European Journal of Medicinal Plants
11(3): 1-10, 2016, Article no.EJMP.18279
ISSN: 2231-0894, NLM ID: 101583475

SCIENCEDOMAIN *international*
www.sciencedomain.org

Susceptibility Pattern of Plasmid-borne Vancomycin-Resistant *Enterococcus faecalis* Strains to Selected Nigerian Medicinal Plants

O. M. David[1*] and O. Famurewa[1]

[1]Department of Microbiology, Ekiti State University, P.M.B. 5363, Ado-Ekiti 360101, Ekiti State, Nigeria.

Authors' contributions

This work was carried out in collaboration between the both authors. Author OF designed the study while author OMD performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/18279
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Daniela Hanganu, Iuliu Hatieganu University of Medicine and Pharmacy, Romania.
(2) Antonello Santini, University of Napoli Federico II, Italy.
Complete Peer review History: http://sciencedomain.org/review-history/12238

Original Research Article

Received 13th April 2015
Accepted 9th August 2015
Published 10th November 2015

ABSTRACT

Objective: To determine whether the presence of the resistance factors (plasmids and *vanA* genes) will negatively aid the susceptibility of vancomycin-resistant *Enterococcus faecalis* (VREf) to medicinal plants.
Materials and Methods: Standard methods were used for the isolation and identification of the *E. faecalis* while antibiotic susceptibility of the isolates was determined by disc diffusion method. Extraction of the plasmid DNA was performed and identification of van genotype *vanA* was carried out by PCR. Anti-enterococcal activities of extracts of ten medicinal screened were determined by agar dilution test.
Results: All the vancomycin-resistant *E. faecalis* strains isolated were resistant to amoxicillin/clavulanic acid, ofloxacin, ciprofloxacin and levofloxaxin. Eight out of the nine VREf starins possessed plasmid with the molecular size ranging from 6557 to 23130 base pairs. Also only four out of the ten test organisms possessed *vanA* gene. All the ten medicinal plants screened had tannins and saponin but lack phlobatanin and cardic glycoside. *Entada africana* followed by

**Corresponding author: E-mail: davidoluwole5@gmail.com;*

Uvaria chamae showed pronounced effect on the isolates while *Sarcocephalus latifolius* had the least effect on the bacteria. Chloroform extracts was the most effective among the three extracts of medicinal plants followed by ethanolic while acetone extracts showed the least effectiveness.
Conclusion: The VREf susceptibility to the medicinal plants seems not to be influenced by the presence of the plasmid and *vanA* gene. The isolation of the anti-enterococcal compounds from the plants and their mode of action are still open to investigation.

Keywords: Vancomycin-resistant; Enterococcus faecalis; medicinal plants; plasmid; phytochemicals; resistance; vanA gene.

1. INTRODUCTION

Medicinal plants as an alternative to conventional medicine is assuming a high dimension and acceptability globally especially in resource-poor nations [1]. Western drugs have not permanently solved the problems of infectious diseases; they leave resistant pathogens as their residues in most cases after their usage. The use of plants for the treatment of infectious diseases is as old as man and has not lost its relevance. Majority of the population of Nigeria, like other developing countries, live in the rural areas. Either out of necessity, poverty and paltry of financial resources they resulted to plant-derived medicine for the treatment of different forms of infections [2-4]. Medicinal plants are cheap, handy and effective in the treatment of infections caused by resistant microorganisms. Traditional and modern medicines, food supplements, pharmaceutical and intermediates for synthetic drugs are all sourced from medicinal plants [5-7]. Extracts of or pure phytochemicals from medicinal plants have been reported to be effective in controlling infectious diseases and/or the growth of antibiotic-resistant pathogenic bacteria [8]. Emergence of drug-resistant microorganisms and the needs to produce more effective antimicrobial agents is of high interest to clinicians and scientists worldwide.

VRE strains are one of the major nosocomial pathogens that have accounted for high morbidity and mortality especially in hospitalized patients and they are resistant to both first line and last resort antibiotics [9]. Vancomycin-resistant enterococci can either be acquired from the hospital or from the community. Vancomycin-resistance enterococci (VRE) have assumed a global emerged pathogen level with increasing widespread and prevalence. Both hospital- and community-acquired enterococcal infections are often lead to treatment failures [10,11].

The recourse to natural products may be the only way out of the prolonged menaces of antibiotic-resistance pathogens, [11-14] hence the purpose of this study. In this study the susceptibility of vancomycin-resistant *Enterococcus feacalis* (VREf) to folkloric medicinal plants used in the south-Western part of Nigeria for the treatment of different infectious diseases was determined. The study also determines whether the presence of the resistance factors (plasmids and *vanA* genes) will negatively affect the susceptibility of VREf to the medicinal plants.

