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Tackling Vancomycin-Resistant *Enterococcus faecium* in Hospital-Acquired Infections: Leveraging qPCR and Isothermal Amplification for *vanA* Gene Detection

Ajab Khan[1], Sania Khalid[2], Ayaz Ahmad[3], Saad Najam[4], Abdul Sami Sandhu[5]

[1]Department of Biotechnology, University of Malakand, Pakistan
[2]Institute of Microbiology, University of Veterinary and Animal Sciences
[3]University of Chinese Academy of Sciences, Beijing, China
[4]Department of Pharmacology, Jinnah Medical and Dental College, Sohail University Karachi Pakistan
[5]Department of Plant Pathology, University of the Punjab, Lahore

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Corresponding Author:
Ayaz Ahmad,
University of Chinese Academy of Sciences,
Beijing China,
ayazmicro0012@gmail.com

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ABSTRACT

Hospital-acquired infections (HAIs) in Enterococcus fecium (VREfm) are primarily caused by the resistant to vancomycin vanA gene, which confers high-level resistance to vancomycin. With an emphasis on quantitative PCR (qPCR) and loop-mediated isothermal amplification (LAMP) for vanA detection, this review delves deeply into the epidemiology, molecular mechanisms, and diagnostic challenges of VREfm. We explore their applications using case studies, mutation analyses, and gene prevalence computational data. The benefits and drawbacks of LAMP and qPCR in clinical contexts are carefully considered. Two tables provide a summary of vanA mutation and VREfm prevalence data from recent studies. By combining data from around the globe to guide infection control and diagnostic approaches, the review tackles the urgent need to address the public health threat posed by VREfm. Future directions like CRISPR-based diagnostics and next-generation sequencing (NGS) are also explored.

1. Introduction

Enterococcus fecium, a gram-positive naturally occurring of the human gastrointestinal tract, has developed into a dangerous nosocomial pathogen as a result of its acquisition of vancomycin resistance, which is primarily mediated by the vanA gene cluster on genetic elements that are mobile such as Tn1546 [1]. Vancomycin-resistant *E. faecium* (VREfm) causes severe HAIs, including bloodstream infections, urinary tract infections (UTIs), endocarditis, and surgical site infections, with mortality rates in immunocompromised patients ranging from 50% to 73% [2, 3]. When the vanA gene alters peptidoglycan precursors, vancomycin loses its binding affinity and loses its effectiveness [4]. The WHO has designated VREfm as a high-priority pathogen due to its extensive distribution and lack of effective treatments [5].

Rapid and accurate diagnostics are necessary to control VREfm outbreaks. VanA can be quickly intervened in because it is highly sensitively targeted by isothermal amplification methods like LAMP and molecular techniques like qPCR [6]. This review examines the epidemiology of VREfm, the mechanisms of resistance, and the diagnostic utility of qPCR and LAMP using case studies and computer modeling information on the vanA, vanB, esp, and hyl genes. We provide two targeted tables, a detailed evaluation of the advantages and disadvantages of these strategies, and a discussion of how to apply them to infection control strategies. The article aims to provide researchers and clinicians with a robust framework to address the growing threat posed by VREfm.

2. Epidemiology of VREfm in Hospital-Acquired Infections

Particularly in high-risk settings like intensive care units (ICUs), oncology wards, and transplant units, VREfm is a major contributor to HAIs. While prevalence rates in Europe can reach 50% in Portugal and 19% in Ireland, VREfm is the cause of 28.5% of enterococcal infections in the United States [7]. Between 2012 and 2020, Thailand's rate rose from 0.7% to 6.9% in Asia due to hospital transmission [8]. Although it is underreported in resource-constrained environments such as sub-Saharan Africa, the average prevalence of VREfm has been reported to be 15% in Nigerian hospitals due to diagnostic limitations [9]. Clonal complex 17 (CC17), which comprises sequence types (ST) ST80, ST17, ST117, and ST761, is the predominant cause of VREfm infections due to its multidrug resistance and hospital adaptation [10].

