



Determination of Resistance of Enterococcal Isolates to Common Chemicals used for Disinfection in Homes and Hospitals

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the resistance Enterococcal isolates to common chemical agents used for disinfection in homes and hospitals.

Place and Duration of the Study: Enugu State University of Technology (ESUT) Teaching Hospital, Parklane and University of Nigeria Teaching Hospital (UNTH), Ituku Ozalla, Enugu State, Nigeria, between July 2012 and June 2014.

Methods: The study analysed antimicrobial products such as Dettol, Hibitane and Jik. Identification of the organism was based on standard procedure and biochemical test.

Results: All the three representative isolates, *E. faecium*, *E. faecalis* and *E. avium* showed luxuriant growth with optical density (OD) range of 0.31 to 0.35 at Sodium Chloride concentration of 0.5%. The growth (OD) reduced as the salt concentration increased. *E. faecium*, *E. faecalis* and *E. avium*

showed no growth at the concentration of 10%. Dettol had MIC of 0.385 mg/ml and MBC of 0.75 mg/ml on *E. faecium*; MIC of 0.75 mg/ml and MBC of 1.5 mg/ml on *E. faecalis*; MIC of 0.193 mg/ml and MBC of 0.385 mg/ml on *E. avium*. Hibitane had MIC of 0.781 mg/ml and MBC of 1.568 mg/ml on *E. faecium*; MIC of 1.563 mg/ml and MBC of 3.125 mg/ml on *E. faecalis*; MIC of 0.781 mg/ml and MBC of 1.563 mg/ml on *E. avium*. Jik had an MIC of 0.547 mg/ml and MBC of 1.094 mg/ml on *E. faecium*; MIC of 1.094 mg/ml and MBC of 2.188 mg/ml on *E. faecalis*; MIC of 0.547 mg/ml and MBC of 1.094 mg/ml on *E. avium*.

Conclusion: The growth of the isolates reduced as the salt concentration increased. The MICs and MBCs values from this study showed that these disinfectants are effective against *Enterococci* sp. in the following order; Dettol (Chloroxylonol), Jik (sodium hypochlorite) and Habitane (Chlorhexidine gluconate). The study therefore showed that concentration of various disinfectants/antiseptics used in hospital settings, medical laboratory section and at home should be of paramount interest for safe elimination of Enterococci.

Keywords: Anti-infective agents; disinfectants; enterococci; infection; minimum inhibitory; concentration; minimum bactericidal concentration.

1. INTRODUCTION

Disinfectants are chemical substances that can kill microorganisms while sterilization kills all forms of life [1,2]. It is usually applied on the surface of non-living things. Endospores forming bacteria and exospores (microbial cyst) are resistant to disinfectants [3]. Also, antiseptics are chemicals that when applied to the surface of living tissue or skin can inhibit the growth or kill microorganisms [4]. Both disinfectants and antiseptics are antimicrobial agents. Examples of disinfectants and antiseptics include propylene glycol, Alcohol, Quaternary ammonium cation, formaldehyde and glutaraldehyde, Hydrogen peroxide, Ozone, Potassium permanganate, Peroxycarboxylic acids, Chloroxylonol, Chlorine and Iodine [4,5].

Disinfectants are widely used in hospital settings, healthcare environment and diagnostic or medical laboratory section to control and prevent nosocomial infections. Also, disinfectants are used in the kitchen, toilet and bathroom. However, the transfer of resistance factors from one *Enterococcus* sp. to another and even from one enterococcal strain to an entirely different bacterial strain (e.g. from VRE to MRSA) makes cure a true part of infection prevention and control [6]. The best way to prevent infection is to prevent transmission.

2. MATERIALS AND METHODS

2.1 Area of the Study

This study was carried out in Enugu State. The two tertiary health institutions used were Enugu State University of Technology (ESUT) Teaching

Hospital, Parklane and University of Nigeria Teaching Hospital (UNTH), Ituku Ozalla in Enugu State, Nigeria.

2.2 Study Design

The study comprised of three categories of patients; 504 in-patients, 504 out-patients and controls (20 male and 20 female volunteers who did not have symptoms of any infection. They were selected from outside the hospital environment).

2.3 Specimen Collection

Sterile universal containers containing boric acid preservative were used for urine sample collection while sputum, stool, aspirates and CSF were collected with sterile plain universal bottles. Sterile swabs were used to collect high vaginal, urethral, wound, nasal, ear, anal sample. For blood culture, five milliliters of blood was collected with syringe and put aseptically into fifty milliliters of sterile brain heart infusion (BHI) broth contained in a bijou bottle.

2.4 Analytical Techniques

Culture/Isolation Considerations [7].

Determination of resistance of isolates to common chemicals used for disinfection in homes and hospitals.

This was determined by subjecting the isolates to different concentrations and degrees of the chemical and physical agents.

Chemical agents assessed include;

Sodium chloride: method as described by Diana –Roxana *et al.* [8]. The colony forming units per ml was also established by using Miles and Misra [9] method. These values were recorded. Log₁₀ cfu/ml was estimated from this value.

Dettol: containing 4.8% W/V chloroxylenol; The resistance of isolates to Dettol was determined by establishing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Dettol on the isolates using double dilution method as recommended by Clinical and Laboratory Standards Institute (CLSI) [10].

The colony forming units per ml was also established by using Miles and Misra [9] method. These values were recorded. Log₁₀ cfu/ml was estimated from this value and recorded. The first dilution in the row where the optical density was 0, was recorded as the minimum inhibitory concentration (MIC) while the first dilution that did not yield any bacterial colony was the minimum bactericidal concentration (MBC).

Hibitane: containing 5% W/V chlorhexidine gluconate). The resistance of isolates to Hibitane (5% chlorhexidine gluconate) was determined by the method as recommended by Clinical and Laboratory Standards Institute (CLSI) [10].