2. MATERIALS AND METHODS

2.1 Isolation and Standardization of Vancomycin Resistant *E. faecalis*

Samples were collected from the wound samples from a tertiary hospital in Ekiti State, Nigeria, using standard procedures. The samples were plated on sterile plates of Bile aesculin agar (Oxoid, Basingstoke, Hampshire, UK) supplemented with 3 µg/ml vancomycin and incubated at 37ºC for 24 h. Colonies with black hallow were subcultured on MacConkey Agar No 2 also supplemented with 3 µg/ml vancomycin and incubated as stated above. The pure isolates were characterized and identified with 24 h old culture using standard methods of Olutiola et al. [15] and Fawole and Oso [16] and the results were interpreted according to Schleifer and Kilpper-Balz [17].

2.2 Antibiotics Susceptibility Testing

Diagnostic Sensitivity Test agar was used for the antibiotic sensitivity testing of the isolates using disc diffusion method as described by CLSI [18]. The isolates were grown at 37ºC in Mueller-Hilton broth (Oxoid) for 16-18 h and diluted to an optical density of 0.1 (0.5 McFarland Standard) at a wavelength of 625 nm and stored at 4ºC. The disc diffusion method was used for susceptibility testing as described by CLSI [18]. The isolates were tested against the following commercially prepared antibiotic (Oxoid Limited)

with the following concentrations (in µg): Gentamicin (10), ofloxacin (30), amoxicillin-clavulanic acid (30) ciprofloxacin (10), levofloxacin (5), and vancomycin (5). The discs were gently but firmly placed on the inoculated plates using sterile forceps. The plates were inverted and incubated at 37ºC for 18 hours after which the plates were examined. The zones of inhibition were interpreted according to CLSI [18].

2.3 Plasmid Analysis

The plasmid analysis of the test isolates was carried out using the method described by Kraft et al. [19]. The extracted plasmids were then separated using a horizontal 1% agarose gel electrophoresis and visualized under ultra violet light using ultra-violet trans-illuminator.

2.4 DNA Preparation and PCR

The *vanA* gene was detected in the vancomycin isolates using *vanA* forward and reveres primer: 5'GGG AAA ACG ACA ATT GC -3' and 5'-GTA CAA TGC GGC CGT TA-3' according to Kariyama et al. [20]. The *vanA* genotype of each of the isolates was identified by the PCR as describe by Olsvik and Strockbin [21]. A 25 µl of PCR amplification mixture contained deionized sterile water, 12.5 µl Green Go *Taq* Master Mix pH 8 (Promega, USA) contained [(50 unit/ml) of Go *Taq* DNA polymerase, (400 Mm) of each dNTPs and (3 mM) of $MgCl_2$], 1pmolfor specific primers (Alpha DNA,Canada). The PCR cycles for *van* genes *(vanA* and *vanB*) were as follow initial denaturation at 95ºC for 5 min, 30 cycles of denaturation at 94ºC for 45 sec, annealing at 54ºC for 45 sec and extension at 72ºC and final extension at 72ºC for 7 min using Gradient PCR (TechNet–500, USA).

2.5 Source and Preparation of Plant Materials

Fresh leaves of *Alchornea cordifolia* Schum, *Alchornea laxiflora* Benth, *Anthocleista vogelii* Planch, *Entada africana* Guill and Perr, *Jateorhiza micrantha*, .Hook F., *Sarcocephalus latifolius* Sm, *Smilax anceps* Wild Thonn, *Albizia coriaria* Welw, *Uvaria chamae* P. Beauv and *Vernonia amygdalina* Del. were collected in various abandon farmlands in Ekiti State, Nigeria and were authenticated at the Herbarium of the Department of Plant Science, Ekiti State University. The fresh plant sample was air-dried and ground to fine powder. A 50 g of ground plant sample was soaked in 500 ml of each of the extractants for 48 h. The sample was then suction-filtered through Whatman Number 1 filter paper and washed with another 200 ml solvent. The filterate was concentrated with Laborata 4000-Efficient (Heldoph, Germany). The dried extract was dissolved in DMSO to make the required concentrations. The reconstituted extracts were filter through 0.45 µm pore (Millipore) size membrane filter for sterility.

2.6 Qualitative Determination of the Phytochemicals

Phlobatannis and flavonoid were detected in the plants samples according the method of Odebiyi and Sofowora [22] while saponin and terpenoinds were detected according to Herborne [23]. The persistent frothing test of Odebiyi and Sofowora [22] was used to detect the presence of the. The methods of Sofowora [24] and Adetuyi et al. [25] were used to determine the presence of flavonoids and cardiac glycosides respectively in the plant samples.

2.7 Determination of Minimum Inhibitory Concentrations (MICs)

Macrobroth dilution method was used for the determination of minimal inhibitory concentration (MIC) of the extract as described by CLSI [18]. Mueller-Hinton broth was used to prepare different concentrations ranging from 0.0977 to 25 mg/ml by serial dilutions. Each prepared concentration in tubes was inoculated with 100 µl of each of the standardized culture of the test bacteria. Tube containing Mueller-Hinton broth without extract was used as negative control. The tubes were incubated aerobically at 37ºC for 18 h. The first tube in the series with no sign of visible growth was taken as the MIC. The *vanA*-negative control, *E. faecalis* ATCC 29212 was used as control.

