Original Research Article

DETERMINATION OF RESISTANCE OF ENTEROCOCCAL ISOLATES TO COMMON CHEMICAL AND PHYSICAL AGENTS USED FOR DISINFECTION IN HOMES AND HOSPITALS.

ABSTRACT

~~Introduction~~

Disinfectants and antiseptics are generally used for the control and prevention of microbial infection. *Enterococci* an emerging virulent pathogen, which can be nosocomially or community acquired and had become a subject of investigation. The study analysed antimicrobial products and physical agents such as Dettol, Hibitane, Jik, temperature and pH. Isolation and identification of the organism was based on standard procedure and biochemical test. All the three ~~(3)~~ representative isolates, *E. facium*, *E. faecalis* and *E. avium* showed luxuriant growth with optical density (OD) range of 0.31 to 0.35 at sodium ~~C~~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 0.1925 mg/ml and MBC of 0.385 mg/ml on *E. faecium*; MIC of 0.385 mg/ml and MBC of 0.75 mglml on *E. faecalis*; MIC of 0.1925 mg/ml and MBC of 0.385 mg/ml on E. *avium*. Hibitane 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*. Jik 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*. The three isolates *E. facium*, *E. faecalis* and *E. avium* recorded the highest growth (optical density) at the temperature of 37^{o}c, followed by the temperature of 35^{o}c. There was no growth (0 optical density) at the temperatures of 0^{o}c and 60^{o}c. Furthermore, *E. facium*, *E. faecalis* and *E. avium* had the highest growth (Optical density) at a pH of 6.5. This was followed by the growth at the pH of 5.5. There was no growth at the pH of 3.0. The study concluded that the 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: antiseptics, disinfectants, *Enterococci*, nosocomial infection, prevention.

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INTRODUCTION

Disinfectants are chemical substance 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 chemical 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 [4, 5] propylene glycol, Alcohol, Quaternary ammonium cation, formaldehyde and glutaraldehyde, Hydrogen peroxide, Ozone, Potassium permanganate, Peroxycarboxylic acids, Chloroxylenol, Chlorine and Iodine.

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.

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MATERIALS AND METHODS

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.

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).

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.

Comment [DG9]: Is there any committee approval number? Please mention.

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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.

Comment [DG11]: Descibe how the strains were isolated. Media used etc. Techniques used for identification of species

Analytical Techniques

Culture/Isolation Considerations [7].

Determination of resistance of isolates to common chemical and physical agents 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. Log_{10}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].

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The colony forming units per ml was also established by using Miles and Misra [9] method. These values were recorded. Log_{10}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].

Physical agents assessed include;

Temperature.

The resistance of isolates to temperature was determined by exposing the isolates to different degrees of temperature 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. Log_{10}cfu/ml was estimated from this value and recorded.

pH

The resistance of enterococcal isolates to pH was determined by exposing the isolates to different pH levels 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. Log_{10}cfu/ml was estimated from these values and recorded.

Result

Statistical analysis of results

The results obtained from this work were analyzed statistically using Student t-test and Chi-square of computer program SPSS version 18 to show significant difference.

Resistance to common chemical and physical agents used in homes and hospitals

The effect of various ~~s~~Sodium Chloride (NaCl) concentrations on the enterococcal isolates.

All the three ~~(3)~~ representative isolates, *E. facium* (Pl 1) *E. faecalis* (Pl 4) and *E. avium* (Pl 5) showed luxuriant growth with optical density (OD) range of 0.31 to 0.35 at sodium ~~C~~chloride concentration of 0.5%. The growth (OD) reduced as the salt concentration increased. *E. faecium* (Pl 1) *E. faecalis* (Pl 4) and *E. avium* (Pl 5) 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% NaCl (MBC). (Figure 1 and 2)

Effect of Dettol (Chloroxylenol) on the enterococcal isolates

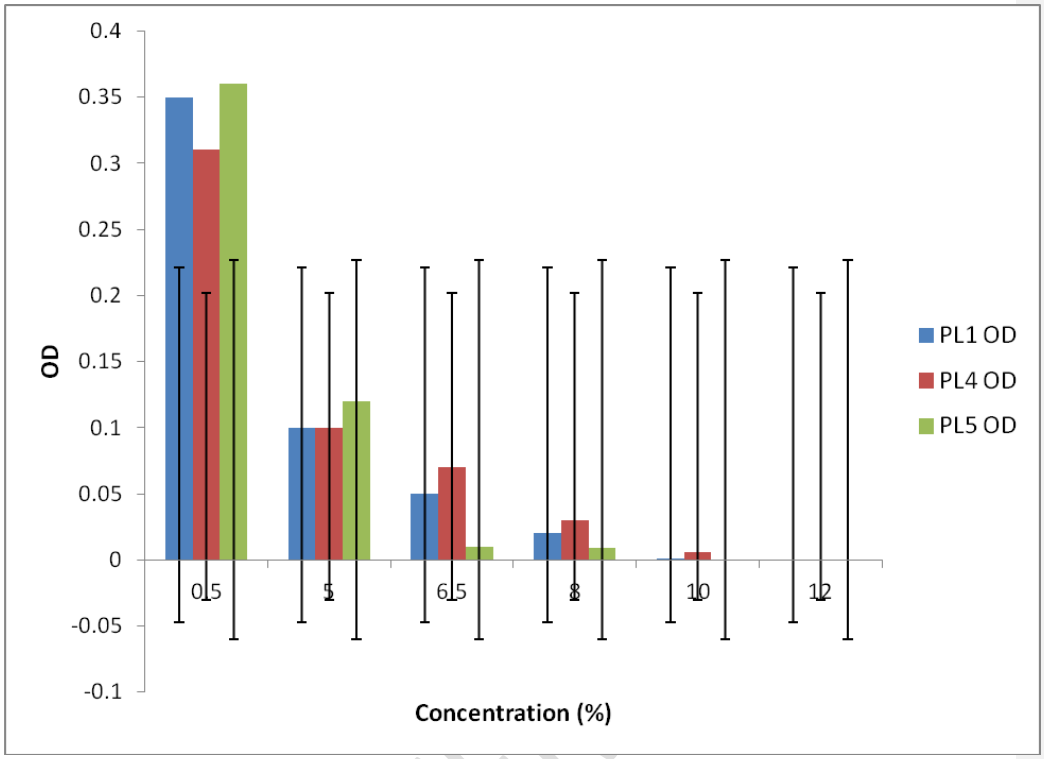
The MIC of Dettol on *E. faecium* (Pl 1) was 0.1925 mg/ml. The MBC of Dettol on *E. faecium* was 0.385 mg/ml. The MIC of Dettol on *E. faecalis* (Pl 4) was 0.385 mg/ml while the MBC was 0.75 mg/ml. The MIC of Dettol on *E. avium* (Pl5) was 0.1925 mg/ml while the MBC was 0.385 mg/ml. (Figure 3 and 4).

