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Evaluation of the Anti-enterococcal Activity of Disinfectants and Medicated Soaps on Vancomycin-resistant *Enterococcus faecalis* Strains

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Authors' contributions

This work was jointly designed and carried out by the authors.

Original Research Article

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ABSTRACT

Background: *Enterococcus faecalis* has intrinsic resistance which aids its spread in the hospital environment. As a nosocomial pathogen with increasing resistance breaking its route of transmission and spread is therefore imperative.
Aims: In this study, six brands of disinfectants and eight medicated soaps commonly used in health care facilities and at homes were investigated for anti-enterococcal activity against eleven strains of *E. faecalis* ten of which are vancomycin-resistant.
Place and Duration of Study: This study was carried out in the Department of Microbiology, Ekiti State University, Nigeria between July, 2009 and February, 2010.
Methodology: Standard microbiological methods were used to determine the effects of the disinfectant and soap samples on the strains of vancomycin-resistant *E. faecalis.*
Results: Two of the disinfectants, NXD and ZGC completely inhibited the test organisms even at the manufacturers' recommended in-use concentrations. ZAL followed by VGL had the least anti-enterococcal property. *Enterococcus faecalis* DMOF 53 and *E. faecalis* DMOF 47 were the most resistant while *E. faecalis* DMOF 21 and *E. faecalis* ATCC 29212 (control) were the least resistant to the disinfectants. Vancomycin-sensitive strain, *E. faecalis* ATCC 29212 (control) was also resistant to some of the disinfectants. This shows

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that there is no correlation between resistance to antibiotics, vancomycin in particular and disinfectants. The disinfectants CRT, RBT, TMS and DTA, in that decreasing order, showed anti-enterococcal property while MSF and NVA showed the least effect on the enterococci. Strains recovered from the surface of the bland soap sample had confluent growth pattern which indicated the ability of the organisms to survive on its surface. Survival was least on CRT soap sample followed by DTL soap sample and most of the strains grew very well on the surfaces of most of the soap samples.

Conclusion: This study shows that most of the disinfectants are not effective at the manufacturers' recommended in-use concentrations, and also that pathogens can be transmitted through the common use or sharing of soaps contaminated with the carriers of antimicrobial-resistant pathogens.

Keywords: Enterococcus faecalis; biocides; disinfectants; medicated soap; infections; vancomycin resistance.

1. INTRODUCTION

Enterococcus species are ubiquitous commensal inhabitants of the gastrointestinal tract of humans, animals and other invertebrates. They are frequently isolated from environmental sources such as soil, surface waters, and raw plant and animal products, where their intrinsic resistance enables them to survive and spread in the environment [1,2]. These organisms acquire antibiotic resistance and spread resistance genes to other species [3]. Multiple antibiotic-resistant enterococci (MRE) have emerged as a global threat to public health which threatens to compromise effective antibiotic treatment [4]. The application of biocides to heavily contaminated environments reduces health care-associated transmission of contagious diseases [5,6]. Health care facilities can be contaminated by nosocomial pathogens in hospitals and during medical practices [7,8].

Biocides are used extensively in hospitals and other health care settings for a variety of purposes. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections [8,9]. A wide variety of active chemical agents or biocides have been used both in antisepsis and disinfection for the prevention of both endemic and epidemic infections and/or diseases [10,11]. Biocides have a broader spectrum of activity than antibiotics, yet less emphasis is laid on biocides compared to antibiotics, as biocides have multiple targets [12]. The widespread use of antiseptic and disinfectant products has led to microbial resistance and cross-resistance to antibiotics in particular.

Most biocides are used singly or in combination and they contain a variety of ingredients which acts on different parts of the target bacteria [13,14,15]. As with antibiotics and other chemotherapeutic agents, acquired resistance to antiseptics and disinfectants can arise either through mutation or the acquisition of genetic material in the form of plasmids or transposons. The role of plasmids in encoding resistance to biocides has been reported [16]. The resistance pattern of vancomycin-resistant *E. faecalis* to some common antimicrobials (biocides) was therefore investigated in this study.

