EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 2
32-49
Year: 2019

PREVALENCE AND CHARACTERIZATION OF ENTEROCOCCAL INFECTIONS IN ENUGU STATE, NIGERIA

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DOI: 10.20959/ejbps20192-6820

Article Received on 25/11/2018 **Article Revised on 15/12/2018** **Article Accepted on 05/01/2019**

ABSTRACT
Enterococcus sp. considered a normal commensal of the intestine of man and other animals, is fast emerging as a pathogen, causing serious and life threatening nosocomial infections. A two year progressive study of this organism was carried out between July, 2012 and June, 2014 in Enugu metropolis and its environs in Eastern Nigeria. A total of one thousand and eight clinical samples of urine, blood, sputum, stool, aspirates and swabs such as high vaginal, urethral, ear, nasal, anal and wound samples and forty non-clinical samples of urine, urethral and high vaginal swabs were examined. Isolation and identification were based on standard procedures and biochemical tests. Nine organisms were isolated in this study: *E. coli* 152(24.1%), *Streptococcus* sp. 95(15%), *Enterococcus* sp. 68(10.8%), *Staph aureus* 64(10.1%), *Pseudomonas* sp. 63(10.0%), *Proteus* sp. 63 (10.0%), *Klebsiella* sp. 50(7.9%), *Candida sp.* 45(7.1%) and coagulase negative staph 32(5.1%). The prevalence of *Enterococcus* sp was 10.8% and was the third most common cause of infection; the first and second being *E. coli* and *Streptococcus* sp. respectively. Three species of *Enterococcus* were identified; *E. faecium* 39(57.4%), *E. faecalis* 25(36.8%) and *E. avium* 4(5.9%). It was observed that the incidence of *Enterococcus* sp was high and showed multiple drug resistance; it is therefore advised that more attention should be given to this organism if nosocomial infections and outbreaks are to be contained.

KEYWORDS: *Enterococci*, infection, prevelance, isolation and identification.

INTRODUCTION
Enterococci are gram positive, catalase negative cocci occurring singly or arranged in pairs or short chains, of size 0.6-2.5 microns, facultative anaerobic (i.e. they are capable of cellular respiration in both oxygen-rich and oxygen-poor environments.[1] Though, they are not capable of forming spores, enterococci are tolerant of high temperatures (10-45^{o}C) with optimum growth at 35^{o}C, pH (4.5-10) and high sodium chloride concentrations of up to 6.5%.[2] They hydrolyse aesculin in the presence of bile salts (Bile-aesculin Test). Because of their ability to ferment carbohydrates to produce lactic acid, the enterococci are referred to as typical lactic acid bacteria. Enterococci typically exhibit gamma haemolysis on sheep blood agar.[3]

Enterococci are usually perceived as commensal bacteria that co-exist with their hosts as part of the normal flora. While not particularly virulent, enterococci can be significant agents of disease in the hospital setting where patients' defenses have been compromised by catheterization, immune deficiencies or both.[4] Important clinical infections caused by enterococci include urinary tract infection, bacteremia, bacterial endocarditis, diverticulitis, meningitis and oesteomyelitis.[1] The virulence of enterococci is lower than that of organisms such as *Staph. aureus*.[5] However, enterococcal infections often occur in debilitated patients and as part of polymicrobial infections. These factors limit the ability of investigators to determine the independent contribution of enterococcal infections to mortality and morbidity.

The direct microscopic examination of Gram-stained smears of normally sterile clinical specimens such as blood may be useful for the diagnosis of enterococcal infections. Direct examination of certain non-sterile specimen may also be informative, but should not be

over-emphasized. However, only presumptive report of the presence of gram-positive cocci should be made as microscopy by itself cannot differentiate the enterococci from other gram positive cocci. Culture and appropriate identification techniques can be performed for confirmation.[5] In the laboratory, enterococci are distinguished by their morphological appearance on Gram stain and culture (gram-positive cocci that grow in chains) and their ability to (i) hydrolyze esculin in the presence of bile, (ii) their growth in 6.5% sodium chloride, (iii) their hydrolysis of pyrrolidonyl arylamidase and leucine aminopeptidase, and (iv) their reaction with group D antiserum. Before they were assigned their own genus, they were classified as group D streptococci.[6] The work of Schierl and Blazevic[7] demonstrated that up to 83% of *Enterococcus* could be identified by the rapid litmus milk reduction test. No false positives were observed. A negative result can be checked by culturing.

Trypticase soy-5% sheep blood agar, brain heart infusion-5% sheep blood agar or any blood agar base containing 5% animal blood supports the growth of *enterococci*.[6] *Enterococci* grow well at 35-37^{0}C and do not require an atmosphere containing increased CO_2, although some strains grow better in this atmosphere.[6] Aesculin, bile and azide containing media are excellent media for selective isolation of *enterococci* from specimens likely to contain gram negative bacteria.