Jik: containing 3.85% W/V sodium hypochlorite. The resistance of isolates to Jik (3.85% sodium hypochlorite) was determined by the method as recommended by Clinical and Laboratory Standards Institute (CLSI) [10].

3. RESULTS

3.1 Resistance to Common Chemicals used in Homes and Hospitals

3.1.1 The effect of various Sodium Chloride (NaCl) concentrations on the enterococcal isolates

All the three representative isolates, *E. faecium*, *E. faecalis* and *E. avium* showed luxuriant growth with optical density (OD) range of 0.31 to 0.36 at Sodium Chloride concentration of 0.5%. The growth (OD) reduced as the salt concentration increased. *E. faecium* and *E. faecalis* showed no growth (0 optical density) at the concentration of 12%. *E. avium* showed no growth (0 optical

density) at the concentration of 10%. When the broth was sub-cultured into 5% sheep blood there was no bacterial growth at the concentration of 10% and 12% NaCl (MBC). (Table 1, Figs. 1 and 2).

3.1.2 Effect of Dettol (Chloroxylenol) on the enterococcal isolates

The MIC of Dettol on *E. faecium* was 0.385 mg/ml. The MBC of Dettol on *E. faecium* was 0.75 mg/ml. The MIC of Dettol on *E. faecalis* was 0.75 mg/ml while the MBC was 1.5 mg/ml. The MIC of Dettol on *E. avium* was 0.193 mg/ml while the MBC was 0.385 mg/ml. (Table 2, Figs. 3 and 4).

3.1.3 Effect of Hibitane (Chlorhexidine gluconate) on the enterococcal isolates

The MIC of Hibitane on *E. faecium* was 0.781 mg/ml while the MBC was 1.568 mg/ml. The MIC of Hibitane on *E. faecalis* was 1.563 mg/ml while the MBC was 3.125 mg/ml. The MIC of Hibitane on *E. Avium* was 0.781 mg/ml while the MBC was 1.563 mg/ml. (Table 2, Figs. 5 and 6).

3.1.4 The effect of Jik (Sodium hypochlorite) on the enterococcal isolates

The MIC of Jik on *E. faecium* was 0.547 mg/ml while the MBC was 1.094 mg/ml. The MIC of Jik on *E. faecalis* was 1.094 mg/ml while the MBC was 2.188 mg/ml. The MIC of Jik on *E. avium* was 0.547 mg/ml while the MBC was 1.094 mg/ml (Table 2, Figs. 7 and 8).

Summary of effects of disinfectants / antiseptic liquids on the enterococcal isolates compared to the manufacture's recommended concentrations for use.

Dettol (Chloroxylenol) had MIC of 0.193 mg/ml and MBC of 0.385 mg/ml on *E. faecium*; MIC of 0.385 mg/ml and MBC of 0.750 mg/ml on *E. faecalis*; MIC of 0.193 mg/ml and MBC of 0.385 mg/ml on *E. avium* and a recommended concentration of 2.9 mg/ml.

Hibitane (Chlorhexidine gluconate) had MIC of 0.1953 mg/ml and MBC of 0.3906 mg/ml on *E. faecium*; MIC of 0.3906 mg/ml and MBC of 0.7813 mg/ml on *E. faecalis*; MIC of 0.1953 mg/ml and MBC of 0.3906 mg/ml on *E. avium* with a recommended concentration of 1.2 mg/ml.

Table 1. The influence of various sodium chloride (NaCl) concentrations on the isolates

CONC. (%)	<i>E. faecium</i> OD	No (log ₁₀ cfu/ml)	<i>E. faecalis</i> OD	No (log ₁₀ cfu/ml)	<i>E. avium</i> OD	No (log ₁₀ cfu/ml)
0.5	0.35	8.6	0.31	7.5	0.36	6.5
5.0	0.1	4.7	0.10	3.3	0.12	3.2
6.5	0.05	3.0	0.07	2.9	0.01	2.1
8.0	0.02	2.5	0.03	2.1	0.009	1.3
10	0.001	1.2	0.006	1.1	0	NG
12.0	0	NG	0	NG	0	NG

Conc. = Concentration; NG = No Growth; OD = Optical Density

Table 2. Summary of effects of disinfectants/antiseptic liquids on the isolates

Disinfectants	<i>E. faecium</i> CONC. (mg/ml)	No (log ₁₀ cfu/ml)	<i>E. faecalis</i> CONC. (mg/ml)	No (log ₁₀ cfu/ml)	<i>E. avium</i> CONC. (mg/ml)	No (log ₁₀ cfu/ml)
Dettol (MIC)	0.385	2.5	0.75	2.7	0.193	2.4
Dettol (MBC)	0.75	NG	1.5	NG	0.385	NG
Habitane (MIC)	0.781	2.1	1.563	3.1	0.781	2.9
Habitane (MBC)	1.568	NG	3.125	NG	1.563	NG
Jik (MIC)	0.547	1.8	1.094	1.1	0.547	1.9
Jik (MBC)	1.094	NG	2.188	NG	1.094	NG