3. RESULTS

The phenotype of the selected nine *E. faecalis* strains used in this study showed all the isolates to be resistant to amoxicillin/clavulanic acid and vancomycin (Table 1). Five out of the isolates were resistant to gentamicin, while all the isolates were susceptible to the remaining five antibiotics. The test organisms were resistant to ofloxacin, ciprofloxacin and levofloxacin. Only *E. faecalis* CIW 44 had intermediate susceptibility to

gentamicin out of the nine test organisms. All isolates except *E. faecalis* CIW 44 had plasmids with the molecular size ranged from 23130 to 6557 base pairs. As shown in Fig. 1, seven out of the plasmid-borne isolates have plasmids with 23130 base pairs. Though all the isolates were resistant to vancomycin, only *E. faecalis* CIW 02, *E. faecalis* CIW 17, *E. faecalis* CIW 22 and *E. faecalis* CIW 26 possessed *vanA* gene among the nine test strains (Fig. 2).

The phytochemical constituents of the medicinal plants screened against VREF showed that all the plants had tannins and saponin while they all lack phlobatanin and cardic glycoside. Alkaloids were not detected in *J. micrantha* and *Ant. vogelii.* Out of the ten plants only *S. anceps*, *U. chamae* and *V. amygdalina* had steroid and terpenoid. Flavonoid was detected in six out of the ten medicinal plants screened (Table 2).

Table 1. The phenotypes of selected VREF strains

E. faecalis	**Antibiotics**					
CIW 02	AMOX/CLAV^{+}	Gen^{+}	Clox^{+}	Ofl^{+}	Cip^{+}	Lev^{+}
CIW 17	AMOX/CLAV^{+}	Gen^{-}	Clox^{+}	Ofl^{+}	Cip^{+}	Lev^{+}
CIW 22	AMOX/CLAV^{+}	Gen^{+}	Clox^{+}	Ofl^{+}	Cip^{+}	Lev^{+}
CIW 26	AMOX/CLAV^{+}	Gen^{-}	Clox^{+}	Ofl^{+}	Cip^{+}	Lev^{+}
CIW 29	AMOX/CLAV^{+}	Gen^{+}	Clox^{+}	Ofl^{+}	Cip^{+}	Lev^{+}
CIW 38	AMOX/CLAV^{+}	Gen^{-}	Clox^{+}	Ofl^{+}	Cip^{+}	Lev^{+}
CIW 40	AMOX/CLAV^{+}	Gen^{+}	Clox^{+}	Ofl^{+}	Cip^{+}	Lev^{+}
CIW 44	AMOX/CLAV^{+}	Gen$^{\pm}$	Clox^{+}	Ofl^{+}	Cip^{+}	Lev^{+}
CIW 49	AMOX/CLAV^{+}	Gen^{+}	Clox^{+}	Ofl^{+}	Cip^{+}	Lev^{+}