Once isolated, the genus *Enterococcus* is subjected to confirmatory tests for speciation. AP1 20 strep identification system is a tool used in speciation of *enterococci and streptococci*. The test systems are stored in reactive tubes. This involves 21 parameters that are phenotypic characteristics of the species.

The present study was set out to assess the prevalence and characterization of enterococcal infection in Enugu Metropolis and environs in Eastern Nigeria.

MATERIALS AND METHODS

Study area

Samples for this study were sourced from:
Enugu State University of Technology (ESUT) Teaching Hospital, Parklane in Enugu Metropolis (ancient coal city) Enugu State, south east of Nigeria: This is a tertiary health institution that receives direct and referred patients from all the local government primary and secondary health facilities in Enugu and environs.

University of Nigeria Teaching Hospital (UNTH), Ituku/Ozalla in Enugu State, Nigeria. The hospital is situated in a boundary between Ituku in Awgu Local Government Area of Enugu State and Ozalla in Nkanu Local Government Area of Enugu State in eastern part of Nigeria about 50 kilometers from Enugu metropolis. It receives direct and referred patients from all the local government areas of the Enugu State and the neighboring Ebonyi, Abia, Imo, Benue and Kogi states. (see maps below).

Study design: This is a cross-sectional study. Three categories of patients were included in the study.

In-patients: 504 in-patients admitted in ESUT Teaching Hospital and University of Nigeria Teaching Hospital both in Enugu who submittedtheir samples of urine, wound swabs, aspirates, sputum, ear swabs, high vaginal swabs, urethral swabs, semen, CSF and blood to the Microbiology Departments for microscopy, culture and sensitivity.

Out-patients: 504 out-patients who visited ESUT Teaching Hospital, Parklane and University of Nigeria Teaching Hospital, Ituku/Ozalla and who submitted clinical samples to the Microbiology Departments for microscopy, culture and sensitivity.

Controls: 20 male and 20 female volunteers who did not have symptoms of any infection. They were selected from outside the hospital environment and were used as controls.

Ethical approval: Ethical approval was obtained from ESUT Teaching Hospital, Parklane and University of Nigeria Teaching Hospital, Ituku/Ozalla. Only patients who gave their informed consent were recruited for the study.

Precautions/Considerations: Patients who were on antibiotic therapy prior to sample collection were excluded to avoid the influence of antibiotic on the growth of the organism.

Samples were cultured within 2 hours of collection to avoid overgrowth by contaminants.

For blood culture, site of venous puncture was cleaned thoroughly with 70% alcohol to avoid contamination with skin flora.

For urine samples, patients were advised to collect midstream urine in order to avoid the isolation of contaminants from the outer surfaces of the urethra.

Female patients were advised to separate the labia before passing the urine after proper cleaning with tissue paper while male patients were also advised to clean the urethral opening with sterile tissue paper.

Sample 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.

Analytical methods

Macroscopy: The colour and appearance of urine, CSF, sputum, aspirates and semen were recorded.

Culture/Isolation Considerations

The urine samples were inoculated into 5% sheep blood agar and cystein lactose electrolyte deficient (CLED) agar plates with quantitative sterile wire loops (0.001 ml). These were incubated for 24 hours at 37^0C aerobically and microaerophilically by incubation in a candle jar. An estimation of colony forming units per millimeter was made and significant bacteriuria established using a colony count of 100,000 (10^5) or more per millimeter[8] for organisms growing in pure culture. Sputum, aspirates, CSF, swabs and semen were inoculated in 5% sheep blood agar and cystein lactose electrolyte deficient (CLED) agar with wire loop, incubated for 24 hours at 37^oC aerobically and microaerophilically in a candle jar.

Blood culture: This was done by aseptically collecting five millilters of blood from the patient with syringe and aseptically inoculating same into fifty milliliters of brain heart infusion broth incubated for 2 days at 37^oC aerobically and microaerophilically in a candle jar. A loopful of blood culture was aseptically withdrawn and sub-cultured on 5% sheep blood agar and cystein lactose electrolyte deficient (CLED) agar incubated for 24 hours at 37^oC aerobically. When applicable, the sub-culturing was done for three times on 2-days intervals to establish that there was no bacterial growth.

Microscopy

Wet preparations of the urine samples were made by centrifuging at 1000 rpm for 5 minutes. The deposit was examined on glass slides covered with cover-slips with x10 and x40 objectives of the microscope. Pus cells, red blood cell, casts, crystals, yeast cells were recorded.