2. MATERIALS AND METHODS

2.1 Source of Disinfectants

Six brands of common disinfectants used in both household and hospitals were purchased from supermarkets in Ado-Ekiti and were coded as follows: DTL, ZAL, NXD, SVN, VGL and ZGC. Seven different germicidal soaps against *E. faecalis* which included CRT, DTA, DTL, MSF, NVA, RBT and TMS were also examined. The active ingredients of the disinfectants and soap used are shown in Tables 1 and 2 respectively.

Table 1. A summary of the various types of disinfectants, their active ingredients, concentration and recommended concentrations

Disinfectant	Active ingredients	Concentrations (%w/v)	Recommended in-use Concentrations (% v/v)
ZGC	Dichlorometaxylenol	2.5	0.20
	Terpineol	10	
DTL	Chloroxylenol	4.8	5.00
	Isopropyl Alcohol	9.43	
	Oleum pini-aromaticum	8.38	
SVN	Chlorhexidine	0.3	6.00
	Cetrimide	3.0	
ZAL	Phenol	27.0	0.50
NXD	Dichlorometaxylenol	2.5	2.0
	Terpineol	10	
VGL	Chlorhexidine	0.3	2.0
	Cetrimide	3.0	

Table 2. Summary of the various test soaps, their active ingredients and concentrations

Soap	Active ingredients	Concentration (%w/w)
MSF	Trichlorocarbanilide	0.1
RBT	Mercuric oxide	1.0
TMS	Monosulfram	5.0
CRT	Trichlorocabanilide,	0.1
	Triclosan	0.1
NVA	Trichlorocabanilide	1.0
DTA	Trichlorocabanilide	0.5
DTL	Chloroxylenol	0.5

2.2 Determination of Susceptibility of *E. faecalis* to Disinfectants

Five dilution levels of each of the disinfectants were prepared with manufacturers' recommended in-use dilutions as median. Two loopfuls of standardized broth of an 18 hour culture (in Müller-Hinton broth, Oxoid, Basingstoke, Hampshire, UK) with approximately 5.0 x 10^7 cfu/ml were introduced into 2 ml of each of the dilution of the disinfectant. After contact for 10 minutes, 5% Tween 80 prepared in deionized water was added to neutralize the action of the disinfectant. The organism was streaked on freshly prepared Müller-Hinton Agar and incubated at 37ºC for 18 h. Both negative and positive control organisms were also similarly treated, plated and incubated as stated above. Development of colonies was taken as positive while plates without sign of growth were taken as negative.

2.3 Determination of Susceptibility of *E. faecalis* to Antiseptic Soaps

A 0.1 ml amount of standardized inoculum of the test organisms was introduced into 1 ml of 1.0% w/v of the soap solution and at time intervals, added to molten Müller-Hinton agar, plated and allowed to solidify. The plates were subsequently incubated at 37ºC for 24 h and evaluated thereafter according to the CLSI guidelines [17].

2.4 Determination of Survival of the Test Organisms on the Surface of the Soap

The ability of the isolates to survive on the surface of the soap samples was determined by inoculating standardized inoculum on the sterile surface (9.0 cm^2) of the soap samples, wrapped up in sterile aluminum foil. and incubated at 37ºC for 72 h. The inoculated surface of the soap was swabbed, plated on Bile aesculin agar (Oxoid), incubated at 37ºC for 24h and subsequently evaluated.

