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Review Article

Laboratory approaches to detect vancomycin-resistant and vancomycin-variable enterococci: Current perspectives

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Abstract

The global spread of vancomycin-resistant enterococci (VRE), particularly *Enterococcus faecium* and *Enterococcus faecalis*, poses a substantial healthcare risk due to limited treatment options and a high potential for nosocomial transmission. Adding to this challenge is the development of vancomycin-variable enterococci (VVE), containing van A or van B genes but appearing susceptible in routine phenotypic tests, leading to diagnostic uncertainty, potential treatment failure, and silent spread. This review critically evaluates current laboratory methods for detecting VRE and VVE, including phenotypic assay, automated systems, and advanced molecular tools such as polymerase chain reaction (PCR). Loop-mediated isothermal amplification (LAMP), lateral flow assay, and whole genome sequencing. The limits of phenotypic approaches in recognising VVE are highlighted, as is the significance of molecular diagnostics in bridging these gaps. This review emphasises the importance of an integrated diagnostic approach to enhance detection accuracy, inform clinical management, and improve antimicrobial resistance surveillance.

Keywords: Vancomycin-resistant enterococci, Vancomycin-variable enterococci, Phenotypic methods, Genotypic methods.

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1. Introduction

Vancomycin is an important antibiotic used to treat Enterococcal infections. However, *E. faecalis* has eventually acquired resistance to vancomycin, potentially due to long-term antibiotic overuse, misuse, cross-infection, or genetic abnormalities. This resistance complicates the treatment of the infection.[1] Vancomycin-resistant Enterococci are frequently multidrug-resistant bacteria that provide a persistent and life-threatening risk to patients in hospital settings.[2] The acquisition of van gene clusters such as vanA, B, C, D, E, G, L, M, and N.[3] And vanA and vanB types are extensively distributed, thus gaining more public health attention.[4] Vancomycin resistance in Enterococcus typically occurs through the vanA gene, which resides on transferable plasmids, or the vanB gene, which is incorporated into the chromosome, in Germany, Europe, and globally. The rising incidence of vancomycin-variable enterococci (VVE) has recently made empirical vancomycin therapy more difficult to administer. VVE are vanA-positive and vancomycin-susceptible; however, they may transform into a vancomycin-resistant phenotype when exposed to vancomycin.[5] VanA tends to be associated with *E. faecium* rather than *E. faecalis*; a few studies report similar genotype-phenotype discrepancies in *E. faecalis. E. faecium* accounts for a significant number of VVE cases reported in the literature.[6] However, identifying VVE is challenging, and as a result, it is frequently misdiagnosed, complicating immediate treatment and contributing to VVE's global spread. As a result, proper VVE identification during clinical screening is essential for selecting the most effective antibiotic treatment and limiting nosocomial spread. Furthermore, it is crucial to monitor these strains.[7] In *E. faecium*, vancomycin resistance

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is typically mediated by the vanA gene located on transferable plasmids or the vanB gene integrated into chromosome.[8]

2. Mechanisms of Vancomycin Resistance

2.1. Vancomycin-resistant enterococci (VRE)

Vancomycin inhibits cell wall production by binding to the terminal D-Ala-D-Ala dipeptide of cell wall precursors, preventing them from converting into peptidoglycan[2]. The change of the D-Ala-D-Ala terminal amino acids of dipeptide monomer subunits to either D-Ala-D-Lac or D-Ala-D-Ser is the primary cause of vancomycin resistance in Enterococci.[9] Enterococci resistance to vancomycin is connected to the operon combinations vanA, B, C, D, E, G, L, M, N, and F.[10] The most prevalent types of clusters are vanA and vanB. Mobile genetic elements typically transfer vancomycin resistance gene clusters, including the transposon Tn1546 and the integrative and conjugative element Tn1549, which carry vanA- and vanB-type resistance, respectively. These components can be added to plasmids and chromosomes.[11] Their decreased susceptibility distinguishes them. The vanA phenotype appears to be far more pathogenic than the vanB phenotype, and the vanC phenotype has recently emerged as the most important.[10] They are categorised into two separate groups based on the ligases they encode. The operons vanA, vanB, vanD, and vanM encode the D-Ala-D-Lac ligase. The operons vanC, vanE, vanG, vanL, and vanN encode D-Ala-D-Ser ligase. VanA enterococci resist a high dose of vancomycin [Minimum Inhibitory Concentration (MIC), 64 mg/mL] and teicoplanin. The presence of either medication fosters resistance. VanB microbes are vancomycin resistant at concentrations ranging from 4 to 11024 mg/ml. They are generally sensitive to teicoplanin and have not been previously observed to cause resistance. VanA and vanB clusters are mainly found in *E. faecalis* and *E. faecium*. VanA and vanB have been detected in fewer enterococcal species.[12] *gallinarum* and *E. casseliflavus* are resistant to vancomycin (MICs of 4-32 μg/ml) and susceptible to teicoplanin.[13] (**Table 1**)