Conc. = Concentration; NG = No Growth

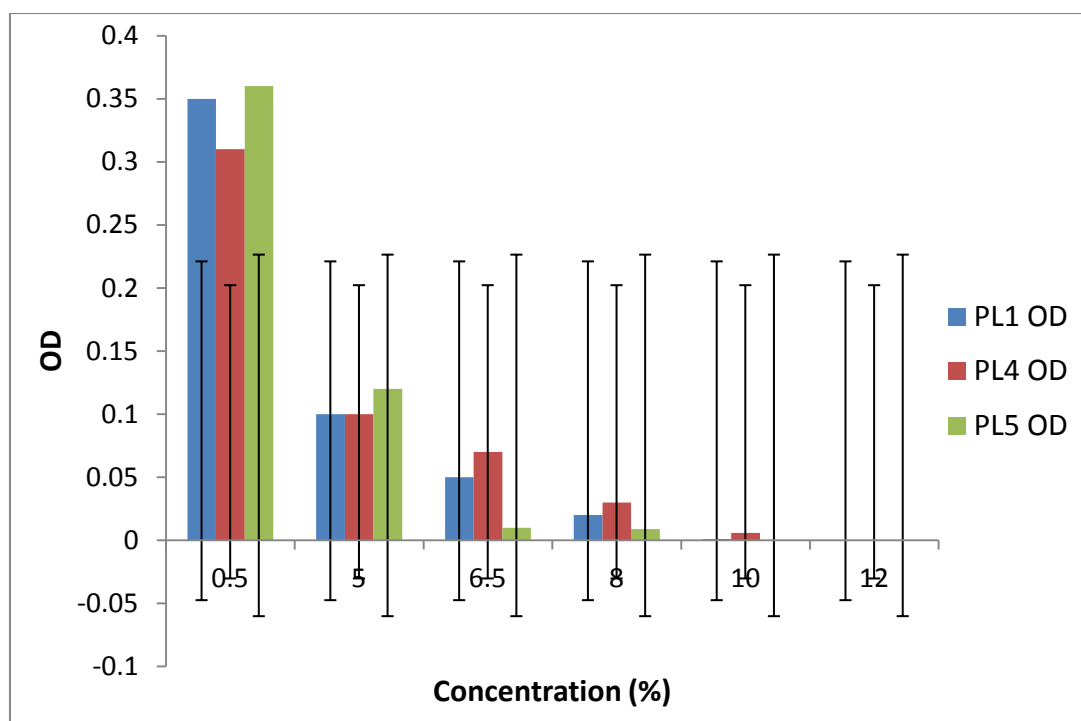


Fig. 1. The effect of various concentrations of Sodium Chloride (NaCl) on the isolates using optical density as a parameter

OD = Optical Density.

PL1 = *E. faecium*.

PL4 = *E. faecalis*.

PL5 = *E. avium*

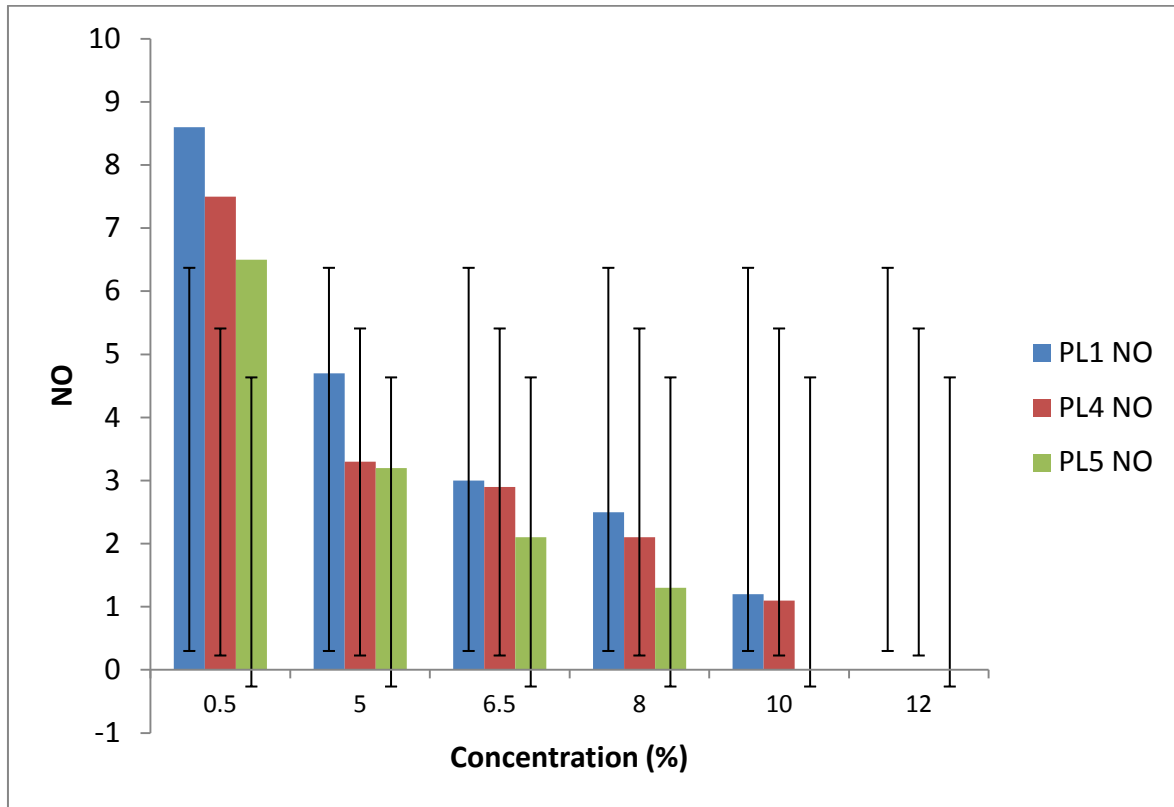


Fig. 2. The effect of various concentrations of Sodium Chloride (NaCl) on the isolates using cell count as a parameter
 NO = $\text{Log}_{10}\text{cfu/ml}$

