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RESEARCH ARTICLE

Virulence profiles of vancomycin-resistant enterococci isolated from surface and ground water utilized by humans in the North West Province, South Africa: a public health perspective

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Abstract

Vancomycin-resistant enterococci (VRE) have been responsible for numerous outbreaks of serious infections in humans worldwide. *Enterococcus faecium* and *Enterococcus faecalis* are the principal species that are frequently associated with vancomycin resistance determinants, thus usually implicated in hospital- and community-acquired infections in humans. The study aim was to determine the antibiotic resistance and virulence profiles of VREs isolated from surface and groundwater samples that are used by humans in the North West Province, South Africa. A total of 170 water samples were collected and analyzed. Eighty-one potential isolates were screened for characteristics of *Enterococcus* species using preliminary biochemical tests, PCR assays and sequence analysis. The antimicrobial resistance profiles of the isolates against nine antibiotics were determined and a dendrogram was generated to access the relatedness of the isolates. The isolates were screened for the presence of antibiotic resistance and virulence genes by multiplex PCR analysis. A total of 56 isolates were confirmed as *Enterococcus* species and the proportion of *E. faecium* (46.9%) was higher than *E. faecalis* (29%) and *E. saccharolyticus* (1.2%). Sequence data of *E. faecium*, *E. faecalis*, and *E. saccharolyticus* isolates revealed 97 to 98% similarities to clinical strains deposited in NCBI Genbank. Large proportions (44; 78.6%) of the isolates were resistant to vancomycin while 16 and 3.6% of the isolates possessed the *vanA* and *vanB* genes respectively. The MAR phenotype Vancomycin-Nalidixic Acid-Streptomycin-Chloramphenicol-Ampicillin-Oxytetracycline-Gentamycin-Nitrofurantoin-Sulphamethoxazole indicated that some isolates were resistant to all of the nine antibiotics tested. Cluster analysis of antibiotic resistance data revealed two major clusters. Sixteen (36.4%), 14 (27.3%), 3 (6.8%), and 2 (4.5%) of the VRE isolates possessed the *gel*, *asa*1, *hyl*, and *esp* virulence genes respectively while the *cylA* gene was not detected in the study. Multiple antibiotic-resistant enterococci were also resistant to vancomycin and possessed virulence determinants indicating that they can pose severe public health complications on individuals who consume contaminated water.

Keywords VREs · Enterococci virulence genes · Vancomycin resistance determinants

Introduction

Enterococci are Gram-positive, facultative anaerobic bacteria that usually occur as normal flora in gastrointestinal and genitourinary tracts of humans and animals (Zirakzadeh and Patel 2006). However, a number of highly pathogenic *Enterococcus* strains have been isolated from food products, clinical samples, and drinking water (Frieden et al. 1993; Goldstein et al. 2014). These strains usually possess intrinsic virulence factors that are associated with a variety of infections as well as resistance traits against a wide range of antimicrobial agents (Mohapi and Ateba 2013; Lisboa et al. 2015). Many species have been identified and fully characterized so far. However,

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strains belonging to the species *Enterococcus faecalis* and *Enterococcus faecium* are the two most frequently identified isolates and they account for the majority of human enterococcal infections worldwide (Ranotkar et al. 2014).

In addition, the public health significance of virulent *Enterococcus* species has been amplified by the constant increase in the prevalence of hospital-acquired enterococcal infections worldwide (Ramsey and Zilberberg 2009). Unfortunately, the success rate achieved with antimicrobial agents is greatly affected by the fact that most virulent *Enterococcus* isolates are highly resistant to commonly recommended drugs, thereby significantly limiting therapeutic options. This has greatly reduced treatment options for infections caused by vancomycin-resistant *Enterococcus* (VRE) strains (Valenzuela et al. 2008).

Access to safe drinking water is a significant human need. Due to the low rate of rainfalls and the increase of the industrialization process in South Africa, the quality of supplied water to the communities has been negatively affected, especially in rural areas (Ateba and Maribeng 2011). Therefore, most individuals from these places rely on water from unprotected alternative sources such as dams, boreholes, and rivers for survival and for use in household activities (Sood et al. 2008). These unprotected sources are usually exposed to fecal microbial contamination of animal and human origin, especially pathogenic strains of *Salmonella*, *E. coli* and *Enterococcus* species (Sood et al. 2008; Ranotkar et al. 2014).

Antibiotic-resistant bacteria, particularly vancomycin-resistant enterococci (VRE), pose a significant challenge to the medical profession even in advanced countries especially when these strains harbor multiple antibiotic resistance determinants (Takeuchi et al. 2005). With the aim of isolating VRE from ground and surface water as well as determining their virulence capabilities, the present study was designed to expand on the previous investigations, to assess the public health implications that these isolates may have on individuals who consume contaminated water in the study area and to generate data that may be of great epidemiological significance.

