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Vancomycin resistant *Staphylococcus aureus* infections: A review of case updating and clinical features

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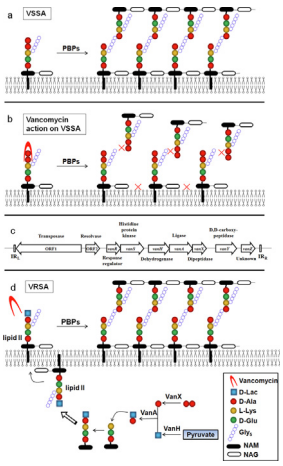
HIGHLIGHTS

- MRSA infection is a global threat to public health.
- Vancomycin is one of the first-line drugs for the treatment of MRSA infections.
- MRSA with complete resistance to vancomycin have emerged in recent years.
- The total number of VRSA isolates is updated in this paper.
- Resistance mechanisms, characteristics of VRSA infections, as well as clinical treatments are reviewed.

GRAPHICAL ABSTRACT



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ABSTRACT

The infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is a global threat to public health. Vancomycin remains one of the first-line drugs for the treatment of MRSA infections. However, *S. aureus* isolates with complete resistance to vancomycin have emerged in recent years. Vancomycin-resistant *S. aureus* (VRSA) is mediated by a *vanA* gene cluster, which is transferred from vancomycin-resistant enterococcus. Since the first VRSA isolate was recovered from Michigan, USA in 2002, 52 VRSA strains have been isolated worldwide. In this paper, we review the latest progresses in VRSA, highlighting its resistance mechanism, characteristics of VRSA infections, as well as clinical treatments.

Introduction

Due to the metabolic versatility and pharmic resistance, *Staphylococcus aureus* is well adapted in varied environments. Approximately 25–30% of healthy individuals are colonized by *S. aureus* on their skins and nasopharyngeal membranes, where *S. aureus* exists as a member of normal microbiota and does not cause

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infections on normal immune status [1]. However, *S. aureus* can cause a variety of serious infections upon invading the bloodstream or internal tissues. *S. aureus* is a leading human pathogen that causes a variety of clinical manifestations ranging from relatively benign skin and soft tissue infections to severe and life-threatening systemic diseases [2], and remains a challenging public health issue due to the emergence and dissemination of multidrug resistant strains, for example, methicillin-resistant *S. aureus* (MRSA) [1,3].

Methicillin resistance is conferred by *mecA* or *mecC* gene, which is located on the staphylococcal chromosomal cassette, and encodes penicillin-binding protein 2A (PBP2A) or $PBP2A_{LGA}$, the enzymes that are responsible for crosslinking the peptidoglycans of bacterial cell wall [4]. Both enzymes show a low affinity for β-lactams, thereby leading to resistance to this category of antibiotics [5,6]. Vancomycin has been one of the first line drugs to treat MRSA infections for decades [7–9]. However, the clinical isolates of *S. aureus* with intermediate and complete resistance to vancomycin have emerged within the past two decades [10,11], and have become a serious public health concern [12]. In this paper, we review the latest advances in the studies of vancomycin-resistant *S. aureus* (VRSA), including resistance mechanism, infection characteristics, and clinical treatments of VRSA infections.

Vancomycin discovery and action mechanism

Vancomycin is one of the oldest antibiotics, and has been in clinical use for nearly 60 years. Dr. Kornield, an organic chemist of Eli Lilly, isolated vancomycin from *Streptomyces orientalis* in the deep jungles of Borneo in 1957 [13]. Vancomycin is active against Gram-positive bacteria, such as *Staphylococci*, *Enterococci*, *Streptococci*, *Pneumococci*, *Listeria*, *Corynebacterium*, and *Clostridia*. Currently, vancomycin is generally used for infections caused by MRSA and for the treatment of patients allergic to semisynthetic penicillin or cephalosporins [13].