Wet preparations of urine deposit and other samples were, made on glass slides, air dried and stained by Gram's Method.[9] Observations were made and recorded.

Identification of organisms

Colonial appearances and gram reactions[9] was the initial steps taken to identify the isolates. Slightly raised white colonies with a diameters of I-2 mm, non-haemolytic (gamma reaction) on blood agar and gram positive cocci that often occured singly or pairs (diplococci) or short chains were suggestive of *Enterococcus sp*.[3] Other organisms were also classified as gram positive or negative, lactose fermenters (coliforms) or non-lactose fermenters, haemolytic or non-haemolytic and speciated accordingly.

Gram Staining Technique

Gram staining technique using method described by Baker *et al.*[9] was carried out and recorded. This differentiates bacteria into Gram-positive and Gram negative based on the physical properties of their cell walls. *Staph aureus* and *E. coli* were used as positive and negative controls respectively.

Motility Test

Hanging Drop slide

A drop of the bacterial suspension was placed on a clean glass cover-slip as described by Cheesbrough.[11] This was inverted over the well of a ground glass and sealed with a ring of Vaseline ointment. The drop was observed with x10 of the microscope. Motile bacteria were seen actively moving while non-motile bacteria were not actively moving. *Pseudomonas aeruginosa* and *Staph aureus* were used as positive and negative controls respectively.

Biochemical Tests

The following distinguishing biochemical tests were carried out to identify the organisms isolated in this study.

These tests were carried out in accordance with standard procedures described by Baker *et al.*,[9], Cheesbrough[10], Devriese *et al*.,[12] and Cheesbrough.[11]

Catalase Test

Catalase test was carried out to differentiate isolates into catalase positive and catalase negative using a method described by Cheesbrough.[10] Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water A drop of hydrogen peroxide was placed on a sterile microscope slide. With the use of a wire loop, a bacteria colony was picked and smeared on the hydrogen peroxide drop. The production of oxygen bubbles (effervescence) indicated positive result while no bubbles indicated negative result. *Staph aureus* and *Streptococcus pyogenes* were used as positive and negative controls respectively.

Coagulase Test

Coagulase test was used to identify *Staph. aureus* which produces coagulase enzyme as described by Cheesbrough.[12]

Slide Test Method (detects bound coagulase)

A drop of distilled water was placed on an end of a sterile slide. A colony of the test organism was emulsified in the drop. A loopful of plasma was added to the suspension and mixed gently. Clumping within 10 seconds indicated presence of bound coagulase. No clumping within 10 seconds indicated no bound coagulase present. *Staph aureus* and *Staph epidermidis* were used as positive and negative controls respectively.

Tube Test Method (Detects free coagulase)

Three 5-ml test tubes were labelled:

T =Test organism (24 hour broth culture)
Pos = Positive control (24 hours *Staph.aureus*broth culture)
Neg = Negative control (24 hours Staph. epidermidis broth culture)

With a pipette 0.2ml of plasma was measured into each tube. Measured 0.8ml of test broth culture was added to tube T. Measured 0.8ml of *Staph. aureus* and *Staph epidermidis*broth cultures were added to tubes labelled 'Pos' and Neg respectively. The three tubes were incubated at 37^{o}C after mixing for 24 hours. Clotting of the T tube contents indicated presence of free coagulase. No clotting indicated negative test.

Citrate utilization test
Slopes of Simmon's citrate agar medium in bijou bottles were prepared as described by Cheesbrough.[10] With a sterile straight wire, the test organism was first streaked on the slope and then stabbed into the butt. This was incubated at 37^{o}C for 48 hours and observations recorded.

Bright blue colour indicated positive citrate test while no colour change indicated negative citrate test. *Klebsiella pneumoniae*and *E. coli*were used as positive and negative controls respectively.

Indole test
This test is important in the identification of enterobacteria which break down the amino acid tryptophan with the release of indole. This was a method described by Baker *et al.*[9] The test organism was inoculated into bijou bottle containing 3 ml of tryptone water, incubated at 37^{o}C for 48 hours. 0.5 ml of Kovac's reagents was added and shaken gently.

Red surface layer indicated positive indole test while no colour change indicated negative indole test. *E. coli* and *Enterobacter aerogenes*were used as positive and negative controls respectively.

Oxidase test
This test was used to identify *Pseudomonas* sp. which produces the enzyme cytochrome oxidase. This was a method described by Cheesbrough.[11]

A piece of filter paper was placed in a clean petri dish and three drops of freshly prepared oxidase reagent (phenylenediamine) was added. Using a piece of glass rod, a colony of the test organism was removed and smeared on the filter paper.

Blue purple colour within 30 seconds indicated positive oxidase test while no blue purple colour indicated negative oxidase test. *Pseudomonas aeruginosa* and *E. coli* were used as positive and negative controls respectively.

