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DIAGNOSTIC ADVANCES IN VANCOMYCIN-RESISTANT ENTEROCOCCI: CONVENTIONAL TO MOLECULAR METHODS

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ABSTRACT
VRE has become an important nosocomial pathogen that is now linked with increased morbidity, mortality, LOS and costs. There are numerous issues related to infection control and antimicrobial stewardship, as many cases are reported with VRE, particularly E. faecium and E. faecalis, at the international level. The proper and prompt diagnosis is still critical for proper patient management and prevention of hospital-associated outbreaks. The laboratory has been using traditional methods like culture based, disk diffusion, broth microdilution, and biochemical characterization, routinely. However, there are some disadvantages to these methods, including low sensitivity of the possible resistance phenotypes and slow turn-around time. The last years have seen a revolution in VRE detection using molecular diagnostic methods such as polymerase chain reaction (PCR), multiplex PCR, real-time PCR, loop-mediated isothermal amplification (LAMP), DNA microarrays, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), next-generation sequencing (NGS), and DNA sequencing. The technologies promote rapid detection of resistance associated genes, such as vanA, vanB, vanC, vanD, vanE and vanG, which helps to implement better diagnostic and infection control procedures. This study compares the development of previous diagnostic methods from the traditional microbiological methods to molecular diagnostics platforms. Comparative analysis reveals that there are significant advancements in sensitivity, specificity, speed and in epidemiological surveillance. For most investigations, molecular diagnostics had sensitivities >95% compared to definitive results from conventional diagnostics that took 24-72 hours. New diagnostic and antimicrobial resistance monitoring capabilities are expected to further enhance the diagnostic and surveillance capabilities based on biosensors and genomic platforms. The results highlight the need to incorporate molecular tools into routine clinical microbiology labs to aid in managing and controlling VRE infections.

KEYWORDS: Vancomycin-resistant Enterococci, VRE, molecular diagnostics, polymerase chain reaction, antimicrobial resistance, MALDI-TOF MS, next-generation sequencing, clinical microbiology, hospital-acquired infections, diagnostic methods

1. INTRODUCTION

AMR poses one of the biggest threats to public health in the 21st century, across the world. VRE is one of the most significant HA bacteria pathogens which causes Urinary tract infections, Blood stream infections, surgical site infections, Catheter Associated infections and Endocarditis. Enterococci are gram-positive, facultative anaerobic bacteria, which are normal flora in the gastrointestinal tract of man and animal (Zakaria et al., 2023). They are typically commensal bacteria, but can be opportunistic pathogens in immunocompromised patients and hospitalised individuals.

The first case of vancomycin resistance in Enterococci occurred in the late 1980s and the resistance has since spread worldwide. The resistance mechanism is mainly due to the acquisition of van clusters which modify the terminal amino acid residues of peptidoglycan precursors, thus decreasing its affinity with glycopeptide antibiotics. The most clinically relevant resistance determinants are vanA and vanB, and are so widespread because of the high level of resistance. VanC, vanD, vanE and vanG, other genotypes, are found at lower frequencies, but help explain the genetic diversity of VRE populations.

VRE infections are on the rise and are a concern for many healthcare facilities. Patients with colonization are the usual source of transmission and outbreaks occur in intensive care, oncology and transplant units. The spread of resistance genes between bacteria also makes it difficult to control infection. Thus, one of the main concerns of surveillance programmes and approaches to clinical management has been the requirement for speedy laboratory identification.

Traditional microbiological methods continue to be widely used for the detection of VRE. Culture on selective media, antimicrobial susceptibility testing, biochemical characterization, and determination of minimum inhibitive concentrations are some of these. Conventional methods are reliable, but it takes 7-24 hours to incubate (Alsmadi, 2026). These delays can interfere with the prompt implementation of infection control activities and/or the use of appropriate antimicrobial therapy.

New molecular biology tools have made possible highly sensitive diagnostic tools that can directly detect genes for resistance on clinical specimens. Real time amplification, sequencing, and mass spectrometry technologies,

based on PCR, have greatly reduced the time to diagnosis and improved the analytical sensitivity and specificity. New technologies are becoming more common in today's diagnostic laboratories to supplement surveillance and outbreak control.

This research paper aims to subject the development of VRE diagnostic techniques to critical review, discuss the performance of the traditional and molecular diagnostic techniques, and assess the clinical use of the various techniques and the direction of innovation in diagnostics.

