THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Antibiotic Sensitivity Pattern of Streptococci Isolated from Different Environment in the Federal University of Technology, Akure, Nigeria

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Abstract:
A study was carried out to isolate streptococci associated with polluted air, water, soil and human samples collected from different sites within the Federal University of Technology, Akure, Ondo State, Nigeria. The samples were analyzed microbiologically using standard microbiological techniques. Twenty-two isolates of twelve species were obtained from the study. The isolates were identified as Streptococcus pneumoniae (HNR), S. pyogenes (ATT), S. agalactiae (SPY), S. anginosus (ARF1), S. salivarius (AWT2), Enterococcus avium (ARF1), E. faecium (WPG1), E. faecalis (WPG3), S. mutans (ABT), S. oralis (AWT1),S. sanguis (AWT3) S. pneumonia (HTR), S. pyogenes (HSK1), S. agalactiae (HSK2), S. anginosus (WSB), S. salivarius (SCM2), S. equi(HER), E. faecium (SCM1),E. faecalis (SGH), S. mutans (HMT), S. oralis (WPG2) and S. sanguis (SUP). The antibacterial activity of 13 commercial drugs against the isolates was assayed by Kirby-Bauer disc diffusion method and the percentage of resistance by Streptococcus sppto the antibiotics are as follows; pefloxacin (18.2 %), gentamicin (13.6 %), septrin (4.5 %), ampicillin (50 %), amoxicillin (50 %), cefuroxime (22.7 %), ceftriaxone (27.2 %), ciprofloxacin (13.6 %), streptomycin (18.2 %), erythromycin (18.2 %) tetracycline (27.2 %), methicillin (40.9 %) and vancomycin (68.3 %). Among the antibacterial drugs tested pefloxacin, ciprofloxacin, gentamicin, streptomycin and septrin showed maximum percentage of inhibition against Streptococcus spp. Vancomycin resistance was observed in the following isolates - E. faecalis (SGH), E. faecium (SCM1), S. oralis (WPG2) and S. salivarius(SCM2)signifying the presence of vancomycin resistant enterococci (VRE) from environmental samples. Enterococcus faecium (WPG1) isolated from ground water source was resistant to a wide range of antibiotics but susceptible to vancomycin.

Keywords: *Antibiotic sensitivity, streptococci, different sources.*

1. Introduction

Streptococcus is a genus of sphericalGram positivebacteria belonging to the phylumFirmicutesand the lactic acid bacteria group (Ryan and Ray, 2004). Cellular division occurs along a single axis in these bacteria and thus they grow in chains or pairs, hence the name from Greek "streptos", meaning easily bent or twisted, like a chain (Facklam, 2002). Most streptococci are oxidase and catalase negative, and many are facultative anaerobes (Tan and File, 2013). Carbohydrates are metabolized fermentatively; lactic acid is the major metabolite. Streptococci produce the enzyme leucine amino peptidase (LAP), which has also been called leucinearylamidase (Musher, 2009).

Many species produce haemolysis when grown on blood agar, due to the production of toxins called haemolysins (Tan and File, 2013). Early attempts to distinguish between pathogenic and commensal streptococci recognized different types of haemolysis around colonies on blood agar plates (Patterson, 1996, Kilian, 2002). Haemolysis is the lysis (bursting) of red blood cells. It may be brought about by bacterial toxins called haemolysins (Ross, 1996, Cheesbrough, 2006). Streptococci are classified in a number of ways on the basis of phenotypic characteristics, but these do not correspond to phylogenetic relationships (Facklam, 2002). Colonies of streptococci belonging to the pyogenic group are generally surrounded by a clear zone, usually several millimeters in diameter, caused by lysis of red blood cells in the agar medium induced by bacterial haemolysins (Greenwood *et al*., 2012). This is called beta (β) haemolysis (Patterson, 1996; Greenwood *et al*., 2012). In contrast, most commensal streptococci give rise to a green discoloration around colonies on blood agar. This phenomenon is termed alpha (α) haemolysis. The factor causing the green discoloration is not a haemolysin, but hydrogen peroxide, which oxidizes haemoglobin to the green methaemoglobin (Ryan and Ray, 2004). Collectively, commensal streptococci are often called 'viridans streptococci' (viridis= green) which refers to their alpha haemolytic property (Ryan and Ray, 2004). This term also includes the few streptococci (e.g. the salivarius and mutans groups of streptococci) that induce neither α- nor β-haemolysis. Moreover, in common usage, the term excludes *Streptococcus pneumoniae*, although this specie is also α-haemolytic. Gamma (γ) haemolytic species cause no hemolysis (Facklam, 2002).

Antibiotic resistance is a form of drug resistance whereby some (or, less commonly, all) sub-populations of a microorganism, usually a bacterial species, are able to survive after exposure to one or more antibiotics; pathogens resistant to multiple antibiotics are considered *multidrug resistant* (MDR) (Guillaume *et al.,* 2011). Some of the common types of drug-resistant bacteria include ESBL (extended spectrum beta-lactamase) and VRE (vancomycin resistant enterococci) (Todar, 2011).

Streptococci are naturally susceptible to penicillin and to a wide range of other antibiotics. However, acquired resistance to other agents has become an increasing problem. Although streptococci are intrinsically resistant to amino glycosides, these agents interact synergic ally with penicillins and the combination is often used in the treatment of streptococcal and enterococcal endocarditis.

Penicillin resistance has never been detected in *S. pyogenes*. Most strains of *S. agalactiae*are susceptible to penicillins, macrolides and glycopeptides. Resistance to penicillin in pneumococci and viridans streptococci, caused by mutations in the target penicillin- binding proteins, is widespread. These mutations have accumulated in strains of *S. mitis*and *S. oralis* and the altered genes have subsequently been transferred by genetic transformation to *S. pneumoniae* (Tan and File, 2013). Penicillin resistance in pneumococci and other viridans streptococci is often linked to resistance to several other antibiotics. Resistance to erythromycin, tetracycline and chloramphenicol are common, and even tolerance to vancomycin has been reported. (Musher, 2009).