The vanA gene, which is commonly carried on conjugate plasmids and facilitates horizontal gene transfer, speeds up the spread of VREfm [11]. Risk factors include prolonged hospital stays, devices that are embedded (like ventilators and catheters), and prolonged contact with antibiotics like vancomycin, cephalosporins, and anti-anaerobic agents [12]. Environmental contamination, such as on bedrails and medical equipment, further perpetuates nosocomial transmission [13]. According to surveillance data, 60–80% of cases have VREfm colonization prior to infection, highlighting the importance of early detection [14].

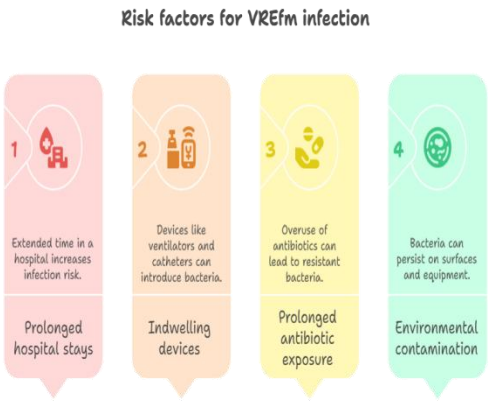


Table 1: Prevalence of VREfm in Hospital-Acquired Infections Across Regions (2015–2023)

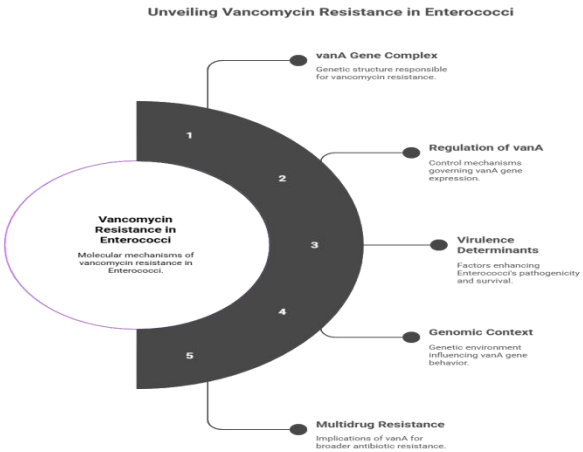
Region	Study Period	Sample Size	VREfm Prevalence (%)	Dominant STs
USA	2015–2020	12,000	28.5	ST17, ST117
Ireland	2016–2019	8,500	19.0	ST80, ST761
Portugal	2017–2020	6,200	50.0	ST117, ST203
Thailand	2012–2020	4,800	6.9	ST17, ST80, ST761
Nigeria	2018–2021	2,100	15.0	ST17, ST412

Note: Data obtained from worldwide surveillance studies; incidence varies by hospital type and diagnostic capabilities [7, 8, 9].

3. Molecular Mechanisms of Vancomycin Resistance

The vanA gene complex, consisting of seven genes (vanRSHAXYZ) on Tn1546 transposon, is the predominant genetic determinant of vancomycin resistance in VREfm [15]. The vanA operon congenerates D-Ala-D-Lac peptidoglycan precursors, which exhibit a 1000-fold lower affinity for vancomycin than native D-Ala-D-Ala [16]. Expression of vanA is mediated by the VanS-VanR two-component system, which senses vancomycin and induces transcription [17]. Mutations in vanS (e.g., G133D) or vanR (e.g., T115A), or deletion in vanX (e.g., 252 bp) can lead to vancomycin-variable *enterococci* (VVEfm), that appear susceptible before treatment, but have the resistance phenotype again during antibiotic) [18, 19]. The less prevalent vanB gene confers low-level resistance and is associated with Tn1549 [20].