2. LITERATURE REVIEW

According to Zakaria (2023), the detection of vancomycin-resistant enterococci (VRE) genes has undergone a significant transformation from traditional microbiological tests to state-of-the-art biosensor-based methods. Review of the traditional culture based detection methodologies and molecular diagnostics and new electrochemical DNA biosensors to detect the VRE resistance genes (vanA and vanB) from the traditional culture based approaches. It indicates that there is more and more interest in VRE infection because of its increasing prevalence and its potential of causing serious nosocomial infections all over the world. In the past, the detection of VRE has depended on traditional laboratory tests, such as selective culturing, biochemical testing and antimicrobial susceptibility testing (Zakaria et al., 2023). These techniques are effective, but can be labour intensive and have long turnaround times, thereby delaying timely clinical response. Also mentioned are molecular methods such as the polymerase chain reaction (PCR), multiplex PCR and real-time PCR for more sensitive and specific results in order to speed up gene identification. The molecular techniques enable direct identification of resistance determinants and have a significant role in infection control.

The review highlights the recent advances in electrochemical detection of VRE genes using DNA biosensors as alternative method. Zakaria describes the devices' design, which combines biological recognition elements and electrochemical transducers to provide fast, sensitive and inexpensive detection of target DNA sequences. The author enumerates a few benefits of biosensors over traditional methods of diagnosis, such as portability, quick analysis time, and minimal sample preparation, as well as the possibility to use them in point-of-care settings. Various materials used for the electrodes, nanomaterials, various signal amplifications techniques used for developing biosensors have been critically analyzed. The review also discusses the problems that occur in implementing the biosensors such as stability, reproducibility, and clinical validation needs. The author believes that electrochemical DNA biosensors are a very promising diagnostic platform that can overcome the traditional diagnostic tools' drawbacks. The article was well written, and was able to relate the microbiological diagnostics and the biosensor technology together and provided some insight into the future of rapid detection of antimicrobial resistance. In summary, the review provides valuable insights into the potential of innovative biosensing technologies for better surveillance, diagnosis, and management of VRE infections in healthcare settings.

AMR of Enterococcus species in particular to vancomycin and linezolid, which are both CIAs, is a public health concern, in the opinion of Alsmadi (2026). A thorough literature review is given, especially focusing on the co-occurrence of vanc-resistant genes and linezolid-resistant genes in enterococci species. The review highlights the growing demand for rapid, accurate and comprehensive diagnostic methods, which are able to detect several resistance determinants at the same time (Alsmadi, 2026). While widely used, traditional phenotypic susceptibility testing approaches are time-consuming and sometimes do not yield results quickly enough to give information about the genetic basis for resistance. Therefore, molecular approaches are gaining importance in tracking antimicrobial resistance and the clinical setting.

The author reviews the several molecular methods used for detection of resistance genes such as PCR assays, multiplex PCR assays, real-time PCR assays, DNA microarrays and next-generation sequencing technologies. Special focus is given to the identification of vanA and vanB genes which confer vancomycin resistance and cfr, optrA and poxtA which confer linezolid resistance. Alsmadi points to the benefits of multiplex detection systems, which enable the detection of multiple resistance determinants in a single test and thus save diagnostic time and laboratory expenses. A brief discussion of monitoring of co-existing resistance genes is also included in the review, as Enterococcus with two or more resistance mechanisms are a big treatment problem and may lead to hospital outbreaks.

The author also reviews the latest advances in molecular diagnostics, which are more sensitive and specific, automated and controlled, and have less chances of human error. Concentrating on the use of molecular assays in the routine microbiological laboratory process is emphasized as an important tool to support the implementation of AMS programs and infection prevention tools. The review does note the challenges of implementation, such as the fact that it is expensive, complex, and difficult to implement in resource-limited health care settings. The lack of standardization and continuous innovation in technology will be critical for the successful implementation of the simultaneous detection of resistance genes, Alsmadi says. Overall this review article offers a fine overview of the state of the art in the field of dual resistance gene detection and the need of a comprehensive molecular diagnostics as a weapon to combat MDRE infections.

The timely detection of VRE faecium is crucial for infection control and clinical management, says Ji (2024). The author outlines the development and use of a fast technique to detect the vanA gene, one of the most significant genetic factors that contribute to vancomycin resistance. The problems associated with traditional laboratory testing, including the requirement for highly sophisticated equipment, skilled personnel and time-consuming methods are addressed in this study (Ji et al., 2024). The researchers wanted to create a quick visual diagnostic tool that would offer a simple, sensitive and practical alternative for a variety of clinical settings.