Material and methods

Area of study

A total of 170 water samples comprising of 119 ground and 51 surface water samples were randomly collected from different villages, rural and urban communities in different areas in the North West Province (Fig. 1) using sterile 500-mL Duran Schott bottles. The number and nature of samples collected from the different points are shown in Table 1. Samples were properly labeled and transported on ice to the laboratory for analysis.

Selective isolation of VRE species

The water samples were immediately analyzed upon arrival in the laboratory. An aliquot of 100 mL from each water sample was filtered through a 0.45-μM filter paper (Whatman® Glass Microfiber GS Filter paper) on a vacuum water pump machine (Model, Sartorius 16824). Using sterile forceps, the membrane filters were placed on Bile Esculin Agar (BEA) (Biolab, South Africa) supplemented with vancomycin (16 μg/mL) to select for VRE. The plates were then incubated aerobically at 37 °C for 24 h and characteristic black colonies were considered as potential VRE species. Pure colonies were preserved in 70% (*v*/*v*) glycerol at − 80 °C for further biochemical identification tests. In the present study, *Enterococcus faecium* (ATCC 700221) was used as a positive control strain and *Staphylococcus aureus* (ATCC 43322) as a negative control strain.

Cellular morphology

The isolates were Gram stained for bacterial identification using standard protocols (Cruickshank et al. 1975). Gram-positive cocci were retained and subjected to both preliminary and confirmatory identification tests for *Enterococcus* species.

Preliminary biochemical identification tests for enterococci

Catalase and oxidase tests were performed according to previously described protocols (Ateba et al. 2013; Ateba and Maribeng 2011). Pure colonies were tested on oxidase paper strips (Whatman International Ltd., Maidstone). Oxidase negative isolates were further considered for catalase test. Oxidase negative isolates were mixed with a drop of 2% (*v*/*v*) hydrogen peroxide (H_2O_2) onto a clean microscope slide and observed for effervescence which is confirmatory for catalase positive organisms. Catalase negative isolates were subjected to further preliminary identification tests.

All the presumptive enterococci were cultured aerobically at 37 °C for 24 h in Falcon tubes containing 10 mL of 6.5% (*w*/*v*) NaCl broth to differentiate them from streptococci (Klein 2003). *Enterococcus faecium* (ATCC 700221) was also used for positive control while an un-inoculated NaCl broth was used as a negative control. Bacterial growth was determined by measuring the optical density at 600 nm using a spectrophotometer (model Helios Epsilon, Merck, South Africa).

Serotyping

The isolates were screened for serological identification of *Enterococcus* species based on the Lancefield grouping of A, B, C, D, F, and G streptococci (Ingram et al. 1983) using