Effect of hibitane (chlorhexidine gluconate) on the enterococcal isolates

The MIC of hibitane on *E. faecium* (Pl 1) was 0.1953 mg/ml while the MBC was 0.7813 mg/ml. The MIC of hibitane on *E. faecalis* (Pl4) was 0.3906mg/ml while the MBC was 0.7813 mg/ml. The MIC of hibitane on *E. Avium* (Pl5) was 0.1953 mg/ml while the MBC was 0.3906mg/ml. (Figure 5 and 6).

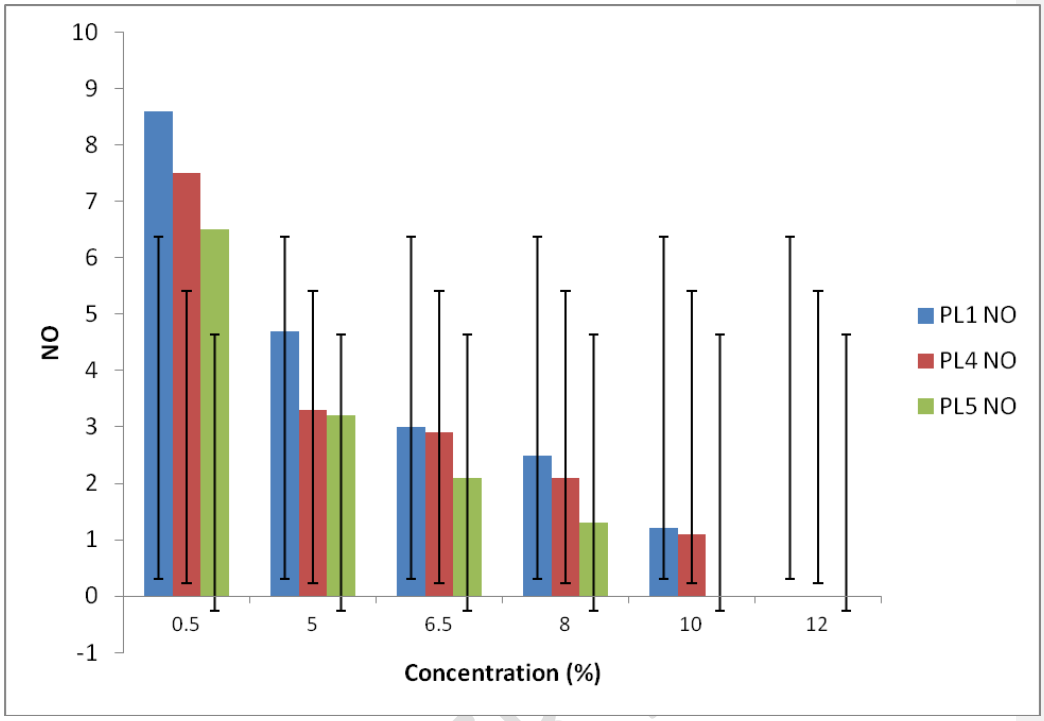
The effect of jik (sodium hypochlorite) on the enterococcal isolates.

The MIC of jik on *E. faecium* was 0.15 mg/ml while the MBC was 0.30 mg/ml. The MIC of jik on *E. faecalis* (Pl4) was 0.15 mg/ml while the MBC was 0.30. The MIC of jik on *E. avium* (Pl5) was 0.15 mg/ml while the MBC was 0.3 mg/ml. (Figure 7 and 8).