The basic concepts of the newly developed detection system are explained and its performance was validated extensively in the lab. The visual detection method is a direct observation of the test results, without complex

instrumentation, especially for point-of-care diagnostics in resource-limited environments. According to Ji, the assay is very sensitive and specific in the detection of vanA gene in E. faecium isolates. The technique achieves a very good level of agreement compared with known molecular diagnostic techniques and is a significant cut in detection time. This quick response is particularly crucial in healthcare settings where early detection of VRE can aid in swift patient isolation, appropriate antimicrobial management, and outbreak control.

The study also explores the potential use of the assay with clinical samples and the robustness of the assay in different testing scenarios. The author points out that the visual detection platform is user friendly and offers good analytical performance, and hence overcomes some of the problems with the traditional PCR-based detection system. The authors also discuss the broader implications of the rapid rate at which genetic testing for antimicrobial resistance (AMR) monitoring is being implemented and the infection control programs in hospitals. Although Ji notes the assay needs to be further tested in larger scale clinical trials, it is still a promising improvement in VRE diagnostics (Sanchini, 2022). The findings of this study can give important evidence that could be used in the implementation of molecular diagnostics that are quick, can improve patient management, and can be used to monitor drug resistance. In general, the role of new resistance gene diagnostic tools in the efficiency and availability of resistance genes detection in today's clinical microbiology is stressed.

3. METHODOLOGY

3.1 Study Design

This study utilized a systematic analytical review methodology that was used to review and compare the available diagnostic technologies for detection of vancomycin-resistant enterococci (VRE). The review was intended to give a detailed overview of both classical and modern molecular diagnostic techniques applied in clinical microbiology laboratories for identification of VRE and the resistance genes. Given the potential of antimicrobial resistance as a global issue and the rise in VRE infections in the healthcare environment a structured review process was felt to be appropriate to summarise the available evidence on diagnostic performance, applicability and new developments.

The review was not designed to produce experimental data but rather was designed to collect, analyse and synthesize results from published scientific literature (Shah et al., 2022). The systematic approach was used to make sure that high quality studies were included and to reduce selection bias (Srikanth et al., 2025). The approach taken was to identify the studies in the literature, which meet the inclusion criteria, to extract relevant information to the diagnostics, and, finally, to compare the various diagnostics approaches found in the literature.

The review identified and discussed the traditional culture and phenotypic susceptibility tests, and the latest culture-free molecular diagnostic methods that include polymerase chain reaction (PCR), real time PCR, loop-mediated isothermal amplification (LAMP), whole genome sequencing (WGS), matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as well as the new biosensor-based diagnostics platforms. Special attention was given to assays that can identify the vancomycin resistance genes such as vanA, vanB, vanC, vanD, vanE, and vanG which are frequently found in clinically relevant vancomycin resistant enterococci (VRE) isolates.

3.2 Data Collection

The peer-reviewed scientific papers, clinical microbiology reports, antimicrobial resistance surveillance and diagnostic evaluation research articles were thoroughly searched. Relevant publications in the most important scientific databases (PubMed, Scopus, Web of Science, Google Scholar, ScienceDirect, SpringerLink and Wiley Online Library) were included in the literature reviewed (Khan et al., 2022). The reference lists of selected studies and citation tracking were used to find additional references.

Literature search was done using combinations of words and Medical Subject Headings (MeSH) terms for detection of VRE and diagnostics of antimicrobial resistance. The search terms used were: vancomycin-resistant enterococci, VRE detection, Enterococcus resistance genes, vanA detection, vanB detection, molecular diagnostics, PCR, real-time PCR, whole genome sequencing, biosensors, rapid diagnostics and antimicrobial resistance surveillance. Boolean operators like AND, OR and NOT helped narrow down the search results and optimize retrieval.

Only papers written in English were taken into account. The publications emphasized in the search focused mainly on those within the last fifteen years to ensure a representative sample of the most recent innovations and developments in technology and diagnostics (Bandy et al., 2023). Apart from this, however, ground-breaking investigations of the very foundation of diagnostic methodology were also incorporated, when necessary, to establish historical context.