2.2. Vancomycin-variable enterococci (VVE)

A cluster of seven genes (vanRSHAXYZ**)** is responsible for mediating VanA-type high-level resistance to both vancomycin and teicoplanin.[14] When glycopeptide antibiotics are given to patients, the two-component regulatory system, which comprises the sensor kinase VanS and the response regulator VanR, is initiated. This activation leads to the upregulation of resistance enzymes—VanH, VanA, VanX, and VanY—The peptidoglycan precursor's terminal dipeptide from D-Ala-D-Ala to D-Ala-D-Lac, thereby reducing vancomycin binding and conferring resistance.[15] In contrast, Vancomycin-Variable Enterococci (VVE), despite carrying the vanHAX operon, typically lack the regulatory genes vanR and vanS, preventing constitutive or inducible resistance expression.[16] As a result, VVE often appear phenotypically susceptible to vancomycin and can escape detection by standard VRE screening methods, posing a risk for underdiagnosis and silent dissemination in clinical settings.[17] This latent potential for resistance, if triggered, compromises therapeutic success and renders VVE a significant reservoir of vancomycin resistance determinants.[18] (**Table 2**)

3. Phenotypic Detection Methods

3.1. VRE selective media (E.G., Chromogenic AGAR)

Chromogenic media are used for detecting and identifying VRE, as it is simple, affordable particularly helpful in surveillance settings. Several authors suggested chromogenic media for identifying VRE. According to Alessa et al. in their study, five types of agar media were tested for detecting vancomycin-resistant enterococci (VRE): ChromID VRE, CHROMagar VRE, Brilliance VRE, VRE Select, and Chromatic VRE. ChromID VRE, CHROMagar VRE, and Brilliance VRE had the maximum sensitivity and specificity after 24 hours of incubation.[20] Gouliouris et al.[19] in their study used VRE Brilliance and Chrom ID. Comparing these two, Brilliance agar showed greater sensitivity and selectivity with a pre-enrichment step and 48 hours of incubation.[20] In contrast, Neil W. Anderson et al and Kling et al. in their studies found high sensitivity in VRE select agar after 24 to 28 hours of incubation.[21,22] But all these authors suggested a combination of clinically relevant diagnostic methods to test vanA/B PCR for *E. faecium* isolates.

4. Culture-Based Techniques

4.1. Agar dilution and broth microdilution

Young Lee et al conducted antimicrobial susceptibility testing (AST) was conducted and vancomycin (VAN). The testing followed CLSI guidelines, and minimum inhibitory concentrations (MICs) were determined using the broth microdilution method. Their study emphasised that relying solely on phenotypic testing would have missed VVE, underscoring the importance of molecular detection of van genes (vanA/vanB).[22] Reena Rajan et al study identified VanA–VanB genotype–phenotype discrepancies, reinforcing that genotypic detection is essential since phenotypic tests alone can miss low-level resistance. It confirms that disk diffusion methods are unreliable for detecting low-level glycopeptide resistance, recommending MIC-based testing (e.g., agar dilution, E-test) and molecular methods (e.g., multiplex PCR) for accurate detection.[23]

4.2. E-Test (Gradient strip)

Few authors demonstrated that the E-test accuracy in detecting VVE. Madhubala Mishra et al. used susceptible isolates and successfully detected VVE strains that failed to be identified by the disc diffusion method.[24] Ritu Shah et al. confirmed 24% of isolates as true vancomycin-resistant, which were initially identified as resistant or intermediate by

disc diffusion. Köhler et al. identified phenotypically susceptible vanA isolates in the disc diffusion test, later tested using the E-test and showed low-level resistance (MIC 1–4 μg/mL). This prevented VSE from misinterpreted and accurately identified VVE.[15] Similar studies were done by Viswanath et al. and Young Yoo et al., according to CLSI criteria.[3,5] In contrast, a study by Deepa Pramod Devhare et al. recommends using disc diffusion as a screening tool and E-test as a confirmatory method, especially in the resource-constrained sector. Their study showed 100% compatibility in all Disc diffusion, E-test, and the BD Phoenix automated system.[25] These studies proved the reliability and serve as a phenotypic confirmation method for detecting VRE and VVE in clinical settings, thereby underscoring its superior accuracy in true identification and highlighting its clinical utility.

