cities with little literature for the main source or origin of these isolates [1,39]. Also, all obtained VRSA isolates were harbored *vanA* gene. These raise the possibility of greatly increased mortality from simple infections and treatment-mediated failures Osman et al. [40], recorded intermediate resistant *S. aureus* in raw meat (51%) which might indicate the spread of vancomycin resistance in the community and imply food safety hazards.

The report of [41] was the first one of the comprehensive identification and confirmation of vancomycin-resistant *S. aureus* (VRSA) harboring *van* genes from meat samples in Egypt. The findings of VRSA in meats are a motive of concern, as they may suggest the spread of such microorganisms or their genetic material outside clinical boundaries. Eight VRSA isolates were obtained, *vanA* gene was detected in 3 isolates which were resistant to vancomycin with  $MIC \geq 128 \mu\text{g/ml}$ , whereas, four isolates carried *vanB* gene with  $MIC \geq 64 \mu\text{g/ml}$ . These results confirmed the criteria, reporting that *vanA*-type strains display high levels of inducible resistance to vancomycin, whereas *vanB*-type strains have variable levels of resistance [23]. Moreover, the absence of *van* genes in one VRSA isolate by PCR even in the presence of phenotypic resistance to vancomycin. As proposed previously, cell wall thickening is the essential contributor for the development of vancomycin resistance. As a result, more vancomycin molecules are trapped in the

peptidoglycan synthesis occurs [42].

The remarkable global concern of antibiotic-resistant pathogens (especially VRSA and MRSA) in the food chain and the potential for these resistant pathogens to spread through the food chain prompted the Codex Alimentarius Commission to establish an ad hoc Intergovernmental Task Force on antimicrobial resistance. The principle errand of this commission is to apply the a complete risk assessment methodology on utilization of antimicrobials belonging to both clinical and veterinary classes with special reference to beef meat that has been implicated to contribute to the emergence of multidrug resistance among humans through the scattering of resistance genes conveyed by resistant pathogens transmitted by contaminated meat [43-45]. However, until today all studies reinforce the development and increase the spread of these pathogens in Egypt resulting from misuse of antibiotics.

### Conclusion And Recommendations

1. The occurrence of VRSA isolates demonstrates that resistance to vancomycin will appear because of overuse. We recommend that this drug should not be used (prescribed) except if it is highly indicated and only after susceptibility tests.

2. Most of the VRSA were profoundly resistant to most antibiotics. This may be because the control of antibiotic use isn't strictly followed by clinicians.

3. The infection-control practices, improved knowledge, and environmental hygiene may aid in controlling the propagation of vancomycin resistance

4. Alternative therapies should be considered to avoid treatment failure of staphylococcal infection.

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