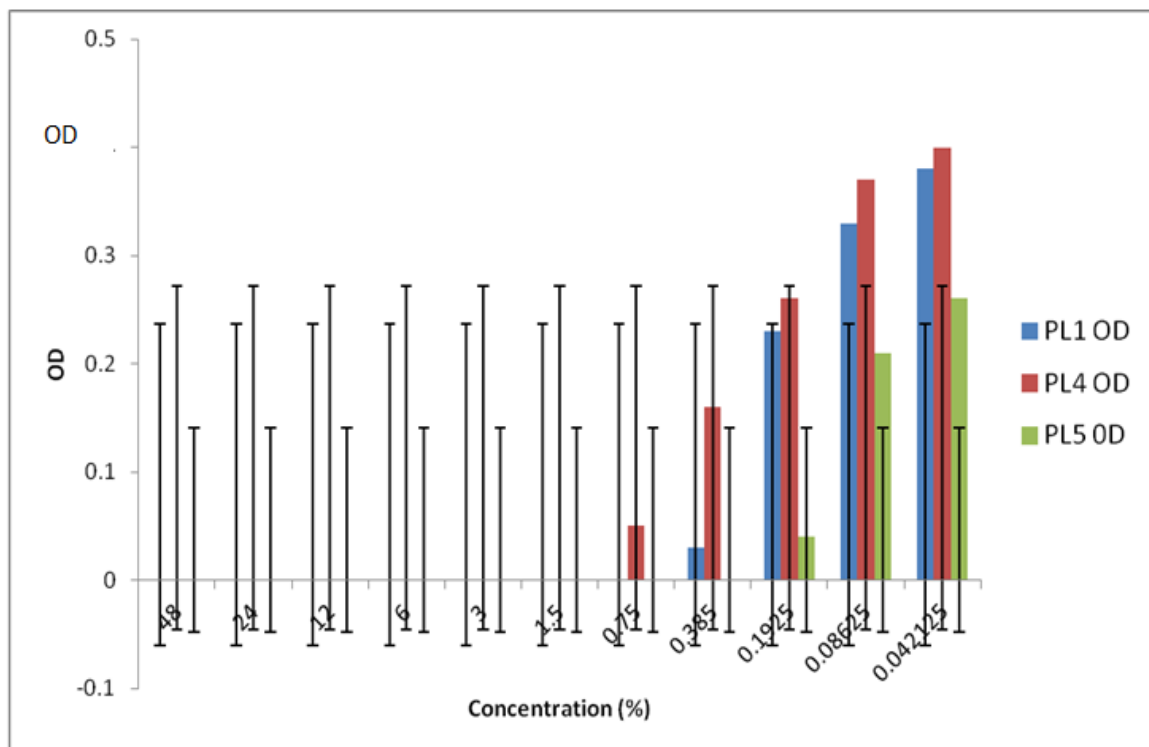


Fig. 3. Effect of Dettol on the enterococcal isolates using optical density as a parameter
 KEY: OD = Optical Density

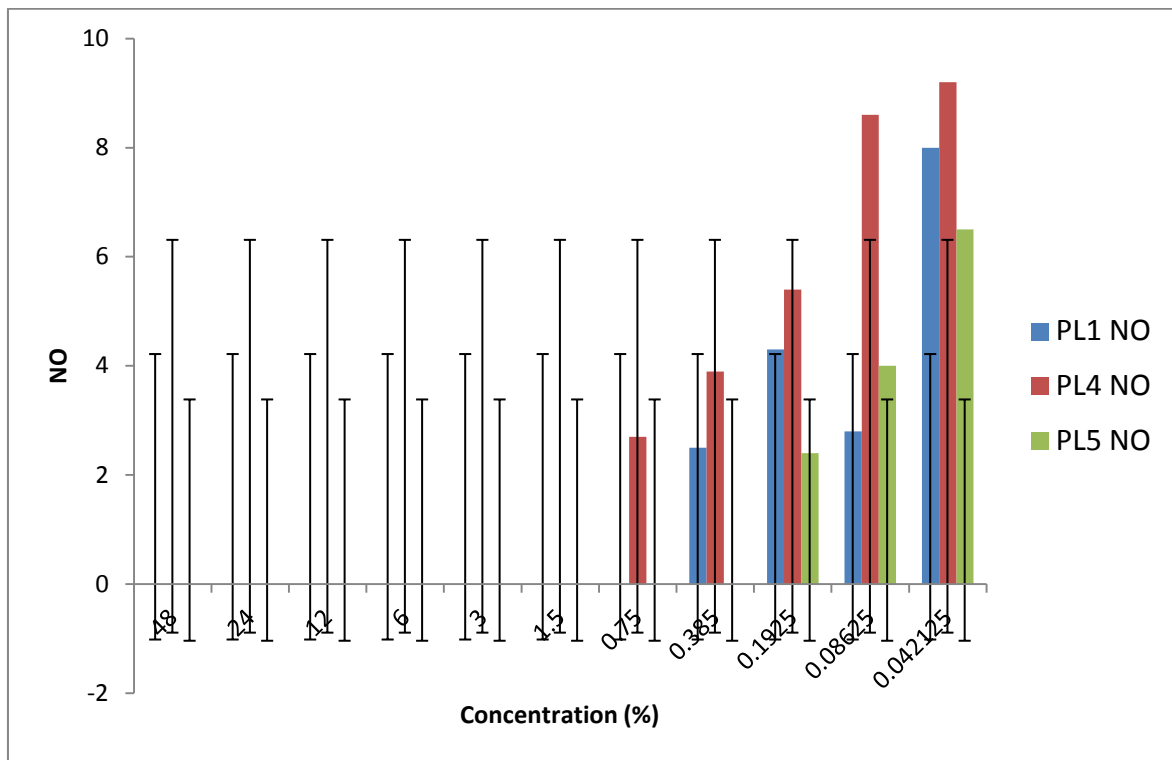


Fig. 4. Effect of Dettol on the enterococcal isolates using cell count as a parameter
 NO = Log₁₀cfu/ml