5. Automated Systems

5.1. Vitek 2

Victoria Jordan et al. reported that 6% of vancomycin-susceptible *E. faecium* isolates harboured the van A gene, revealing VVE undetected by VITEK2, highlighting the need for genotypic testing.[26] Walker et al**.** in their study, VITEK2 failed to consistently detect vanB-mediated vancomycin resistance in *E. faecium*, including strains with high-level MICs, thus posing a risk for treatment failure and unnoticed transmission. And he also suggests that doing additional diagnostic methods, such as chromID VRE agar and PCR, is necessary to reliably identify VRE, especially occult VRE.[27] The VITEK 2 automated system is widely employed to detect multidrug-resistant pathogens, namely methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum β-lactamase (ESBL) producers, and carbapenemase-producing organisms. However, it is not able to consistently detect vancomycin-variable enterococci (VVE), as these organisms usually demonstrate phenotypic susceptibility to vancomycin despite carrying resistance genes such as vanA or vanB. For precise detection of VVE, molecular techniques are required.

5.2. Phoenix

The BD Phoenix™ system has demonstrated consistent reliability in detecting *Enterococcus faecium* strains with phenotypically expressed vancomycin resistance (VRE), as confirmed in studies by Rocha et al.[28] According to Victoria Jordan et al., 6% of VSE isolates included vancomycin resistance genes. Although genotypic testing may identify resistance genes left out by standard susceptibility approaches, like as Phoenix, more study is needed to prove its clinical significance and confirm the approach used.[26] Viswanath et al. show that automated systems like Phoenix failed to detect VVE in India, but it doesn't assess Phoenix specifically.[5]

5.3. Microscan

The MicroScan automated system (Beckman Coulter) is widely used for phenotypic antimicrobial susceptibility testing (AST), including detection of vancomycin resistance in *Enterococcus* spp. However, several studies have reported its limitations in accurately finding vancomycin-variable enterococci (VVE), which carry van genes yet exhibit phenotypic resistance. While MicroScan is successful at detecting VRE, numerous studies highlight its limits in identifying vancomycin-variable enterococci (VVE). Semra Bilen et al. reported false-positive VRE results by MicroScan that were sensitive to vancomycin by E-test and PCR. Santona et al. [30] showed that MicroScan could not identify a vanB2-*positive E. faecium*, indicating the presence of an occult [VRE30]. Viswanath et al. identified that MicroScan missed % of phenotypically susceptible E. faecium isolates containing van A, which is consistent with the VVE phenotype.[5]

6. Genotypic Detection Methods

6.1. PCR-based detection

Coccitto et al. in their study used both conventional and qPCR; they found removals in vanR/S and noticed that resistance reappeared following vancomycin exposure due to promoter activation and increased plasmid copy.[17] The findings highlight the importance of molecular screening in accurately detecting VVE and preventing therapy failure. Yoo et al. also state the same.[3] Elizabeth Osadare et al. used Multiplex real-time PCR for detecting van A, van B using species-specific markers. These procedures have been suggested for routine screenings, particularly in high-risk clinical settings.31 Lee et al. developed a one-step multiplex qPCR technique for the concurrent identification of vanB and vanA. This method provides quick and accurate identification of VRE in a clinical setting. A.L. Dahl et al.[32] Enrichment-based real-time PCR gave increased specificity and sensitivity, and an accurate and fast technique for the identification of vanB-positive VRE. Hozan Muhammed Abdullah et al. An easy, economical real-time PCR allowed for the quick detection of vanA/vanB genes directly from cultures, showing a vancomycin-variable *E. faecium* outbreak.[7]