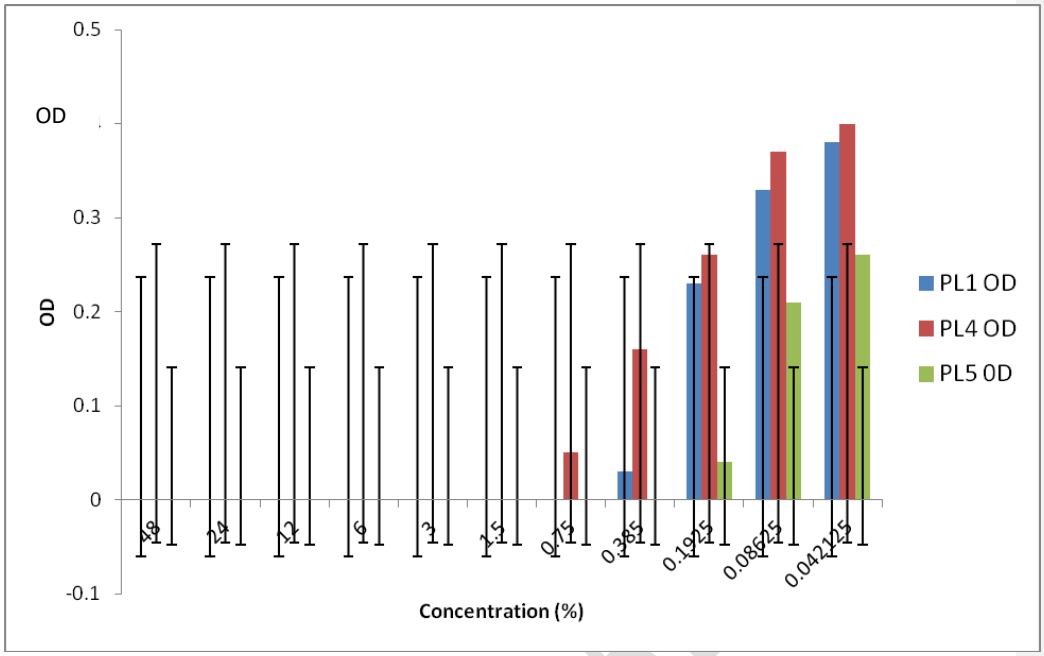
OD = Optical Density.

Figure 1: The effect of various concentrations of sodium chloride (NaCl) on the isolates using optical density as a parameter.



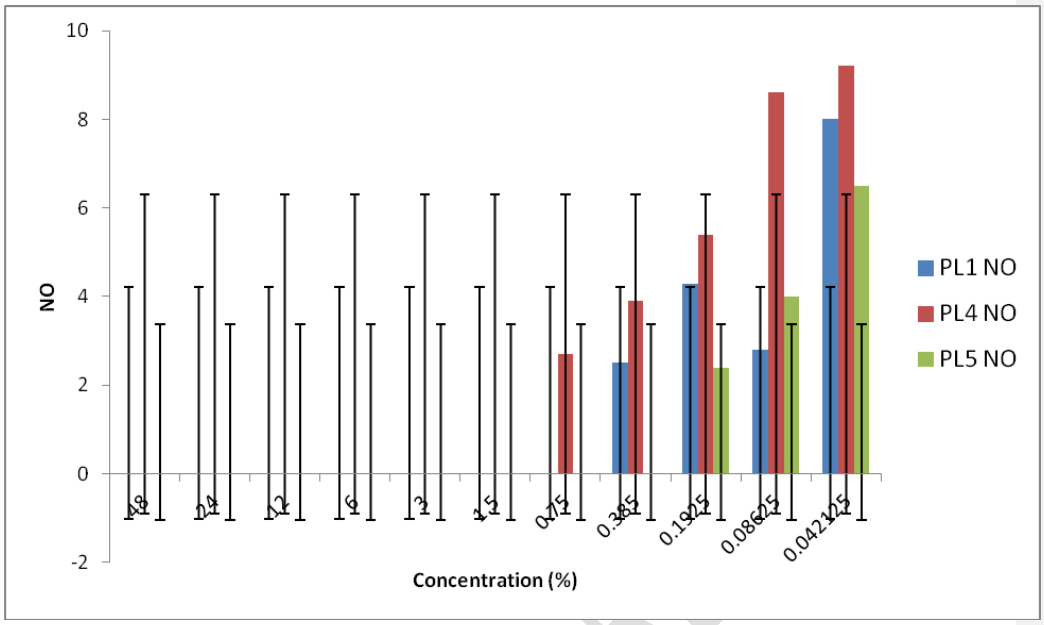
NO = Log_{10}cfu/ml

Figure 2: The effect of various concentrations of sodium chloride (NaCl) on the isolates using cell count as a parameter.



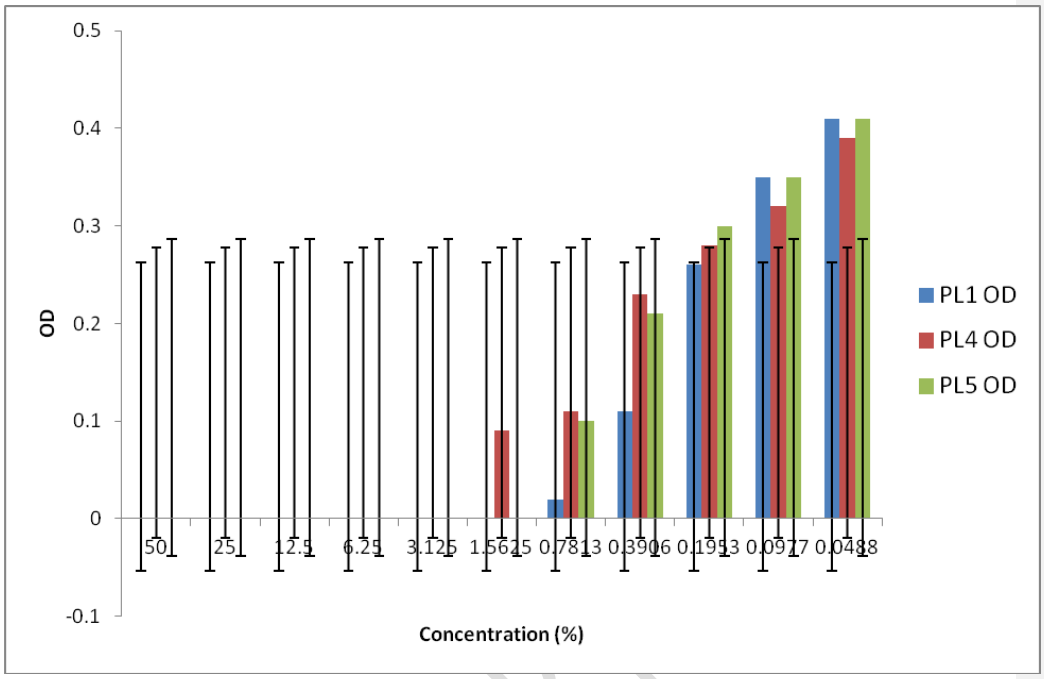
KEY: OD = Optical Density

Figure 3: Effect of dettol on the enterococcal isolates using optical density as a parameter.