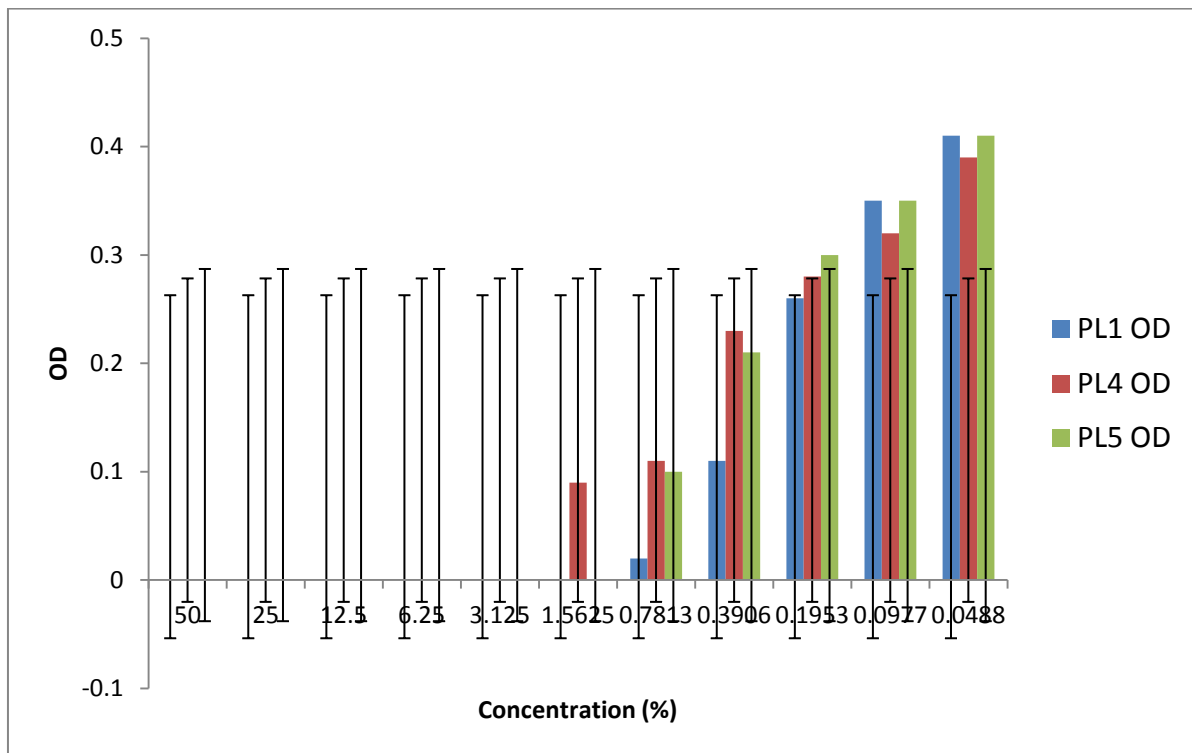


Fig. 5. Effect of Hibitane on the isolates using optical density as a parameter
 KEY: OD = Optical Density

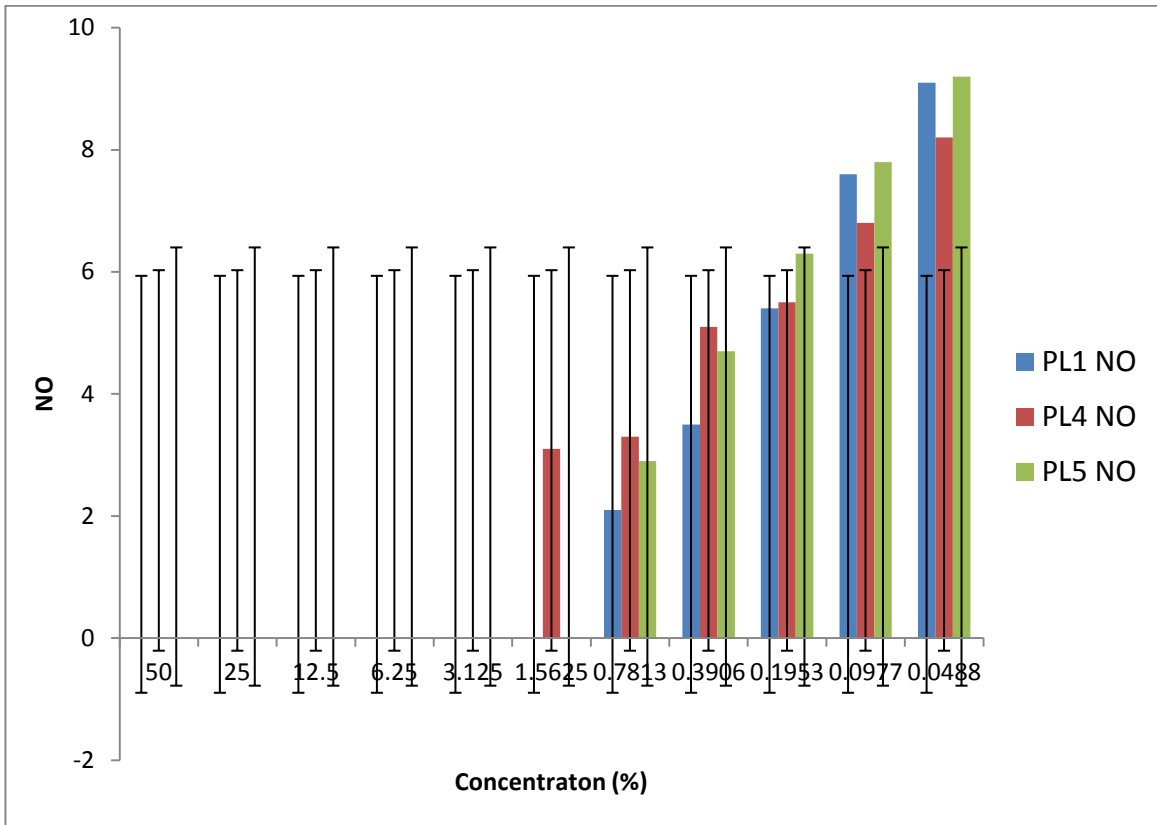


Fig. 6. The effect of Hibitane on the isolates using cell count as a parameter
NO = Log₁₀cfu/ml