Vancomycin exerts its bactericidal action via interrupting proper cell wall synthesis in the susceptible bacteria. Most bacterial membranes are coated with a cell wall structure that protects cells from swelling and bursting due to intracellular high osmolarity. During propagation, the cell wall structure including peptidoglycan needs to be enlarged. To do that, the precursor lipid II is added to the nascent peptidoglycan via transglycosylation and transpeptidation by the penicillin-binding proteins (PBPs) (Fig. 1a). The hydrophilic molecule of vancomycin can form hydrogen bond interactions with the terminal D-alanyl-D-alanine (D-Ala–D-Ala) moieties of the precursor lipid II. The binding of vancomycin leads to conformational alteration that prevents the incorporation of the precursor to the growing peptidoglycan chain and the subsequent transpeptidation, thereby leading to cell wall decomposition and bacterial lysis (Fig. 1b) [14]. However, the complex structure of vancomycin blocks its penetration through the outer membrane of Gram-negative bacteria, and exerts limited bactericidal effect on Gram-negative bacteria.

Vancomycin resistance and mechanism in *S. aureus*

The isolates of *S. aureus* with reduced susceptibility to vancomycin are classified into three groups by the Clinical and Laboratory Standards Institute. These are vancomycin-susceptible *S. aureus* (VSSA) with MIC $\leq$ 2 μg/ml, vancomycin-intermediate *S. aureus* (VISA) with MIC of 4–8 μg/ml, and VRSA with MIC $\geq$ 16 μg/ml. In confirming whether an isolate belongs to VRSA, the presence of *vanA* or other *van* resistance determinants should be demonstrated by molecular methods [15].

VISA

Isolates of VISA, which are associated with hospitalization, persistent infection, prolongation, and/or failure of vancomycin therapy, have been recovered worldwide [12]. VISA strains are generally believed to be initiated from heterogeneous vancomycin-intermediate *S. aureus* (hVISA), which is defined as an *S. aureus* strain with a vancomycin MIC within the susceptible range ($\leq$2 μg/ml) determined by conventional methods, while a cell subpopulation is in the vancomycin-intermediate range ($\geq$4 μg/ml) [16,17].

The molecular mechanisms underlying VISA development are incompletely defined [14,18,19]. However, many efforts have been made to identify genetic determinants associated with vancomycin-intermediate resistance via comparative genomics, transcriptomics, and proteomics of VISA and its isogenic VSSA, and led to the identification of multiple mutations in genes responsible for VISA formation [14,20,21]. It is generally accepted that VISA is a result of the gradual mutation accumulation of the VISA-associated genes [22]. Of particular importance are the genes encoding two-component regulatory systems, such as WalKR [23–25], GraSR [26], and VraSR [14,26,27]. Although their genetic lineages varied, VISA strains generally display common phenotypes, including thickened cell wall, reduced autolytic activity, and decreased virulence [12]. The mechanism linking the diverse mutant genes to the VISA common phenotypes is unclear and should be investigated further.

VRSA

Vancomycin became a therapeutic agent for the treatment of serious infections caused by MRSA in the late 1980 s [28,29]. Almost at the same time, vancomycin-resistant *enterococci* (VRE) were first identified in Europe [30–33], and quickly became endemic in hospital intensive care units. Vancomycin resistance in VRE was mediated by transposons mainly found on plasmids, which raised considerable concern about the risk of dissemination of vancomycin-resistant determinants to universally susceptible microorganisms of medical importance, especially *S. aureus* [33,34]. This concern was subsequently confirmed by the successful transfer of the *van* element from *Enterococcus faecalis* to a MRSA strain in mix-infected mice [35].

In 2002, the first VRSA strain was recovered in Michigan, USA [11,36]. In the same year, the second VRSA strain was isolated in Pennsylvania, USA [37]. Since then, a total of 52 VRSA strains carrying *van* genes have been reported (Table 1), including 14 isolated in USA [37–40], 16 in India [41–43], 11 in Iran [44–46], 9 in Pakistan [47,48], 1 in Brazil [49,50], and 1 in Portugal [51].