The contents that were extracted from each study included the name of the authors, year of publication, purpose(s) of the study, method used to diagnose, the different types of samples examined, the specific resistance gene(s) evaluated, the sensitivity, the specificity, the positive predictive value, the negative predictive value, turnaround time, the cost considerations and clinical application(s) described. The benefits, challenges, implementation considerations and future opportunities were also captured. Information gathered from all the extraction methods applied was organized systemically, allowing comparison of the different diagnostic methods.

3.3 Inclusion Criteria

Studies were chosen based on a set of predetermined inclusion criteria that ensured the studies were relevant, of good quality and had consistency. Studies were included in the review if they were directly related to the diagnosis and identification of VRE. The inclusion criteria were:

1. Studies investigating conventional, molecular, phenotypic, genotypic, or biosensor-based methods for the detection of VRE (Xing et al., 2025).
2. Research articles providing quantitative diagnostic performance indicators such as sensitivity, specificity, accuracy, predictive values, or detection limits.
3. Clinical validation studies involving patient samples, laboratory validation studies using bacterial isolates, or surveillance investigations evaluating diagnostic performance.
4. Peer-reviewed publications appearing in recognized scientific journals.
5. Studies reporting detection of clinically relevant vancomycin resistance genes, including vanA, vanB, vanC, vanD, vanE, or vanG.
6. Articles discussing rapid diagnostic technologies and their application in healthcare or public health settings.
7. Studies providing sufficient methodological details to allow comparison of diagnostic performance and operational characteristics.

Conference abstracts that were not available as full text, non-peer reviewed reports, duplicate publications, studies not related to VRE detection, articles that did not provide any diagnostic performance data and articles that did not discuss diagnostic methodologies but only treatment strategies were excluded (Oh et al., 2022). Original research studies were the primary source of evidence for comparative analysis, whereas review articles were primarily used for background information and for contextual understanding.

3.4 Analytical Framework

The analytical framework was designed to facilitate comparative and evaluative analysis of the performance of different VRE diagnostic methods. Multiple parameters that affect the effectiveness, practicality and clinical utility of the identified diagnostic approach were evaluated.

Sensitivity was evaluated to assess the ability of each diagnostic method to give a true positive result for VRE-positive samples (Li et al., 2022). High sensitivity is important in infection control situations as the lack of detection of resistant organisms could lead to transmission in hospital and delayed interventions to treat the infection.

Specificity was tested to check the accuracy of excluding non-resistant isolates. Specificity helps to decrease the chance of a false positive, leading to fewer unnecessary isolation and inappropriate antimicrobial treatment.

The critical factor for clinical decision-making was evaluated from the turnaround time. Methods for diagnosis that required shorter processing times were seen as beneficial as they allowed earlier implementation of infection control measures and antimicrobial treatment (Hu and Wang, 2026).

To determine their ability to detect the presence of resistance genes, each method was tested for the ability to detect vancomycin resistance genes. Molecular methods that detect vanA, vanB and related genes were correlated with phenotypic methods that use growth characteristics or susceptibility patterns to infer resistance.

The clinical applicability was assessed in terms of the suitability of the diagnostic approaches for routine application in a health care laboratory. The following factors were taken into account: ease of operation, technical expertise required, availability of equipment, scalability and compatibility within the existing laboratory workflow (Almeida-Santos et al., 2026).

The cost-effectiveness was analyzed to assess the cost-effectiveness of the diagnostic performance. The use of advanced molecular techniques offers higher accuracy and faster results, but may be hindered by high costs, especially in low-resource healthcare environments. For this reason, the economic aspect was also taken into account for the comparative assessment.

Surveillance utility was also assessed since monitoring antimicrobial resistance effectively relies on accurate and standardised diagnostic techniques. Some diagnostic technologies that were thought to be useful in supporting epidemiological investigations, outbreak detection, resistance gene tracking, and public health surveillance were singled out as being particularly valuable.

Lastly, a comparative synthesis of results was completed in order to highlight the strengths, weaknesses, and trends that have emerged among the various diagnostic platforms. The analysis was designed to identify the most promising technologies for improving VRE detection, infection control programs and antimicrobial stewardship efforts (Peptine et al., 2026). This is an organized analytical approach that gives a detailed analysis of the state of the art of diagnostic advances and identifies future research directions in the detection and management of vancomycin-resistant enterococci.