Voges-Proskauer test
This test was used to detect acetoin production during sugar fermentation in a bacterial broth culture and was described by Cheesbrough.[11] A tube containing 5 ml of Voges-Proskauer's broth was inoculated with a pure colony of the test organism and incubated at 37^{o}C for 48 hours. After incubation, 1 ml of the VP broth culture was measured into a clean test tube into which 0.6 ml of 5% alpha-naphthol was added. This was followed by the addition of 0.2 ml of 40% potassium hydroxide. The tube was shaken to expose the medium to atmospheric oxygen and allowed to stand for 10 to 15 minutes. Red colour development indicated positive result while no red colour indicated negative result. *Klebsiella pneumoniae* and *E. coli* were used as positive and negative controls respectively.

Methyl red test
It is used to identify bacteria producing stable acid by mechanism of mixed acid fermentation of glucose and was described by Cheesbrough.[11] The methyl red test is used to identify enteric bacteria based on their pattern of glucose metabolism. An isolate was inoculated into a tube containing 5 ml of one percent glucose with sterile loop. The tube was incubated at 37^{o}c for 2 days. After incubation, 2.4 ml of medium was transferred to another tube. Five drops of the pH indicator methyl red was added to the tube. The tube was gently rolled between the palms to disperse the methyl red. The bacteria that metabolized pyruvic acid to other acids lower the pH to 4.2. At this pH, methyl red turned to red. The red colour represented a positive test. The bacteria that metabolized pyruvic acid to neutral end- points lower the pH of the medium to only 6.0. A yellow colour represented a negative test. *E. coli* and *Enterobacter aerogenes* were used as positive and negative controls respectively.

Sugar fermentation tests
The ability of different organisms to ferment certain carbohydrates is used in their identification.[9] An aqueous solution containing 1 gram of trypticase, 1 gram of carbohydrate, 0.5 gram of NaCl, and 0.0189 gram of phenol red all in 100 ml of distilled water was prepared in conical flask. This was sterilized at 115^{o}c for 15 minutes. This was dispensed in screw capped bottles containing Durham's tubes in 5 ml aliquots. The Durham's tubes was inverted and filled completely with the medium. Each broth was aseptically inoculated with 0.1 ml of 0.5 MacFarland standard of bacterial suspension and incubated at 37^{o}c for 24 hours. A change in colour from red to yellow indicated organic acid production but no colour change indicated no fermentation and no acid production. Any trapped bubble of gas indicated gas production.

Litmus Milk Decolorization Test
This was carried out as described by Cheesbrough[11] and colour change from mauve to white or pale yellow was suggestive of enterococci.

Measured 0.5ml of sterile litmus milk was inoculated with heavy inoculums of the test organism and incubated at 37°C for up to 4 hours.

White or pale yellow-pink colour indicated litmus milk reduction while no colour change indicated negative litmus milk test. *Enterococcus faecalis* and *Streptococcus pyogenes* were used as positive and negative controls respectively.

Aesculin Hydrolysis

Bile aesculin test is based on the hydrolysis of aesculin into glucose and esculetin (6, 7- dihydroxy-coumarin) by a micro-organism that produces an enzyme aesculinase. aesculetin reacts with an iron salt (ferric citrate) in the medium to form a phenolic iron complex which produces a dark brown or black/grey colour. This was performed by a method described by Cheesbrough[11] by streaking the organism on a plate of bile-aesculin agar incubated at 37°C overnight. Colonies of organisms and their surroundings turning black/grey indicates positive test. No colour change means a negative test. *Enterococcus faecalis* and *Staph. aureus* were used as positive and negative controls respectively.

Chromagar medium

This is a chromogenic medium that makes it possible to identify bacteria on basis of their colour and appearance after incubation. Typical appearances of microrganisms on chromagar orientation are as follows: *E.coli* → dark pink to reddish. *Enterococcus* → turquoise blue. *Klebsiella,* → metallic blue. *Proteus* → brown and halo. *Pseudomonas* → cream and translucent. *S.aureus* → golden, opaque and small. *S.saprophyticus* → pink, opaque and small (Appendix VI).

This confirmed the identity of *Enterococcus* species and was performed by streaking the organism on chromagar plate and incubating at 37^{0}c overnight. Colonies of *Enterococcus* speciesappeared turquoise blue.

Plate 1: One of the *Enterococcus* sp on chromagar medium.