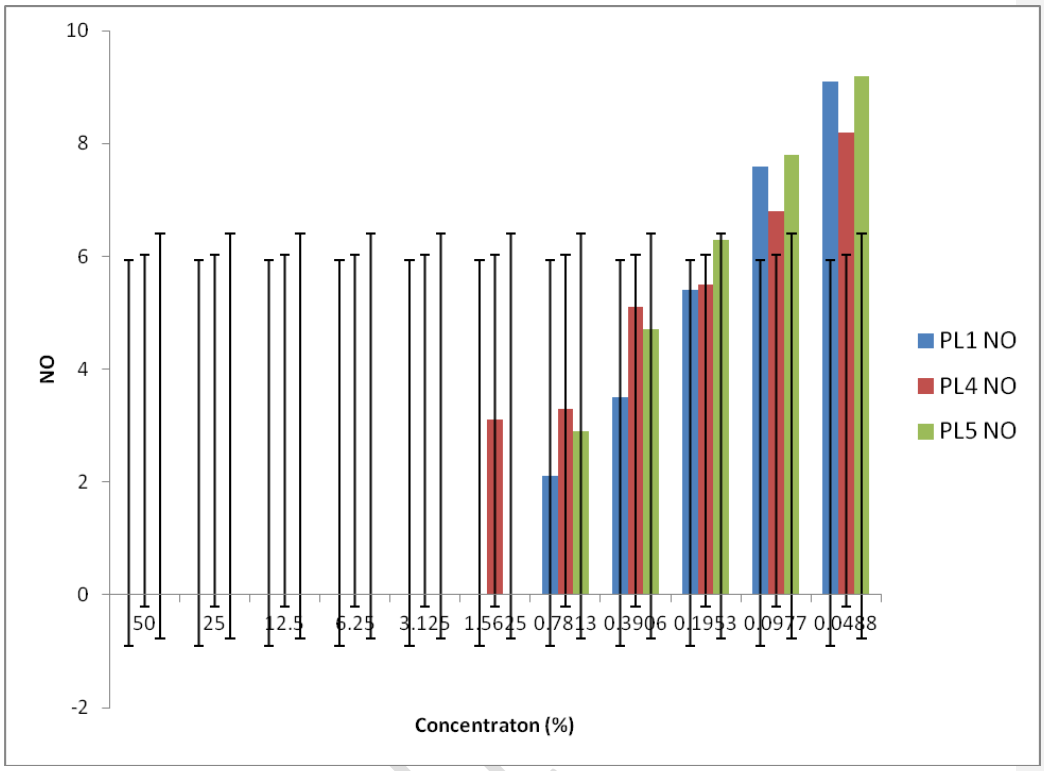
NO = Log_{10}cfu/ml

Figure 4: Effect of dettol on the enterococcal isolates using cell count as a parameter.



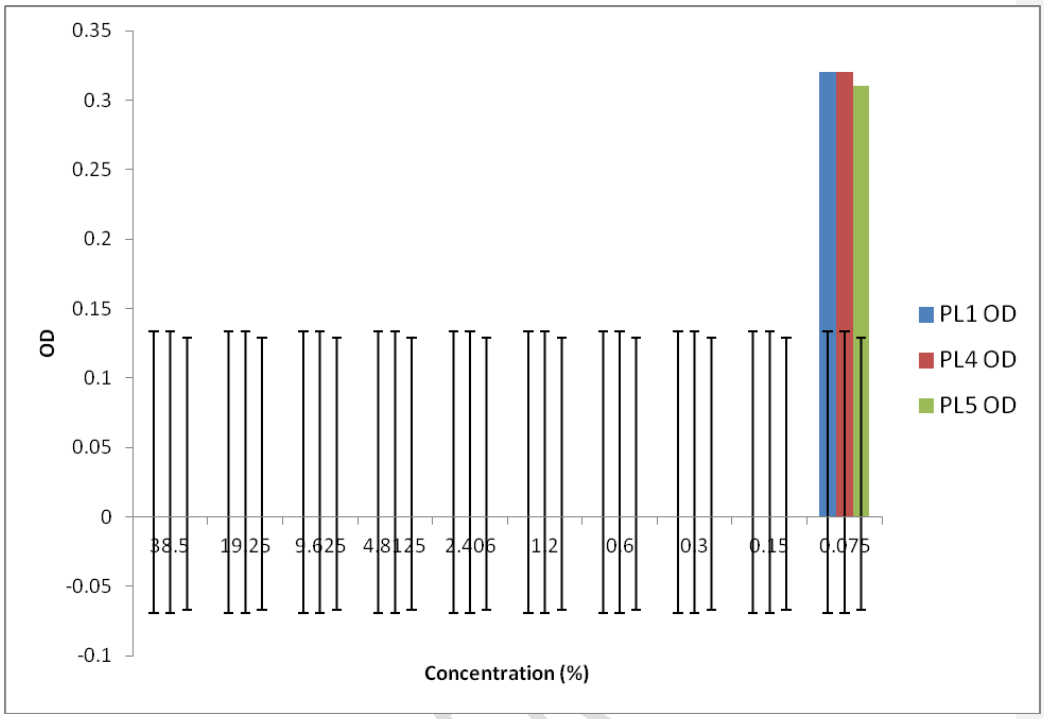
KEY: OD = Optical Density

Figure 5: Effect of hibitane on the isolates using optical density as a parameter