Mechanism underlying vancomycin resistance of VRSA

Vancomycin resistance in bacteria is mediated by *van* gene clusters that are found in pathogens (such as *E. faecalis*, *E. faecium*, *S. aureus*, and *Clostridium difficile*), glycopeptide-producing actinomycetes (such as *Amycolotopsis orientalis*, *Actinoplanes teichomyceticus*, and *Streptomyces toyocaensis*), anaerobic bacterium of the human bowel flora (such as *Ruminococcus* species), as well as a biopesticide *Paenibacillus popilliae* [52–56]. Vancomycin resistance is classified into several gene clusters based on the DNA sequence of the ligase *van* gene homologues that encode the key enzyme for the synthesis of D-alanyl–D-lactate (D-Ala–D-Lac) or D-alanyl–D-serine (D-Ala–D-Ser). At least 11 *van* gene clusters that confer vancomycin-resistance, responding for VanA, VanB, VanD, Van F, VanI, VanM, VanC, VanE, VanG, VanL, and VanN phenotypes,

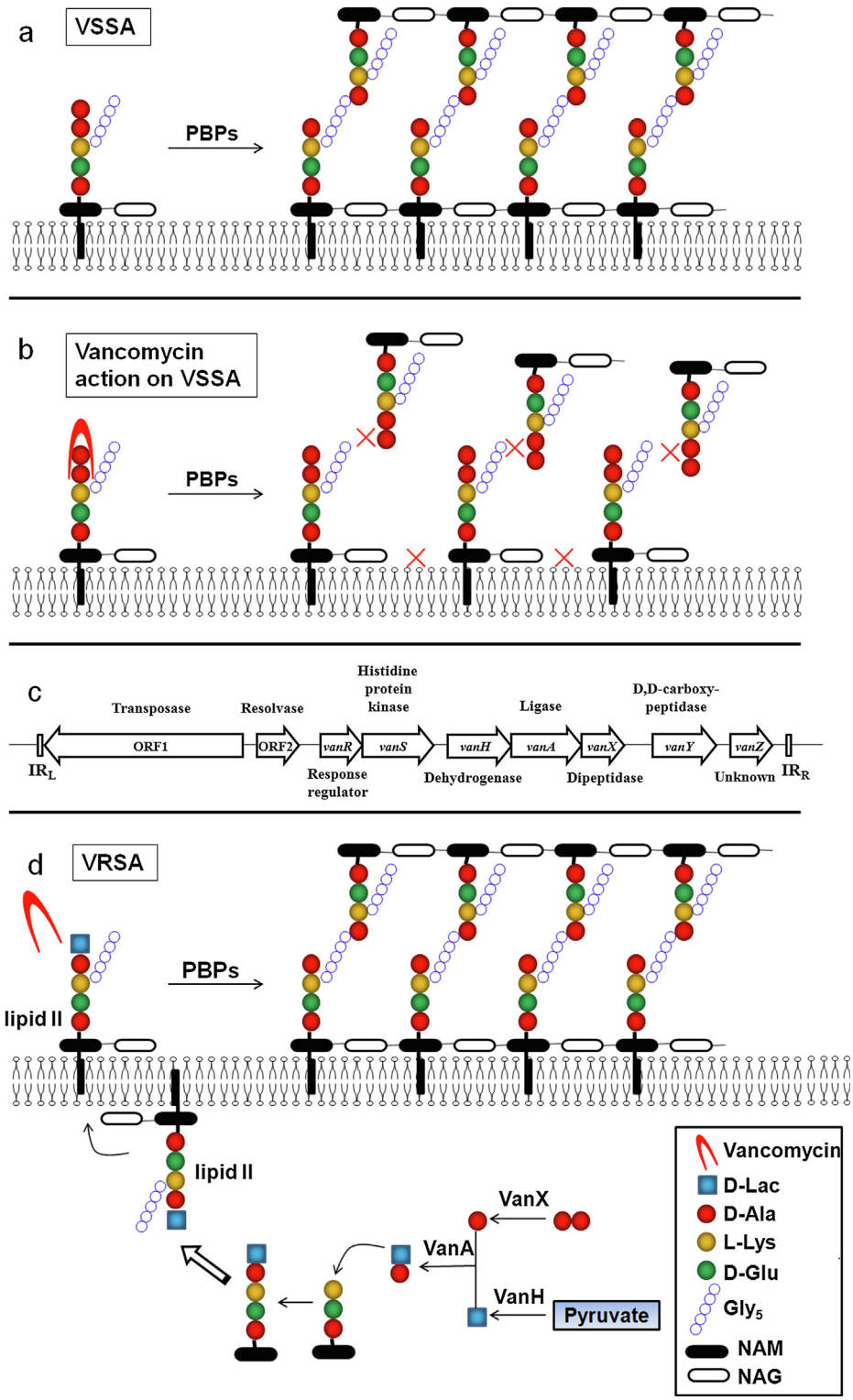

Fig. 1. Resistant mechanism of vancomycin-resistant *Staphylococcus aureus*. (a) Schematic diagram of normal peptidoglycan synthesis. (b) Schematic diagram of vancomycin action. (c) Organization of the *vanA* cluster. (d) Resistant mechanism of vancomycin-resistant *S. aureus*. D-Ala: D-Alanine; D-lac: D-lactate; L-Lys: L-Lysine; D-Glu: D-glutamate; Gly_5: Pentaglycine; NAM: N-acetylmuramic acid; NAG: N-acetylglucosamine.

have been described to date [53,57–60]. The organization of these *van* clusters are shown in Supplementary Fig. 1. The genes that encode D-Ala:D-Lac ligases, such as *vanA*, *vanB*, *vanD*, *van F*, *vanI*, and *vanM*, often result in high-level vancomycin resistance with MICs > 256 mg/ml, while the genes that encode D-Ala:D-Ser ligases, including *vanC*, *vanE*, *vanG*, *vanL*, and *vanN*, generally result in low-level resistance with MICs of 8–16 mg/ml [61].

Acquired vancomycin resistance is most prevalent among *Enterococcus* and is still rare in other pathogenic bacteria [15]. The vancomycin-resistant mechanism has been elucidated in *Enterococcus* species, which are also the main reservoir of acquired vancomycin resistance [15]. Although 11 *van* gene clusters have been discovered to confer vancomycin resistance, only the *vanA* gene cluster is responsible for the isolated VRSA strains [15].

Table 1
Characteristics of VRSA isolates.

