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SHORT REPORT
New type F lineage-related Tn*1546* and a *vanA*/*vanB* type vancomycin-resistant *Enterococcus faecium* isolated from patients in Dammam, Saudi Arabia during 2006–2007

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SUMMARY

Knowledge regarding vancomycin-resistant enterococci (VRE) from Middle Eastern countries is scarce. We therefore investigated the antimicrobial resistance profiles and genetic relationships of VRE *Enterococcus faecium* isolates obtained from patients attending the King Fahad Specialist Hospital, Dammam, during 2006–2007. The predominant VRE comprised 20 *vanB*, five *vanA* and one *vanA*/*vanB* type isolates, which tended to fall into two genetic clusters that were identifiable phenotypically by their susceptibility to tetracycline. Multi-locus sequence typing of a random selection of isolates showed that they were part of clonal cluster 17, showing the importance of this genotype in nosocomial VRE infections in Saudi Arabia. Further analysis showed that four of the *vanA* genotype isolates possessed a new type F Tn*1546* transposon, associated with IS*1216V* and IS*1251*. Finally, *E. faecium vanA*/*B* isolates are rarely reported in the clinical setting including in Saudi Arabia.

Key words: Antibiotic resistance, enteric bacteria.

Enterococci are normal commensals of the human and animal gut that are particularly associated with nosocomial infections, as well as community carriage. Vancomycin-resistant enterococci (VRE) express high-level resistance to glycopeptide and aminoglycosides, resistance having been promoted via (i) the extensive use of vancomycin in healthcare environments, and (ii) the use of the animal growth promoter avoparcin. Several different VRE phenotypes have been described in the literature, including VanA, VanB, VanC, VanD, VanE, VanG, VanL and VanM, of which *vanA*, *vanB*, *vanD* and *vanM* genes code for a D-alanyl- D-lactate ligase, and *vanC*, *vanE*, *vanG* and *vanL* genes for a D-alanyl-D-serine ligase. Recently a new transferable VanN-type resistance, with homology to VanL-type vancomycin resistance has been reported [1].

In the nosocomial situation, the VanA phenotype is most prevalent and isolates are characterized by the expression of both high-level and inducible resistance to vancomycin [minimum inhibitory concentration (MIC) 64–1024 mg/l], and teicoplanin (MIC 16–512 mg/l), a phenomenon facilitated by the carriage of the transposon Tn*1546*. Surveys have shown the worldwide distribution of this phenotype [2–4]. The VanB phenotype is also common in nosocomial infections and confers lower levels of acquired resistance to vancomycin, but not to teicoplanin. To date, few reports have indicated the presence of clinically relevant

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vanA/*B Enterococcus faecium* isolates [5]. The prevalence and genotypic relationships of VRE isolates have been documented in many parts of the world but relatively few studies have investigated the Middle East further and characterized VRE from hospitals in Saudi Arabia [4]. Therefore, the aim of the study was to expand the phenotypic and molecular characterization of Saudi Arabian VRE isolates, by investigating all vancomycin-resistant *E. faecium* isolates recovered from the King Fahad Specialist Hospital, Damman, Saudi Arabia between February 2006 and December 2007, and to characterize any *vanA* Tn*1546* transposons present. The hospital is a tertiary-care centre with 230 beds which treats about 6000 patients annually and is situated in the eastern province of Saudi Arabia, about 375 km from the capital city of Riyadh.

During 2006–2007, 26 vancomycin-resistant *E. faecium* isolates were cultured from clinical specimens from hospitalized patients (Table 1). Isolates were confirmed as *E. faecium* species by polymerase chain reaction (PCR) [6]. Antibiotic susceptibility testing for ampicillin, ciprofloxacin, tetracycline, quinupristin/dalfopristin and linezolid was performed using the VITEK 2 automated identification and susceptibility system (bioMérieux, France), and E tests (AB Biodisk, Sweden) were used for vancomycin and teicoplanin.