$^{+}$= Resistant, $^{-}$ = susceptible, $^{\pm}$ = Intermediate

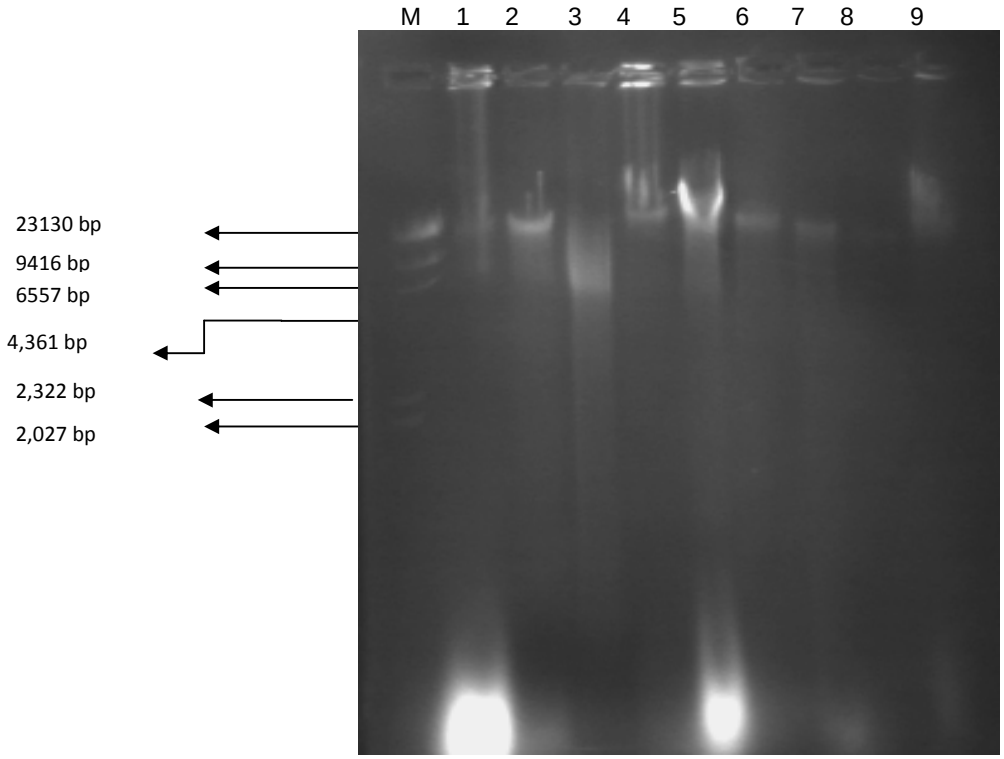

Fig. 1. Gel electrophoresis results of *E. faecalis*

M = HIND III digest of λ-DNA (DNA molecular weight marker). Lane 1 = E. faecalis CIW 02, Lane 2 = E. faecalis CIW 17, Lane 3 = E. faecalis CIW22, Lane 4 = E. faecalis CIW 26, Lane 5 = E. faecalis CIW 29, Lane 6 = E. faecalis CIW 38, Lane 7 = E. faecalis CIW 40, Lane 8 = E. faecalis CIW 44 and Lane 9 = E. faecalis CIW 49

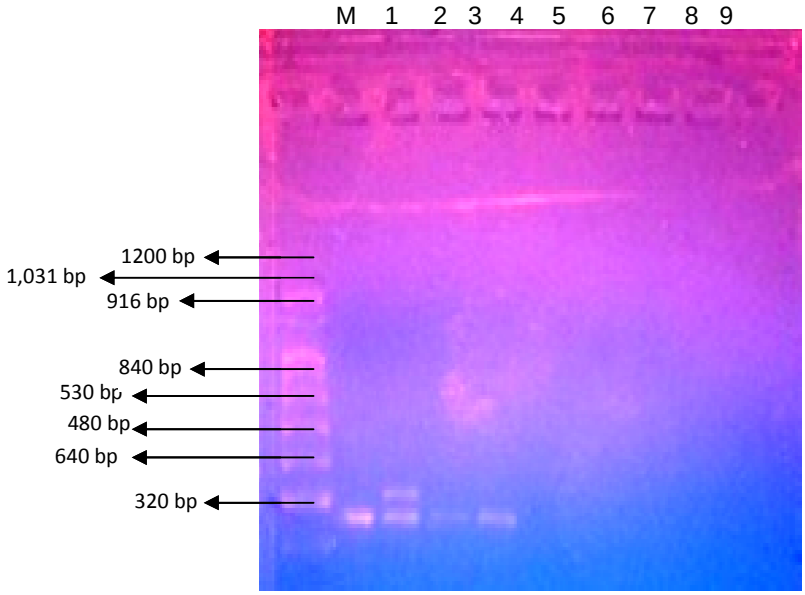

Fig. 2. Agarose gel showing PCR pattern of *vanA* gene from vancomycin-resistant *E. faecalis*

Lane 1 = E. faecalis CIW 02 (showing vanA band), Lane 2 = E. faecalis CIW 17 (showing vanA band), Lane 3 = E. faecalis CIW22 (showing vanA band), Lane 4 = E. faecalis CIW 26 (showing vanA band), Lane 5 = E. faecalis CIW 29, Lane 6 = E. faecalis CIW 38, Lane 7 = E. faecalis CIW 40, Lane 8 = E. faecalis CIW 44 and Lane 9 = E. faecalis CIW 49

Table 2. The phytochemical constituents of medicinal plants screened against VREF

Plant species	Phytochemical constituents							
	Alk	Tan	Sap	Ste	Phl	Ter	Fla	Car
U. chamae	+	+	+	+	-	+	+	-
S. anceps	+	+	+	+	-	+	+	-
J. micrantha	-	+	+	-	-	-	+	-
V. amygdalina	+	+	+	+	-	+	+	-
Alc laxiflora	+	+	+	-	-	-	+	-
Ant. vogelii	-	+	+	-	-	-	-	-
E. africana	+	+	+	-	-	-	-	-
Sar. latifolius	+	+	+	-	-	-	+	-
Alc. cordifolia	+	+	+	-	-	-	-	-
Alb. coriaria	+	+	+	-	-	-	-	-

Alk=Alkaloids, Tan=Tannins, Sap=Saponin, Ste=Steroid, Phl=Phlobatanin, Ter=Terpenoid, Fla=Flavonoi, Car= Cardic glycoside, += Present, -= Absence

Enterococcus faecalis ATCC 29212 was the most susceptible among the test organisms to ethanolic extract of the medicinal plants followed by *E. feacalis* CIW 26 while *E. faecalis* CIW 40 was the most resistant among the test organisms. *Entada africana* followed by *Uvaria chamae* showed pronounced antibacterial effect on the isolates. Out of the ten plants screened *S. latifolius* had the least effect on the organisms with its MIC greater than 5.00 mg/ml when tested against *E. feacalis* CIW 02, *E. feacalis* CIW 17, *E. feacalis* CIW 29, *E. feacalis* CIW 40 and *E. feacalis* CIW 44. The MIC of the ethanolic extract of the plant against *E. feacalis* ATCC 29212 was higher (2.50 mg/ml) than *E. feacalis* CIW 49 with 1.25 mg/ml.