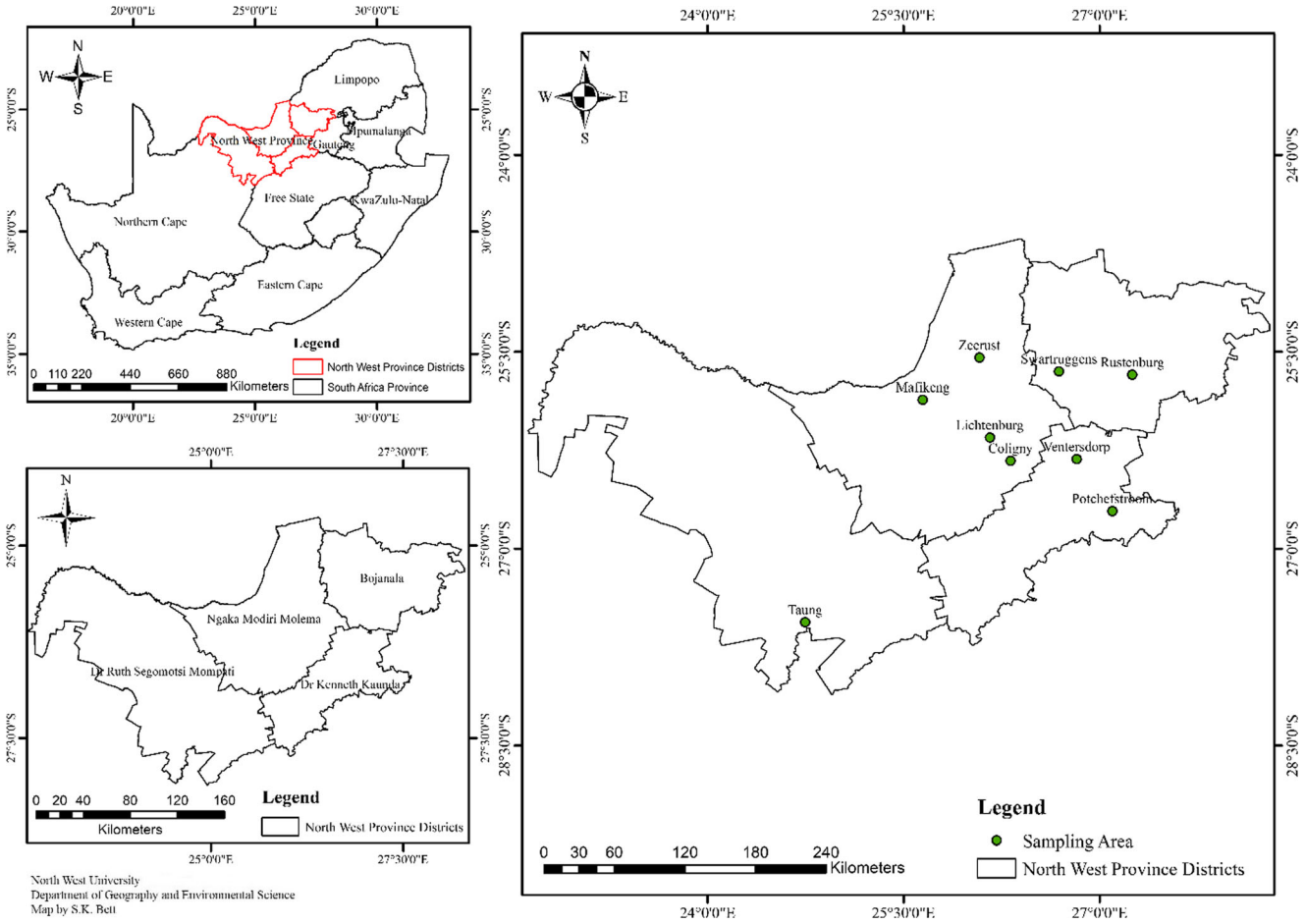

Fig. 1 Rural and urban communities in different areas in the North West Province from which water samples were collected during the study

A SLIDEX® Strepto Plus Latex agglutination test kit (BioMérieux, South Africa). All the isolates which showed positive result (agglutination) were subjected to molecular identification tests.

Table 1 Areas from which water samples were collected

District	Sampling area	Number of groundwater samples	Number of surface water samples
Bojanala Platinum District	Rustenburg	Borehole = 16	Dam = 10
	Swarstruggens	Borehole = 3	River = 0
Ngaka Modiri Molema District	Mafikeng	Borehole = 55	Dam = 12
	Zeerust	Borehole = 7	Dam = 3
			Spring = 3
	Coligny	Borehole = 5	Dam = 5
	Lichtenburg	Borehole = 3	River = 0
Dr Ruth Segomotsi Mompati	Taung	Borehole = 14	Dam = 2
			River = 2
Dr Kenneth Kaunda	Potchefstroom	Borehole = 10	Dam = 7
			River = 7
	Ventersdorp	Borehole = 6	0
Total		119	51
		170	

Molecular identification of enterococci using PCR analysis

Genomic DNA was extracted from all presumptive VRE isolates using the hot cetyltrimethyl ammonium bromide (CTAB), polyvinyl pyrrolidone (PVP) extraction protocol (Doyle 1990). The genomic DNA was quantified using a nano-drop lite spectrophotometer (Model 1558, Thermo Scientific, USA). Presumptive *Enterococcus* isolates that satisfied both preliminary and confirmatory biochemical tests were subjected to bacterial 16S rRNA gene PCR. The 16S rRNA PCR was performed using oligonucleotide primer combinations and cycling conditions described previously by Mohapi and Ateba (2013). The amplified 16S rRNA gene fragments were sequenced by Inqaba Biotech, South Africa. Sequence data was subjected to BLAST search on the NCBI WebTool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the identities of the amplified sequences as well as the isolates. The identities of the presumptive *E. faecalis* and *E. faecium* isolates were determined using a multiplex PCR assay designed to amplify species-specific sequence *ddl* gene that code for D-alanine: D-alanine ligase (*ddl*) in *E. faecalis* and *E. faecium* (Depardieu et al. 2004). Molecular identification of *E. saccharolyticus* was achieved through amplification of a 370 bp fragment using primer sequences SA1 (5′-AAAC ACCATAACACTTATGTG-3′) and SA2 (5′-GTAG AAGTCACTTCTAATAAC-3′) based on a previous protocol (Jackson et al. 2004).

Antibiotic resistance susceptibility test

The antibiotic resistance profiles of the isolates were determined using the Kirby-Bauer disc diffusion technique (Kirby et al. 1966). Isolates were screened against a panel of nine antimicrobial agents that appear in Table 2 and obtained from Mast Diagnostics, UK. Cluster analysis of antibiotic susceptibility data was determined by using Ward's algorithm and Euclidean distances on Statistica version 7.0 software (Statsoft, US) and the results were efficiently expressed as a dendrogram.