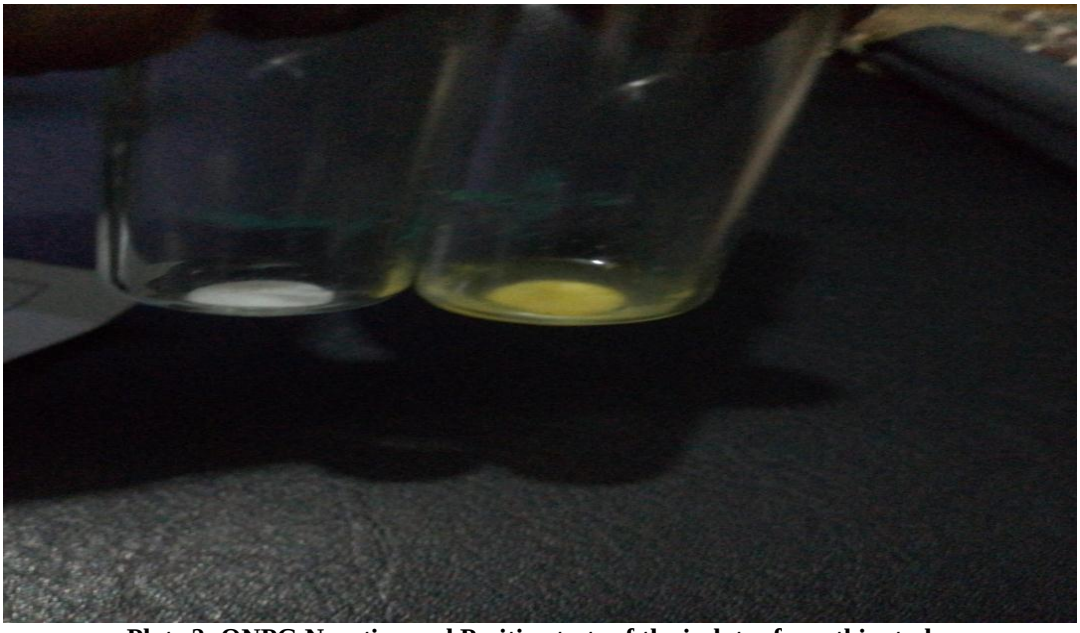

Plate 2: ONPG Negative and Positive tests of the isolates from this study.

Beta (β)-GALACTOSIDASE (ONPG) TEST

This is a test to identify the ability of the bacteia to produce the enzyme beta-galactosidase and was described by Cheesbrough.[11] Lactose utilization requires a couple of enzymes, one of which is beta-galactosidase. In this test, a molecular decoy called ONPG (Ortho-nitrophenyl-b-D-galactopyranoside) that will turn to a yellow color in the presence of this enzyme is used. ONPG is an anologue of lactose that the enzyme can break down to produce a yellow coloured end-product, O-nitrophenol. Since this enzyme is made only in the presence of the lactose substrate, there was need to be sure to grow this organism on media high in lactose. *E. coli* and *Proteus mirabilis* were used as positive and negative controls respectively.

Organism was made to be growing on lactose broth (to induce the production of the galactosidase enzyme). Into a sterile tube, 0.5ml of saline was pipetted. Measured 0.1 ml of 0.5 Macfarlane standard suspension of the bacteria was aseptically inoculated and the ONPG disc added with sterile forceps to the tube. This was incubated at 37^{o}C for 4 hours**.** The fluid and disc will turn any shade of yellow if positive for galactosidase enzyme.

API 20 Strep by Biomerieux

This was for species identification[13] using the identification software. Reference type *E. faecalis* strain (ATCC 29212) was used as control. The API 20 strep strip consists of 20 microtubes containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of sugars. The enzymatic test strips were inoculated with a dense suspension of organisms, made from a pure culture, which was used to reconstitute the enzymatic substrates. During incubation, metabolism produced colour changes that were either spontaneous or revealed by the addition of reagents. The fermentation tests were inoculated with an enriched medium which rehydrates the sugar substrates. Fermentation of carbohydrates was detected by colour changes. The reactions were read according to the reading table provided by Biomereieux and the identification was obtained by using the identification software.

Interpretation

Determination of the numerical profile: On the result sheet, the 21 parameters which included the 20 tests and haemolytic reaction (as the 21st parameter) were separated serially into groups of three and a value 1, 2 or 4 was indicated for the 1st 2nd or 3rd positive parameter in each group. By adding together the values corresponding to positive reactions within each group, a seven digit profile number called numerical profile was obtained.

Identification: The identification was done using database (V.7.0) with APIWEB identification software provided by Biomereieux. The 7 digit numerical profile was manually entered via the keyboard. The name of the specie appeared on the screen as the specie identification. This was recorded.