The general overview of the susceptibility of the test organisms to the extracts to the *E. feacalis* ATCC 29212 was most susceptible among the organisms this is closely followed by *E. feacalis* CIW 49. Chloroform extracts was the most effective among the three medicinal plants followed by ethanol while acetone extract showed the least effectiveness on the isolates. The susceptibility order of the isolates to acetone extract was observed as followed: *E. feacalis* CIW 44 > *E. feacalis* CIW 02 > *E. feacalis* CIW

38 > *E*. *feacalis* CIW22 > *E*. *feacalis* CIW 17 > *E*. *feacalis* CIW 40, while the least susceptibility was observed in *E*. *feacalis* CIW 49 followed by *E*. *feacalis* ATCC 29212 (Tables 3, 4 and 5). Resistance to the medicinal plants seems not to be mediated by the presence of the plasmid. There is no clear evidence that the possession of *vanA* coded for the resistance to the medicinal plants.

Relatively, *E. feacalis* CIW 44 exhibited the least susceptibility to all the extracts of ten medicinal plants tested. *Enterococcus feacalis* CIW 49 with plasmid showed the least resistance to the extracts.

4. DISCUSSION

Enterococcus faecalis is one of the leading antibiotic-resistant bacterial pathogens causing both nosocomial- and community-acquired infections. It is resistant to an array of antibiotics including the antibiotic of last resort [26]. Borhani et al. [27] reported that 100% and 98% vancomycin resistant enterococci isolated from Tehran were resistant to ciprofloxacin and gentamicin, respectively. In this study also 100% of the isolates were resistant to ciprofloxacin while only 55.56% were resistant to gentamicin. The use of broad-spectrum antibiotics especially third-generation cephalosporins have been reported to encourage intestinal overgrowth of enterococci. The spread of the vancomycin resistance genes especially *vanA* among enterococci and other organisms has been identified in various bacterial species like *S. aureus.*

All isolates except *E. faecalis* CIW 44 had plasmid with the molecular size range from 6557 to 23130 base pairs. Seven out of the plasmid-borne isolates have plasmids with 23130 base pairs. Though all the isolates were resistant to

Table 3. Minimum inhibitory concentrations (mg/ml) of chloroform extracts of ten medicinal plants screened against vancomycin resistant *E. faecalis*

Medicinal plants	*Enterococcus faecalis*									
	CIW 02	**CIW 17**	**CIW 22**	**CIW 26**	**CIW 29**	**CIW 38**	**CIW 40**	**CIW 44**	**CIW 49**	**ATCC 29212**
U. chamae	>5.00	>5.00	>5.00	1.25	>5.00	0.625	2.50	>5.00	>5.00	<0.156
S. anceps	>5.00	2.50	>5.00	1.25	2.50	5.00	1.25	>5.00	>5.00	0.625
J. micrantha	5.00	>5.00	5.00	0.625	2.50	0.625	2.50	2.50	>5.00	0.156
V. amygdalina	0.625	0.625	>5.00	0.156	0.625	2.50	2.50	5.00	0.625	0.156
Alc. laxiflora	1.25	0.625	>5.00	1.25	2.50	0.625	2.50	2.50	0.625	<0.156
Ant. vogelii	>5.00	>5.00	>5.00	5.00	2.50	<0.156	>5.00	2.50	>5.00	0.625
E. africana	1.25	0.312	1.25	2.50	<0.156	<0.156	2.50	>5.00	0.156	<0.156
Sar. latifolius	0.625	1.25	0.156	5.00	2.50	1.25	>5.00	>5.00	0.625	<0.156
Alc. cordifolia	2.50	0.312	0.312	<0.156	<0.156	0.625	2.50	5.00	<0.156	2.50
Alb. coriaria	<0.156	<0.156	<0.156	2.50	>5.00	>5.00	0.625	2.50	2.50	0.312

Table 4. Minimum inhibitory concentrations (mg/ml) of acetone extracts of ten medicinal plants screened against vancomycin resistant *E. faecalis*