Country (No.)	City/State	Patient (Sex/age)	Isolation time	Isolation site of VRSA	Typing and clonal complex	Vancomycin use in previous 3 months	*Enterococci* co-isolated	References*
US (1)	Michigan	Female/40	2002.06	Foot ulcer, catheter exit-site, catheter tip	USA100, t062, CC5	Yes	VR *E. faecalis*	[29,35]
US (2)	Pennsylvania	Male/70	2002.09	Foot ulcer	USA100, t002, CC5	No (exposure in 1997.09)	–	[29,37,75,81]
US (3)	New York	Female/63	2004.03	Urine	USA800, t002, CC5	No (exposure in 2003.11)	VR *E. faecium*	[29,39,75]
US (4)	Michigan	Male/78	2005.02	Toe wound	USA100, t002, CC5	Yes	VR *E. faecalis*	[29,75]
US (5)	Michigan	Female/58	2005.10	Postpanniculectomy surgical infection	USA100, t002, CC5	Yes	VR *E. faecalis*	[29,75]
US (6)	Michigan	Male/48	2005.12	Sole wound	T062, CC5	Yes	VR *E. faecalis* VR *E. avium*	[29,75]
US (7)	Michigan	Female/43	2006.10	Triceps wound	USA100, t002, CC5	Yes	–	[29,75]
US (8)	Michigan	Female/48	2007.10	Plantar foot wound	USA100, t002, CC5	Yes	–	[75,79,89]
US (9)	Michigan	Female/54	2007.12	Plantar foot wound	USA100, t002, CC5	Yes	VR *E. faecalis*	[75,79,89]
US (10)	Michigan	Female/53	2009.12	Sole wound	USA100, t002, CC5	NA	VR *E. faecalis* VR *E. galinarum* VR *E. raffinosus* VR *E. faecium*	[75]
US (11)	Delaware	Female/64	2010.04	Surgical wound drainage	USA100, t002, CC5	NA	VR *E. faecalis*	[75]
US (12)	Delaware	Female/83	2010.08	Vaginal swab	t045, CC5	NA	VR *E. galinarum*	[75]
US (13)	Delaware	Male/70	2012.06	Foot wound	USA1100, t019, CC30	Yes	VR *E. faecalis*	[75]
US (14)	Delaware	NA	2015.02	Toe wound	USA100, t002, CC5	No	VR *E. faecalis*	[76]
India (1)	Kolkata	NA	2002.01–2005.12	Outpatient pus sample	NA	NA	NA	[41]
India (2–6)	Uttar Pradesh	NA	NA	Postoperative wound	NA	NA	NA	[90]
India (7)	Andhra Pradesh	NA	2008.03–2008.10	Wound swab	NA	NA	NA	[42]
India (8)	Andhra Pradesh	NA	2008.03–2008.10	Wound swab	NA	NA	NA	[42]
India (9)	Andhra Pradesh	NA	2008.03–2008.10	Urine	NA	NA	NA	[42]
India (10)	Andhra Pradesh	NA	2008.03–2008.10	Ear swab	NA	NA	NA	[42]
India (11)	Andhra Pradesh	NA	2008.03–2008.10	Blood	NA	NA	NA	[42]
India (12)	Andhra Pradesh	NA	2008.03–2008.10	Throat swab	NA	NA	NA	[42]
India (13–16)	Bangalore	NA	2003.04–2007.12	Swabs from healthy individuals	NA	NA	NA	[91]