Conventional Biochemical Characterization /identification of the isolates

Conventional biochemical phenotypic tests were carried out to challenge and confirm the API 20 strep identification of the isolates. Reference type *E. faecalis* strain (ATCC 29212) was used as control. These include:

Tests for sugars such as mannitol, sorbose, arabinose, sorbitol, raffinose and sucrose. This was as described by Faklam *et al.*[15] An aqueous solution containing 1 gram of trypticase, 1 gram of carbohydrate, 0.5 gram of Nacl, and 0.0189 gram of phenol red all in 100 ml of distilled water was prepared in conical flask. This was sterilized at 115^{o}c for 15 minutes. This was dispensed in screw capped bottles containing Durham's tubes in 5 ml aliquots. The Durham's tubes was inverted and filled completely with the medium. Each broth was aseptically inoculated with 0.1 ml of 0.5 MacFarland standard of bacterial suspension and incubated at 37^{o}c for 24 hours. A change in colour from red to yellow indicated organic acid production but no colour change indicated no fermentation and no acid production. Any trapped bubble of gas indicated gas production.

Arginine dehydrolase test: An inoculum of 0.1 ml of 0.5 MacFarland standard bacterial suspension from a pure culture was transferred aseptically to 5ml sterile arginine dehydrolase broth as described by Faklam *et al.*[15] This was incubated for 24 hours at 37^{o}c and re-incubated for another 24 hours at 37^{o}c. A change in colour from purple to yellow after the first incubation and back to purple from yellow after the second incubation indicated positive test for arginine dehydrolase. Failure to turn yellow at 24 hours or to revert back to purple at 48 hours indicated negative result. *Enterococcus* sp. and *Streptococcus* sp. were used as positive and negative controls respectively.

Motility test: This was carried out by hanging drop method. A drop of the bacterial suspension was placed on a clean glass cover-slip. This was inverted over the well of a ground glass and sealed with a ring of Vaseline ointment. The drop was observed with x10 of the microscope. Motile bacteria were seen actively moving while non-motile bacteria were not actively moving. *Pseudomonas* sp. and *Enterococcus* sp. were used as positive and negative controls respectively.

Pigment production: This was observed on the solid blood and CLED agar plates as colour change around the colonies of the isolates as described by Faklam *et al.*[15]

Statistical analysis

The results obtained from this work were analyzed statistically using a computer program SPSS version 18. The values were expressed in percentages.

RESULTS

Wet preparation microscopic result of clinical specimens that yielded growth of micro-organisms processed in this study was displayed in table 1. Of the 139 samples of

urine, 125 (89.9%) had pus cells; 21 (15.1%) had red blood cells; 34 (24.5%) had casts; 48 (34.5%) had crystals; 89 (64.0%) had epithelial cells. There was none (0%) with ova of schistosomes.

Of the 133 samples of high vaginal swabs (HVS), 108 (81.2%) had pus cells; none had red blood cells; 133 (100%) had epithelial cells; 45 (33.8%) had yeast cells. Of the 22 sample of cerebrospinal fluid (CSF), 22 (100%) had pus cells; 5 (22.7%) had red blood cells.

Table 1: Result of wet preparation microscopy of the clinical samples that yielded organisms.

Clinical samples	Pus cells	RBCS	Casts	Crystals	Epith cells	Yeast cells	Ova of schistosomes
Urine(n=139)	125(89.9)	21(15.1)	34(24.5)	48(34.5)	89(64)	0 (0)	0 (0)
HVS (n=133)	108(81.2)	0 (0)	NA	NA	133(100)	45(33.8)	NA
CSF (n=22)	20(90.9)	2(22.7)	NA	NA	NA	NA	NA

Key: RBCS= Red blood cells
Epith cells= Epithelial cells.
HVS= High vaginal swabs.
CSF= Cerebrospinal fluids.
NA= Not applicable.

Clinical samples of urine and CSF were grouped according to the number of pus cells per high power field (x40 objective lens) of light as shown in table 2. Of the 139 urine samples, 14 (10%) had pus cells 0-1 per high power field (/HPF); 26 (18.7%) had 2-3 pus cells PHF; 21 (15.1%) had 4-6 pus cells/HPF; 14 (10%) had 7-9 pus cells/HPF; 20 (14. microscope 4%) had 10-13 pus cells/HPF; 21 (15.1%) had many pus cells/HPF; 23 (16.5%) had numerous pus cells/HPF.

Table 2: Number of pus cells per high power field (X40 objective lens of light microscope) of the urine and CSF wet mount.