Medicinal plants	*Enterococcus faecalis*									
	CIW 02	**CIW 17**	**CIW 22**	**CIW 26**	**CIW 29**	**CIW 38**	**CIW 40**	**CIW 44**	**CIW 49**	**ATCC 29212**
U. chamae	2.50	2.50	1.25	1.25	0.625	>5.00	0.312	>5.00	1.25	<3.175
S. anceps	>5.00	5.00	>5.00	2.50	5.00	5.00	>5.00	2.50	1.25	0.312
J. micrantha	>5.00	>5.00	>5.00	0.625	2.50	>5.00	5.00	>5.00	5.00	0.625
V. amygdalina	2.50	0.625	0.625	0.625	2.50	5.00	>5.00	>5.00	0.625	<3.175
Alc. laxiflora	>5.00	>5.00	>5.00	5.00	<3.175	3.175	>5.00	>5.00	5.00	1.25
Ant. vogelii	0.625	3.175	0.625	<3.175	3.175	<3.175	2.50	2.50	1.25	3.175
E. africana	>5.00	0.312	>5.00	>5.00	>5.00	5.00	5.00	>5.00	2.50	2.50
Sar. latifolius	>5.00	>5.00	>5.00	0.312	3.175	0.625	2.50	5.00	0.312	<3.175
Alc. cordifolia	3.175	<3.175	<3.175	2.50	0.625	<3.175	0.625	1.25	0.312	<3.175
Alb. coriaria	3.175	<3.175	2.50	2.50	1.25	3.175	0.625	2.50	2.50	1.25

Table 5. Minimum inhibitory concentrations (mg/ml) of ethanol extracts of ten medicinal plants screened against vancomycin resistant *E. faecalis*

Medicinal plants	*Enterococcus faecalis*									
	CIW 02	CIW 17	CIW22	CIW 26	CIW 29	CIW 38	CIW 40	CIW 44	CIW 49	ATCC 29212
U. chamae	1.25	<3.175	0.625	0.625	0.625	1.25	2.50	>5.00	>5.00	0.625
S. anceps	>5.00	>5.00	2.50	>5.00	2.50	>5.00	2.50	>5.00	2.50	0.312
J. micrantha	2.50	2.50	2.50	0.312	2.50	>5.00	5.00	>5.00	2.50	0.312
V. amygdalina	0.312	3.175	3.175	1.25	0.312	5.00	1.25	1.25	3.175	<3.175
Alc. laxiflora	<3.175	<3.175	<3.175	5.00	2.50	1.25	2.50	2.50	<3.175	<3.175
Ant. vogelii	2.50	<3.175	<3.175	0.625	2.50	<3.175	>5.00	>5.00	0.625	0.312
E. africana	0.312	0.625	2.50	2.50	0.312	0.312	1.25	1.25	0.312	<3.175
Sar. latifolius	>5.00	>5.00	5.00	5.00	>5.00	1.25	>5.00	>5.00	1.25	2.50
Alc. cordifolia	>5.00	0.312	>5.00	0.625	2.50	0.625	>5.00	>5.00	3.175	0.625
Alb. coriaria	<3.175	<3.175	0.625	2.50	>5.00	0.625	0.625	1.25	<3.175	<3.175

vancomycin, only four of the isolates possessed *vanA* gene among the nine test strains. In this study we observed that only 44.44% had *vanA* gene among the VREf strains. This report was lower than the 100% recorded by Borhani et al. [27] among the VRE isolates they screened. Vancomycin-resistant enterococci can transfer genetic (resistant) factor to closely and distantly related bacteria. This phenomenon has been responsible for the development of vancomycin resistance in *S. aureus* and *S. epidermidis* [28,29].

Detection of *vanA* determinant in four out of the VRE, demonstrates that the *vanA* gene cluster located on mobile elements is able to disseminate between different species. The *vanA* gene can be carried in by different species of *Enterococcus;* the most common carriers being *E. faecalis, E. faecium* and *E. durans.* Other types of vancomycin resistance genes may be present in them. The *vanA* determinant was not detected in five out of the strains in this study. Apart from *vanA, vanB* and *vanD.* The *vanE* and *vanG* genes have both been reported in *E. faecalis* [30].

Vancomycin-resistant enterococci have been reported to be associated with infections with treatment failures [10,31,32]. However, natural plant products have showed pronounced activity against them [14]. The phytochemical constituents of the medicinal plants screened against VREf in this study had tannins and saponin while they all lack phlobatanin and cardiac glycoside. Alkaloids were not detected in *J. micrantha* and *Ant. vogelii.* Phytochemical constituents of the plants are responsible for their anti-enterococcal activity either singly or in combination. The quality and quantity of biologically active compounds in plant extracts are largely dependent on the type of solvent used in the extraction process [33-35].

Phytochemical analyses revealed the presence of various metabolites. These metabolites have been reported to contribute to the anti-enterococcal action. The phytochemicals in *Withania somnifera* were reported to aid its effects on the VRE [36]. The most of the MICs of the extracts of the plants screened for anti-enterococci were within the effective range of 100–1000 µg/mL reported by Ahmed et al. [37]. Plant phytochemicals are often show considerable activity against Gram-positive bacteria compared to Gram-negative bacteria and yeast. This is due to the inability of their outer membrane serving as an ineffective barrier for amphipathic compounds [37]. Their single membrane is more easily permeated by the amphipathic phytochemicals [37]. Chloroform extracts was the most effective among the three medicinal plants followed by ethanol while acetone extract showed the least effectiveness on the isolates. This finding agrees with the report of Parekh et al. [38] and Masoko and Eloff [39] that reported that most bioactive phytochemicals are not water soluble and biologically active. The susceptibility order of the isolates to acetone extract was observed as followed: *E*. *feacalis* CIW 44 > *E*. *feacalis* CIW 02 > *E*. *feacalis* CIW 38 > *E*. *feacalis* CIW22 > *E*. *feacalis* CIW 17 > *E*. *feacalis* CIW 40, while the least susceptibility was observed in *E*. *feacalis* CIW 49 followed by *E*. *feacalis* ATCC 29212. Vancomycin resistant Gram-positive bacteria have been reported to have thicker cell wall than the vancomycin susceptible strains [40,41]. Cui et al. [40] reported the thick cell wall to significantly reduce the penetration of antimicrobials through the cell wall. This may account for the high susceptibility of the