Virulence determinants such as esp (enterococcal surface protein) and hyl (hyaluronidase) increase VREfm's virulence by encouraging biofilm development and tissue invasion, respectively [21]. Bioinformatics studies indicate vanA co-occurs with tetM (tetracycline resistance) and ermB (erythromycin resistance) in 60–80% of strains, signifying multidrug resistance [22]. WGS research reveals vanA's genomic flexibility, with plasmids harboring other resistance genes such as *aac(6')-aph(2") (aminoglycoside resistance) in 50% of VREfm strains [23].



4. Molecular Diagnostics: qPCR and Isothermal Amplification

4.1 Quantitative PCR (qPCR)

With a detection limit of approximately 100 fg/μL and 100% specificity, qPCR is the gold standard diagnostic method for identifying vanA in clinical samples [24].

It completes assays in one to two hours and monitors real-time amplification using fluorescent probes (such as TaqMan) or SYBR Green [25]. The simultaneous detection of vanA, vanB, and vanC by multiplex qPCR assays enhances diagnostic efficacy in mixed infections [26]. A qPCR assay for vanA in 500 rectal swabs was validated in 2020, with 98.7% sensitivity and 99.2% specificity [27]. In the assay, a 25 µL reaction was first denaturated for 4 minutes at 95°C. This was followed by 35 cycles of denaturation (95°C, 30 s), annealing (55°C, 1 min), and extension (72°C, 1 min). The quantitative results of qPCR are useful for monitoring the spread of resistance and directing antibiotic stewardship [28].

4.2 Loop-Mediated Isothermal Amplification (LAMP)

Using a strand-displacing polymerase and four to six primers, LAMP amplifies vanA at a steady 65°C, reaching a sensitivity of 100 pg/µL in 25 minutes [29]. Because it is isothermal, thermocyclers are not necessary, which makes it perfect for point-of-care testing in environments with limited resources. A LAMP assay for vanA in 300 urine samples was optimized in a 2019 study, resulting in 100% concordance with qPCR and allowing for visual detection through color change [30]. Rapid screening in outbreak situations is supported by LAMP's portability and affordability (reagents ~$1-2 per test), especially in hospitals with limited resources [31].

4.3 Pros of qPCR for *vanA* Detection

Quantitative PCR is widely accepted for its ability to determine low bacterial loads in complex clinical samples like blood, stool, or urine because of its remarkable sensitivity, which can detect as little as 100 fg/µL of vanA DNA [24]. Clinicians can evaluate the degree of colonization or infection and track resistance trends over time due to its quantitative nature, which yields precise evaluations of vanA copy numbers [27]. In situations where enterococcal populations are diverse, qPCR's support for multiplex assays, which concurrently detect multiple resistance genes (vanA, vanB, and vanC) in an individual reaction, speeds up diagnostics and increases efficiency [26]. The approach is dependable for clinical decision-making and epidemiological surveillance due to its high specificity (99–100%), which reduces false positives [28]. Additionally, qPCR's reproducibility across labs is guaranteed by its compatibility with automated systems and standardized protocols, which increases its value in hospital networks and extensive research [32].

4.4 Cons of qPCR for *vanA* Detection

Despite its advantages, qPCR has serious disadvantages. The requirement for expensive thermocyclers (costing across $20,000 and $50,000) and concentrated laboratory equipment limits its use in resource-constrained environments, particularly in low-income countries where VREfm prevalence is rising [33]. Because sample preparation, primer design, and data analysis must be handled by skilled personnel, the method increases operational costs and complexity [34]. Because inhibitory samples, like blood elements or fecal matter, can result in false negative results, robust DNA extraction techniques are required [24]. Additionally, the cost of reagents for regular testing in high-burden environments may be prohibitive due to the use of fluorescent dyes or probes in qPCR, which increase the cost of reagents by approximately $5 to $10 per test [35]. The need for cold-chain reagent storage complicates its deployment in remote areas [36].