Concentrations of antibiotics used and the inhibition zone measurements (in mm) that were used to classify organisms as resistant, intermediate resistant, and susceptible to a particular antibiotic are shown in Table 2.

Multiplex PCR for screening and confirmation of VRE and virulence genes

The presence of vancomycin resistance determinants (*vanA*, *vanB*, v*anC*) in VRE strains were determined using a multiplex PCR analysis with specific primer sets previously described by Depardieu et al. (2004). The virulence determinants of VRE isolates were determined through the amplification of the *asa1*, *cylA*, *esp*, *gel*, and *hyl* gene sequences using chromosomal DNA extracted from the isolates (Molale and Bezuidenhout 2016).

Amplicons were separated by electrophoresis on a 1.5% (*w*/*v*) agarose gel (containing ethidium bromide 0.0 01 μg/ml) using 1× TAE (40 mM Tris (pH 7.6), 20 mM acetic acid, 1 mM EDTA) at 80 V for 15 min and later at 60 V for 4 h. A ChemiDoc Imaging System (Bio-RAD ChemiDoc™ MP Imaging System, Hercules, California, USA) was used to capture the image using Gene Snap (version 6.00.22) software. Each gel contained a 100-bp or 1-kb molecular weight marker (BioLab, New England).

Results

Occurrences of *Enterococcus* species in ground and surface water

A total of 170 samples (ground and surface water) were collected from villages and rural communities within the North West Province. Eighty-one potential *Enterococcus* isolates were obtained based on differences in their colonial morphologies. All the 81 isolates were Gram-positive cocci and also satisfied the preliminary identification for *Enterococcus* species. However, only 74.1% (60/81) of the isolates were positively identified as *Enterococcus* species based on

Table 2 The details of antibiotics used in this study

Class	Antibiotic used	Concentration (μg)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Penicillin/β-lactamase	Ampicillin	10	≤ 16	–	≥ 17
Aminoglycoside	Streptomycin	10	≤ 6	7–9	≥ 10
	Gentamycin	10	≤ 12	13–14	≥ 15
Nitrofurans	Nitrofurantoin	100	≤ 14	15–16	≥ 17
Tetracycline	Oxytetracycline	30	≤ 14	15–18	≥ 19
Quinolone	Nalidixic acid	30	≤ 13	14–18	≥ 19
Sulphonamides	Sulphamethoxazole	25	≤ 10	11–15	≥ 16
Chloramphenicol	Chloramphenicol	30	≤ 12	11–17	≥ 18
Glycopeptides	Vancomycin	30	≤ 14	15–16	≥ 17

Table 3 The number of samples collected from various sources in villages and rural communities, the total number of *Enterococcus* isolates isolated, and the names of the *Enterococcus* species identified

Area	Sample source	No. samples collected	No. samples positive	*Enterococcus* species identified	No. positive for the different species in samples
Mafikeng	GW, Boreholes	55	18	*E. faecium*	12
				E. faecalis	5
				E. saccharolyticus	1
	SW, Dams	12	4	*E. faecium*	1
				E. faecalis	3
Rustenburg	GW, Boreholes	16	4	*E. faecium*	6
	SW, Dams	10	7	*E. faecium*	6
				E. faecalis	4
Potchefstroom	GW, Boreholes	10	1		0
	SW, Dams	7	1	*E. faecium*	1
	SW, Rivers	7	2	*E. faecium*	2
Taung	GW, Boreholes	14	0		
	SW, Dams	2	0		
	SW, Rivers	2	1	*E. faecium*	1
				E. faecalis	1
Zeerust	GW, Boreholes	7	1	*E. faecium*	2
	SW, Dams	3	0		
	SW, Spring	3	3	*E. faecium*	4
				E. faecalis	2
Coligny	GW, Boreholes	5	0		
	SW, Dams	5	3	*E. faecium*	3
				E. faecalis	2
Ventersdorp	GW, Boreholes	6	0		
Swartruggens	GW, Boreholes	3	0		
Lichtenberg	GW, Boreholes	3	0		
Total		170	45	*E. faecium*	38
				E. faecalis	17
				E. saccharolyticus	1
				TOTAL	56

GW ground water, *SW* surface water

agglutination test, while confirmatory *ddl* gene PCR analysis revealed 69% (56/81) as enterococcal isolates. Detailed results, including the proportion of *Enterococcus* species, are shown in Table 3.