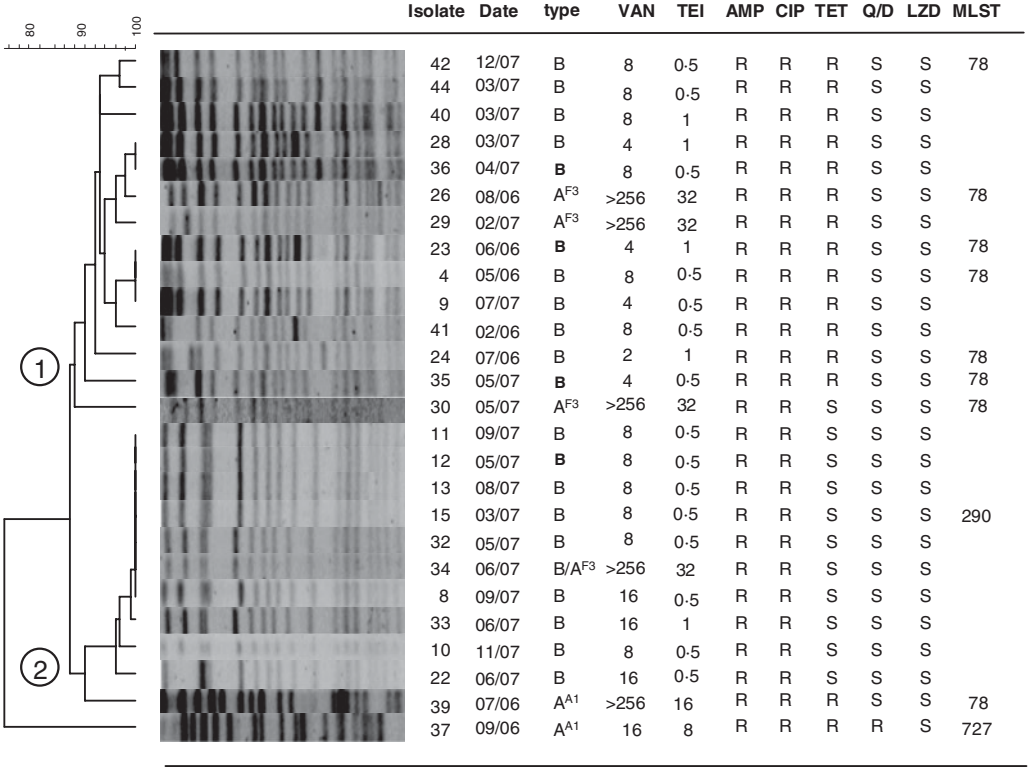
Genotyping of isolates was performed using pulsed-field gel electrophoresis (PFGE) of *Sma*I digests of chromosomal DNA. Fragments were separated in 0·8% agarose gel at a constant voltage of 6 V/cm at 14 °C with pulse times of 3·5–25 s increased linearly over 12 h, and 1–5 s over 8 h. Gel profiles (48·5–339·5 kb) were analysed using BioNumerics v. 6·0 software (Applied Maths, Belgium) with gel lanes normalized against a lambda DNA ladder (BioRad, USA) with band tolerance of 1·0%. Multi-locus sequence typing (MLST) was performed on 10 randomly selected isolates representative of PFGE types according to the protocol described at http://efaecium.mlst.net/misc/info.asp. The presence of *vanA* or *vanB* genes, and the characterization of Tn*1546* elements was determined by PCR with published primer pairs [7], using a touchdown PCR protocol comprising an initial annealing temperature of 70 °C, which was reduced by 1 °C per cycle over 15 cycles and followed by 20 cycles of amplification at 55 °C [4]. Characterization of Tn*1546* elements was further achieved using primer pairs primers *vanS1.seq* (5′-TGCTCGTTCTTCCGATAC-3′), *H1.seq-i* (5′-AGCGCAAGAAGAATAGAGGC-3′); *vanX1. seq* (5′-ACGTTTGCGCTCCATCAT-3′); *vanY1. seq-i* (5′-CACACTTTCTTGGCGAACAG-3′); IS*1216VD* (5′-TGGGATTCCCAATAATACC-3′); IS*1216VA* (5′-GGAAAGCAATTTCAGCAG-3′); IS*1251*.f (5′-GCCCACTTGAATGTCTGA-3′); and IS*1251*.r (5′-GGATGCATTCTTGGGCTGTA-3′). PCR amplicon sizes were compared with those calculated theoretically using the Tn*1546* reference sequence BM4147 (GenBank accession no. M97297) and the location of insertion sequences within the transposons was determined by DNA sequencing of relevant PCR products using the primers above.