Unlike other streptococci, enterococci are intrinsically resistant to cephalosporins. Sensitivity to penicillins and other antibiotics varies widely, and clinical isolates must be tested for their susceptibility. Vancomycin resistance has been observed in enterococci and is a problem in high-dependency areas of some hospitals (Ruhe*et al.,* 2004). The emergence of antibiotic resistant bacterial pathogens has become a major public health concern (Cheng *et al.,* 2004; Mafu*et al.,* 2009).

Faecal coliforms and fecal streptococci (including the sub-group enterococci) are traditionally used as the indicator organisms to guarantee microbiological safety of drinking water, natural water resources (Sidhu and Toze, 2009).

The objectives of this research were to:

- isolate streptococci from different environments i.e. air, water, soil and human bodies;
- determine the antibiotic sensitivity pattern of these isolates.

2. Materials and Methods

2.1. Isolation of Streptococci from the Samples.

The samples from soil and water were microbiologically analyzed using the pour plate method (Fawole and Oso, 2004; Cheesbrough, 2006)). The bacterial counts were thereafter, enumerated. Individual colonies were identified by morphological and biochemical techniques using the taxanomic scheme of Beygey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Morphological and biochemical characteristics included Gram Staining**,** Determination of haemolysis of *Streptococcus* on blood agar in the presence and absence of CO_2, catalase test, coagulase test, starch hydrolysis test, growth in 6.5% NaCl, bile solubility test, bile esculin hydrolysis test, pyrrolidonylarylamidase test (PYR), optochin sensitivity test, bacitracin sensitivity test, Voges-Proskauer Test (VP) and sugar fermentation test (arabinose, sucrose, mannitol, lactose and glucose).

2.2. Determination of Lactic Acid Production

Using the method of Saranya and Hemashenpagam (2011), the test organisms were grown on MRS broth for 72 hours and samples taken at 12 hours' interval. To 25 ml of broth culture of organisms, 3 drops of phenolphthalein were added as indicator. From the burette, 0.1N NaOH were slowly added to the sample until pink colour appeared. Eachml of 0.1 NaOH is equivalent to 90.08mg of lactic acid (AOAC, 2006).

2.3. Determination of Antibiotic Sensitivity Pattern of Streptococcus spp

The antibiotic sensitivity pattern of *Streptococcus*spp was evaluated using the Modified Kirby-Bauer technique of disc diffusion. *Streptococcus*spp were adjusted according to 0.5 McFarland standard which was prepared by adding 0.05ml of barium chloride ($BaCl_2$) (1.17% w/v $BaCl_2.2H_2O$) to 9.95ml of 0.18M H_2SO_4 (1.0%w/v) with constant stirring. The inoculums of test strains were adjusted to 1.5 x 10^8cfu/ml equal to that of the 0.5McFarland standard by adding sterile distilled water. The antimicrobial sensitivity of the test strains to 13 antibacterial drugs was determined by Kirby-Bauer disc diffusion method (Cheesbrough, 2006). Twenty milliliters of Muller Hinton agar melted and cooled at 45^0C and supplemented with five percent horse blood was poured into sterile petri plates and allowed to solidify completely. A lawn of test pathogen was prepared by evenly spreading 100µl inoculums (1.5 x 10^8cfu/ml) onto the entire surface of agar plate. The plates were allowed to dry before applying antibiotic disc. The discs were firmly applied to the surface of agar plates within 15 minutes of inoculation and incubated at 37^0C for 24hrs. The zones of inhibition were measured and compared with CLSI (2013) interpretative chart of zone sizes for *Streptococcus* species. The antibiotics used were erythromycin, tetracycline, ampicillin, amoxicillin/clavulanate, cetriaxone, cefuroxime, septrin, gentamicin, streptomycin, pefloxacin, vancomycin, flucoxacillin (methicillin) and ciprofloxacin discs.

2.4. Data Analysis

Data are presented as mean ± SE (standard error). Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using SPSS window 7 version 17 software. For all the tests, the significance was determined at the level of $P<0.05$ and 95% confidence limits.

3. Results

3.1. Population of Streptococcusspp

Table 1 shows that from the air source, the highest number of microbial load was obtained from dumpsite (27.50×10^2cfu/ml) while least was from the roof (12.92×10^2cfu/ml). The highest microbial load from human source was obtained from mouth (20.75×10^2cfu/ml) and the least from the ear (12.42×10^2cfu/ml). Table 2 shows that the highest microbial load from water samples was from surface water (24.92×10^5cfu/ml) while the least was from ground water (12.58×10^5) while the highest microbial load from soil samples was 25.25×10^5cfu/g from soil polluted with hydrocarbon and the least from unpolluted soil (11.75×10^5cfu/g).

3.2. Biochemical and Morphological Characterictics of Streptococcusspp

The biochemical characteristics are shown in Table 2. The isolates were identified as *Streptococcus pneumoniae* (HNR), *S. pyogenes* (ATT), *S. agalactiae* (SPY), *S. anginosus* (ARF1), *S. salivarius* (AWT2), *Enterococcus avium* (ARF1), *E. faecium* (WPG1), *E. facials* (WPG3), *S. mutans*(ABT), *S. oralis*(AWT1), *S. sanguis* (AWT3) *S. pneumoniae* (HTR), *S. pyogenes*(HSK1), *S. agalactiae*(HSK2), *S. anginosus*(WSB), *S. salivarius*(SCM2), *S. equi* (HER), *E. faecium* (SCM1), *E. faecalis* (SGH), *S. mutans*(HMT), *S. oralis*(WPG2) and *S. sanguis*(SUP). All the twenty-two (22) isolates were Gram-positive cocci. All the isolates grew on blood agar with grey to cream colonies. There was acid production and no gas production during sucrose and lactose fermentation for all isolates except for *Streptococcus equi* which exhibited no change.