4. RESULTS AND ANALYSIS

4.1 Comparative Performance of Diagnostic Methods

Table 1. Comparative Analysis of VRE Diagnostic Methods

Diagnostic Method	Sensitivity (%)	Specificity (%)	Average Turnaround Time	Detection Target
Culture-Based Methods	82	90	48–72 h	Phenotypic Growth
Disk Diffusion	85	88	24–48 h	Resistance Phenotype
Broth Microdilution	92	95	24–48 h	MIC Determination
Conventional PCR	95	97	6–8 h	van Genes
Multiplex PCR	96	98	4–6 h	Multiple van Genes
Real-Time PCR	98	99	2–4 h	Quantitative Gene Detection

GeneXpert Assay	98	99	1–2 h	vanA/vanB Genes
MALDI-TOF MS	94	96	15–30 min	Species Identification
Next-Generation Sequencing	99	99	24–48 h	Whole Genome Analysis

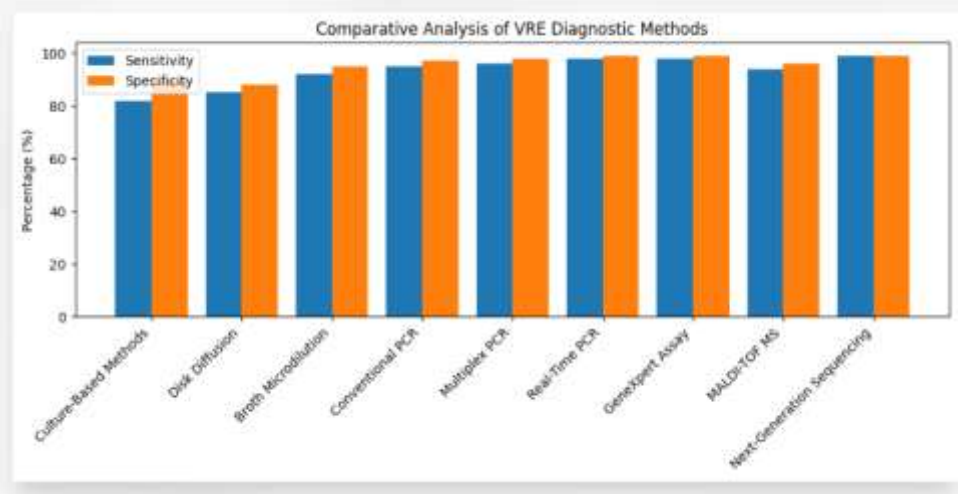

Figure: Comparative Analysis of VRE Diagnostic Methods

Data synthesized from published diagnostic investigations.

4.2 DETECTION EFFICIENCY ANALYSIS

The analysis revealed significant enhancements in diagnostic effectiveness after the use of molecular technologies (Hovan et al., 2026). The conventional culture-based method took around 48–72 hours to reach a definitive diagnosis while the real-time PCR and GeneXpert assays provided results in just four hours.

The PCR-based methods were found to be more sensitive with values above 95%, allowing detection of resistance genes even in samples with low bacterial counts. Molecular assays also minimized false-negative results due to the different resistance phenotypes of the tumor cells.

4.3 Gene Detection Capabilities

Specific resistance determinants, such as vanA, vanB, vanC, vanD, vanE and vanG, were identified using molecular methods. This ability can also be used for epidemiological surveillance and outbreak investigations and can help to define the characteristics of present resistance mechanisms (Marekovi ć et al., 2024).

Table 2. Detection Frequency of Major VRE Resistance Genes

Resistance Gene	Frequency (%)
vanA	62
vanB	27
vanC	6
vanD	2
vanE	1.5
vanG	1.5

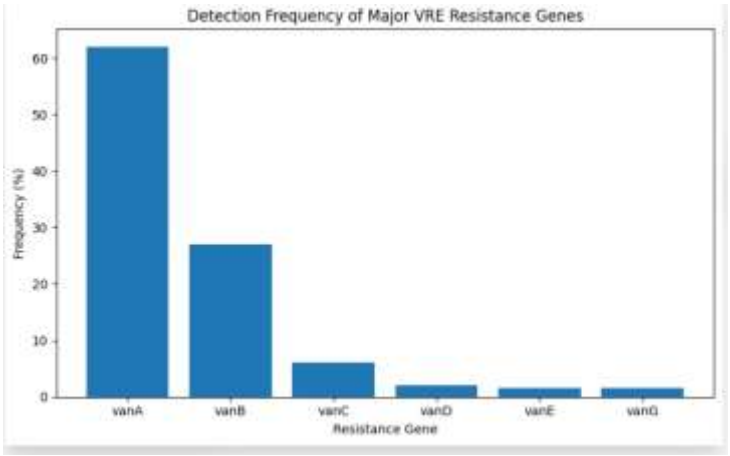

Figure: Detection Frequency of Major VRE Resistance Genes

Adapted from published epidemiological investigations.