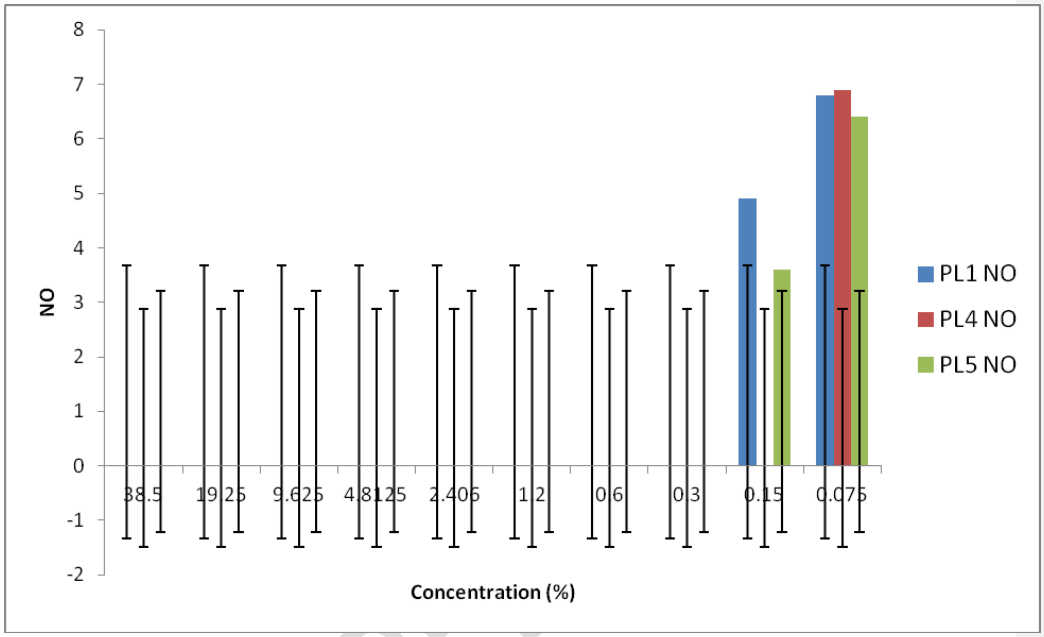
NO = Log_{10}cfu/ml

Figure 6: The effect of hibitane on the isolates using cell count as a parameter.



OD = Optical Density

Figure 7: Effect of jik on the enterococcal isolates using optical density as a parameter



NO = Log_{10}cfu/ml

Figure 8: Effect of jik on the enterococcal isolates using cell count as a parameter.

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

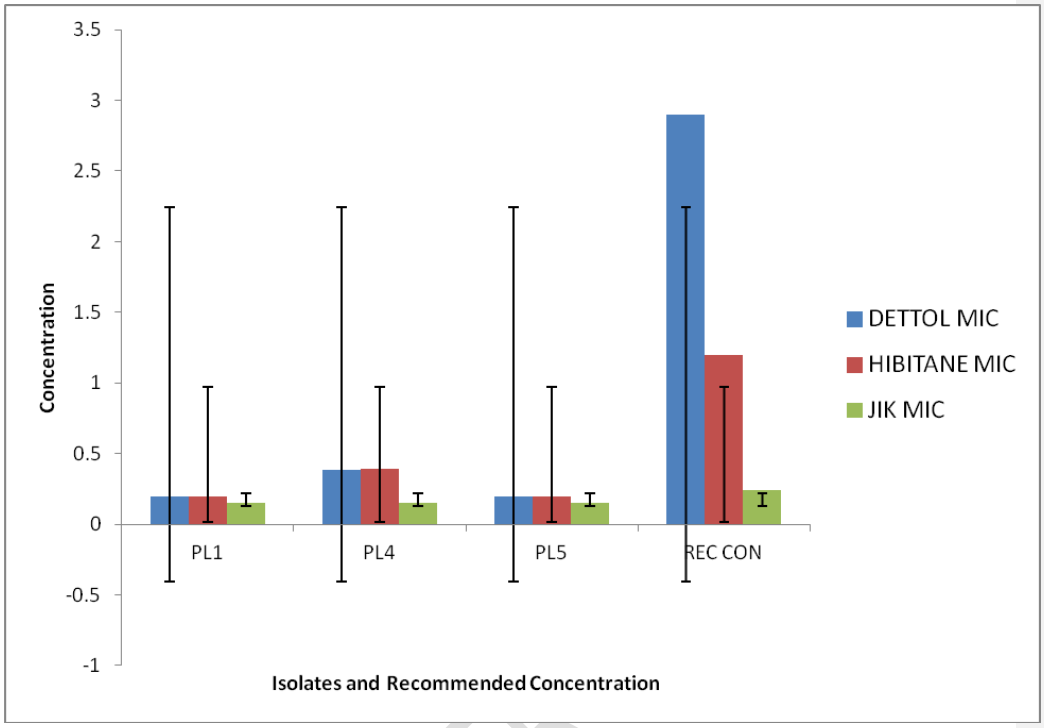
Comment [DG13]: What reference has been used for the recommended concentration?

Dettol (Chloroxylenol) had MIC of 0.1925 mg/ml and MBC of 0.385 mg/ml on *E. faecium* (Pl 1); MIC of 0.385 mg/ml and MBC of 0.75 mglml on *E. faecalis*; MIC of 0.1925 mg/ml and MBC of 0.385 mg/ml on E. *avium* and a recommended concentration of 2.9 mg/ml.