3. RESULTS AND DISCUSSION

Six brands of disinfectants commonly used in the health care facility and in homes were tested against strains of *E. faecalis*. Eleven strains of *E. faecalis* were used in this study (Table 3) out of which ten were vancomycin-resistant while vancomycin-sensitive *E. faecalis* ATCC 29212 served as control. The results of the present study indicate that the test organisms showed diverse resistance to the test agents; not all the disinfectants inhibited the growth of the test organisms. Dichlorometaxylenol and terpineol were present in two of the disinfectants, viz. ZGC and NXD which completely inhibited the test organisms at the manufacturers' recommended in-use concentrations. ZAL followed by VGL had the least anti-enterococcal effects. The finding in this study corroborates the report of Karatzas et al. [18] which showed that *E. faecalis* can adapt and develop resistance to extensively used antiseptics and disinfectants. Many reports of resistance often have parallel issues including inadequate cleaning, incorrect product use, or ineffective infection control practices, which cannot be overemphasized [19]. Hence the control of infectious diseases is inevitable and largely depends on the approaches employed to break the transmission chain [20].

Table 3. Antibiotic resistance pattern of selected *Enterococcus faecalis*

Strains	Antibiotic Resistant Pattern
E. faecalis DMOF 21	Nor^+, Van^+, Pef^+, Cip^+
E. faecalis DMOF 67	Nor^+, Van^+, Pef^+, Cip^+
E. faecalis DMOF 47	Nor^+ ,Van^+, Ofl^+, Cip^+
E. faecalis DMOF 81	Nor^+, Van^+, Ofl^+, Cip^+
E. faecalis DMOF 97	Nor^+, Van^+, Ofl^+, Cip^+
E. faecalis DMOF 53	Nor^+, Van^+, Ofl^+, Cip^+
E. faecalis DMOF 04	Nor^+, Van^+, Ofl^+, Cip^-
E.faecalis DMOF 69	Nor^+, Van^+, Ofl^+, Cip^+
E. faecalis DMOF 89	Nor^+, Van^+, Ofl^+, Cip^+
E. faecalis DMOF 26	Nor^+, Van^+, Ofl^+, Cip^+
E. faecalis ATCC 29212	Standard strain

Nor Norfloxacin, Van Vancomycin, Pef Pefloxacin, Cip Ciprofloxacin, Ofl Ofloxacin, $^+$ resistant, $^-$ susceptible

Except NXD and ZGC, the recommended in-use concentrations of the disinfectants had no observable effect on the test organisms (Table 4). The nature of the active ingredients and particularly their concentrations in biocides may explain why some are inhibitory while some are not [11,14]. Quite an array of chemical agents are presented or marketed as chemical floor disinfectants, each with different composition, activity and mode of action [15,21]. *Enterococcus faecalis* DMOF 53 followed by *E. faecalis* DMOF 47 were the most resistant strains while *E. faecalis* DMOF 21 showed the least resistance to the disinfectants. Vali et al. [16] and Noguchi et al. [22] reported that some acquired mechanisms of resistance have been shown to have clinical significance and disinfectants if not carefully selected may be carrier/reservoir of outbreak of nosocomial infections [8,23]. Except CRT, all the soap brands had just one active ingredient. *Enterococcus faecalis* DMOF 53 was the most resistant strain tested, followed by *E. faecalis* DMOF 47 while *E. faecalis* DMOF 21 and *E. faecalis* ATCC 29212 showed the least resistance to the disinfectants. This probably indicates that the active ingredients in the soap may be used to control the spread of the isolates investigated in this study.

The ability of the isolates to grow in the soap solutions is presented in Table 5. The effect of the soap samples on the growth of vancomycin-resistant *E. faecalis* investigated revealed that CRT, RBT, TMS and DTA possessed anti-enterococcal property in decreasing order. Table 6 depicts the ability of the test organisms to survive on the surface of the soap samples. The organisms survived on the surface of the bland soap [LXX] sample while survival was least on CRT followed by DTL. MST and NVA among the medicated soaps had the least effect on the enterococci strains. This finding indicates that soap samples that are unable to inhibit the microorganisms are only probably good for social purposes [24], which may also be a pointer to the substantial level of their (residual) antimicrobial potentials.