The majority (23/26) of the isolates were recovered from patients in the adult intensive care unit (AICU), whose underlying conditions included cancer ($n=7$) and septic shock ($n=2$) among others. Five (19%) of the 26 VRE isolates were of the VanA phenotype and *vanA* gene-positive, with 20 (77%) isolates of the VanB phenotype and *vanB* gene-positive, a single VanA phenotype isolate was positive for both *vanA* and *vanB* genes by PCR. All 26 isolates were resistant to vancomycin, ampicillin and ciprofloxacin, and all but one (isolate 37) were susceptible to linezolid and quinupristin/dalfopristin (Fig. 1). From Table 1, it can be seen that nine rectal swabs were positive for vancomycin-resistant *E. faecium*, and were identified as part of the ICU screening process for all patients upon admission. The remaining 17 isolates were obtained from clinical samples as part of work-up for patients with fever and clinical signs of sepsis. In these cases, treatment targeting VRE was initiated accordingly. Both routine screening and clinical samples contained both *vanA*- and *vanB*-positive *E. faecium* isolates from both of the major genetic clusters identified. This finding points to a possible source for the vancomycin-resistant *E. faecium* infections, i.e. isolates were most probably brought into the hospital by patients, particularly as the King Fahad Specialist Hospital in Dammam is a referral hospital, with patients admitted to the ICU usually arriving from other ancillary hospitals and where patients have usually been exposed to antibiotics for at least 1 week prior to admission. Further, environmental samples obtained as part of outbreak investigations identified no environmental source of these resistant *E. faecium*. Microbial analysis of drains showed that the most common co-pathogens were *E. coli* and *Klebsiella* spp., although in 2007 these organisms [including extended spectrum β-lactamase (ESBL)-producing variants] were not a big problem.

Table 1. *Medical history of patients ranked according to their* E. faecium *PFGE genotypes*

Isolate no.	Ward	Age (yr)	Sex	Specimen	Date of specimen	Transferred from another hospital	Diagnosis	Previous admission	Previous AICU admission	Antimicrobials in previous 3 months	Parenteral nutrition	Central line	Date of death
42	AICU	90	M	RS	03.12.2007	Yes	Septic shock	1–7 days	No	Unk.	No	Yes	20.06.2006
44	AICU	69	M	BLD	28.03.2007	Yes	Septic shock	Unk.	No	Yes	No	Yes	n.a.
40	AICU	81	M	PUS	18.03.2007	No	Sepsis	None	No	Yes	No	No	n.a.
28	AICU	80	F	ETT	26.03.2007	Yes	Respiratory failure	>7 days	Yes	Yes	No	Yes	n.a.
36	AICU	85	M	TLC	04.03.2007	No	Acute myocardial infarction	None	No	No	Unk.	Yes	04.11.2007
26	AICU	51	F	RS	16.08.2007	No	Breast cancer	None	No	No	No	No	n.a.
29	AICU	80	F	RS	21.02.2007	Yes	Community-acquired pneumonia	>7 days	No	Yes	No	Yes	20.02.2007
23	AICU	75	M	SAC	08.06.2007	Yes	Rectal cancer	>7 days	No	Yes	No	Yes	n.a.
4	HBW	37	M	CBD	12.05.2007	Yes	Cholangio cancer	None	No	No	No	No	n.a.
9	AICU	7	F	RS	14.07.2007	Yes	Adenotonsillectomy	None	No	No	No	No	n.a.
41	AICU	62	M	URI	14.02.2007	No	Pyelonephritis	1–7 days	No	Yes	No	Yes	03.05.2006
24	MM	75	M	URI	18.07.2007	No	Obstructive neuropathy	>7 days	No	Unk.	No	No	n.a.
35	MS	60	M	BED	15.05.2007	Yes	Periampullary cancer	>7 days	Yes	No	Yes	Yes	n.a.
30	AICU	84	F	TTD	22.05.2007	Yes	Peritonitis	>7 days	Yes	Yes	No	Yes	18.07.2007
11	AICU	61	M	RS	23.09.2007	No	Rectal cancer	>7 days	Yes	Yes	Yes	Yes	n.a.
12	AICU	40	F	ABD	09.05.2007	Yes	Peritonitis	1–7 days	No	Yes	No	Yes	28.11.2007
13	AICU	51	M	RS	21.08.2007	Yes	Pancreatic cancer	1–7 days	Yes	Yes	No	Yes	n.a.
15	AICU	82	M	BLD	11.03.2007	No	Acute renal failure	>7 days	Yes	Yes	No	Yes	n.a.
32	AICU	75	F	BLD	25.05.2007	No	Pneumonia	>7 days	No	Yes	No	Yes	06.04.2007
34	AICU	15	M	TRA	16.06.2007	No	Hypokalaemia	1–7 days	No	No	No	Yes	13.07.2007
8	AICU	26	F	RS	28.09.2007	Yes	ARDS	1–7 days	No	No	No	Yes	n.a.
33	AICU	79	M	RS	05.06.2007	Yes	ARDS	>7 days	Yes	Yes	No	No	30.10.2007
10	AICU	72	M	RS	08.11.2007	No	Cholangitis	>7 days	Yes	Yes	Yes	Yes	17.08.2007
22	AICU	54	F	TRA	07.06.2007	Yes	Healthcare-associated pneumonia	>7 days	Yes	Yes	No	Yes	08.07.2007
39	AICU	59	M	URI	03.07.2006	Yes	Bladder cancer	>7 days	No	Unk.	No	No	23.12.2006
37	AICU	80	F	TLC	07.09.2006	Yes	Congestive heart failure	>7 days	No	Unk.	No	Yes	28.07.2007