4.5 Pros of LAMP for *vanA* Detection

LAMP offers a number of advantages for vanA detection, chief among them being its accessibility and ease of use. LAMP can be utilized for testing at the point of care in hospitals with restricted funds because it only requires a heat block or water bath to maintain a constant temperature of 65°C [29]. Its 25-minute turnaround time allows for rapid VREfm identification, which is crucial for outbreak control [30]. Even untrained staff can interpret results using LAMP's visual detection techniques, like color change or turbidity, without the need for complicated equipment [31]. The method's low cost (reagents ~$1-2 per test) as well as lack of cold-chain demands make it economically viable for widespread use in low-resource settings [37]. The reliability of LAMP for direct testing from urine or rectal swabs is increased by its resistance to inhibitors in clinical samples [38].

4.6 Cons of LAMP for *vanA* Detection

The main drawback of LAMP is its lower sensitivity (~100 pg/μL) in comparison to qPCR, which could lead to missed detections in samples with low bacterial loads [29]. Due to the technique's dependence on four to six primers, assay design becomes more complex, and there is a chance that non-specific amplification will result in false positives in mixed samples [39]. LAMP's usefulness in tracking resistance dynamics is limited by its primarily qualitative nature and lack of qPCR's quantitative precision [40]. Subjectivity may be introduced by the method's reliance on visual interpretation, especially when used by novices or in low light [31]. Furthermore, LAMP's use in detecting multiple resistance genes at once is limited by its inability to readily support multiplex assays, which is a crucial requirement in multidrug-resistant VREfm infections [41].

5. Case Studies and Reports

5.1 Case Study: VREfm Bacteremia Outbreak in a US Oncology Ward

A VREfm bacteremia outbreak in an oncology ward was reported by a Boston tertiary care hospital in 2005 [42]. Bloodstream infections occurred in 15 patients (median age 60, 75% neutropenic), and 12 isolates had vanA. In 80% of cases, clonal spread (ST17) was confirmed by pulsed-field gel electrophoresis (PFGE) [43]. Gastrointestinal colonization ($p < 0.001$) and previous vancomycin use (odds ratio 3.8, $p = 0.008$) were risk factors. By identifying vanA in 14/15 patients through qPCR screening of rectal swabs, contact precautions were able to reduce transmission by 70% in just four months. Rapid isolation protocols were supported by LAMP, which was piloted in five samples and confirmed vanA in 20 minutes [44].

5.2 Case Study: VVEfm Emergence in Denmark

A vancomycin-variable E. faecium (VVEfm) strain (ST1421) was found in 65 patients from four hospitals in a Danish study conducted in 2018–2019 [45]. The strain was susceptible to vancomycin (MIC ≤ 4 mg/L) due to a 252 bp deletion in vanX. After being exposed to vancomycin, 25% of isolates showed reversion to resistance (MIC >256 mg/L) according to WGS and qPCR, which were caused by vanS mutations or plasmid amplification [46]. Bypassing culture delays, LAMP identified vanA in 93% of rectal swabs. The diagnostic difficulty of VVEfm and the importance of molecular tools in detecting silent vanA carriers are highlighted by this case [47].

5.3 Case Study: VREfm Transmission in Thailand

Eighty VREfm isolates from ICU patients with UTIs were examined in a 2024 study conducted in Northeastern Thailand [8]. All isolates carried vanA, tetM, and ermB and were members of CC17 (ST80, ST17, and ST761). Within 90 minutes, qPCR identified vanA in 97% of urine samples, whereas LAMP reached 94% sensitivity in 25 minutes. According to genomic analysis, biofilm formation was correlated with esp and hyl in 62% and 48% of isolates, respectively [48]. Over a six-month period, infection control strategies informed by molecular diagnostics decreased the incidence of VREfm by 55% [8].

5.4 Case Study: VREfm in a German Transplant Unit

50 patients had VREfm colonization, and 22 of them developed bacteremia, according to a 2021 study conducted in a German transplant unit [49]. 92% of isolates had ST117 and vanA detected by WGS, with 68% having esp co-occurring. Early isolation was made possible by the discovery of vanA in 46 out of 50 patients through qPCR screening of stool samples. Within 30 minutes, LAMP verified vanA in 12 cases, showing 100% concordance with qPCR. Routine molecular screening was predicted by computational modeling to reduce transmission by 35% [50].