Table 4 Proportion of isolates from the different stations that were resistant to the antibiotics tested

Sampling area	VAN (30*)	NA (30*)	STR (10*)	CHIL (30*)	AMP (10*)	OXY-TET (30*)	GEN (10*)	NIT (100*)	SMX (25*)
Mafikeng	16 (72.7%)	17 (77.3%)	5 (22.7%)	6 (27.3%)	8 (36.4%)	8 (36.4%)	4 (18.2%)	11 (50%)	0 (0%)
Rustenburg	13 (81.3%)	14 (87.5%)	4 (25%)	4 (25%)	11 (68.8%)	8 (50%)	0 (0%)	8 (50%)	3 (18.6%)
Potchefstroom	2 (66.7%)	2 (66.7%)	1 (33.3%)	1 (33.3%)	2 (66.7%)	2 (66.7%)	1 (33.3%)	2 (66.7%)	2 (66.7%)
Taung	2 (100%)	2 (100%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	2 (100%)	2 (100%)	1 (50%)
Zeerust	7 (87.5%)	7 (87.5%)	2 (25%)	2 (25%)	2 (25%)	5 (62.5%)	0 (0%)	5 (62.5%)	0 (0%)
Coligny	4 (80%)	5 (100%)	4 (80%)	3 (60%)	1 (20%)	4 (80%)	2 (40%)	3 (60%)	1 (20%)
Total	44 (78.6%)	47 (83.9%)	17 (30.4%)	17 (30.4%)	25 (44.6%)	28 (50%)	9 (16.1%)	31 (55.4%)	7 (12.5%)

VAN vancomycin, *NA* nalidixic acid, *STR* streptomycin, *CHIL* chloramphenicol, *APM* ampicillin, *OXY-TET* oxytetracycline, *GEN* gentamicin, *NIT* nitrofurantoin, *SMX* sulphamethoxazole

*Indicates the concentrations of antibiotics that were present in the discs

Table 5 Predominant multiple antibiotic resistance phenotypes for vancomycin-resistant *Enterococcus* (VRE) isolated from different sampling areas

Sample area	Phenotype	No. observed
Mafikeng	VAN-NAL-AMP	3
NT = 22, NO = 18	VAN-NAL-OXYTET	2
	VAN-CHIL-NIT	1
	VAN-NAL-STR-AMP-OXYTET-GEN-NIT	1
	VAN-NAL-AMP-OXYTET-GEN-NIT	1
	VAN-NAL-AMP-OXYTET-NIT	1
	VAN-STR-NIT	1
	NAL-STR-NIT	1
	VAN-NAL-CHIL-OXYTET	1
	VAN-NAL-STR-CHIL-OXYTET-NIT	1
	VAN-NAL-OXYTET-GEN-NIT	1
	VAN-NAL-CHIL-NIT	1
	VAN-NAL-STR-CHIL-AMP-GEN-NIT	1
	VAN-NAL-CHIL-AMP-OXYTET	1
	STR-CHIL-GEN-NIT	1
Rustenburg	VAN-NAL-AMP-OXYTET-NIT	2
NT = 16, NO = 11	VAN-NAL-AMP-OXYTET	2
	VAN-NAL-AMP	1
	VAN-NAL-STR-CHIL-NIT	1
	NAL-STR-CHIL-AMP-OYTET-NIT-SMX	1
	NAL-CHIL-AMP-NIT	1
	VAN-NAL-STR-AMP-OXYTET-NIT-SMX	1
	VAN-NAL-STR-AMP-OXYTET-NIT	1
	VAN-STR-CHIL-AMP-OCYTET-NIT-SMX	1
Potchefstroom	VAN-NAL-AMP-OXYTET-NIT-SMX	1
NT = 3, NO = 2	VAN-NAL-STR-CHIL-AMP-OXYTET-GEN-NIT-SMX	1
Taung	VAN-NAL-STR-CHIL-AMP-OXYTET-GEN-NIT-SMX	1
NT = 2, NO = 2	VAN-NAL-GEN-NIT	1
Zeerust	VAN-NAL-OXYTET-NIT	3
NT = 8, NO = 8	VAN-NAL-CHIL	1
	NAL-STR-CHIL-AMP	1
	VAN-NAL-OXYTET	1
	VAN-STR-OXYTET-NIT	1
	VAN-NAL-AMP-NIT	1
Coligny	VAN-NAL-STR-CHIL-AMP-OXYTET-GEN-NIT-SMX	1
NT = 5, NO = 4	VAN-NAL-STR-CHIL-OXYTET-NIT	1
	NAL-NAL-STR-CHIL-NIT-OXYTET	1
	NAL-STR-OXYTET-GEN-NIT	1
Total NT = 56, NO = 45		45

VAN vancomycin, *NA* nalidixic acid, *STR* streptomycin, *CHIL* chloramphenicol, *APM* ampicillin, *OXY-TET* oxytetracycline, *GEN* gentamicin, *NIT* nitrofurantoin, *SMX* sulphamethoxazole, *NT* number tested, *NO* number observed