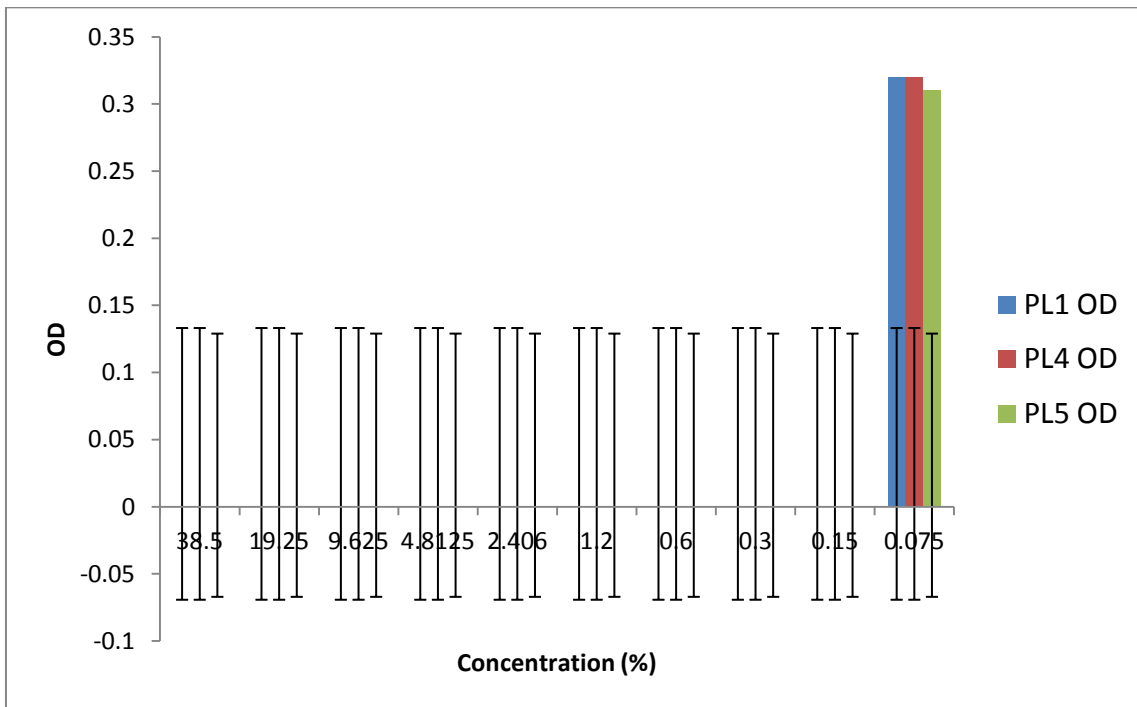


Fig. 7. Effect of Jik on the enterococcal isolates using optical density as a parameter
OD = Optical Density

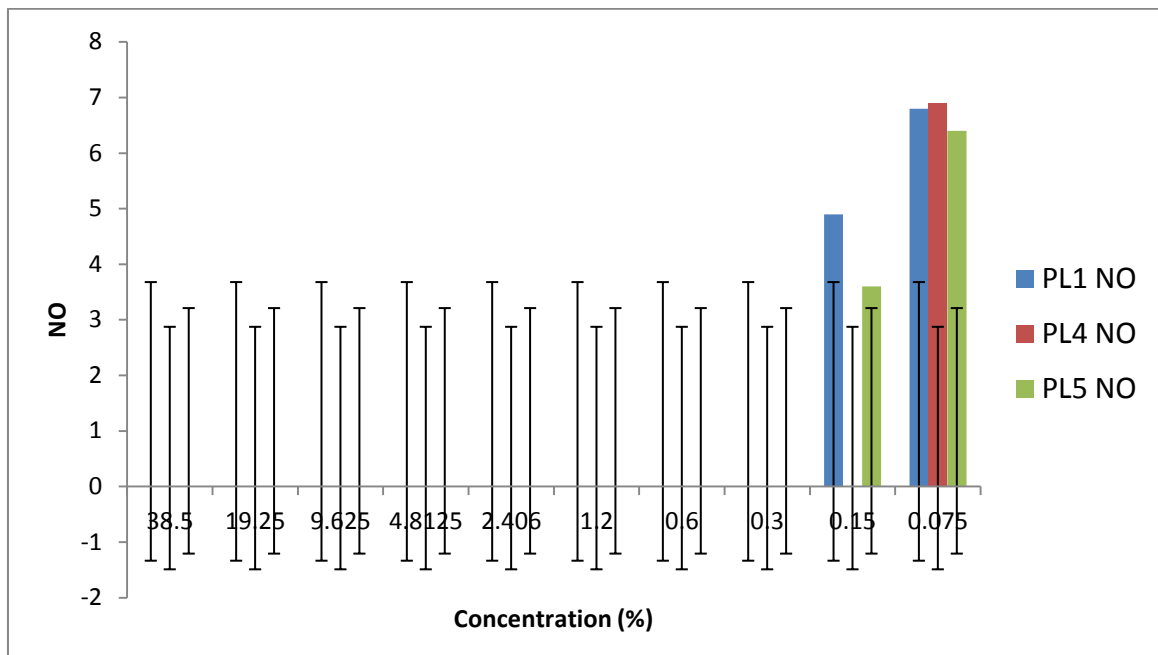


Fig. 8. Effect of Jik on the enterococcal isolates using cell count as a parameter
 NO = $Log_{10}cfu/ml$

Jik (sodium hypochloride) had an MIC of 0.15 mg/ml and MBC of 0.30mg/ml on *E. faecium*; MIC of 0.15 mg/ml and MBC of 0.30 mg/ml on *E. faecalis*; MIC of 0.15 mg/ml and MBC of 0.30

mg/ml on *E. avium* with a recommended concentration of 0.241 mg/ml. This is lower than the MBC of jik and cannot ensure total killing of the bacteria.