vancomycin-susceptible (control strain) *E. faecalis* ATCC 29212.

5. CONCLUSION

The presence of the plasmids and *vanA* gene in the VREf strains seems not to affect the susceptibility of the isolates to the extracts of the medicinal plants. Isolation and characterization of bioactive phyto-compounds with proven anti-enterococcal properties have to be studied. Toxicity and mechanisms of action of the screened medicinal plants is still open to investigation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization (WHO) WHO traditional medicine strategy 2002-2005. World Health Report, World Health Organization, Geneva; 2002.
2. Zhang X. Traditional medicine: Its importance and protection. In: Twarog S, Kapoor P, (Eds). Protecting and promoting traditional knowledge: Systems, national experiences and international dimensions. Part 1. The role of traditional knowledge in healthcare and agriculture. New York: United Nations. 2004;3–6.
3. World Health Organization (WHO). Traditional medicine strategy 2002–2005. Geneva: World Health Organization; 2005.
4. Ramzi AA, Mothana SA, Abdo A, Hasson MN, Althawab SAZ, Ulrike L. Antimicrobial, antioxidant and cytotoxic activities and phytochemical screening of some Yemeni medicinal plants. eCAM Adv Access Published. 2008;1:1–8.
5. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Applied Microbiol. 1999;86(6):985.
6. Baris O, Gulluce M, Sahin F, Ozer H, Kilic H, Ozkan H, Sokmen M, Ozbek T. Biological activities of the essential oil and methanol extract of *Achillea Biebersteinii* Afan. (*Asteraceae*). Turk J Biol. 2006;30: 65-73.
7. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J Biotech. 2008;7(12):1797-1806.
8. Tanaka JCA, Da Silva CC, De Oliveira AJB, Nakamura CV, Dias Filho BP. Antibacterial activity of indole alkaloids from *Aspidosperma ramiflorum*. Braz J Med Biol Res. 2006;39(3):387-391.
9. Austin DJ, Bonten MJ, Weinstein RA, Slaughter S, Anderson RM. Vancomycin-resistant Enterococci in intensive-care hospital settings: Transmission dynamics, persistence, and the impact of infection control programs. Proc Natl Acad Sci. 1999;96:6908-13.
10. Menichetti F. Current and emerging serious Gram-positive infections. Clin Microbiol Infect. 2005;11(Suppl. 3):22–28.
11. Rivera AM, Boucher HW. Current concepts in antimicrobial therapy against select Gram-positive organisms: Methicillin-resistant *Staphylococcus aureus* penicillin-resistant pneumococci, and vancomycin-resistant enterococci. Mayo Clin Proc. 2011:86;1230–1243.
12. Chusri S, Voravuthikunchai SP. Damage of staphylococcal cytoplasmic membrane by *Quercus infectoria* G. Olivier and its components. Lett Appl Microbiol. 2011;52: 565–572.
13. Mishra BB, Tiwari VK. Natural products: An evolving role in future drug discovery. Eur J Med Chem. 2011;46:4769-4807.
14. Leejae S, Taylor PW, Voravuthikunchai, SP. Antibacterial mechanisms of rhodomyrtone against important hospital-acquired antibiotic-resistant pathogenic bacteria J Med Microbiol. 2013:62;78–85.
15. Olutiola PO, Famurewa O, Sonntag HG. An Introduction to General Microbiology. Hygiene-Institut der Universität Heidelberg, Federal Republic of Germany. 2000;267.
16. Fawole MO, Oso BA. Laboratory Manual of Microbiology. Spectrum Books Limited, Ibadan. 2001;127.
17. Schleifer KH, Kilpper-Balz R. Transfer of *Streptoccus faecalis* and *Streptococcus faecium* to the genus *Enterococcus* nom. rev. as *Enterococcus faecalis* comb. nov.;

and *Enterococcus faecium* comb. Nov. Int J Syst Bacteriol. 1984;34:31-4.