Iran (1)	Tehran	Male/67	2005	Post-heart surgery wound	NA	NA	NA	[44]
Iran (2)	Tehran	Female/51	2008.01	Foot ulcer	NA	NA	NA	[45]
Iran (3)	Mashhad	Male/26	2011.09–2011.12	Bronchial aspirate	*agr* group I, ST1283, t037	No	–	[46]
Iran (4)	Tehran	Male/>35	2014.12–2015.09	Urine	NA	Yes	NA	[92]
Iran (5)	Tehran	Female/>35	2014.12–2015.09	Urine	NA	Yes	NA	[92]
Iran (6)	Tehran	NA	2014.03–2017.02	Throat	ST5, t002, CC5	Not sure (exposure within previous 11 months)	NA	[93]
Iran (7)	Tehran	NA	2014.03–2017.02	bronchial aspirate	ST239, t037, CC8	Not sure (exposure within previous 11 months)	NA	[93]
Iran (8)	Tehran	NA	2014.03–2017.02	Wound	ST239, t037, CC8	Not sure (exposure within previous 11 months)	NA	[93]
Iran (9)	Kerman	Female/76	2015.02	Bronchial aspirate	t030	NA	NA	[94]
Iran (10)	Kerman	Female/66	2015.04	Bronchial aspirate	t030	NA	NA	[94]
Iran(11)	Guilan	NA	2017	Blood	t030	NA	NA	[95]
Pakistan (1)	Karachi	NA	NA	Blood	NA	NA	NA	[47]
Pakistan (2–9)	Faisalabad	NA	2016.03–2016.07	Pus from wounds, ear and skin	NA	NA	NA	[48]

Table 1 (*continued*)

Country (No.)	City/State	Patient (Sex/age)	Isolation time	Isolation site of VRSA	Typing and clonal complex	Vancomycin use in previous 3 months	*Enterococci* co-isolated	References*
Portugal (1)	Lisbon	Female/74	2013.05	Toe amputation wound	ST105	Yes	VR *E. faecalis*	[51]
Brazil (1)	São Paulo	Male/35	2012.08	Blood, VR-MRSA; Blood, VR-MSSA	ST8, t292, CC8	Yes	VR *E. faecalis*	[49,50]

NA: not available; -: Negative.

Five proteins encoded by the *vanA* gene cluster, VanS, VanR, VanH, VanA and VanX are essential for vancomycin resistance [62]. The original *vanA* gene cluster is carried in a transposon Tn1546 (Fig. 1c). VanS and VanR form a two-component system, and upregulate the expression of the cluster genes in the presence of vancomycin. VanH, VanA, and VanX modify the precursors from the native D-Ala-D-Ala to the resistant D-Ala-D-Lac. To do that, VanH serves as a dehydrogenase that reduces pyruvate to form D-Lac. VanX acts as a D,D-dipeptidase that hydrolyzes the native D-Ala–D-Ala to prevent it being used in the synthesis of peptidoglycan. VanA ligates D-Lac to D-Ala to produce the resistant D-Ala-D-Lac, which replaces D-Ala–D-Ala in the synthesis of peptidoglycan (Fig. 1d).