3.3. Lactic Acid production

All the isolates were found to produce lactic acid and the concentration of lactic acid produced increased with time. Figure 1 shows the concentration of lactic acid produced in MRS broth after 72 hours of incubation by isolates from air. *Streptococcus mutans* had the highest concentration (1.24g/L), *E. avium* (0.59g/L), *S. oralis* (0.73g/L), *S. salivarius* (0.68g/L), *S. pyogenes* (0.63g/L), while *S. sanguis* had the least concentration (0.54g/L). Figure 2 shows the concentration of lactic acid produced by isolates from water after 72 hours of incubation. *Streptococcus faecalis* produced the highest concentration (1.21g/L), *S. oralis* (0.70 g/L) *and S. anginosus* (0.76g/L) while *S. faecium* (0.58g/L) produced the least.

Figure 3 shows the lactic acid concentration produced by isolates from the soil samples; *S. agalactiae* had the highest concentration of 1.08g/L, *E. faecalis* (0.63g/L), *E. faecium* (0.90g/L), *S. salivarius*(0.59g/L) while *S. sanguis* had the least (0.54g/L).

Figure 4 shows the concentration of lactic acid produced by isolates from the human body; S. equi produced the highest (0.98g/l) concentration of lactic acid, *S. pneumoniae* (0.72g/L), *S. pyogenes* (0.67g/L), *S. pnemoniae* (0.68g/L), *S. mutans* (0.85g/L) while *S. agalactiae* produced the least (0.59g/L).

SAMPLE	Mean colony count
ARF	$12.92\pm2.21^{a}\times10^2$cfu/ml
ABT	$16.25\pm4.77^{b}\times10^2$cfu/ml
ATT	$16.33\pm3.22^{d}\times10^2$cfu/ml
AWT	$27.50\pm1.79^{d}\times10^2$cfu/ml
HER	$12.42\pm1.75^{a}\times10^2$cfu/ml
HNR	$15.42\pm1.90^{d}\times10^2$cfu/ml
HSK	$13.84\pm1.72^{d}\times10^2$cfu/ml
HTR	$13.84\pm1.61^{d}\times10^2$cfu/ml
HMT	$20.75\pm4.07^{d}\times10^2$cfu/ml
WPG	$12.58\pm2.00^{a}\times10^5$cfu/ml
WSB	$24.92\pm3.26^{d}\times10^5$cfu/ml
WTP	$13.75\pm2.22^{a}\times10^5$cfu/ml
SPY	$17.16\pm2.75^{c}\times10^5$cfu/g
SCM	$25.25\pm4.61^{d}\times10^5$cfu/g
SUP	$11.75\pm2.44^{a}\times10^5$cfu/g

Table 1: Microbial Load of samples

Data are presented as mean ± SE of three replicates with significant increases and the alphabets showed that mean ± SE in the same row with different superscript are significantly changed. ($p < 0.05$).

- KEY:

ARF: air from roof
AWT-air from dumpsite
ATT-air from toilet
ABT-air from bathroom
HER-ear
HNR-nose
HSK-skin
HTR-throat
HMT-mouth
WPG-exposed ground water WSB-polluted surface water
WTP-tap water
SGH-soil from generator house SCM-soil with hydrocarbon waste
SCM-soil with hydrocarbon waste
SPY-soil with human waste SUP-unpolluted soil
SUP-unpolluted soil

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
TESTS	**ARF1**	**ARF2**	**AWT1**	**AWT2**	**AWT3**	**ATT**	**ABT**	**WPG1**	**WPG2**	**WPG3**	**WSB**	**SGH**	**SCM1**	**SCM2**	**SUP**	**SPY**	**HER**	**HNR**	**HSK1**	**HSK2**	**HTR**	**HMT**
Haemolysis	β,γ	α	α,β	γ	α,β	β	α,β	α,γ	α,β	β,γ	β,γ	β,γ	α,γ	γ	α, β	β	β	α	β	β	α	α,β
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CO_2 For Growth	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	+/-	+	-	+/-	+
6.5% NaCl	-	+	-	-	-	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-
Bile Esculin Hydrolysis	-	+	-	-	-	-	-	+	-	+	-	+	+	-	-	+	-	+	-	+	+	-
Starch Hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bile Solubility	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
VP	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	+	-	+
PYR	-	+	-	-	-	+	-	+	-	+	-	+	+	-	-	-	-	-	+	-	-	-
Phosphatase	+	-	+	+	-	+	-	-	+	-	+	-	-	+	-	+	+	-	+	+	-	-
Bacitracin Sensitivity	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-
Optochin Sensitvity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
Catalase	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Coagulase	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+
Mannitol	+	+	-	-	-	+	+	+	-	+	+	+	+	-	-	-	-	+	+	-	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Arabinose	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-

Table 2: Biochemical characteristics of Streptococcus species isolated from air, soil, water and human samples.

- KEYS:

+-positive, -negative 1. *S. anginosus*2. *E. avium* 3. *S. oralis* 4. *S. salivarius* 5. *S. sanguis* 6. *S. pyogenes* 7. *S. mutans* 8. *E. faecium* 9. *S. oralis*

10. *E. faecalis* 11. *S. anginosus*12. *E. faecalis* 13. *E. faecium*14. *S. salivarius* 15. *S. agalactiae*16. *S. sanguis* 17. *S. equi*18. *S. pneumoniae*

19. *S. pyogenes*20. *S. agalactiae*21. *S. pneumoniae*22. *S. mutans*

ARF: air from roof, AWT-air from dumpsite, ATT-air from toilet, ABT-air from bathroom, WPG-ground water, WSB-surface water, SGH-soil from generator house, SCM-soil with hydrocarbon waste, SPY-soil with human waste, SUP-unpolluted soil**,** HER-ear, HNR-nose,