Clinical samples	0	1-2	2-3	4-6	7-9	10-`14	15-20	21 and above
Urine PC (n=139)	20(14.4)	14 (10)	26 (18.7)	21 (15.1)	14(10)	20 (14.4)	21 (15.1)	23 (16.5)
Urine EC (n=139)	50(36.0)	10(7.3)	10 (7.3)	13 (9.3)	12 (8.6)	16 (11.5)	15 (10.8)	13 (9.3)
CSF (n=22)	2(9.0)	1(4.5)	5(22.7)	3(13.6)	3(13.6)	4(18.2)	3(13.6)	1(4.5)

Key: CSF= Cerebrospinal fluid. PC= Pus cell. EC= Epithelial cell

Out of one thousand and eight (1008) samples processed in this study, six hundred and thirty two (632) (58.5%) yielded different species of bacteria and fungi, the prevalence of which are displayed in Table 3 as follows: *E. coli* 152 (24.1%), *Streptococcus* sp 95 (15%), *Enterococcus* sp 68 (10.8%), *Staph aureus* 64 (10.1%), *Pseudomonas* sp.63 (10.0%), *Proteus* sp. 63 (10.0%), *Klebsiella* sp 50 (7.9%), *Candida* sp. 45 (7.1%) and Coagulase negative staphylococci 32 (5.1%).

Table 3: Characterization of organisms isolated from clinical samples during this study.

Gram rxn	Catalase	Coagulase	LM	AH	Oxidase	Citrate	Urease	VP	Methyl Red	Indol	Motility	ONPG	Glucose	surose	lactose	co	6.5% NACL	Indentification	total	%
-rods	NA	NA	NA	NA	NA	V	-	+	+	+	+	+	+	+	+	Dark pink to reddish	-	Escherichia sp	152	24.1
+cocci in long chain	-	NA	-	-	NA	NA	NA	NA	NA	NA	-	NA	NA	NA	+	NA	-	Streptococcus sp	95	15
+cocci in short chain	-	NA	+	+	NA	NA	NA	+	NA	NA	-	NA	NA		+	Torquoise	+	Enterococcus sp	68	10.8
+cocci in clusters	+	+	NA	NA	NA	NA	NA	NA	NA	NA	-	NA	NA	NA	+	Golden opaque small	-	Staph.aureus	64	10.1
-Rods	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	+	NA	NA	NA	-	NA	-	Pseudomonas sp	63	10
-Rods	NA	NA	NA	NA	-	NA	+	NA	NA	+	+	NA	NA	NA	-	Brown halo	-	Proteus sp	50	10
-Rods	NA	NA	NA	NA	-	+	+	+	-	-	-	+	+	+	+	Dark pink to reddish	-	Kiebsiella sp	45	7.9
+cocci in clusters	+	-	NA	NA	NA	NA	NA	NA	NA	NA	-	NA	NA	NA	-	Pink to opaque small	-	Coagulase staph	32	7.1
+budding yeast cells	NA	NA	NA	NA	NA	-	NA	NA	NA	NA	-	NA	NA	NA	NA	NA	-	Candida sp.		5.1

NA= Not applicable. + =Positive. - = Negative.

The isolates were grouped according to the specimens from which they were isolated as in Table 4.

Urine. Total number of isolates from urine was 139. These consist of 46 (33.1%) isolates of *E. coli*, 13 (9.4%) isolates of *Streptococcus* sp.; 24 (17.3%) isolates of *Enterococcus* sp.; 10 (7.2%) isolates of *Staph aureus*; 11 (7.9%) isolates of *Pseudomonas* sp.; 12 (8.6%) isolates of *Proteus* sp.; 16 (11.5%) isolates of *Klebsiella* sp.; no *Candida* sp. was isolated; 7 (5.0%) isolates of coagulase negative staphylococci.

High vaginal swabs (HVS). Total number of isolates was 133 with 30 (22.6%) isolates of *E. coli*; 9 (6.8%) isolates of *Streptococcus* sp; 12 (9.0%) isolates of *Enterococcus* sp.; 10 (7.5%) isolates of *Staph aureus*; 6 (4.5%) isolates of *Pseudomonas* sp; 6 (4.5%) isolates of *Proteus* sp; 9 (6.8%) isolates of *Klebsiella* sp; 45 (33.8%) isolates of *Candida* sp; and 6 (4.5%) isolates of coagulase negative staphylococci.

Ear swabs. Total number of isolates was 69 with 20 (29.0%) isolates of *E. coli*; 1 (1.4%) isolate of *Streptococcus* sp.; 7 (10.1%) isolates of *Enterococcus* sp.; 6 (8.7%) isolates of *Staph aureus*; 12 (17.4%) isolates of *Pseudomonas* sp.; 10 (14.5%) isolates of *Proteus* sp.; 6 (8.7%) isolates of *Klebsiella* sp.; no *Candida* sp. was isolated; 7 (10.1%) isolates of coagulase negative staphylococci.