6.2. Whole genome sequencing (WGS)

Whole-genome sequencing (WGS) is a diagnostic method that identifies the entire DNA sequence of an organism's genome in just a single step, allowing for extensive investigation of chromosomes, plasmids, and mobile genetic components. Whole-genome sequencing was crucial in identifying a clonal outbreak. Hozan Muhammed Abdullah et al.[7] used WGS to confirm a clonal outbreak of vancomycin variable *E. faecium* (ST1421-CT1134) carrying vanA, despite phenotypic susceptibility. Raspail Carrel Founou et al. used WGS to show that VRE strains in South Africa carry complex resistance genes, virulence factors, and mobile

elements, underscoring the need for genomic surveillance to inform infection control and antibiotic stewardship.[33] Coccitto et al. proved that VVE strains frequently carry vanA with phenotypic sensitivity, with WGS identifying structural deletions and promoter mutations that show reversible resistance.[17] Yoo et al. reported similar findings.[3] Sobkowiak et al. used long-read WGS and plasmid typing to distinguish outbreak-related VREfm isolates from sporadic cases, enhancing infection control measures.[34]

6.3. Detection challenges in vve

Mutations in the vanA gene cluster lead to genotypic-phenotypic mismatches, resulting in VVE, which enhances the possibility of resistance spreading and treatment failures. The silent transmission of VVE in a nosocomial setting occurs because it bypasses the traditional Antibiotic Susceptibility test. VVEs have frequently been described as clusters producing hospital outbreaks. Following vancomycin exposure, VVE shift to VRE both in vivo and in vitro. The transmissible gene cluster and diagnostic challenges often make VVE undetected, hindering treatment and facilitating its spread. Accurate detection and surveillance are vital for control. As a result, further genotypic methods are required to precisely identify all vanA- and vanB-positive VREfm in clinical practice.[35]

7. Emerging and Alternative Methods

7.1. Loop-mediated isothermal amplification (LAMP)

LAMP is a quick, sensitive, and specific nucleic acid amplification process that runs at a constant temperature (60-65 °C) without thermal cycling, making it Suitable for low-resource and point-of-care situations.[36] A study by Ikenaga et al used the LAMP method that specifically detected van A and van B in VRE isolates and reference strains, showing detection limits comparable to conventional PCR but with faster and simpler execution, making it suitable for a clinical setting. Azizi et al.[37] used duplex LAMP for the first time in their investigation to identify E. faecalis and the vanA gene, which was followed by culture and multiplex-PCR. The technique has great sensitivity and specificity, is inexpensive, but time-consuming, with a low Limit of Detection (LOD) in comparison to other processe.[38] Huang et al.'s method of identification has a high potential for clinical use because it can identify the vanA gene through a clinical sample.[39] Baek et al used Multiplex LAMP shows high specificity and sensitivity, and it can be used in clinical settings.[36]

7.2. Lateral flow assay

Researchers have done various studies to find the effectiveness of lateral flow-based tests for the quick detection of vancomycin resistance in *E. faecium*. Oueslati et al. used the NG-Test LFIA for the identification of vanB VREs. This equipment-free test is basic, rapid (15 minutes), and appropriate for regular clinical workflows. Hence, it could be simply implemented into the routine procedures by most clinical testing laboratories as an additional confirmation for VanB-VREs.[40] Combined with the NG-Test VanA, Panpru et al. designed a Recombinase Polymerase Amplification with Lateral Flow (RPA-LF) assay focusing on *ddl*, *vanA*, and *vanB.* Compared to PCR, LFA provides 10 times higher detection with 100% sensitivity and specificity within 30 minutes at 37°C.[41] Similarly, Tuo Ji et al. demonstrated 100% specificity in the MIRA-LFS assay for vanA-type VREfm; it is also 100% reliable with PCR, hence it is appropriate for use in low-supply, point-of-care settings.[42]

7.3. Maldi-TOF MS

Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is a commonly used instrument for bacterial detection in clinical microbiology.[43] new research has investigated its potential for detecting vancomycin-resistant *Enterococcus faecium* (VRE), especially when linked with machine learning.[44] Wang et al.in their research, the raw MALDI-TOF data with MS spectrum were immediately turned to the CNN model. Susceptibility or resistance to vancomycin can be predicted in seconds and reported to clinical practitioners. Candela et al. also demonstrated that MALDI-TOF MS combined with deep learning or classifiers like support vector machine (SVM) and partial least squares-discriminant analysis (PLS-DA) can distinguish VRE from susceptible strains using protein spectra but fail to detect VVE, which harbour silent vanA genes without phenotypic resistance.[45] Kling et al.' approach with Spectra™ VRE medium and MALDI-TOF MS detects phenotypic VRE but not VVE.[22] Schmidt Santiago et al. conducted a thorough review that reaffirms the potential of MALDI-TOF paired with machine learning for resistance prediction while also noting present limitations, particularly in the detection of VVE.[44]