Antibiotic resistance profiles of isolates

A total of 56 isolates that were genotypically detected as VRE by PCR were subjected to the disc diffusion antimicrobial susceptibility test. This test was done to evaluate the resistance patterns of isolates from the different sampling sites. The data in Table 4 indicate the percentage of resistance against a panel of nine antimicrobial agents. Larger proportions of the isolates were resistant to NAL (83.9%) and VAN (78.6%). In addition, the majority (68.8–87.5%) of the isolates from Mafikeng and Rustenburg were resistant to VAN, NAL, and AMP. Similarly, a majority (60–100%) of the isolates from Potchefstroom and

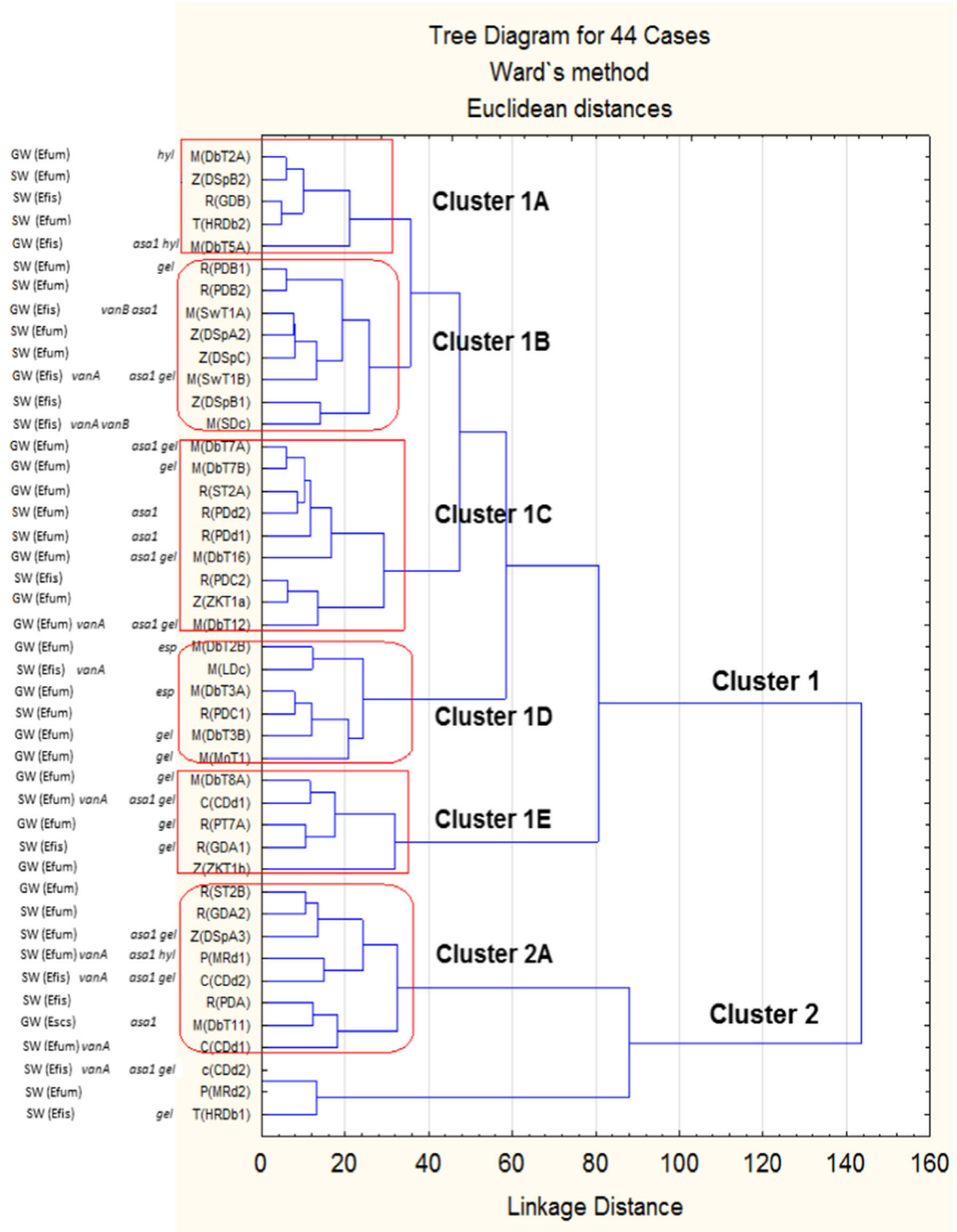

Fig. 2 Dendrogram showing the relationship between vancomycin-resistant *Enterococcus* isolates from ground and surface water samples obtained in the different locations. Bacterial designation prefixes are based on sampling station origin and sample type. Designation: M, Mafikeng; R, Rustenburg; P, Potchefstroom; T, Taung; Z, Zeerust; C, Coligny; GW, ground water; SW, surface water; Efum, *E. faecium*; Efis, *E. faecalis*; Escs, *E. saccharolyticus*

