

ORIGINAL ARTICLE

# Comparative Study of Real Time Quantitative PCR for vanA Gene with the Conventional Culture-based Method for the Detection of Vancomycin-resistant Enterococci

<sup>1</sup>Safaa M. EL-AGEERY\*, <sup>2</sup>Howayda N. Mahran

<sup>1</sup>Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Egypt; Medical Laboratories Technology Department, Faculty of Applied Medical Sciences, Taibah University, Saudi Arabia

<sup>2</sup>Medical Laboratories Technology Department, Faculty of Applied Medical Sciences, Taibah University, Saudi Arabia; Medico-legal Department, Ministry of Justice, Alexandria, Egypt

## ABSTRACT

### Key words:

Vancomycin resistant enterococci,  
Conventional culture based assay,  
Real time quantitative PCR for vanA gene

**Background:** Vancomycin-resistant enterococci (VRE) are considered third leading cause of nosocomial infection. So, an effective, perfect and timely detection of VRE is required to start proper therapy and thus better patient outcome. **Objectives:** To compare the performance of real time quantitative PCR (Q-PCR) for vanA gene with the conventional culture-based method for the detection of VRE. **Methodology:** A total of 170 strains of enterococci; 95 vancomycin resistant strains and 75 vancomycin sensitive enterococci (VSE) isolated from different clinical specimens were detected by conventional culture based assay, at the Bacteriology Lab of King Fahd Hospital in Al-Madinah Al-Monawarah, KSA. All the strains were further tested for Van A gene by Q-PCR. **Results:** sensitivity and specificity of Q-PCR for VanA genotype were found to be 100% and 98.66% respectively. **Conclusions:** Q-PCR is a rapid and accurate technique to detect vanA in enterococci, thus it could be useful to settle on the treatment modalities of infections caused by VRE.

## INTRODUCTION

Enterococci are amongst the common microbiota of the gastrointestinal tract of humans and animals. The members of genus *Enterococcus* are also considered opportunistic pathogenic bacteria and have emerged as major cause of hospital acquired infections worldwide<sup>1,2</sup>. Two species accountable for majority of human infections are *Enterococcus faecalis* and *Enterococcus faecium*<sup>3</sup>.

Vancomycin-resistant enterococci (VRE) are ever more challenging worldwide health care with morbidity, mortality, and expense. Infection, resulting from VRE, is now one of the most common causes of nosocomial infections in the United States<sup>4</sup>. In India, some investigators found that vancomycin-resistant enterococci are third principal cause of nosocomial infection<sup>5</sup>.

Considered high-risk organisms in hospital setting, enterococci can transfer vancomycin resistance to other bacteria (including methicillin-resistant *Staphylococcus aureus*), thus making them a subject of close scrutiny by infection prevention practitioners in high-risk units such as intensive care units (ICUs), oncology units, and transplant units<sup>6</sup>. Any surface can be contaminated by VRE. Moreover, VRE is stable in dry environments with a prolonged survival time (>1 week) and this could increase the risk of passage from one patient to another by health care workers<sup>6,7</sup>.

Glycopeptide resistance is controlled by six different vancomycin resistance (van) gene operons. vanA and vanB are the most clinically relevant of the van genes as they are associated with transposons and easily transferred from one to other organisms<sup>8</sup>. Phenotypically, the vanA gene mediates inducible, high-level resistance to vancomycin (minimum inhibitory concentrations [MICs], 64 to >1024 µg/ml) and teicoplanin (MICs, 16-512 µg/ml) while the vanB gene confers low to moderate-level resistance to vancomycin (MICs, 32-64 mg/ml)<sup>9</sup>.

Many reports are available regarding the identification of vancomycin-resistant enterococci (VRE) using conventional standard microbiological methods, which require time, resources, and space<sup>10</sup>.

### \*Corresponding Author:

Safaa M. EL-AGEERY

Medical Laboratories Technology Department, Faculty of Applied Medical Sciences, Taibah University, Saudi Arabia;

Medical Microbiology and Immunology Department,

Faculty of Medicine, Mansoura University, Egypt

E-mail: [safageery@gmail.com](mailto:safageery@gmail.com); Tel: +201090888263; +966504772970

These standard methods are labour-intensive and require 48-72 h to give the result. Therefore, management of VRE infection relies on rapid and sensitive detection<sup>11,12</sup>.

Also, VRE can be detected by conventional polymerase chain reaction (PCR), although an improvement over conventional microbiological tests, lack absolute quantitation and requires time-consuming post-PCR analysis. Real time quantitative polymerase chain reaction (Q-PCR) can quantify the presence of microorganisms in clinical specimens and improve the precision and sensitivity of conventional PCR so that the target gene can be detected and quantified without post-reaction analysis<sup>13,14</sup>.

Since this method (Q-PCR) is more rapid than other conventional culture-based method which is considered the standard method. Therefore, there is need to study which technique is better among these detection methods. Therefore, in the current study, we compared the performance of real time Q-PCR for vanA gene with the conventional culture-based method for the detection of VRE.

## METHODOLOGY

### Study design

A total of 170 strains of enterococci; 95 vancomycin resistant strains and 75 vancomycin sensitive enterococci were isolated from different clinical specimens (blood, urine, wound, and ascetic fluid). The strains were isolated by conventional culture based assay, at the Bacteriology Lab of King Fahd Hospital in Al-Madinah Al-Monawarah, KSA. All the strains were further tested for VanA gene by Q-PCR. The study period was four months, from the January 2014 to June 2014.