Comment [DG14]: mg/ml

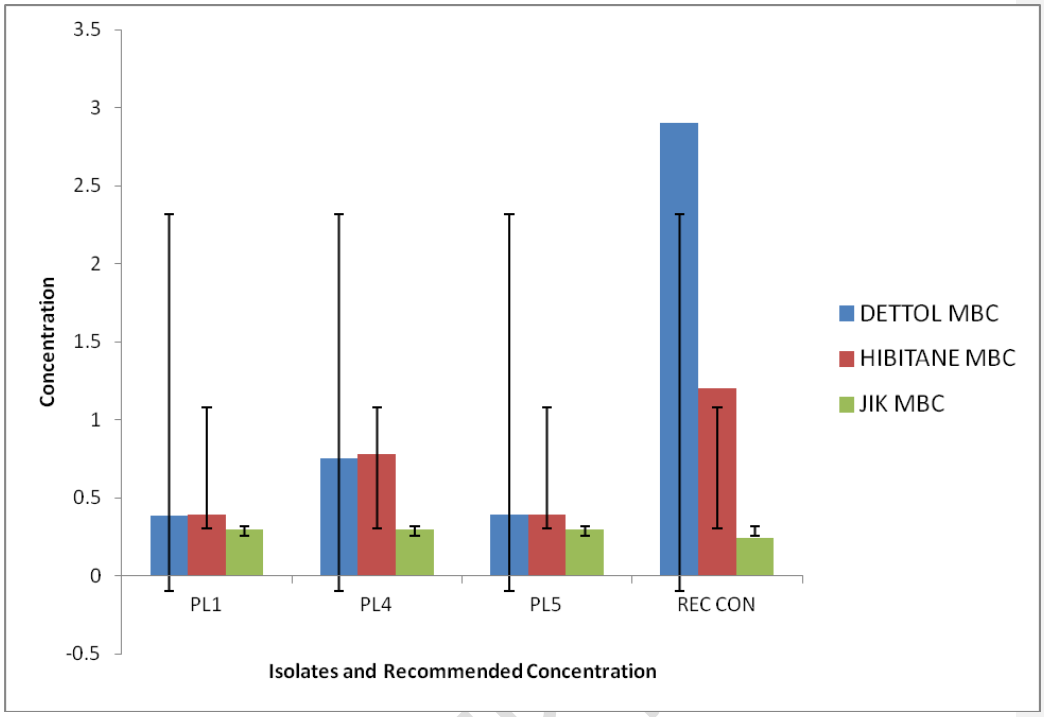
Hibitane (Chlorhexidine gluconate) had MIC of 0.1953 mg/ml and MBC of 0.3906 mg/ml on *E. faecium*; MIC of 0.3906 mg/ml and MBC of 0.7813 mg/ml on *E. faecalis*; MIC of 0.1953 mg/ml and MBC of 0.3906 mg/ml on *E. avium* with a recommended concentration of 1.2 mg/ml.

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.



REC. CONC. = Recommended concentration

Figure 9: Summary of MICs of disinfectants/antiseptic liquids on the isolates compared to the recommended concentration



REC. CONC. = Recommended concentration

Figure 10: Summary of MBCs of disinfectants/antiseptic liquids on the enterococcal isolates compared to the recommended concentration.

The effect of temperature on the enteroccoccal isolates

E. faecium (Pl 1) recorded the highest growth (optical density) at the temperature of 37°c, followed by the temperature of 35°c. There was no growth (0 optical density) at the temperatures of 0°c and 60°c. *E. faecalis* (Pl 4) had the highest growth (optical density) at the temperature of 37°c followed by the temperature of 35°c. There was no bacterial growth (0 optical density) at the temperatures of 0°c and 60°c. *E. avium* (Pl 5) had the highest growth (optical density) at the temperature of 37°c followed by the temperature of 35°c. There was no bacterial growth (0 optical density) at the temperatures of 0°c and 60°c. (Figure 11 and 12).

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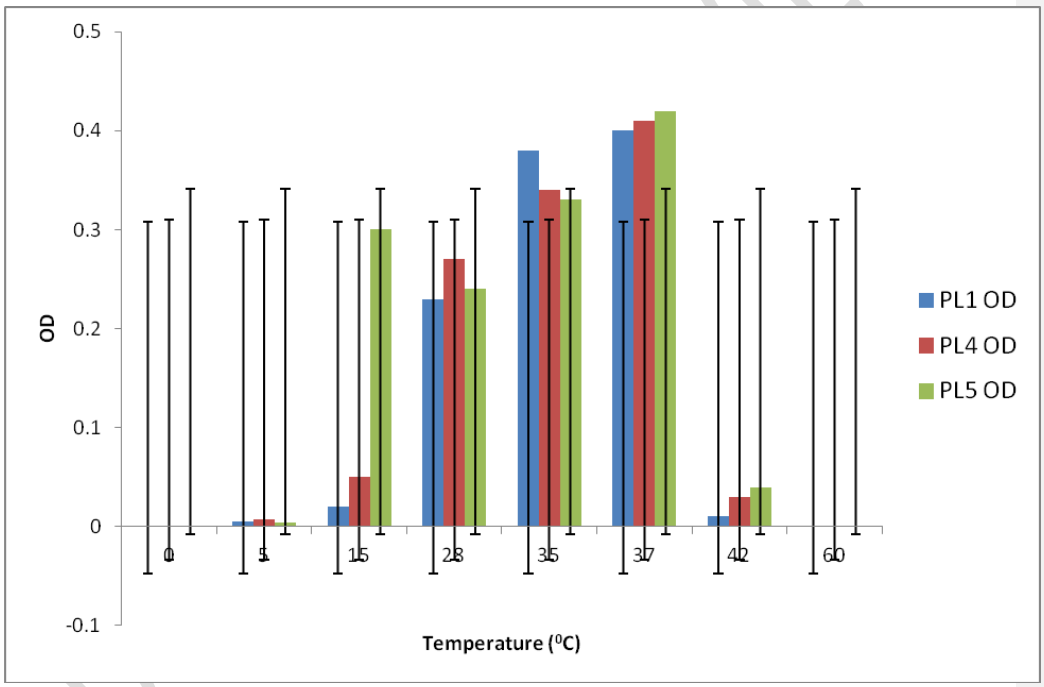
The effect of pH variations on the enterrococcal isolates.