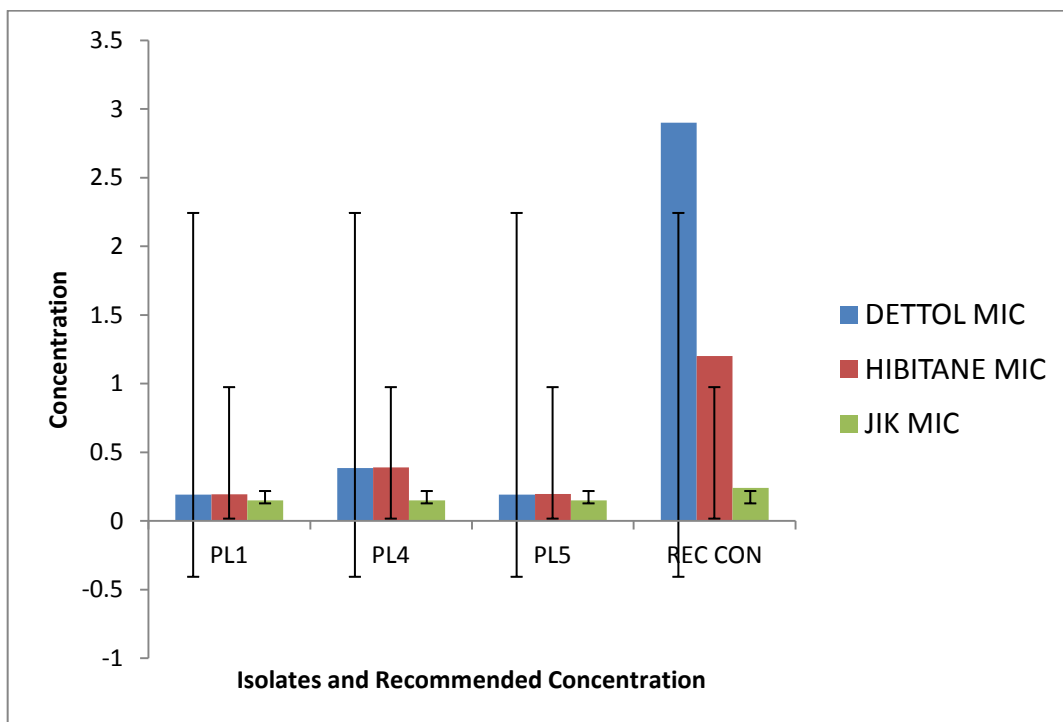


Fig. 9. Summary of MICs of disinfectants/antiseptic liquids on the isolates compared to the manufacturer's recommended concentration
 REC. CONC. = Recommended concentration

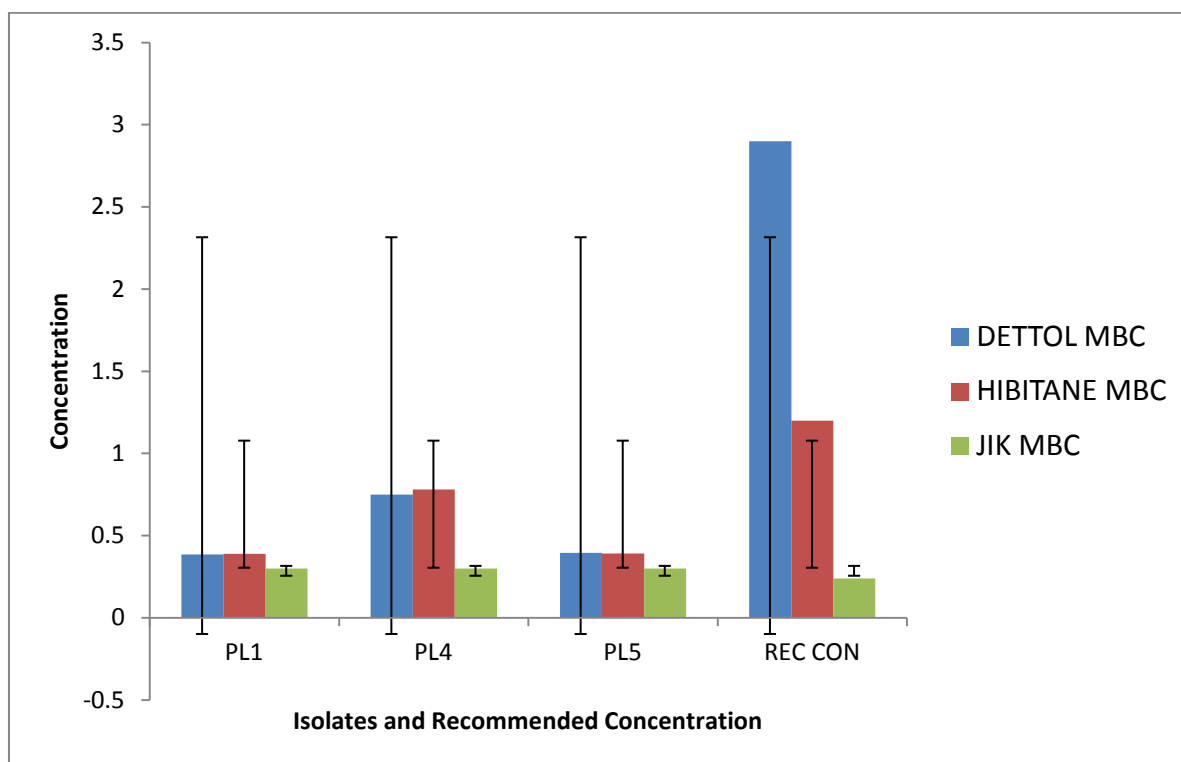


Fig. 10. Summary of MBCs of disinfectants/antiseptic liquids on the enterococcal isolates compared to the recommended concentration
REC. CONC. = Recommended concentration