7.4. Clinical and infection control implications

Correct detection of VVE during laboratory tests is critical for choosing the right antibiotic treatment and controlling nosocomial spread. Furthermore, surveillance for these strains is necessary. Enterococci should be tested by both phenotypic and genotypic methods, as vancomycin use in VVE infection may induce resistance, causing treatment failure and public health impact.[18] CLSI 2022 and EUCAST 2025 recommended reporting Enterococcus isolates as vancomycin-resistant if van A is detected or vancomycin resistance is observed. EUCAST also advises reporting them as teicoplanin. This cautious approach addresses possible VVE and testing approaches. Train all healthcare personnel on infection prevention and control (IPC) measures, VRE and VVE. To decrease the spread of HA-VREfm/VSEfm clones, stricter preventive actions, such as Proper hand hygiene, isolating affected patients and improving disinfection procedures, are recommended.[30] Incorporating all of this can lead to a more comprehensive and effective strategy for combating the spread of diseases.[46] (**Table 3**)

Table 1: Phenotypic, genotypic and clinical characteristics of VAN A vs VAN B

Features	vanA Operon	vanB Operon	Reference
Genetic Location	Usually, on plasmids, transposon Tn1546	Typically chromosomal, transposon Tn1549/Tn5382	[8,11]
Inducibility	Inducible by vancomycin or teicoplanin	Inducible only by vancomycin	[12]
Host Species	Generally seen in *E. faecium* and *E. faecalis.*	More common in *E. faecalis*, *E. faecium*	[13]
Clinical Impact	Widespread, causes outbreaks; harder to treat	Often undetected, silent in VVE cases	[17]
Resistance Level	Increased vancomycin and teicoplanin resistance	Vancomycin resistance is variable; however, teicoplanin is sensitive.	[12]
Vancomycin MIC (μg/mL)	>64	4–64	[12]
Teicoplanin Resistance	Resistant	Susceptible	[12]
Terminus of Cell Wall Precursor	D-Ala-D-Lac	D-Ala-D-Lac	[6]
Regulation System	Two-component system (VanR–VanS)	Two-component system (VanR–VanS)	[15]
Detection Challenges	Detectable by PCR; phenotypic tests match genotype	Phenotypically variable; may test susceptible while harbouring *vanB*	[6,17]

Table 2: VRE vs VVE diagnostic and clinical features

Features	VRE (Vancomycin Resistant Enterococci)	Van B Operon (Vancomycin Variable Enterococci	Reference
Definition	Enterococci having consistent, high-level resistance to vancomycin	Enterococci with vanA gene but variable vancomycin resistance expression(49)	[19]
Common genotype	vanA, vanB, vanD, etc.	Mostly vanA, occasionally vanB	[5]
Phenotype	High vancomycin resistance (MIC ≥32 μg/mL)	Inconsistent or fluctuating resistance; can appear vancomycin-susceptible	[12]
Clinical implication	Known resistant vancomycin is ineffective	May be misclassified as a susceptible risk of treatment failure	[17]
detection	Constitutive (always expressed), detected by both genotypic (PCR for vanA/vanB) and phenotypic (MIC, disk diffusion) methods	Variable, often silent or inducible expression of vanA may be missed by phenotypic tests unless genotypic testing is done	[6,17]
Risk	Spreads in healthcare settings; difficult to treat	The risk of emergent resistance during therapy is underdiagnosed	[7,10]
Example species	*E. faecium*, *E. faecalis*	*E. faecium*, rarely *E. faecalis*	[6,12]

Table 3: Comparative table for each diagnostic method

Diagnostic method	Cost	Accuracy (sensitivity /Specificity)	Turnaround time	Accessibility	Reference
Chromogenic agar (ChromID VRE, CHROM agar VRE, Brilliance VRE, VRE Select	Approximately Rs.1000 per isolate	90-95% after 24-48 hrs, but false negatives are possible without enrichment	1-2 days	Widely available in routine microbiology labs	[1,3]
Conventional culture +MIC (Agar dilution /Broth microdilution)	Approximately Rs.800	High for phenotypically resistant VRE, but can miss low-level VVE	1-2 days	Standard in most tertiary labs	[4,5]