Unk., Unknown; n.a., not applicable; ARDS, acute respiratory distress syndrome.
Wards: AICU, adult intensive care unit; HBW, hepatobiliary ward, MM, male medical ward, MS, male surgical ward.
Specimens: RS, rectal swab; BLD, blood; PUS, pus; ETT, endotracheal tube; TLC, triple lumen catheter; SAC, sacral sore; CBD, common bile duct stent; URI, urine; BED, bed sore; TTD, T-tube drain; ABD, abdominal drain; TRA, tracheal aspirate.



Isolate	Date	Van type	E test VAN	E test TEI	AMP	CIP	TET	Q/D	LZD	MLST
42	12/07	B	8	0·5	R	R	R	S	S	78
44	03/07	B	8	0·5	R	R	R	S	S	
40	03/07	B	8	1	R	R	R	S	S	
28	03/07	B	4	1	R	R	R	S	S	
36	04/07	**B**	8	0·5	R	R	R	S	S	
26	08/06	A^{F3}	>256	32	R	R	R	S	S	78
29	02/07	A^{F3}	>256	32	R	R	R	S	S	
23	06/06	**B**	4	1	R	R	R	S	S	78
4	05/06	B	8	0·5	R	R	R	S	S	78
9	07/07	B	4	0·5	R	R	R	S	S	
41	02/06	B	8	0·5	R	R	R	S	S	
24	07/06	B	2	1	R	R	R	S	S	78
35	05/07	**B**	4	0·5	R	R	R	S	S	78
30	05/07	A^{F3}	>256	32	R	R	S	S	S	78
11	09/07	B	8	0·5	R	R	S	S	S	
12	05/07	**B**	8	0·5	R	R	S	S	S	
13	08/07	B	8	0·5	R	R	S	S	S	
15	03/07	B	8	0·5	R	R	S	S	S	290
32	05/07	B	8	0·5	R	R	S	S	S	
34	06/07	B/A^{F3}	>256	32	R	R	S	S	S	
8	09/07	B	16	0·5	R	R	S	S	S	
33	06/07	B	16	1	R	R	S	S	S	
10	11/07	B	8	0·5	R	R	S	S	S	
22	06/07	B	16	0·5	R	R	S	S	S	
39	07/06	A^{A1}	>256	16	R	R	R	S	S	78
37	09/06	A^{A1}	16	8	R	R	R	R	S	727

Fig. 1. PFGE patterns obtained from 26 vancomycin-resistant *E. faecium* isolates originating from the King Fahad Specialist Hospital, Damman, Saudi Arabia between 2006 and 2007. Superscript letters indicate Tn*1546* lineage (based on reference 9). AMP, Ampicillin; CIP, ciprofloxacin; TET, tetracycline; Q/D, quinupristin/dalfopristin; LZD, linezolid; R, resistant; S, susceptible. Numbers in circles represent the two major genetic clusters observed.