18. CLSI. Performance standards for antimicrobial susceptibility testing.15th informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2012;M100-S15.
19. Kraft R, Tardiff J, Krauter KS, Leinwand LA. Using mini-prep plasmid DNA for sequencing double stranded template with sequences. Biotechniques. 1988;6:544-546.
20. Kariyama R, Mitsuhata R, Chow JW, Clewell DB, Kumon H. Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant enterococci. J Clin Microbiol. 2000;38(8):3092–3095.
21. Olsvik O, Strockbin NA. PCR detection of heat-stable, heat-label and shiga-like toxin genes in *Escherichia coli*. In. Persing DH, Smith TF, Tenover FC, White TJ. Diagnostic Molecular Microbilogy. 9th ed. American Society for Microbiology. Washington, DC; 1993.
22. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medical plants II Lloydia. 1978;41:2234–246.
23. Harborne JB. Phytochemical methods, London, Chapman and Hall Ltd. 1973; 49-188.
24. Sofowara A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria. 1993;289.
25. Adetuyi AO, Oyetayo VO, Popoola AV, Lajide L. Phytochemical and antibacterial screening of ethanol extracts of six dye plants. Biosci Biotechnol Res Asia. 2004; 2(1):41-44.
26. McNeil SA, Clark NM, Chandrasekar PH, Kauffman CA. Successful treatment of vancomycin–resistant *Enterococcus faecium* bacteremia with linezolid after failure of treatment with synercid (quinupristin/dalfopristin). Clin Infect Dis. 2000;30:403–404.
27. Borhani K, Ahmadi A, Rahimi F, Pourshafie MR, Talebi M. Determination of vancomycin resistant *Enterococcus faecium* diversity in tehran sewage using plasmid profile, biochemical fingerprinting and antibiotic resistance. Jundishapur J Microbiol. 2014;7(2):1-5.
28. Garrett DO, Jochimsen E, Murfitt K, Hill B, McAllister S, Nelson P. The emergence of decreased susceptibility to vancomycin in *Staphylococcus epidermidis*. Infect Control Hosp Epidemiol. 1999;20(3):167-170.
29. Juarez-Verdayes MA, Reyes-Lopez MA, Cancino-Diaz ME, Munoz-Salas S, Rodriguez-Martinez S, De la Serna FJ. Isolation, vancomycin resistance and biofilm production of *Staphylococcus epidermidis* from patients with conjunctivitis, corneal ulcers, and endophthalmitis. Rev Latinoam Microbiol. 2006;48(3-4):238-246.
30. Fines M, Perichon B, Reynolds P, Sahm DF, Courvalin P. *VanE*, a new type of acquired glycopeptide resistance in *Enterococcus faecalis* BM4405. Antimicrob Agents Chemother. 1999;43:2161-2164.
31. Cunha BA. Methicillin-resistant *Staphylococcus aureus*: Clinical manifestations and antimicrobial therapy. Clin Microbiol Infect. 2005;11(Suppl. 4):33–42.
32. Moore CL, Lu M, Cheema F, Osaki-Kiyan P, Perri MB, Donabedian S, Haque NZ, Zervos MJ. Prediction of failure in vancomycin-treated methicillin-resistant *Staphylococcus aureus* bloodstream infection: A clinically useful risk stratification tool. Antimicrob Agents Chemother. 2011;55:4581–4588.
33. Tada M, Ishimaru K. Efficient ortho-oxidation of phenol and synthesis of anti-MRSA and anti-VRE compound abietaquinone methide from dehydroabietic acid. Chem Pharm Bull. 2006;54: 1412-1417.
34. Selim SAH, El-Alfy SM, Aziz MHA, Mashait M, Warrad MF. Antibacterial activity of selected Egyptian ethnomedicinal plants. Mal J Microbiol. 2013;9(1):111-115.
35. Rijo P, Duarte A, Francisco AP, Semedo-Lemsaddek T, Simoes MF. *In vitro* antimicrobial activity of royleanone derivatives against gram-positive bacterial pathogens. Phytother Res. 2014;28(1): 76-81.
36. Chandoria RK, Chaudhary HS, Yadav A, Gupta A, Yadav JP, Shrivastava AR. Effect of *Withania somnifera* leaf extracts as antibacterial agent against multidrug resistant bacteria. Inter J Phytomed. 2012; 4:237-242.
37. Ahmed I, Aqil F, Owais M. Modern phytomedicine - Turning medicinal plants to drug. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim; 2006.
38. Parekh J, Karathia N, Chanda S. Screening of some traditionally used

medicinal plants for potential antibacterial activity. Ind J Pharmcol Sci. 2006;68(6): 832- 834.

39. Masoko P, Eloff JN. Bioautography indicates the multiplicity of antifungal compounds from twenty-four Southern African *Combretum* species (Combretacea). Afr J Biotechnol. 2006; 5(18):1625-1647.
40. Cui L, Ma X, Sato K, Okuma K, Tenover FC, Mamizuka EM, Gemmell CG, Kim M N, Ploy MC. Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. J Clin Microbiol. 2003;41:5–14.
41. Howden BP, Johnson PDR, Ward PB, Stinear TP, Davies JK. Isolates with low-level vancomycin resistance associated with persistent methicillin-resistant *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother 2006;50: 3039–3047.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12238