4. DISCUSSION

This chemical commonly called common salt apart from serving as important additive to our food, is a preservative that was very popular even before the advent of freezers. It is therefore necessary to ascertain the effects of various concentration of this salt on *Enterococcus* sp. At a low concentration of 0.5% sodium chloride, there was a luxuriant growth. The growth rate decreased as the concentration of salt increased. The isolates survived the concentration of 8% sodium chloride but at the concentration of 10% and above, there was no bacterial growth. Fisher and Philips [6] demonstrated that *Enterococcus* sp. was capable of growth at a high sodium chloride concentration of up to 6.5%. This is one of the distinguishing characteristics between *Enterococcus* sp. and *Streptococcus* sp. which does not survive 6.5% of sodium chloride [6]. Experiments have shown that the presence of sodium chloride in the growth medium induces more heat tolerance in enterococci than the cells grown in normal medium [11].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) are

tests used to determine the efficacy and sensitivity of disinfectants and antiseptics against micro-organisms [12]. MIC is the lowest concentration that inhibits the growth of tested micro-organism, while MBC is the lowest concentration of the disinfectant that kills the microorganism [12,13]. If MIC and MBC values of a disinfectant are small enough, the disinfectant is stronger, potent, and suitable compared to other disinfectants [12]. In most cases, MIC values are usually less than MBCs because higher concentrations of an antibacterial agent required for bactericidal compared to bacteriostatic effects [12].

Dettol is widely used in homes and healthcare settings for various purposes including disinfection of skin, objects and equipments, as well as environmental surfaces. With prior cleaning before application, the number of microorganisms colonizing the skin and surfaces are greatly reduced [14]. The antimicrobial properties of chloroxylenol, the main chemical constituent of Dettol and other chlorinated phenols have been extensively studied [15]. The antimicrobial properties of the disinfectant against some pathogenic bacteria have earlier

been reported [16]. The MICs and MBCs of Dettol (chloroxylenol) as demonstrated in this work are lower than the recommended concentration for use and so are safe and effective at this concentration. This in effect is not a threat to effective control of infection in hospital setting.

Chlorhexidine gluconate (Hibitane) is an antiseptic skin cleansing agent that is used in soaps, cleansers and oral solutions. Chlorhexidine acts to remove surface bacteria on the skin and is often recommended for managing acne, rosacea, eczema and other bacteria-related skin conditions, as well as fungal infections such as athlete's foot. It's also commonly used as a disinfecting ingredient in wound cleansers and in the soaps that surgeons scrub with before surgery. *Enterococcus faecium* strains showing high level resistance to vancomycin and gentamicin or both are not more resistant to chlorhexidine or other non-antibiotic agents [17]. Furthermore, despite the extensive dental use of chlorhexidine, strains of *Streptococcus mutans* remain sensitive to it [18]. Up to 1993, therefore there was little or no evidence of plasmid associated resistance of non-staphylococcal gram positive bacteria to antiseptics and disinfectants [18]. In this study, hibitane (chlorhexidine gluconate) had MIC of 0.195 mg/ml and MBC of 0.391 mg/ml on *E. faecium*; MIC of 0.391 mg/ml and MBC of 0.781 mg/ml on *E. faecalis*; MIC of 0.195 mg/ml and MBC of 0.391 mg/ml on *E. avium* with a recommended concentration of 1.2 mg/ml. The MICs and MBCs of chlorhexidine as demonstrated in this work are lower than the recommended concentration for use and so are safe and effective at this concentration. This, in effect, is not a threat to effective control of infection.

Jik (sodium hypochlorite) is one of the most important chlorine releasing agents (CRAs). Others CRAs are chlorine dioxide and N-Chloro compounds. Sodium hypochlorite solutions are widely used for hard surface disinfection (household bleach) and can be used for disinfecting spillages of blood containing human immunodeficiency virus (HIV) or hepatitis B virus (HBV). In water, sodium hypochlorite ionizes to produce Na^+ and the hypochlorite ion OCl^- which establishes equilibrium with hypochlorous acid, HOCl [19]. Between pH 4 and 7 chlorine exists as HClO , the active moiety, whereas, above pH 9, OCl^- predominates. Although, CRAs have been predominantly used as hard surface

disinfectants, novel acidified sodium chloride (a two compound system of sodium chlorite and mandelic acid) has been described as an effective antiseptic [19]. In this study, the MIC of Jik is lower than the recommended concentration for use. But the MBC of Jik (sodium hypochlorite) is higher than the recommended concentration for use and so the recommended concentration may not ensure safe elimination of enterococci. This, in effect, is a threat to effective control of enterococcal infection if this is used.

5. CONCLUSION

In conclusion, the growth of the isolates reduced as the salt concentration increased. The MICs and MBCs values from this study showed that these disinfectants are effective against *Enterococci sp.* in the following order; Dettol (Chloroxylenol), Jik (sodium hypochlorite) and Habitane (Chlorhexidine gluconate). The study therefore showed that concentration of various disinfectants/antiseptics used in hospital settings, medical laboratory section and at home should be of paramount interest for safe elimination of Enterococci.

CONSENT AND ETHICAL CONSIDERATION

Ethical clearance from the ethical committees of two institutions (Enugu State University of Technology (ESUT) Teaching Hospital, Parklane and University of Nigeria Teaching Hospital (UNTH), Ituku/Ozalla in Enugu State, Nigeria.) and informed consent from the patients were obtained.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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