Table 4. Effect of different disinfectants on the growth of vancomycin-resistant *Enterococcus faecalis*

Disinfectant		*E. faecalis* test strains										
Name	**Dilutions**	**DM OF 21**	**DM OF 67**	**DM OF 47**	**DM OF 81**	**DM OF 97**	**DM OF 53**	**DM OF 04**	**DM OF 69**	**DM OF 89**	**DM OF 26**	**ATCC 29212**
ZAL	4 X R	--	+	--	+	--	--	--	--	--	+	--
	2 X R	+	+	+	+	+	+	+	+	+	+	--
	R	+	+	+	+	+	+	+	+	+	+	--
	½ X R	+	+	+	+	+	+	+	+	+	+	+
	¼ X R	+	+	+	+	+	+	+	+	+	+	+
NXD	4 X R	--	--	--	--	--	--	--	--	--	--	--
	2 X R	--	--	--	--	--	--	--	--	--	--	--
	R	--	--	--	--	--	--	--	--	--	--	--
	½ X R	--	--	--	+	+	+	+	--	--	--	+
	¼ X R	--	--	+	+	+	+	+	--	--	--	+
SVN	4 X R	+	+	--	--	--	+	+	--	--	--	+
	2 X R	+	+	+	+	--	+	+	+	--	--	+
	R	+	+	+	+	--	+	+	+	+	--	+
	½ X R	+	+	+	+	+	+	+	+	+	+	+
	¼ X R	+	+	+	+	+	+	+	+	+	+	+
DTL	4 X R	--	--	--	--	--	--	--	+	--	+	--
	2 X R	--	--	+	--	--	--	+	+	+	+	--
	R	--	--	+	--	--	--	+	+	+	+	--
	½ X R	--	--	+	+	+	--	+	+	+	+	+
	¼ X R	+	+	+	+	+	--	+	+	+	+	+
VGL	4 X R	--	+	--	--	--	+	+	--	--	--	+
	2 X R	+	+	+	+	--	+	+	+	--	--	+
	R	+	+	+	+	--	+	+	+	+	+	+
	½ X R	+	+	+	+	+	+	+	+	+	+	+
	¼ X R	+	+	+	+	+	+	+	+	+	+	+
ZGC	4 X R	--	--	--	--	--	--	--	--	--	--	--
	2 X R	--	--	--	--	--	--	--	--	--	--	--
	R	--	--	--	--	+	--	+	--	--	--	--
	½ X R	+	+	+	--	+	+	+	+	+	+	+
	¼ X R	+	+	+	+	+	+	+	+	+	+	+

R= Manufacturers' in use concentrations, + = growth, -- = no growth

Table 5. Growth rate of VREF (10 x cfu/ml) in solutions of test soap samples

Soap Samples	*E. faecalis* strains											
	Time (mins)	DMOF 21	DMOF 67	DMOF 47	DMOF 81	DMOF 97	DMOF 53	DMOF 04	DMOF 69	DMOF 89	DMOF 26	ATCC 29212
CRT	2	3	6	4	11	-	10	3	-	2	2	3
	4	7	8	9	12	-	14	1	2	6	7	
	6	2	3	14	7		11	-	5	8	4	5
	8	-	3	5	3	7	4	-	6	7	-	8
	10	-	-	-	-	12	1	-	9	4	-	7
RBT	2	2	4	3	7	3	2	-	6	5	3	3
	4	4	2	9	12	8	8	4	6	17	7	3
	6	6	8	4	14	11	9	3	9	14	11	4
	8	8	11	4	15	7	7	7	8	13	9	-
	10	10	5	-	13	6	8	9	9	13	8	-
DTL	2	2	11	8	7	3	9	7	-	4	11	2
	4	4	13	9	10	5	8	10	3	7	14	4
	6	6	17	21	19	6	3	4	8	21	9	13
	8	8	27	17	24	9	1	4	11	23	5	17
	10	11	29	27	26	12	-	1	14	32	2	2
DTA	2	2	4	-	6	3	7	1	3	6	4	9
	4	4	21	3	11	8	13	5	8	7	8	13
	6	6	15	12	14	11	18	9	14	12	11	11
	8	8	14	12	17	18	11	10	17	9	13	6
	10	12	12	7	22	12	5	14	13	8	13	2
MSF	2	2	21	14	19	8	12	28	21	5	17	8
	4	4	29	27	24	14	17	31	23	12	22	8
	6	6	17	34	18	19	30	34	27	14	19	12
	8	9	7	38	15	17	34	8	28	11	18	16
	10	12	3	38	13	12	37	38	28	14	13	24
TMS	2	2	14	6	5	9	3	6	8	17	19	14
	4	4	11	19	7	16	7	9	12	15	16	13
	6	6	7	15	8	22	8	13	14	8	11	8
	8	8	5	14	7	20	11	7	9	7	4	5
	10	10	5	11	4	13	17	2	5	7	2	2