HSK-skin, HTR-throat, HMT-mouth

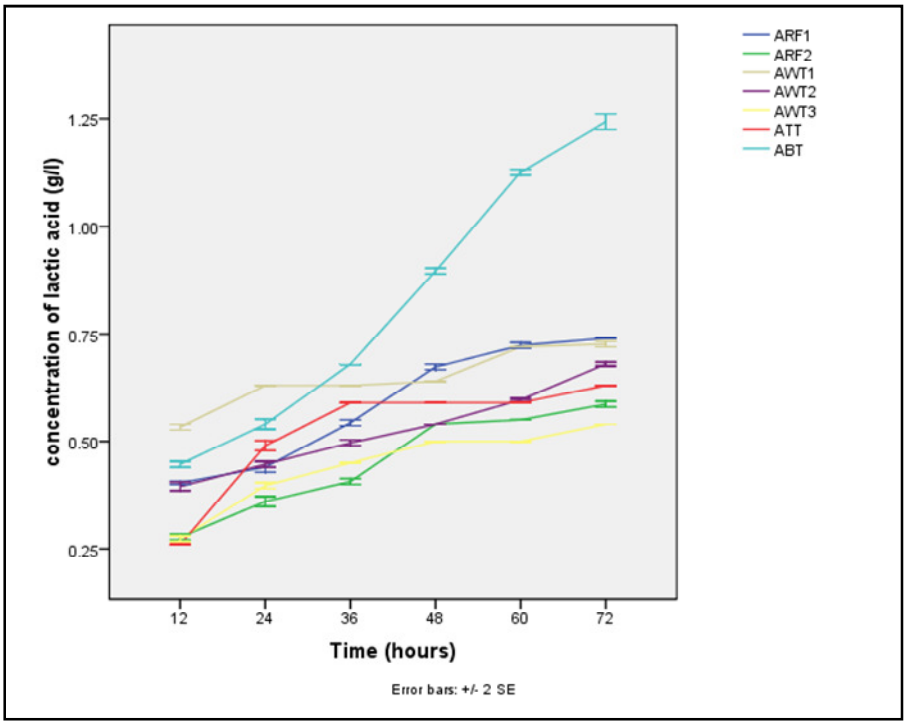

Figure 1: Concentration of Lactic Acid Produced By Isolates from Air

- KEYS:

ARF (Roof):1-*S. anginosus*, 2.-*E.avium*
AWT (Dump):1.*S.oralis* 2. *S. salivarius*3.*S. sanguis*
ATT (Toilet): *S. pyogenes*
ABT (bathroom): *S. mutans*

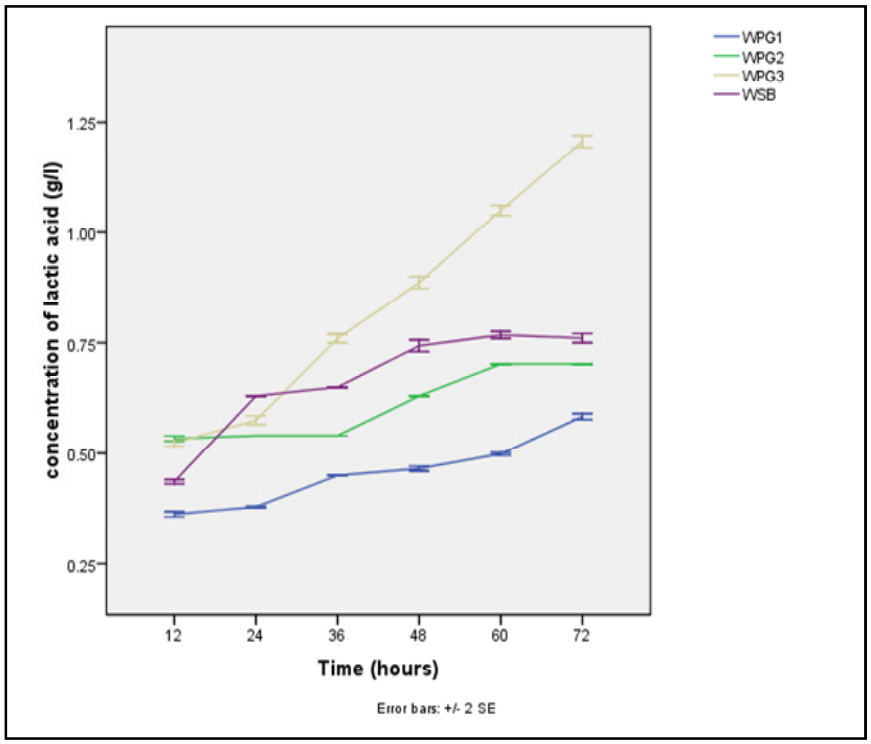

Figure 2: Concentration of Lactic Acid Produced By Isolates from Water

- KEYS:

WPG (ground water):1. *E. faecium* 2. *S. oralis* 3. *E. faecalis*
WSB (surface water): *S. anginosus*

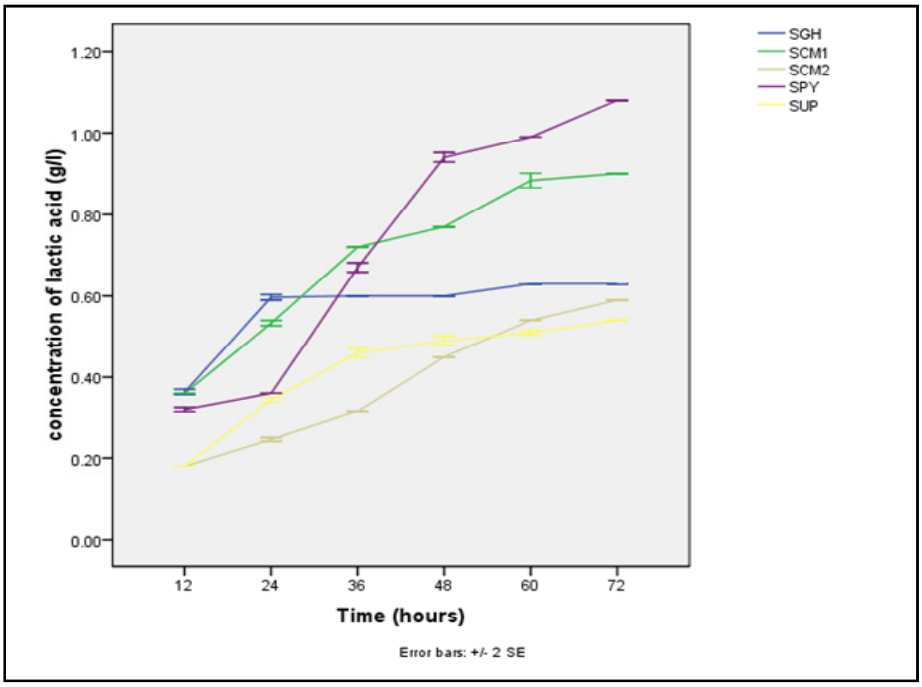

Figure 3: Concentration of Lactic Acid Produced By Isolates from Soil

- KEYS:

SGH (soil from generator house) - *E. faecalis*,
SCM (soil with hydrocarbon) - 1. *E. faecium* 2. *S. salivarius*
SPY (soil with human waste)-*S. agalactiae,*
SUP (unpolluted soil)-*S. sanguis*

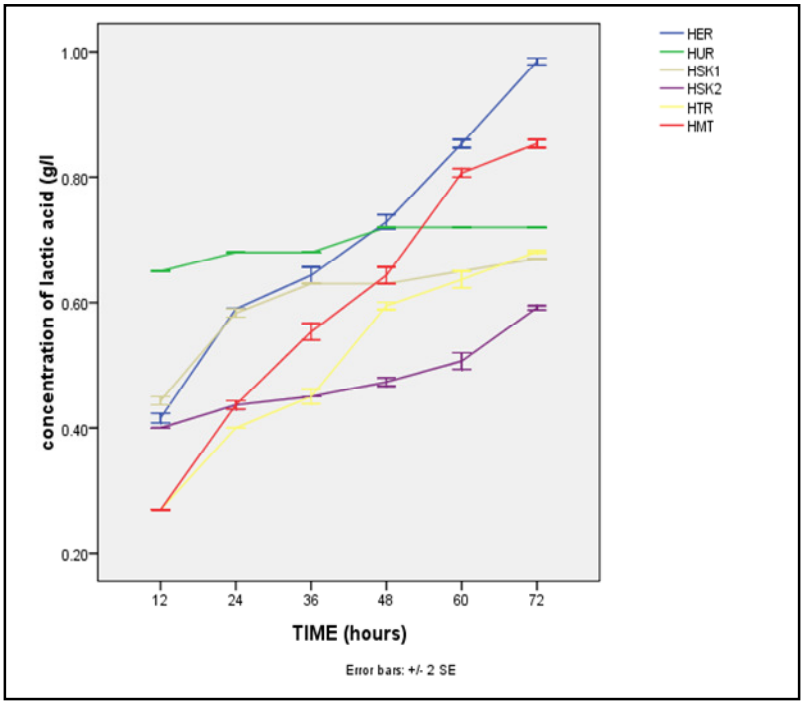

Figure 4: Concentration of Lactic Acid Produced By Isolates from Human Sources

- KEY:

HER (ear)-*S. equi*
HNR (nose)-*S. pneumoniae*
HSK (skin)-1. *S. pyogenes* 2. *S. agalactiae*
HTR (throat)-*S. pneumoniae*
HMT (mouth)- *S. mutans*

4. Antibiotic Sensitivity Pattern

Table 3a and 3b show the mean diameter zones of inhibition of commercial antibiotics against *Streptococcus* isolates. The antibacterial activity of 13 commercial drugs was assayed by Kirby-Bauer disc diffusion method and data on the diameter of inhibition zones produced by *Streptococcus spp s*hows that the mean range of diameter inhibition zones observed were pefloxacin (12.20-26.60mm), gentamicin (9.73-22.50mm), septrin (8.00-24.17mm), ampicillin (10.00-24.00mm), amoxicillin (12.07-23.00mm), cefuroxime (12.03-27.00mm), ceftriaxone (15.13-27.67mm), ciprofloxacin (9.03-25.37mm), streptomycin (9.47-24.07mm), erythromycin (11.03-26.63mm) tetracycline (12.10-26.63mm), methicillin (13.80-22.10mm) and vancomycin (12.53-25.20mm). Among the antibacterial drugs tested pefloxacin, cefuroxime, ceftriaxone tetracycline and vancomycin showed maximum zone of inhibition against *Streptococcus* spp.

Table 4 shows the antibiotic sensitivity pattern of commercial antibiotics against *Streptococcus* isolates. *Streptococcus agalactiae* isolated from soil contaminated with human waste was susceptible to all antibiotics while *Enterococcus faecium* isolated from a ground water source was resistant to nine (9) of the antibiotics but was susceptible to vancomycin.

Table 5 shows that no antibiotic had 100% susceptibility from all the isolates but pefloxacin, septrin, gentamicin, streptomycin and ciprofloxacin had the highest susceptibility (77.3%, 17/22) and vancomycin had 68.3% (15/22) from the isolates. Tetracycline, ampicillin and amoxicillin had the least susceptibility (50%).