As above-mentioned, the action target of vancomycin is the terminal D-Ala–D-Ala moieties of the precursor lipid II, with which vancomycin forms hydrogen bond interactions and prevent subsequent transglycosylation and transpeptidation. However, the modified D-Ala–D-Lac leads to an almost 1000-fold decrease in affinity with vancomycin [63]. Therefore, vancomycin loses its bactericidal effect on strains with modified peptidoglycan precursor [15] (Fig. 1d).

Deletion of any one of these Van components leads to recovery of vancomycin activity, making them promising targets for new drug development [64]. For example, hydroxyethylamines, posphinate and phosphonate transition-state analogues have been demonstrated to be inhibitors of VanA [65,66]. Phosphinate based, covalent inhibitors, and sulfur containing compounds have been explored to be inhibitors of VanX [67]. These inhibitors can be used in combination with vancomycin to prevent the decreased binding affinity of vancomycin with its target.

Characteristics of VRSA infections

Up to date, there have been reports of 52 VRSA isolates with definite determination of the *vanA* gene via PCR assay. Their main characteristics are listed in supplementary Table S1. The analysis of these cases yielded the following common characteristics.

Co-infection and co-colonization of VRE and MRSA Vancomycin resistance is achieved via the transfer of resistance determinants from a donor, usually VRE, to a recipient, usually MRSA. Therefore, co-infection and co-colonization of VRE with MRSA are common in clinical cases. In many cases, VRE strains were isolated along with VRSA (Table 1). VRE and MRSA co-infection and co-colonization are particularly common in the intensive care unit and long-term cared patients [68–71]. Indwelling device use, recent antibiotic use, diabetes, and open wounds are believed to predict the co-colonization situation [72–74].

Prior use history of vancomycin In most cases, patients had a history of vancomycin use within three months prior to VRSA isolation. Vancomycin apparently serves as a selective pressure for drug resistance conferred by the *vanA* gene cluster.

Precursor diseases for VRSA infection Patients infected by VRSA generally suffered from several precursor diseases, including diabetes, end-stage renal failure, and gangrenous wound or surgical wound. Biofilms formed in wound or catheter facilitated the transfer of vancomycin-resistant plasmids from VRE to *S. aureus* [39].

Majority of VRSA isolates belongs to clonal complex 5 (CC5) lineage The molecular types of most VRSA isolates with typing data are categorized to CC5 phylogenetic lineage. Notably, in 14 characterized US VRSAs, 13 strains belong to the CC5 lineage [75,76], which is the most prevalent clones causing hospital-associated infections in the Western Hemisphere [77]. The mechanisms underlying the predominant role of CC5 lineage in VRSA formation have not been determined to date.

Effective antibiotic for VRSA Fortunately, VRSA isolates are susceptible to multiple antibiotics [78] (Table S1), which made antibacterial therapy an effective option in the clinical treatment of VRSA infections. Datomycin and linezolid are two commonly selected antibiotics for VRSA infection treatment (Table S1).

Treatment of VRSA infections

Due to the scarcity of the VRSA infection cases, no treatment guideline is currently available. Out of all reported VRSA cases, only a few provided detailed clinical data. According to these treatment experiences and CDC recommendations, the treatment of VRSA infections in general should include, but not be limited to the following.

Systemic antimicrobial therapy

Except for vancomycin resistance, VRSA isolates commonly remain susceptible to multiple antimicrobial agents (Table S1). It was reported that >90% of 13 VRSA isolates were susceptible to ceftaroline, daptomycin, linezolid, minocyline, tigecycline, rifampin, and trimethoprim/sulfamethoxazole [78]. Therefore, a systemic antimicrobial therapy with effective antibiotics is generally implemented upon VRSA isolation determination by a clinical laboratory [29,36,79,80].