Comment [DG16]: enterococcal

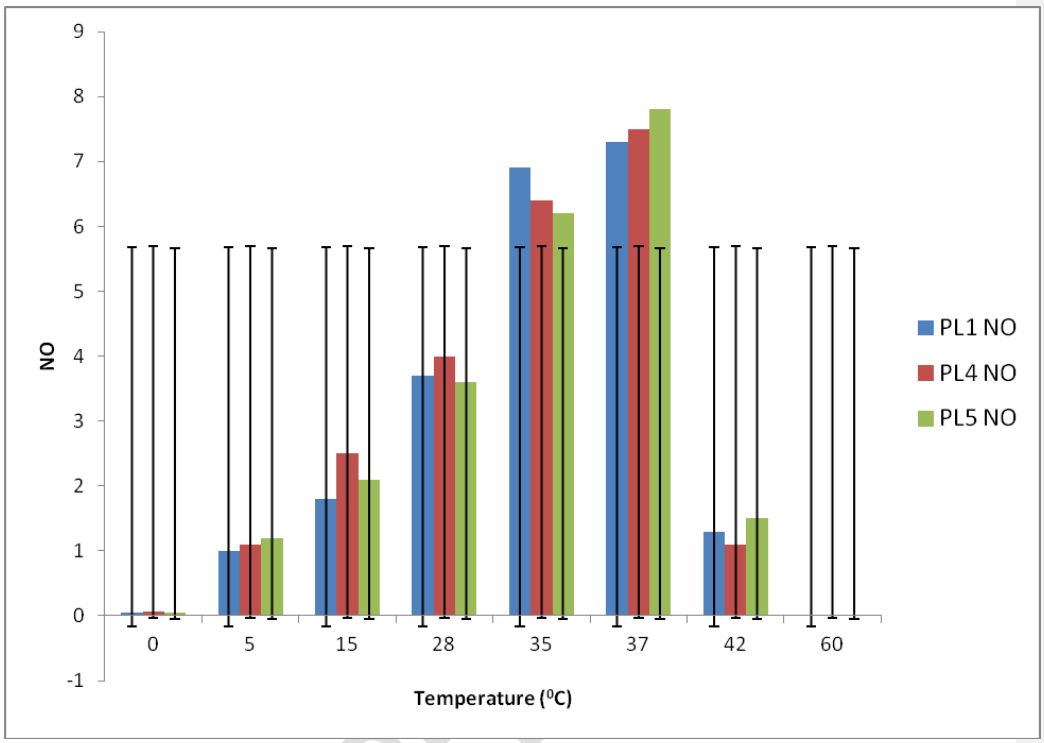
E. faecium (Pl 1) had the highest growth (Optical density) at a pH of 6.5. This was followed by the growth at the pH of 5.5. There was no growth at the pH of 3.0. *E. faecalis* (Pl 4) had the highest growth (optical density) at a pH of 6.5. This was followed by the growth at the pH of 5.5, there was no growth at the PH of 3.0. *E. avium* (Pl 5) had the highest growth (optical density) at a pH of 6.5. This was followed by the growth at the pH of 5.5. There was no growth at the pH of 3.0. (Figure 13 and 14).

Comment [DG17]: pH



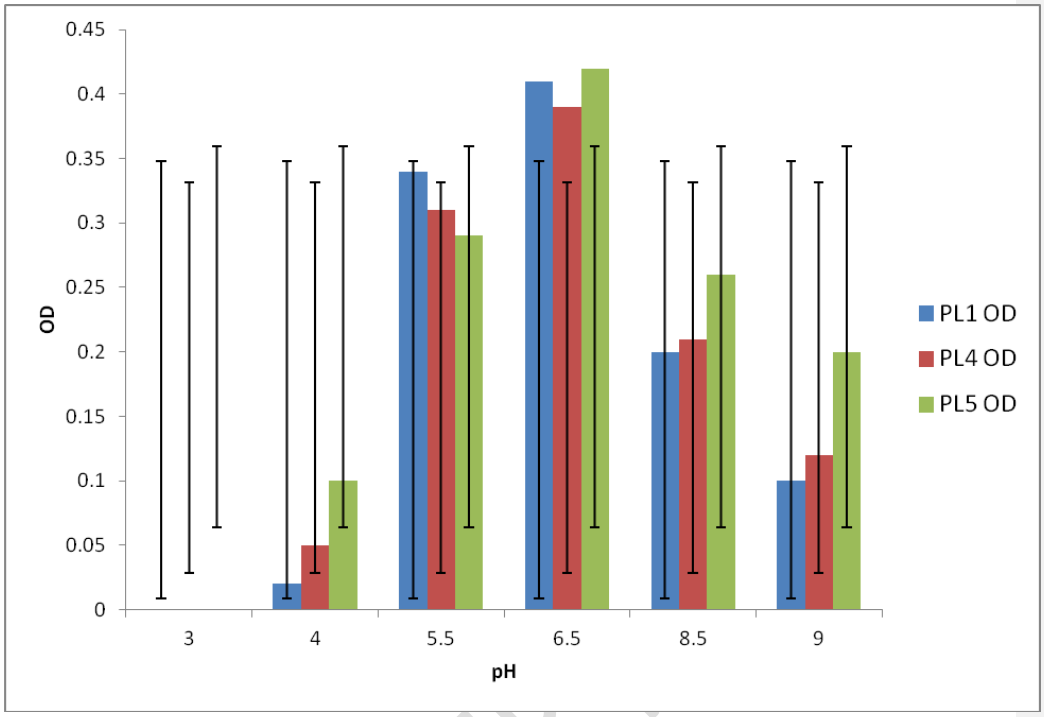
KEY: OD = Optical Density

Figure 11: The effect of temperature on the enterococcal isolates using optical density as a parameter.