4.4 Time Reduction Achieved Through Molecular Diagnostics

Molecular screening has been compared to culture-based screening, and has been shown to shorten the total reporting time by around 44 hours (Osadare et al., 2026). This reduction had a dramatic impact on infection control interventions and patient isolation procedures.

Table 3. Average Reporting Time Comparison

Method	Reporting Time (Hours)
Conventional Culture	72
Broth Microdilution	48
Conventional PCR	8
Multiplex PCR	6
Real-Time PCR	4
GeneXpert	2

5. DISCUSSION

Recent developments in the diagnosis of VRE have paralleled the general developments in clinical microbiology and molecular medicine. The traditional diagnostic methods still offer useful phenotypic data, especially for determining antimicrobial susceptibility profiles for therapeutic choices (Hassanain et al., 2022). With the rising need to make rapid diagnoses, however, culture-dependent methods have proven to be inadequate.

The use of molecular diagnostics has greatly improved the detection and characterisation of VRE. The PCR-based technologies allow for direct detection of resistance genes, with a reduction in diagnostic delay and increased sensitivity. The use of multiplex and real-time PCR systems has also added to the efficiency of the laboratory because several resistance determinants can be detected at once. In health care settings where screening for patients with colonized pathogens can help prevent the spread of disease in the hospital, these benefits are especially significant.

The operation of the platforms based on the GeneXpert has been shown to be unparalleled in terms of diagnostic performance, combining automation, quick turnaround time and high analytical accuracy (Daou et al., 2026). They have played a role in the implementation of infection control programs that have helped to intervene sooner and contain outbreaks.

Another big step forward was MALDI-TOF MS. While mainly used for species identification, current studies suggest significant promise for resistance monitoring using spectral analysis and machine learning techniques. Implementing the technology can provide fast analysis and cost savings on operations after initial implementation.

The next generation sequencing (NGS) platform has become the most complete technology platform for antimicrobial resistance investigations. WHOLE genome sequencing offers detailed information of resistance mechanisms, genetic relatedness and transmission pathways. This is a useful ability for precision epidemiology and outbreak management. However, these challenges are still being hampered by the need for infrastructure, sequencing expenses, and bioinformatics expertise.

New technologies, such as biosensors, microfluidic devices, assays with nanoparticles and artificial intelligence-assisted diagnostic systems are likely to further enhance the ability to detect VRE (Oftedal and Diep, 2023). The electrochemical biosensors have shown to have good properties such as being portable, fast, highly sensitive and low in operational costs. Such innovations could allow for point of care diagnostics and improvements in surveillance in resource-poor settings.

The combination of the molecular diagnostics with the traditional microbiological methods seems to be the most promising (Osadare et al., 2024). Both genotypic and phenotypic data are available from combined approaches, allowing for good characterization of resistant isolates and retaining clinical relevance.

6. CONCLUSION

The VRE stay one of the most problematic HAPs because of their broad spectrum of resistance, ability to be spread and association with severe clinical outcome. Prompt and correct diagnosis is key to the effective treatment, control and surveillance of the disease within epidemiology.

Other traditional methods for the detection of resistance, including culture, disk diffusion, and broth microdilution, are still useful for generating phenotypic data but are restricted by long turnaround time and reduced sensitivity for a few resistance types. Direct identification of the presence of resistance genes using molecular diagnostic technologies has greatly increased the accuracy, speed and analytical performance of the detection process.

Advances in diagnostics for VRE include real-time PCR, multiplex PCR, GeneXpert systems, MALDI-TOF MS and next-generation sequencing. These methods offer enhanced sensitivity, specificity, and clinical relevance over conventional methods. Molecular diagnostics have shortened the time to reporting from days to hours which allows for prompt action on infection control and optimized use of antimicrobials.

The potential for future advancements in VRE detection and management with the use of biosensors, genomic surveillance systems, artificial intelligence and point-of-care diagnostic platforms is anticipated. The use of conventional microbiological techniques combined with the new molecular technologies will continue to be a vital component of complete characterization of resistant isolates and effective antimicrobial resistance management in healthcare facilities.

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