NVA	2	2	18	17	15	9	12	24	7	13	11	21
	4	4	28	22	19	14	7	31	19	16	13	25
	6	6	25	23	19	21	21	33	23	21	24	28
	8	8	27	31	21	25	27	36	26	22	24	31
	10	12	29	34	24	30	27	38	31	29	29	36

Data are the modal values of three determinations

Table 6. Survival of *E. faecalis* strain on the surface of tested soap samples

Soap	***E. faecalis* strains**										
	DM OF 21	**DM OF 67**	**DM OF 47**	**DM OF 81**	**DM OF 97**	**DM OF 53**	**DM OF 04**	**DM OF 69**	**DM OF 89**	**DM OF 26**	**ATCC 29212**
CRT	-	-	-	-	-	-	-	+	-	++	-
RBT	++	++	+++	++	+++	+++	+++	++	+++	+++	+
DTL	++	++	-	+++	+++	+++	-	-	+++	+++	++
DTA	++	+++	-	-	+++	+++	+++	+++	+++	+++	-
MSF	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
TMS	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++
NVA	+++	+++	++	+++	+++	+	++	+++	+++	+++	+++
Bland (LXX)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

+++ very many colonies (≥5.9 x 10^3 cfu/cm^2), ++ many colonies (between 5.9 x 10^2 and 5.9 x10^3cfu/cm^2), + few colonies (between 30 and 5.8 x 10^2 cfu/cm^2), - scanty or no growth (<30 cfu/cm^2)

The bacterial population increased with time reaching a maximum at the end of the experiment (10^{th} hour) when tested against NVA and DTL. The organisms grew on the surface of most of the soap samples but the microbial load was lower in samples (i.e MSF, CRT, NVA and DTA) containing trichlorocabanilide. This confirms the broad spectrum effectiveness of the ingredients against bacteria as reported previously [19,25]. The ability of the organisms to survive on the surface of the soap samples indicates that they can be transmitted through sharing of soaps. This may however explain the need for the use of additional disinfectants and not restrict usage to only one agent in medical practices.

Among the soap samples tested CRT followed by DTL were very effective against the test organisms. Sharing soaps in health care facilities could lead to the spread of potentially harmful bacteria. Manufacturers' recommended in-use concentrations of most of the disinfectants were not effective, and hence would most probably not control proliferation and spread of the test organisms. The versatile nature, ability of the vancomycin-resistant *E. faecalis* to become resistant to adverse environmental conditions, possession of a large arsenal of pathogenic factors, intrinsic resistance to many antimicrobial agents and high propensity to transfer genetic materials, may as well explain resistance to the biocides investigated. This claim is however still open to further investigation.

CONCLUSION

We conclude that most of the disinfectants (biocides) examined were not effective at the in-use concentrations that were recommended by their respective manufacturers. Ability of most test organisms on the surface of soap samples also shows their poor activity and ineffectiveness against the test organisms. The use of biocides with poor efficacy may only give a false sense of security but represent potential reservoirs and or sources of transmission of antimicrobial-resistant pathogens.

COMPETING INTERESTS

The authors declare no competing interests.

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