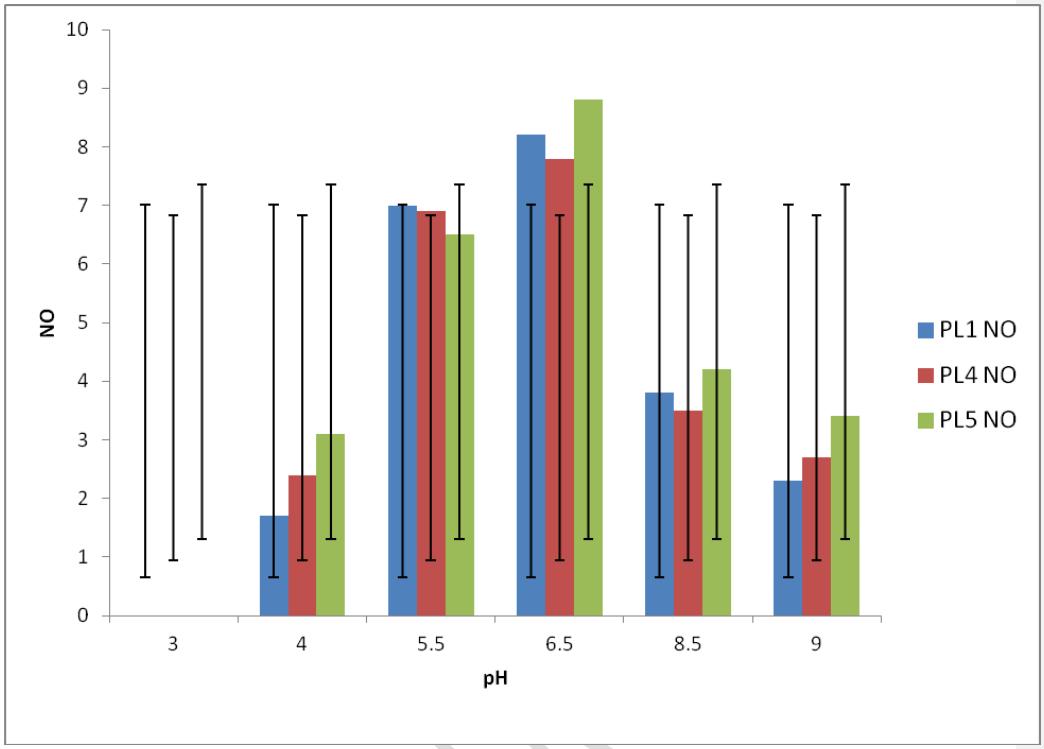
NO = Log_{10}cfu/ml

Figure 12: Effect of temperature on the enterococcal isolates using cell count as a parameter.



KEY: OD = Optical Density

Figure 13: Effect of pH variations on the enterococcal isolates using optical density as a parameter



NO = Log_{10}cfu/ml

Figure 14: Effect of pH on the enterococcal isolates using cell count as a parameter.

Discussion

This chemical commonly called common salt apart from serving as important additive to our food, is a preservative that is 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

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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].

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 [12]. The antimicrobial properties of chloroxylenol, the main chemical constituent of Dettol and other chlorinated phenols have been extensively studied [13]. The antimicrobial properties of the disinfectant against some pathogenic bacteria have earlier been reported [14]. 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 [15]. Furthermore, despite the extensive dental use of chlorhexidine, strains of *Streptococcus mutans* remain sensitive to it [16]. Up to 1993, therefore there was little or no evidence of

plasmid associated resistance of non- staphylococcal gram positive bacteria to antiseptics and disinfectants [16]. In this study, 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. 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 an equilibrium with hypochlorous acid, $HOCl^-$ [17]. 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 [17]. 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.

All the three representative isolates of *E. faecium, E. faecalis* and *E. avium*, recorded the highest growth (optical density) at the temperature of 37^0c followed by the temperature of

35^0c. There was no growth of bacteria at the temperature of 0^0c and 60^0c. This is comparable to the report of Fisher and Philips [6] which stated that though enterococci are not capable of forming spores, they are tolerant of high temperature range of 10^0c to 45^0c with optimum growth at 35^0c.The ability to grow at 10°C and 45°C, to initiate growth in 6.5% NaCl broth at pH 9.6 and to survive at 60°C for 30 min, are commonly used to separate enterococci from other streptococci [18, 19].

All the representative isolates of *E. faecium, E. faecalis* and *E. avium* had the highest growth at the pH of 6.5 followed by the growth at pH of 5.5. There was mild growth at the pH of 9 (alkaline pH). There was no growth at the pH of 3.0 (acid pH) for all the representative isolates. This is comparable to the report of Fisher and Philips [6] which demonstrated that though enterococci were non-spore forming, they grow at the pH range of 4.5-10

Conclusion

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.

Comment [DG20]: Conclusion is very vague. What do you conclude of your study? What are your suggestions for use or improvement in usage of these disinfectants/antiseptics?

References

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