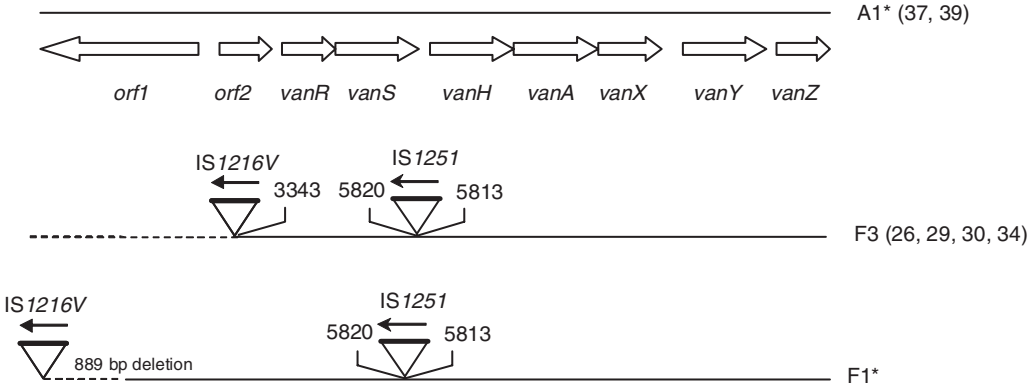


Fig. 2. Genetic map of Tn*1546* types associated with six VanA *E. faecium* isolates originating from Dammam, Saudi Arabia. The unbroken black lines represent reference Tn*1546* sequence BM4147. Unfilled block arrows represent the positions of genes and open reading frames (*orf1* and *orf2*). Filled boxes represent IS elements and arrows their direction of transcription. The positions of the first nucleotide upstream and downstream of the insertion sequence are shown. Group definitions (right) are based on those published by Willems *et al.* [9]. Numbers in parentheses indicate isolates belonging to each Tn*1546* grouping. * The new Tn*1546* lineage type F3 is closely related to the previously described Group F1 Tn*1546* lineage.

PFGE of the 26 *E. faecium* isolates (Fig. 1) showed the presence of two major clusters (isolates 42–35 and 11–22), based on an approximate similarity cut-off value of 90%, as well as a single unrelated genotype (isolate 37). Resistance to tetracycline was a distinguishing feature between the two clusters in the majority of isolates except for one (isolate 30). The clusters were not associated with Van A or Van B

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phenotypes or year of isolation. However, all representatives of cluster 2 originated from the AICU whereas cluster 1 was more widespread in the hospital. Figure 1 shows that eight of the 10 isolates typed by MLST were of sequence type (ST) 78 and seven of these grouped in cluster 1. Single isolates of ST290 and a novel type ST727 were also identified; the latter sequence type showed a single allelic difference from previously described types (ST16 and ST182). The three sequence types found here are associated with the global clonal cluster (CC) 17 which is often associated with outbreaks of hospital infections worldwide [8].

Of the six isolates positive for *vanA*, Tn*1546* PCR amplicon size analysis showed that two (isolates 26 and 29) contained no major insertions or deletions compared to the reference Tn*1546* BM4147 sequence. However, in four *vanA* isolates (30, 34, 37, 39), both insertion elements IS*1251* and IS*1216V* were found (Fig. 2), with the IS*1251* element being associated with a duplication of an ATAATTTT motif, as described previously [9]. Moreover, although related to a previously described group F Tn*1546* lineage [2, 9], the resultant Tn*1546* variant had not been previously described in the literature. The presence of two new Tn*1546* lineage types in *E. faecium* isolates from Riyadh isolated between 2000 and 2003 has previously been noted [4]. Interestingly, these workers also found a new Tn*1546* type *vanA E. faecium* associated with nosocomial infections, which suggests that there may be yet more *vanA* Tn*1546* variants circulating in Saudi Arabian hospitals. The question remains as to how important these different lineages are in epidemiologically linking endemic and epidemic nosocomial infections of VRE in Saudi hospitals, as well as the relationship between VanA Tn*1546* variants and clinical resolution of infection with successful antibiotic therapy in Saudi Arabia.

Previous reports have shown that nosocomial infections due to enterococci are not new to Saudi Arabian hospitals, for example Tayfour *et al*. [10] reported finding two *E. faecium*, four *E. faecalis* and 13 *Enterococcus* spp. in a total of 366 surgical site infections over 1 year (2003–2004) at the King Fahad Hospital, Al-Baha. Further, a more recent publication from a hospital in the Riyadh area in 2009–2010 also documented the emergence of VRE (3·9% in 206 enterococcal isolates) and increased rates of multidrug resistance [11]. Consistent with our observations, Al-Otaibi *et al*. [12] found that a significant number of patients presenting with enterococcal bacteraemia between 2001 and 2002 at the King Khalid University Hospital, Riyadh ($n=60$), had previously stayed in an ICU, and our results also show the significance of these units in Saudi nosocomial enterococcal infections. Indeed at that time, vancomycin and imipenem consumption was not controlled, but as a result of the increased cases of VRE observed, an antibiotic stewardship programme was initiated and guidelines for treatment were adopted within the King Fahad Specialist Hospital, Dammam.

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DECLARATION OF INTEREST

None.

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