Wound care

Bacterial colonization is the first step in development of infections. As described in the previous section, co-infection and co-colonization of VRE and MRSA is favorable for the development of VRSA. Wound is the most common source of VRSA isolates and provides an environment for co-infection and co-colonization of MRSA and VRE. Therefore, aggressive wound care is generally implemented in the VRSA infection treatment (Table S1) [29,36,80]. Wound management not only contributes to the removal of VRSA but also blocks further possible plasmid delivery via eliminating the favorable environment for co-colonization.

Prevention of hospital transmission

Hospital-acquired infections are commonly associated with high morbidity and mortality. In order to prevent hospital transmission, infection control precautions, including patient isolation, contact investigation, decolonization (when necessary), and prompt notification to infection control and local health authorities, should be initiated promptly upon VRSA determination in a laboratory [29,36,37]. Fortunately, no in-hospital communication has been reported so far.

In cases with detailed clinical data, clinical treatments were effective in part [36,79] but have failed in several cases due to the deterioration of the primary diseases [46,49,81]. Accumulation of more cases is necessary for recommendation of a treatment guideline for VRSA infections.

Will VRSA be widespread in future?

The emergence of vancomycin resistance has ended the dominant status of vancomycin in the therapy of Gram-positive bacterial infections. Fortunately, the occurrence of VRSA infection remains rare. This phenomenon is prominent, especially in comparison with the rapid emergence and spread of vancomycin resistance in *Enterococci*. This phenomenon may be explained by a few possible reasons listed as following.

Role of staphylococcal restrictive modification

In *S. aureus* cells, two restrictive modification systems, namely, SauI and type III-like restriction system, block horizontal gene transfer into *S. aureus* from other species and control the spread of resistant genes between the isolates of different *S. aureus* lineages [82,83].

Restriction of vancomycin use

Prior vancomycin treatment was involved in most cases of VRSA infection. The use of vancomycin is a risk factor for infection and colonization with VRE [84,85], and can increase the emergence of VRSA. Current controlled use of vancomycin (from the first line of antibiotics down to the final line of antibiotics against Gram-positive bacteria) reduces the selection pressure of vancomycin resistance.

Limitation of VRSA transmission in S. aureus

In most reported cases of VRSA infection, VRSA isolates arose from the independent transfer of the *van* gene cluster on a conjugative element from a VRE donor to a *S. aureus* recipient [75,86,87]. Epidemiologic and laboratory investigations have been used to assess the risk for transmission of VRSA to other patients, healthcare workers, and close family members. No VRSA transmission has been observed to date, and the mechanism underlying this limitation is of interesting for further investigation.

Instability of Tn1546 transposon carried plasmid in VRSA

The vancomycin-resistant phenotypes of several VRSA isolates are unstable. For example, an isolate designated as VRSA 595, which was recovered from a 63-year-old female patient at a long-term care facility in New York (US 3), reverted to a vancomycin-susceptible phenotype after two subcultures [39]. The VRSA isolate recovered in Pennsylvania (US 2) is genetically instable with a high rate of spontaneous loss of the vancomycin resistance determinant, thereby resulting in low-level vancomycin-resistant phenotype [88].

Conclusions

1. Vancomycin has been an effective agent against the MRSA infections for decades. It is likely remains domination as long as resistance to vancomycin is under control, as well as new antibiotic with superior performance are not available.
2. Although the case number of VRSA infection is limited, VRSA is still a potential threat to public health. Intensive surveillance of vancomycin-resistance, proper use of antibiotics, and adherence to infection control guidelines in health-care settings are essential for preventing emergence and dissemination of VRSA strains.
3. The identification and study of new resistant determinants are important for surveillance of vancomycin-resistance.
4. The modification of the terminal dipeptide moieties of the precursor lipid II is the main cause of failure of vancomycin action. Future research should focus on how to reverse the modification.
5. Most patients with VRSA infection suffered from underlying diseases, vaccination for susceptible populations may be a feasible way to reduce the infections caused by *S. aureus* including VRSA.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

Disclosure statement

No potential conflict of interest was reported by the authors.

Declaration of Competing Interest

The authors have declared no conflict of interest

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2019.10.005.

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