E-test (Gradient Strip)	ApproximatelyRs.1,200	Higher sensitivity for low-level resistance compared to disc diffusion; detects MIC 1-4 g/mL	1-2 days	Available in most tertiary labs	[6,7]
Disc Diffusion	ApproximatelyRs.200	Lower accuracy for VVE (misses vanA/vanB-positive phenotypically susceptible isolates)	18-24h	Widely available	[5,8]
Automated AST (VITEK 2, BD Phoenix, MicroScan)	Approximately Rs 5000 per card	Variable -can miss VVE despite genotypic positivity; false positivity /negatives reported	6-18h	Common in tertiary /reference labs	[9,11]
Conventional PCR (vanA/vanB genes)	Approximately Rs1,500-2000	>95% if target genes intact; detects genotype regardless of phenotype	4-6h	Available in molecular diagnostic labs	[12,13]
Multiplex PCR	Approximately 2,500-3,000	High; allows simultaneous detection of multiple van genes	4-6h	Advanced microbiology labs	[13,15]
Real-time PCR (qPCR)	Approximately Rs 3,000-3,500	>98% quantifies gene copies; detects directly from culture or specimen	2-4h	Reference/Molecular labs	[14,16]
Whole-genome sequencing (WGS)	Approximately Rs 50,000-1,00,000	Highest possible; detects all resistance /virulence genes, mobile elements, and clonal relationship	2-5days	Only in advanced research /reference centres	[17-19]

Table 4: Data sharing, surveillance, and cross-border implications of VVE spread

S.No	Year	Country	Numeric VVE estimate	Denominator (what the % refers to)	Infection type reported	Reference
1.	2019-2022	South Korea	5.9%	All blood stream (Kor Glass 2017-2022)	Bloodstream infection.	[7]
2.	2015-2016	Canada	47%	Van A positive sterile site isolates (study collection)	Primarily, bacteremia/ sterile site isolates.	[16]
3.	2020-2022	India	1.5%	Phenotypically vancomycin-susceptible *E. faecium* (multi-centre tertiary collection)	Bloodstream infection.	[5]
4.	2017-2024	Taiwan /Southern hospital series	4-7%	Usually, VSE or hospital collection denominators (local studies)	Bloodstream infection, other hospital infections	[3,49]
5.	2018-2024	Italy	4-5%	Putative VVE ~ 4.6% of clinical enterococcal isolates in a hospital collection (vanA +, phenotypically susceptible; local hospital series).	Clinical isolates, including blood	[18]
6.	2019	Denmark	-	High proportion within van A isolates -emerging ST1421 VVE clone dominated some regional collection (44% of van A /van B	Bloodstream infection, other hospital-associated infections	[50]

				/vanA-vanB isolates in Q1 2019 were that clone); VVE widespread surveillance /genomic reports		
7.	2018	Australia	-	Multiple VVE reported (outbreaks /surveillance); AGAR reported VVE among van A carriers (numbers variable; several single-centre and national reports	Bloodstream infection and hospital outbreaks	[51]
8.	2014-2016 (outbreaks & reports)	Other European countries (Italy, Norway, UK, Denmark, broadly).	-	Presence reported in outbreaks or hospital series; prevalence varies widely, and many papers report presence rather than large prevalence estimates	Hospital-associated Bloodstream infection.	[6,46,52,53]

7.4. Case-based scenarios showing diagnostic challenges and treatment outcomes

Several case reports and series have highlighted the diagnostic and therapeutic challenges posed by vancomycin-resistant enterococci (VRE). Coburn et al described a single case of *E. faecium* bacteraemia in a 67-year-old male with acute myeloid leukaemia, where an initially vancomycin-susceptible isolate harbouring the van A gene developed high-level resistance during therapy.[47] Downinh et al. reported a 54-year-old male with alcohol related cirrhosis and spontaneous bacterial peritonitis who developed but became resistant in vivo.[48] Lakshmi Shree et al. Documented 11 cases from India, with patients ages ranging from 23 to 68 years, including diagnoses such as UTI, bloodstream infections, and intra-abdominal sepsis. These cases collectively emphasise the potential for VVE to emerge during treatment, often leading to delayed recognition of resistance and necessitating changes in antibiotic therapy. **(Table 4)**

8. Discussion

Emphasis on the identification and management of vancomycin-resistant enterococci (VRE) has been strongly recommended as a part of antimicrobial resistance (AMR) strategies in all healthcare settings. Despite this, the strategies are often facing a threat due to the emergence of vancomycin-variable enterococci (VVE), the isolates which are phenotypically susceptible but carry the VAN gene.