Coligny were resistant to VAN, NAL, and NIT. It was observed that significant proportions of the isolates from some of these sampling sites were resistant to GEN (0–40%), CHL (27–50%), and SMX (0–66.7%). Although isolates from other areas showed moderate resistance to these antibiotics, small proportions (0–25%) of the isolates from Mafikeng, Rustenburg, and Zeerust were highly susceptible to SMX, GEN, STR, and CHL.

Multiple antibiotic resistance (MAR) phenotypes of VRE species isolated from surface and ground water

The multiple antibiotic resistance phenotypes of 56 confirmed *Enterococcus* isolates were generated for isolates showing resistance to three or more antibiotics. Values obtained were expressed as percentages and results are as shown in Table 5. A large proportion (80.4%) of the isolates obtained in this study showed MAR phenotypes and among these, 38 (84.4%) were resistant to vancomycin. Despite the fact that several phenotypes were observed among isolates from Mafikeng, MAR phenotypes VAN-NAL-AMP-OXYTET-NIT and VAN-NAL-AMP were predominant among these isolates. Phenotype VAN-AMP-NIT was dominant among isolates from Mafikeng and Rustenburg. A cause for concern was the fact that one isolate each from Potchefstroom, Taung, and Coligny was resistant to all the nine antibiotics that were tested.

Phenotypic relationship between vancomycin-resistant *enterococcus* isolates obtained from surface and ground water based on clustering patterns using the antibiotic inhibition zone diameter data

A total of 44 (78.6%) isolates showed phenotypic resistance to vancomycin and these were considered for clustering patterns and based on their inhibition zone diameters. A total of 44 VRE strains, isolated from different locations sampled were subjected to phenotypic clustering. A dendrogram was generated and detailed results are shown in Fig. 2. The dendrogram was analyzed for associations of isolates from the different sampling sites and results are shown in Table 6.

Two clusters (cluster 1 and cluster 2) were generated. Cluster 1 was subdivided into five sub-clusters (cluster 1A–1E), while cluster two was subdivided into only one sub-cluster and three non-clustered isolates. Furthermore, all the sub-clusters were analyzed for patterns of association of isolates from different sources and/or locations (Fig. 2). The largest sub-cluster was sub-cluster 1C, comprised of isolates derived from ground water samples. The second largest sub-cluster (sub-cluster 2A) contained isolates from all the sampling areas, except Taung. In addition, large proportions (75%) of isolates in this sub-cluster (sub-cluster 2A) were derived from surface water samples and most of the isolates observed were *E. faecium* species. On the contrary, the results revealed three isolates in cluster 2 were not grouped into any of the sub-clusters. Moreover, only isolates from Mafikeng and Rustenburg were represented across all the clusters and large proportions (40%) of isolates present in sub-cluster 1C were from Mafikeng.

Detection of vancomycin resistance and virulence determinants in *enterococcus* isolates by multiplex PCR analysis

A total of 56 confirmed *Enterococcus* isolates were subjected to a multiplex PCR analysis in order to amplify *vanA*, *vanB*, and *vanC21/2* that code for resistance to vancomycin in enterococci (Depardieu et al. 2004). Results indicated that a small portion of 9 (16%) isolates possessed the *vanA* resistance gene while 2 (4%) isolates possessed the *vanB* resistance gene. On the contrary, none of the isolates possessed the *vanC* resistance gene. It was also identified that an isolate from Mafikeng possessed both the *vanA* and *vanB* resistance genes.

The results of virulence gene PCR analysis indicated that 16 (36.4%), 14 (27.3%), 3 (6.8%), and 2 (4.5%) of the isolates possessed the *gel*, *asa1*, *hyl*, and the *esp* virulence genes

Table 6 The number of VREs isolates obtained from different areas within the various clusters

Sampling area	Source	Cluster 1A $N=5$	Cluster 1B $N=8$	Cluster 1C $N=9$	Cluster 1D $N=6$	Cluster 1E $N=5$	Cluster 2A $N=8$	Non-cluster 2 $N=3$
Mafikeng	GW	2	2	4	4	1	1	0
	SW	0	1	0	1	0	0	0
Rustenburg	GW	0	0	1	0	1	1	0
	SW	1	2	3	1	1	2	0
Potchefstroom	GW	0	0	0	0	0	0	0
	SW	0	0	0	0	0	1	1
Taung	GW	0	0	0	0	0	0	0
	SW	1	0	0	0	0	0	1
Zeerust	GW	0	0	1	0	1	0	0
	SW	1	3	0	0	0	1	0
Coligny	GW	0	0	0	0	0	0	0
	SW	0	0	0	0	1	2	1

