ELSEVIER

International Journal of Antimicrobial Agents 31 (2008) 549–554

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

Short communication

Genetic diversity of Tn*1546*-like elements in clinical isolates of vancomycin-resistant enterococci

Kidon Sung, Saeed A. Khan *, Mohamed S. Nawaz

Division of Microbiology, National Center for Toxicological Research/FDA, 3900 NCTR Road, Jefferson, AR 72079, USA

Received 27 November 2007; accepted 31 January 2008

Abstract

We have investigated the genetic diversity of Tn*1546* among 17 vancomycin-resistant enterococci (VRE) isolates of *Enterococcus faecium*. Most of these multidrug-resistant strains harboured plasmids of 2 kb to >300 kb in size. The vancomycin resistance marker *vanA* was located on both the plasmid and the chromosomal DNA. VRE isolates 18 and 22 failed to amplify the *orf1*-IR_R and *orf2*-IR_R but contained the *orf1* and *orf2*. VRE3 failed to amplify the *orf1*, *orf2*, *vanR* and *vanS*, but still yielded a larger than expected (4.4 kb vs. 2.3 kb) *vanSH* amplicon. VRE9, 10, 21 and 22 also yielded larger (5.5 kb) *vanSH* amplicons; all others yielded 4.0 kb *vanSH* amplicons. Sequence analysis of the *vanSH* amplicons from VRE9, 10, 21 and 22 revealed the presence of IS*1251* between the *vanS* and *vanH* genes in these isolates. The observed *vanSH* amplicon from VRE3 contained *orf31*, *orf30* and *orf29* of the plasmid pRUM followed by the *vanHAXYZ* region of Tn*1546*. Translocation of Tn*1546* to a pRUM-like plasmid in VRE3 resulted in the loss of its *orf1*, *orf2*, *vanR* and *vanS* elements and a loss of the *orf32* of pRUM, leading to a unique structural arrangement of *vanA* elements that is hitherto unknown.
Published by Elsevier B.V. on behalf of International Society of Chemotherapy.

Keywords: *Van* operon; Hybridisation; Insertion element; Transposon

1. Introduction

Vancomycin-resistant enterococci (VRE) are the third most common cause of bloodstream infections among hospitalised patients [1,2]. However, their presence in non-human sources such as the faeces of farm animals, raw meat and sewage is also reported [2,3]. VRE from any of these sources could act as reservoirs of vancomycin resistance markers and pose a threat to public health, especially if they carry *vanA*/*B*-type transferable resistance markers. Of the two transferable markers, *vanA* confers a high level of resistance to vancomycin (minimal inhibitory concentration (MIC) ≥128 μg/mL) and is present on a 10.8 kb transposon, Tn*1546* [4]. Resistance to vancomycin is expressed via the action of several genes. The *vanR* and *vanS* genes regulate the expression of vancomycin resistance determinant genes *vanH*, *vanA* and *vanX*. The *vanY* gene codes for an accessory protein and *vanZ* confers resistance to teicoplanin. All these genes that are present on Tn*1546* are together known as *vanA* structural elements. Arrangement of these genes is often interrupted by insertion elements that confer heterogeneity to Tn*1546* [5].

Genetic heterogeneity in Tn*1546* is also caused by mutations in the *vanR* and *vanS* genes, and deletions and movement of insertion sequence (IS) elements within Tn*1546* [6–8]. Specific IS elements are present in defined geographical locations. For example, insertion elements IS*1216V*, IS*3* and IS*1542* appear to be more common in Europe [6,7]. US isolates contain mostly IS*1216V* and IS*1251* [7]. However, a report of IS*1251* in Europe [9] suggests an intercontinental spread of the *vanA* gene cluster, most probably due to increased travel, and further emphasises an epidemiological need to monitor and track the changes occurring in VRE. DNA polymorphism among Tn*1546* can be exploited for tracing routes of transmission, monitoring the dissemination of *vanA* elements and investigating the evolution of VRE. Pulsed-field gel electrophoresis (PFGE), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and hybridisation [10] are commonly used to study polymorphism in Tn*1546*. Whilst PFGE can differentiate the

* Corresponding author. Tel.: +1 870 543 7197; fax: +1 870 543 7307.
E-mail address: saeed.khan@fda.hhs.gov (S.A. Khan).

0924-8579/$ – see front matter. Published by Elsevier B.V. on behalf of International Society of Chemotherapy.
doi:10.1016/j.ijantimicag.2008.01.030

strains based upon their restriction profiles, isolates with identical PFGE types may contain different *vanA* elements that can only be seen by overlapping PCR and PCR-RFLP. The latter two techniques offer certain advantages over PFGE but have their own limitations. Overlapping PCR can only demonstrate the presence or absence of an insertion element within a sequence; it can not show point mutations. Similarly, PCR-RFLP can suggest point mutations only if the mutations are within the restriction enzyme recognition sites.