PROBABLE ISOLATES	Pefloxacin	Gentamicin	Septrin	Ampicillin	Amoxicillin	Cefuroxime	Ceftriaxone	Ciprofloxacin	Streptomycin	Erythromycin	Tetracycline	Methicillin	Vancomycin
S. anginosus (ARF1)	20.23±0.12^{b}	18.17±0.09^{a}	20.3±0.15^{b}	21.03±0.^{d}07^{c}	22.17±0.07^{c}	24.17±0.12^{e}	25.07±0.07^{e}	22.07±0.09^{c}	20.17±0.12^{b}	22.17±0.10^{c}	23.17±0.09^{e}	15.17±0.09^{a}	19.17±0.12^{a}
E. avium (ARF2)	21.07±0.10^{c}	18.07±0.10^{b}	19.10±0.07^{c}	15.07±0.07^{a}	14.10±0.10^{a}	25.13±0.13^{d}	26.07±0.07^{d}	24.07±0.07^{d}	18.37±0.12^{b}	21.10±0.06^{c}	20.07±0.07^{c}	17.27±0.15^{b}	15.17±0.09^{a}
S. oralis (AWT1)	20.23±0.12^{b}	19.13±0.09^{a}	21.00±0.00^{c}	20.13±0.13^{b}	23.10±0.06^{c}	26.07±0.03^{d}	26.03±0.10^{d}	23.10±0.10^{a}	16.27±0.15^{d}	24.17±0.09^{c}	21.13±0.13^{c}	20.23±0.12^{b}	25.20±0.06^{d}
S. salivarius(AWT2)	16.20±0.12^{b}	10.17±0.17^{a}	17.00±0.00^{b}	14.03±0.03^{a}	16.07±0.07^{b}	19.10±0.10^{c}	24.10±0.06^{d}	21.00±0.00^{c}	22.23±0.12^{d}	21.10±0.06^{c}	19.07±0.07^{c}	13.80±0.12^{a}	22.00±0.06^{d}
S. sanguis (AWT3)	19.07±0.07^{b}	11.10±0.10^{a}	18.00±0.00^{b}	16.93±0.03^{a}	15.00±0.00^{a}	24.83±0.17^{d}	25.00±0.00^{d}	22.13±0.07^{c}	19.10±0.06^{b}	24.97±0.09^{d}	24.13±0.09^{c}	22.07±0.03^{c}	20.03±0.09^{b}
S. pyogenes (ATT)	21.93±0.07^{c}	20.13±0.09^{b}	19.03±0.03^{a}	20.20±0.10^{b}	22.07±0.07^{c}	26.10±0.10^{d}	24.93±0.07^{d}	19.03±0.03^{a}	22.13±0.09^{c}	23.03±0.03^{d}	24.10±0.06^{d}	16.07±0.07^{a}	21.00±0.00^{b}
S. mutans (ABT)	22.07±0.07^{c}	17.07±0.07^{a}	20.17±0.09^{b}	12.00±0.06^{a}	20.07±0.03^{b}	24.13±0.07^{c}	17.96±0.03^{a}	20.10±0.10^{b}	23.10±0.06^{c}	12.13±0.13^{a}	14.93±0.03^{a}	20.00±0.00^{b}	23.06±0.03^{c}
E. faecium (WPG1)	13.07±0.07^{c}	21.03±0.03^{d}	8.00±0.00^{a}	10.00±0.00^{a}	12.07±0.07^{b}	25.00±0.00^{d}	27.07±0.07^{d}	9.03±0.03^{a}	10.07±0.07^{b}	11.03±0.03^{b}	13.07±0.07^{c}	20.10±0.10^{c}	21.07±0.07^{d}
S. oralis (WPG2)	22.03±0.03^{c}	19.00±0.00^{b}	12.07±0.03^{a}	24.00±0.00^{d}	21.03±0.03^{c}	20.03±0.03^{c}	24.03±0.03^{d}	19.07±0.07^{b}	17.03±0.03^{a}	23.03±0.03^{d}	18.10±0.10^{b}	20.03±0.03^{c}	13.07±0.07^{a}
E. faecalis (WPG3)	24.10±0.10	20.13±0.13^{b}	22.13±0.09^{c}	20.13±0.09^{b}	23.00±0.00^{d}	17.10±0.10^{a}	25.00±0.00^{d}	22.17±0.09^{c}	12.07±0.07^{a}	22.00±0.00^{c}	21.00±0.06^{c}	13.07±0.07^{a}	16.10±0.06^{a}
S. anginosus(WSB)	21.07±0.07^{c}	22.17±0.12^{d}	24.17±0.03^{d}	13.13±0.07^{a}	15.00±0.00^{b}	12.03±0.03^{a}	15.13±0.07^{b}	16.07±0.07^{c}	11.07±0.03^{a}	25.13±0.09^{d}	12.10±0.10^{a}	15.10±0.10^{b}	16.00±0.00^{a}
E .faecalis (SGH)	20.67±0.07^{c}	22.50±0.06^{a}	13.07±0.07^{b}	14.40±0.06^{c}	14.00±0.00^{b}	22.13±0.07^{f}	16.07±0.07^{d}	22.00±0.06^{d}	23.93±0.07^{d}	13.97±0.03^{e}	22.10±0.06^{b}	21.10±0.06^{e}	14.07±0.03^{a}
E. faecium (SCM1)	19.30±0.06^{c}	13.57±0.07^{a}	18.33±0.07^{b}	20.70±0.06^{c}	14.10±0.06^{b}	25.50±0.06^{f}	27.67±0.03^{f}	21.40±0.06^{d}	21.33±0.07^{d}	21.87±0.03^{e}	16.13±0.03^{b}	22.10±0.06^{e}	13.50±0.06^{a}
S. salivarius (SCM2)	25.10±0.00^{d}	21.20±0.00^{c}	16.00±0.00^{a}	20.00±0.00^{b}	21.00±0.00^{c}	27.00±0.00^{a}	26.07±0.07^{a}	24.17±0.03^{d}	20.77±0.03^{c}	14.20±0.06^{a}	25.60±0.06^{c}	17.10±0.06^{b}	12.53±0.03^{a}
S. agalactiae (SPY)	22.43±0.03^{a}	19.20±0.06^{b}	19.50±0.06^{a}	22.70±0.06^{a}	20.17±0.09^{a}	24.13±0.03^{b}	25.20±0.06^{b}	23.43±0.03^{a}	19.07±0.07^{a}	24.10±0.06^{a}	23.83±0.03^{a}	22.10±0.00^{a}	25.10±0.10^{a}
S. sanguis (SUP)	26.60±0.10^{c}	17.40±0.06^{a}	11.07±0.03^{a}	10.77±0.03^{a}	20.00±0.00^{b}	23.83±0.03^{c}	26.53±0.03^{c}	20.10±0.06^{b}	18.67±0.03^{b}	12.10±0.06^{a}	24.07±0.03^{c}	21.20±0.10^{b}	24.13±0.13^{c}
S. equi (HER)	23.10±0.06^{a}	20.10±0.06^{b}	19.27±0.03^{a}	20.13±0.03^{b}	21.43±0.03^{a}	24.87±0.09^{a}	27.47±0.07^{a}	25.37±0.03^{a}	21.10±0.06^{a}	25.10±0.06^{a}	26.17±0.09^{a}	22.17±0.03^{a}	23.90±0.06^{a}
S. pneumoniae (HNR)	14.20±0.06^{b}	13.10±0.06^{a}	20.17±0.09^{a}	12.20±0.06^{a}	13.60±0.06^{a}	13.37±0.07^{a}	16.10±0.06^{a}	17.13±0.03	9.47±0.03^{a}	26.63±0.13^{a}	23.23±0.13^{b}	14.07±0.07^{b}	22.50±0.06
S. pyogenes (HSK1)	20.07±0.03^{d}	18.27±0.03^{c}	13.17±0.03^{a}	20.67±0.03^{b}	22.17±0.03^{a}	22.33±0.03^{a}	19.60±0.07^{b}	23.43±0.03^{a}	16.13±0.07^{b}	24.40±0.06^{a}	24.67±0.03^{a}	20.53±0.03^{a}	22.97±0.33^{c}
S. agalactiae (HSK2)	12.20±0.10^{a}	17.23±0.03^{c}	18.20±0.06^{d}	14.57±0.03^{a}	15.63±0.03^{b}	17.23±0.03^{c}	26.00±0.00^{e}	22.20±0.12^{d}	13.53±0.07^{a}	17.70±0.06^{c}	15.37±0.03^{b}	15.13±0.03^{b}	19.13±0.07^{d}
S. pneumoniae (HTR)	12.90±0.06^{c}	9.73±0.03^{a}	18.00±0.00^{b}	21.03±0.03^{b}	15.13±0.07^{d}	26.07±0.07^{c}	25.03±0.03^{f}	12.27±0.03^{a}	18.30±0.06^{d}	24.47±0.03^{e}	23.47±0.03^{c}	16.70±0.00^{b}	22.17±0.03^{b}
S. mutans (HMT)	19.47±0.03^{a}	18.67±0.03^{a}	16.50±0.06^{c}	16.20±0.06^{b}	17.27±0.03^{f}	25.23±0.03^{b}	15.17±0.03^{a}	16.17±0.03^{b}	18.47±0.03^{a}	18.13±0.03^{a}	24.27±0.03^{a}	22.10±0.06^{b}	23.07±0.03^{a}