### Conventional organism identification and vancomycin susceptibility testing

Cultural characteristics, Gram's stain, catalase test, and subculture on bile esculin agar (Oxoid, England) were used to identify the strains to the genus level<sup>15</sup>. Gram-positive cocci that were catalase negative and produced blackening of the medium were further tested for a susceptibility test using vancomycin (0.016–256 µg/mL) E-Test strip (Bio-Mérieux, France) on Mueller–Hinton agar (Oxoid, England). Those organisms with a vancomycin MIC value of >32 µg/mL were defined as VRE according to Clinical and Laboratory Standards Institute (2008) guidelines<sup>16</sup>.

### Real time quantitative polymerase chain reaction (Q-PCR)

After a 6 h incubation in BHI broth, 200 µL were collected and used for DNA extraction with the QIAamp DNA Mini Kit (Qiagen, Germany), in accordance with the manufacturer's instructions. Two mL of the extracted DNA were used in a total volume of 20mL of PCR mix containing SYBR Green II (Invitrogen, USA). A set of primers was used to amplify the VanA gene (169bp): VAN-A1: 5'AGCTGTACTCTCGCCGGATA-3' and VAN-A2: 5'CGCAGCCTACAAAAGGGATA-3 (Promega, USA). Real time PCR were performed using the Light Cycler platform (Roche, Switzerland) and VanA gene was achieved by melting curve analysis (VanA: 79°C)<sup>5</sup>.

### Statistical Methods

Evaluation of qualitative test performance was performed according to CLSI (2000)<sup>17</sup>. Sensitivity (agreement with positive results) and specificity (negative agreement were calculated for vanA assay, in comparison to culture based assay (standard method).

## RESULTS

### Comparison of conventional culture based results and Q-PCR

On the basis of culture based tests, 95 strains were vancomycin resistant while 75 strains were considered vancomycin sensitive. Q-PCR for vanA genotype confirmed that all VRE isolates were positive. However one vancomycin sensitive isolate was found to be positive by vanA genotype, Table I.

### Sensitivity and specificity of Q-PCR

The sensitivity and specificity of Q-PCR compared to culture based tests are presented in table II. The molecular method Q-PCR was found to be 100% sensitive and 98.66% specific. Q-PCR confirmed that all VRE isolates were vanA type (100% sensitive). Furthermore, one VSE isolate was found to be negative by both vanA genotype (98.66% specific).

**Table I.** Comparison of conventional culture based results and Q-PCR.

Culture based results	Q-PCR		Total no.
	Positive	Negative	
Positive	95	0	95
Negative	1	74	75
Total no.	96	74	170

**Table II.** Sensitivity and specificity of Q-PCR assay.

Method	True positive	False positive	True negative	False negative	Sensitivity	Specificity
Q-PCR	95	0	74	1	100%	98.66

## DISCUSSION

Vancomycin-resistant enterococcus is becoming the causative agent in an increasing number of worldwide health-care associated infections in the last decade<sup>18,19</sup>. Nowadays, VRE is reaching Middle East countries like Saudi Arabia<sup>20</sup>.

There are various laboratory and molecular methods for the identification of VRE which can differ in speed, sensitivity, and specificity<sup>10-12</sup>. The results of the present study suggested that the Q-PCR detection assay is considerably good in comparison to conventional culture based methods for VRE isolation from different clinical specimens which required 48-72 h to give the result.

In the present study the diagnosis of VRE by Q-PCR revealed 100% sensitivity and 98.66% specificity. Tripathi et al<sup>5</sup> showed a higher level of concurrence between the two methodologies; Q-PCR and culture based technique as they found that Q-PCR has have sensitivity and specificity of 100%. False positive result (molecular screening positive and culture negative) in our study was also found by other investigators and may be due to nonviable or non culturable enterococci<sup>21</sup>.

In the present study, Q-PCR was done rather than conventional PCR. Some microbiologists recorded that conventional PCR and Q-PCR gave equal response as regard sensitivity and specificity<sup>5</sup>. However, conventional PCR method is qualitative but not quantitative. Real-time chemistries allow for the detection of PCR amplification during the early phases of the reaction. Measuring the kinetics of the reaction in the early phases of PCR provides a different gain over traditional PCR detection. Agarose gels are required for traditional methods to detect PCR amplification which is the time consuming post PCR reaction step<sup>22</sup>.

The present study used Q-PCR for vanA genotype to detect VRE. It was found that vanA and vanB types of VRE have plasmid encoded transferable vancomycin resistant gene and both are the highly prevalent genotypes in patients with hospital acquired infection<sup>23</sup>. Previous studies suggested that in vitro or in vivo transfer potential of vanA is higher than vanB, which indicates that nosocomial spread of vanA gene in health-care settings is a major problem. The dominance of vanA type VRE in our population demands the rapid and sensitive identification of such VRE strains<sup>24</sup>. Moreover, it was mentioned that the dominant resistance factor in enterococci is vanA. VanA is an acquired gene that encodes production of a cell wall-altering ligase, which modifies the vancomycin binding target and reduces its affinity for vancomycin<sup>25</sup>.

In summary, the results of the present study suggest that vanA specific Q-PCR is accurate and more rapid technique than the standard culture dependent methods for detection VRE. In the era of emerging drug

resistance with limited treatment options Q-PCR appears to be a valuable method not only for the detection of VRE but also guiding appropriate therapy in order to provide better patient care in future.

### Ethical approval:

Ethical Committee of King Fahd Hospital in Al-Madinah Al-Monawarah and scientific research of Taibah University approved the study.

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