Considering the pros and cons of the most common techniques used for studying polymorphism in Tn*1546*, we have explored the heterogeneity among 17 *vanA*-type *Enterococcus faecium* isolates by PFGE, overlapping PCR, hybridisation, cloning and sequence analysis to see whether the variations in Tn*1546* structural elements occur within the USA. The data presented here suggest the translocation of *vanHAXYZ* elements from Tn*1546* to a pRUM-like plasmid [11], thereby generating a new kind of *vanA* structural arrangement that has never been reported.

2. Materials and methods

2.1. Bacterial strains

Seventeen *vanA*-type human clinical *E. faecium* isolates from San Antonio, TX, Las Vegas, NV and Biloxi, MS, obtained from the Brooks Air Force Base, San Antonio, TX, were used in this study (Table 1).

2.2. Antibiotic susceptibility testing and MIC determination

Disk diffusion assays for antibiotic susceptibility and broth dilution assays for the determination of MICs for vancomycin (Sigma Chemical Co., St Louis, MO) were performed using guidelines from the Clinical and Laboratory Standards Institute [12,13]. The antibiotics used for disk diffusion assays included nalidixic acid (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), tetracycline (30 μg), kanamycin (30 μg), gentamicin (120 μg), erythromycin (15 μg), bacitracin (10 μg), sulfamethoxazole/trimethoprim (SXT) (23.75/1.25 μg), chloramphenicol (30 μg), streptomycin (300 μg) and vancomycin (30 μg). The concentration range of vancomycin used in MIC assays was 32–1024 μg/mL.

2.3. PCR amplification

Amplification of the *vanR-vanS* (*vanRS*), *vanS-vanH* (*vanSH*), *vanH-vanX* (*vanHAX*), *vanX-vanY* (*vanXY*) and *vanY-vanZ* (*vanYZ*) of Tn*1546* was carried out using previously described primers [10]. Amplification of the *orf1*, *orf2*, *vanR* and *vanS* was carried out using the primers listed in Table 2. Sequences between *orf1*-IR_R and *orf2*-IR_R were amplified using IR_R and IR_L primers described by Palepou et

Table 1
Hybridisation profile of plasmid and total DNA, minimal inhibitory concentration (MIC) values, missing/mutated Tn*1546* structural elements and variability of *vanSH* amplicons in vancomycin-resistant enterococci (VRE) isolates

Isolate #	Location	Vancomycin MIC (μg/mL)	Plasmid DNA[a]			Total DNA[b]		*vanSH* (kb)	IS element[c]	Missing/mutated Tn*1546* elements
			Intact	*Sal*I	*Sma*I	*Sma*I	*Sal*I			
VRE3	San Antonio, TX	512	130	20	25, 40	25	11	4.4	None	(*orf1* to *vanS*)[d]
VRE9	San Antonio, TX	256	145	20	35	25	12	5.5	IS*1251*	
VRE10	San Antonio, TX	256	150	20	40	23	16	5.5	IS*1251*	
VRE21	Biloxi, MS	512	150	24	42	31	14	5.5	IS*1251*	
VRE22	Biloxi, MS	512	145	14	40	23	13	5.5	IS*1251*	(IR_R)[e]
VRE4	Las Vegas, NV	512	210	24	80	23	25	4.0	None	
VRE5	Las Vegas, NV	256	162	24	55	23	20	4.0	None	
VRE6	Las Vegas, NV	256	205	22	61	15	23	4.0	None	
VRE7	Las Vegas, NV	256	200	35	58	27	22	4.0	None	
VRE11	Las Vegas, NV	256	200	22	58	23	22	4.0	None	
VRE13	Las Vegas, NV	256	205	23	61	23	23	4.0	None	
VRE14	Las Vegas, NV	256	205	22	61	25	22	4.0	None	
VRE16	Las Vegas, NV	128	205	22	61	23	22	4.0	None	
VRE17	Las Vegas, NV	256	205	22	61	19	22	4.0	None	
VRE18	Las Vegas, NV	256	205, 190	24	58	19	20, 23	4.0	None	(IR_R)[e]
VRE26	Las Vegas, NV	256	205	22	61	25	22	4.0	None	
VRE29	Las Vegas, NV	256	205	22	61	25	21	4.0	None	

[a] Size in kb of the intact and *Sal*I- and *Sma*I-digested plasmid DNA after hybridisation with the *vanA* probe.
[b] Size in kb of the *Sal*I- and *Sma*I-digested total DNA after hybridisation with the *vanA* probe.
[c] Insertion sequence elements present within Tn*1546*.
[d] Region of Tn*1546* that was missing.
[e] Region of Tn*1546* that contained a mutation.