Table 3: Diameter zones of inhibition of antibacterial drugs against Streptococcus isolates (in millimeters)

Data are presented as mean ± SE of three replicates with significant increases and the alphabets showed that mean ± SE in the same row with different superscript are significantly changed. ($p < 0.05$)

KEYS: SGH-soil from generator house, SCM-soil with hydrocarbon waste, SPY-soil with human waste, SUP-unpolluted soil
HER-ear HNR-nose , HSK-skin, HTR-throat, HMT-mouth

PROBABLE ISOLATES	PEF	CN	SXT	AMP	AMC	CXM	CRO	CIP	S	E	TE	MET	VA
***S. anginosus* (ARF1)**	S	S	S	S	S	S	S	S	S	S	S	R	S
***E. avium* (ARF2)**	S	S	S	R	R	S	S	S	S	S	I	R	I
***S. oralis* (AWT1)**	S	S	S	S	S	S	S	S	S	S	I	S	S
***S. salivarius*(AWT2)**	I	R	S	R	R	R	S	S	S	S	I	R	S
***S. sanguis* (AWT3)**	S	R	S	R	R	S	S	S	S	S	S	S	S
***S. pyogenes* (ATT)**	S	S	S	S	S	S	S	S	S	S	S	R	S
***S. mutans* (ABT)**	S	S	S	R	S	S	S	S	S	R	R	S	S
***E. faecium* (WPG1)**	R	S	R	R	R	S	R	R	R	R	R	S	S
***S. oralis* (WPG2)**	S	S	I	S	S	S	S	S	S	S	R	S	R
***E. faecalis* (WPG3)**	S	S	S	S	S	R	S	S	R	S	I	R	I
***S. anginosus*(WSB)**	S	S	S	R	R	R	R	I	R	S	R	R	I
***E. faecalis* (SGH)**	S	S	I	R	R	I	R	S	S	R	I	S	R
***E. faecium* (SCM1)**	S	I	S	S	R	S	S	S	S	S	R	S	R
***S. salivarius* (SCM2)**	S	S	S	S	S	S	S	S	S	I	S	R	R
***S. agalactiae* (SPY)**	S	S	S	S	S	S	S	S	S	S	S	S	S
***S. sanguis* (SUP)**	S	S	I	R	S	I	S	S	S	R	S	S	S
***S. equi* (HER)**	S	S	S	S	S	S	S	S	S	S	S	S	S
***S. pneumoniae* (HNR)**	R	I	S	R	R	R	R	R	R	S	S	R	S
***S. pyogenes* (HSK1)**	S	S	I	S	S	I	R	S	S	S	S	S	S
***S. agalactiae* (HSK2)**	R	S	S	R	R	R	S	S	I	I	R	R	S
***S. pneumoniae* (HTR)**	R	R	S	S	R	S	S	R	S	S	S	R	S
***S. mutans* (HMT)**	S	S	S	R	R	S	R	I	S	I	S	S	S

Table 4: Antibiotic sensitivity pattern of Streptococcus isolates

KEYS: R- resistant to antibiotics, I- intermediate (bacteriostatic) to antibiotics, S- susceptible to antibiotics, ARF-air from roof; AWT-air from dumpsite; ATT-air from toilet; ABT-air from bathroom; WPG-ground water; WSB-surface water; SGH-soil from generator house;
SCM-soil with hydrocarbon waste; SPY-soil with human waste; SUP-unpolluted soil;
HER-ear; HNR-nose; HSK-skin; HTR-throat; HMT-mouth;
PEF-pefloxacin; CN-gentamicin; SXT-septrin; AMP-ampicillin; AMC-amoxicillin;
CXM-cefuroxime; CRO-ceftriaxone; CIP-ciprofloxacin; S-streptomycin; E-erythromycin;
TET-tetracycline; MET-methicillin; VA-vancomycin.
With reference to CLSI (2013) interpretative chart of zone sizes.