GW ground water, *SW* surface water, *N* number of isolates within the cluster

Table 7 Proportion of isolates that were positive for *Enterococcus* virulence genes multiplex PCR analysis

Sampling area (*n*)	No. tested/no. positive	*asa1*	*Esp*	*Gel*	*Hyl*
Mafikeng (*n* = 16)	NP	7 (43.8%)	2 (12.5%)	7 (43.8%)	2 (12.5%)
Rustenburg (*n* = 13)	NP	2 (15.4%)	0 (0%)	4 (30.8%)	0 (0%)
Potchefstroom (*n* = 2)	NP	1 (50%)	0 (0%)	0 (0%)	1 (50%)
Taung (*n* = 2)	NP	1 (50%)	0 (0%)	1 (50%)	0 (0%)
Zeerust (*n* = 7)	NP	0 (0%)	0 (0%)	1 (14.3%)	0 (0%)
Coligny (*n* = 4)	NP	3 (75%)	0 (0%)	3 (75%)	0 (0%)
Total	NT/	44	44	44	44
	NP	14 (31.8%)	2 (4.5%)	16 (36.4%)	3 (6.8%)

n number tested, *NP* number positive

respectively (Table 7). On the contrary, none of the isolates possessed *cylA* gene which is responsible for cytolysin production.

Discussion

Similar observations in which *E. faecium* and *E. faecalis* were the two predominant species isolated from both ground and surface water bodies have been reported (Cattoir and Leclercq 2013; Da Silva et al. 2006; Goldstein et al. 2014). Microbial resistance to glycopeptides has recently increased and VRE traits are frequently detected among *Enterococcus* species despite the fact that this antibiotic is no longer used in human and veterinary medicine (Roberts et al. 2016). Vancomycin resistance determinants have the ability to be transferred among various related and unrelated bacterial strains (Borgen et al. 2001; Cattoir and Leclercq 2013). Mobile genetic elements such as plasmids and transposons have been implicated in the transmission of vancomycin resistance determinants among bacteria strains (Cetinkaya et al. 2000). It is therefore suggested that strains harboring these genes as well as other resistance genes may serve as potential hosts for the dissemination of antibiotic resistance traits within a given area.

The impact and contribution of vancomycin resistance determinants in enhancing severity of enterococcal infections in humans still remain a controversial issue. This is based on the fact that findings from a previous study indicated that bacteremia caused by *Enterococcus* species that possess vancomycin resistance determinants was associated with refractory infection, serious morbidity, and ultimately death in patients (Fowler et al. 2015).

Detection of MAR *Enterococcus* isolates in unprotected water samples reveals that these isolates may not only have severe effects on the treatment of human infections but may also serve as vectors for the transmission of antibiotic resistance determinants to related and unrelated bacterial strains with whom they share a common ecological niche. The linkage on cluster analysis indicated that isolates shared similar antibiotic resistance phenotypes and this link is an indication that isolates from the different areas may have been exposed to the comparable antimicrobial agents.

The great similarities in the antibiotic resistance profiles of VRE isolates from the different locations may have resulted from the indiscriminate and frequent use of these antibiotics in humans and animals. It is therefore suggested that studies designed to determine relatedness of different isolates based on clustering of their antibiogram data may provide an understanding of the evolution of newer antibiotic resistance profiles. Considering these facts, such data may be of great epidemiological significance and therefore be very useful in identifying the source of contamination.

The presence of *vanA* and *vanB* genes in the isolates proves that surface and ground water sources are reservoirs of VRE strains and this is considered as a cause of concern. Dissemination of carriers of both resistance and virulence determinants in environmental water exposes individuals to severe health risks, especially in rural communities and hospital settings.

A wise use of antimicrobial agents is therefore vital in human medicine, animal husbandry as well as in veterinary medicine. Ground and surface water intended for recreational and agricultural use should be frequently tested for the existence of VRE determinants. The enforcement of strategic and effective intervention methods will also improve water quality and human health.

Conclusion

Virulent VRE species were detected and confirmed in ground and surface water samples. The identification of multiple antibiotic-resistant enterococci was a serious cause for concern. Moreover, the detection of both the *vanA* and *vanB* gene determinants indicated that these isolates in rural areas could pose a serious public health challenge especially to the medical and

veterinary professions. Moreover, these may cause waterborne infections to consumers of contaminated water. It is therefore suggested that metagenomics analysis be performed to characterize these species as well as to identify the pattern of resistance genes transmission among these in the environment.

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