This review elaborates on the shortcomings in the timely and accurate identification of VVE despite the availability of elaborate diagnostic procedures, including automated systems like VITEK 2, BD Phoenix, and MicroScan, and phenotypic methodologies like chromogenic agar and E-tests. As VVE exhibits no observable resistance in the phenotypic approaches, identification is completely reliant upon the response to exposure to antibiotics. For that reason, there are several missed cases, delay in diagnosis, misidentification, treatment failure and undiagnosed nosocomial transmission.

Molecular tests such as PCR (including real-time and multiplex formats), LAMP, and WGS can be employed for the identification of the virulent genes (vanA and vanB), which hold a higher sensitivity and specificity. VVE phenotypes can be tracked using the molecular and automated tools by tracking hidden resistance, trailing the spread of clones, uncovering mutations or deletions in the regulatory genes. Outbreak surveillance and the conception of the evolution of resistance mechanisms have been made possible using WGS. Regardless of the benefits of such methods, downsides including low-resource environments, high cost and longer turnaround time exist. Consequently, using both genotypic and phenotypic tools will provide a well-rounded, economical, and clinically useful diagnostic pathway.

The precision of identification of VVE phenotypes can be augmented by using chromogenic media for initial screening, MIC calculation and confirmation by PCR or LAMP testing.

If the newer techniques like MALDI-TOF MS and lateral flow assays are combined with AI or machine learning, it can expedite prompt point-of-care testing. Still, their usefulness in identifying VVE is restricted and requires more development and verification. Failing to detect VVE clinically can lead to inappropriate treatment, ongoing vancomycin use in patients who do not respond, adding to the spread of resistance. Considering the possibility of this phenotypic variability, CLSI and EUCAST have upgraded their guidelines, necessitating the reporting of vanA-positive isolates as resistant, irrespective of their MIC. Hence, while formulating the protocols for infection control, including staff sensitisation, patient isolation and targeted screening, VVE phenotypes should also be taken into consideration.

8.1. Implementation barriers: In rural or resource-limited settings

8.1.1. Realistic assessment

Acknowledges the significant gap between advanced molecular diagnostics recommended in the review and the reality of resource-constrained healthcare settings.

8.1.2. Tiered approach

Proposes a three-level implementation strategy that aligns diagnostic complexity with facility capacity and resources.

8.1.3. Technology adaptations

Focuses on promising point-of-care solutions mentioned in the review (LAMP, lateral flow assays) that could be more feasible in rural settings.

8.1.4. Practical solutions

Emphasizes cost-effective strategies like enhanced phenotypic methods (E-test) and selective molecular testing rather than comprehensive molecular diagnostics for all cases.

8.1.5. Systems strengthening

Addresses broader challenges including training, quality assurance, supply chain, and financing mechanisms.

8.2. Meta analytical commentary

8.2.1. Review strengths

The comprehensive coverage across detection methods, strong clinical relevance, and technical depth spanning traditional to emerging technologies.

8.2.2. Methodological limitations

The lack of systematic quality assessment, absence of formal meta-analysis, and heterogeneity in study designs and populations.

8.2.3. Clinical significance

The commentary highlights the critical finding that VVE represents a "silent threat" in healthcare settings, with up to 6% of phenotypically susceptible isolates harbouring resistance genes.

8.2.4. Research gaps

Identifies the need for standardised reference methods, prospective outcome studies, and health economic analyses.

9. Conclusion

For the prompt and precise detection of VVE and VRE, Effective clinical management, critical AMR surveillance, and stringent infection control practices are mandatory. In this regard, to counter this expanding clinical issue, a tiered diagnostic strategy including targeted molecular assays and conventional phenotypic diagnostic tools is needed. The potential research arena is open for augmenting detection algorithms for such atypical phenotypes like VVE. Easier access to genomic technologies and streamlining molecular workflows can foster the early detection of cases.

10. Conflict of Interest

We declare no conflict of interest.

11. Ethical Approval

There is no ethical issue.

12. Acknowledgement

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