S/N	Antibiotics	Disc Potency (µg)	Resistant No (%)	Intermediate No (%)	Susceptible No (%)
1	Pefloxacin	10	4(18.2)	1(4.5)	17(77.3)
2	Erythromycin	15	4(18.2)	3(13.6)	15(68.3)
3	Septrin	10	1(4.5)	4(18.2)	17(77.3)
4	Gentamicin	10	3(13.6)	2(9.1)	17(77.3)
5	Streptomycin	30	4(18.2)	1(4.5)	17(77.3)
6	Cefuroxime (Zinnacef)	30	5(22.7)	3(13.6)	14(63.7)
7	Ceftriaxone (Rocephin)	30	6(27.2)	0(0)	16(72.8)
8	Ciprofloxacin	10	3(13.6)	2(9.1)	17(77.3)
9	Ampicillin	30	11(50)	0(0)	11(50)
10	Amoxicillin	30	11(50)	0(0)	11(50)
11	Tetracycline	30	6(27.2)	5(22.7)	11(50)
12	Flucoxacillin(methicillin)	15	9(40.9)	0(0)	13(59.1)
13	Vancomycin	30	4(18.2)	3(13.6)	15(68.3)

Table 5: Percentage distribution of antibiotic sensitivity pattern

5. Discussion and Conclusion

The microbial count of streptococcal isolates from air sources was lower as compared with those from other environmental sources (soil and water). *Streptococcus mutans* (ATT) was the most effective producer of lactic acid while *S. sanguis* (AWT3 and SUP) were the least lactic acid producers. *Streptococcus mutans* from oral cavity was also an effective producer of lactic acid Streptococcal isolates was able to produce lactic acid within the period of incubation at various concentrations due to differences in species and growth. The lactic acid produced is a by-product that could cause a decline in the bacterial population after 72 hours of incubation.

This by-product has been known to inhibit the growth of pathogenic microorganisms in other studies (Misra*et al.,* 2012; Saranya and Hemashenpagam, 2011).

Misra*et al*. (2012) isolated *S. mutans* from clinical sources with the highest lactic acid production of 0.010g/L and was used to inhibit the growth of *Candida tropicalis* which is lower than the highest produced by *S. mutans* (1.24g/L) in this study. This is to say, with further studies on lactic acid production by *Streptococcus* species, it could serve as another source of inhibitory agent against some microorganisms. The ability of these isolates to produce lactic acid when utilizing carbohydrates as carbon source through the homofermentative pathway shows that they possess inherent genes for this activity despite their low rank among lactic acid bacteria.

As patterns of antibiotic use change, so do bacterial patterns of antibiotic resistance. Musher (2009) addressed apparent risk factors for the acquisition of antibiotic-resistant streptococcal isolates which include previous antibiotic use and recent streptococcal infections. In this study the antibiotics used belong to eight different classes; the penicillins (ampicillin, amoxicillin, flucloxacillin which is a variant of methicillin), the cephalosporins (cefuroxime, ceftriazone), the glycopeptide (vancomycin), aminoglycosides (gentamicin, streptomycin), macrolides (erythromycin), tetracycline, the quinolones (ciprofloxacin, pefloxacin) and sulphonamide and trimethoprim (septrin). Antibiotics were selected because of their widespread use in animals and/or humans: amoxicillin (AMX), ampicillin (AMP), flucoxacillin (MET), cefuroxime (CXM), ceftriazone (CRO), erythromycin (E), gentamicin (GN), streptomycin (S), ciprofloxacin (CIP), pefloxacin (PEF), septrin (SXT), tetracycline (TET) and vancomycin (VAN). All these drugs are used in both humans and animals except vancomycin which is used in humans only (Wiggins *et al*., 1999).

Most of the streptococci isolates were susceptible to pefloxacin, ciprofloxacin, gentamicin, streptomycin and erythromycin. Some were bacteriostatic to vancomycin, septrin and tetracycline. The most resistant isolate to antibiotics was *Enterococcus faecium* isolated from ground water while the most susceptible to antibiotics was *Streptococcus agalactiae* isolated from soil polluted with human waste (urine). Resistance to penicillins and tetracycline was higher when compared to the other six groups of antibiotics. Resistance to antibiotics of at least three different groups has been defined as multiple drug resistance (Kandakai and Dido, 2009). Multiple antibiotics resistance was high in this study which is similar when compared with those in some other studies, at least 41% of the isolates showed multiple drug resistance. The relatively high frequency of resistance of the isolates to antibiotics limits the choice of antimicrobials that can be used for the treatment of streptococcal infections. Streptococcal susceptibility to macrolides is high (about 68 percent) among isolates contrary to Kandakai and Dido (2009) which state that resistance to macrolides is very common in streptococci but prevalence varies from location to location (Although ampicillin and amoxicillin used in this study were only effective against 50% of the isolates, they were reported to be effective for treatment of oral infections caused by streptococci (Archana*et al*., 2011). Methicillin (penicillin) resistance was observed in *S. pyogenes*.

In this study, all the antibiotics used were effective against *Streptococcus agalactiae* and *S. equi*, these species are known to cause human and animal infections and thus these antibiotics can be recommended for the treatment of such infections (Roberts, 2002). Erythromycin has been recommended as alternative options for patients who are allergic to penicillin and are also widely used for antibiotic prophylaxis of endocarditis (Addy and Martin, 2005).

Varying rates of resistance to quinolones (even same generation) by *Streptococcus* have been reported (Agwu*et al.,* 2004). Therefore, the usual practice in some health establishments where susceptibility test is carried out on one quinolone and another is used for treatment either due to cost constraints or availability should be discontinued (Agwu*et al.,* 2004). None of the antibiotics tested was effective against all the isolates which makes antibiotic susceptibility testing imperative for all isolates of streptococci isolated from the environmental setting before treatment. The existence of multiple resistances poses a greater threat for the management of diseases caused by these bacteria (Kandakai and Dido, 2009).

There is continuous emergence of multiple antibiotic resistant streptococci from sources other than clinical samples. Vancomycin resistance was observed in isolates from soil and water samples- *E. faecalis*(SGH), *E. faecium*(SCM1), *S. oralis* (WPG2) and *S. salivarius* ((SCM2)signifying the presence of vancomycin resistant enterococci (VRE) from environmental samples. Sensitivity testing among isolates of same species is important to identify specific drug combinations with higher efficacy to inhibit such organisms.

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