6. Computational Data: Gene Mutations and Diagnostic Targets

Critical information about resistance mechanisms and potential diagnostic targets is obtained through computational analyses of vanA and related genes. VanA mutations in vanS (G133D, 12%) and vanR (T115A, 9%), linked to increased MICs (>512 mg/L), were found in 2,500 VREfm genomes sequenced in 2023 [51]. By increasing promoter activity, these mutations increase the synthesis of D-Ala-D-Lac. VVEfm phenotypes are associated with the vanX 252 bp deletion, which is found in 6% of isolates [45]. Treatment becomes more difficult when vanA co-occurs with esp and hyl, as this is associated with increased virulence [52].

Table 2: Computational Analysis of *vanA* Mutations in VREfm (2020–2023)

Mutation	Gene	Frequency (%)	Impact on MIC (mg/L)	Diagnostic Target	Reference
G133D	*vanS*	12.0	>512	qPCR, LAMP	[51]
T115A	*vanR*	9.0	>256	qPCR	[51]
252 bp deletion	*vanX*	6.0	≤4 (VVEfm)	qPCR, LAMP	[45]
Promoter SNPs	*vanA*	14.0	>512	qPCR	[52]

Note: Prokka and FastTree were used to identify mutations in 2,500 VREfm genomes [53, 54].

Clonal dissemination is supported by the findings of a FastTree phylogenetic analysis, which showed that ST17 and ST117 form separate clades with high vanA prevalence [54]. Based on plasmid and transposon signatures, machine learning models trained on the Comprehensive Antibiotic Resistance Database (CARD) have a 93% accuracy rate in predicting the presence of vanA [55]. The slight diagnostic advantage of qPCR was confirmed by receiver operating characteristic (ROC) curves from a 2022 meta-analysis (n = 4,500 samples), which showed that the area under the curve (AUC) for qPCR was 0.99 and that of LAMP was 0.97 for vanA detection [56].

7. Clinical and Infection Control Implications

For quick VREfm identification that shortens the time needed for isolation and

treatment, molecular diagnostics such as qPCR and LAMP are essential. The portability of LAMP facilitates outbreak management in environments with limited resources, while the quantitative precision of qPCR helps track low-level carriers [57]. According to a 2022 study, qPCR-based screening reduced VREfm transmission in intensive care units by 45% [58]. The high cost of qPCR and the complexity of primer design for LAMP present challenges that could restrict scalability [59]. Diagnostic workflows could be improved by combining the two techniques—qPCR in centralized labs and LAMP in field settings.

8. Future Directions

While NGS finds new resistance determinants, emerging technologies like CRISPR-based diagnostics (e.g., SHERLOCK) provide single-molecule sensitivity for vanA detection [60]. When combined with resistome databases, machine learning models have the potential to forecast VREfm outbreaks with greater than 90% accuracy [61]. Point-of-care diagnostics may be improved by creating multiplex LAMP assays for vanA, vanB, and virulence genes (e.g., hyl). Addressing the changing resistance patterns of VREfm will require standardizing procedures across international healthcare systems [62].

9. Conclusion

Due to clonal spread and vanA-mediated resistance, VREfm is still a dangerous nosocomial pathogen. LAMP and qPCR provide complementary diagnostic solutions; LAMP is more accessible, while qPCR is more sensitive. Computational data and case studies demonstrate their influence on patient outcomes and outbreak control. Healthcare systems can lessen the worldwide burden of VREfm by utilizing emerging technologies and incorporating these tools into infection control frameworks. Staying ahead of this changing threat requires ongoing research into the mechanisms of resistance and diagnostic advancements.

Author Contributions

Conceptualization, methodology, writing, and data curation were performed by all authors. All authors reviewed and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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