Wound swabs. Total number of isolates was 74 with 10 (13.5%) isolates of *E. coli*; 10 (13.5%) isolates of *Streptococcus* sp.; 7 (9.5%) isolates of *Enterococcus* sp.; 8 (10.8%) isolates of *Staph aureus*; 14(18.9%) isolates of *Pseudomonas* sp.; 15 (20.3%) isolates of *Proteus* sp.; 5 (6.8%) isolates of *Klebsiella* sp.; no *Candida* sp. was isolated; 5 (6.8%) isolates of coagulase negative staphylococci.

Urethral swabs (US). Total number of isolates was 81 with 30 (37.0%) isolates of *E. coli*; 10 (12.3%) isolates of *Streptococcus* sp.; 4 (4.9%) isolates of *Enterococcus* sp.; 10 (12.3%) isolates of *Staph aureus*; 11 (13.6%) isolates of *Pseudomonas* sp.; 11 (13.6%) isolates of *Proteus* sp.; 2 (2.5%) isolates of *Klebsiella* sp.; no isolate of *Candida* sp.; 3 (3.7%) isolates of coagulase negative staphylococci.

Blood. Total number of isolates was 19 with 5 (26.3%) isolates of *E. coli*; 5 (26.3%) isolates of *Streptococcus* sp.; 4 (21.1%) isolates of *Enterococcus* sp.; 3 (15.8%) isolates of *Staph aureus*; 2 (10.5%) isolates of *Pseudomonas* sp. Other organisms were not isolated.

Aspirates. Total number of isolates was 45 with 3 (6.7%) isolates of *E. coli*; 15 (33.3%) isolates of *Streptococcus* sp.; 4 (8.9%) isolates of *Enterococcus* sp.; 10 (22.2%) isolates of *Staph aureus*; 2 (4.4%) isolates of Pseudomonas sp.; 3 isolates of *Proteus* sp.; 4 (8.9%) isolates of *Klebsella* sp.; no isolate of *Candida* sp.; 4 (4.9%) isolates of coagulase negative staphylococci.

Cerebrospinal fluid (CSF). Total number of isolates was 22 with 2 (9.1%) isolates of *E. coli*; 10 (45.5%) isolates of *Streptococcus* sp.; 4 (18.2%) isolates of *Enterococcus* sp.; 3 (13.6%) isolates of *Pseudomonas* sp.; no isolate of *Proteus* sp.; 2 (9.1%) isolates of *Klebsiella* sp..; no isolate of *Candida* sp.; no isolate of coagulase negative staphylococci.

Sputum. Total number of isolates was 50 with 6 (12%) isolates of *E. coli*; 22 (44%) isolates of *Streptococcus* sp.; 2 (4.0%) isolates of *Enterococcus* sp.; 4 (8.0%) isolates of *Staph aureus*; 4 (8.0%) isolates of *Pseudomonas* sp.; 6 (12.0%) isolates of *Proteus* sp.; 6 (12.0%) isolates of *Klebsiella* sp.; no isolates of *Candida* sp. and coagulase negative staphylococci.

Total number of isolates in this study was 632 with prevalence (%) of *E. coli* 152 (24.1%) *Streptococcus* sp. 95 (15%), *Enterococccus* sp 68 (10.8%) *Staph aureus*64 (10.1%), *Pseudomonas sp.* 63 (10.0%), *Proteus* sp. 63 (10.0%) *Klebsiella* sp. 50 (7.9), *Candida* sp. 45 (7.1%) and coagulase negative staphylococci 32 (5.0%).

Of the 40 nonclinical samples collected in this study as control, 7 (10.5%) yielded bacterial and fungal isolates as follows:
Urine: Of the 20 samples of urine, total number of isolates was 3 with 2 isolates of *E. coli* and 1 isolate of coagulase negative *Staphylococcus*. No *Enterococcus* sp. was isolated.

Urethral smear: Of the 10 nonclinical samples of urethral smear, total number of isolats was 2 isolats of *Staph aureus*. No *Enterococcus* sp. was isolated.

High vaginal swabs (HVS): Of the 10 nonclinical samples of high vaginal swabs, total number of isolates was 2 with 1 isolate of *Staph aureus* and 1 isolate of *E. coli*. No *Enterococcus* sp. was isolated.

Escherichia coli: This grew aerobically and at the temperature of 37^0C within 24hours of incubation on blood agar. Non-haemolytic, 1-4mm diameter colonies after overnight incubation at 37^0C. Some strains were haemolytic.

CLED agar: fermented lactose producing smooth yellow colonies on CLED agar. Some strains were non – lactose fermenters.

Gram staining – Gram negative rods (bacilli).

Biochemical reactions – indole positive, citrate utilization negative, ureas negative, voges proskauer positive, methyl red positive, glucose positive, sucrose positive and appeared dark pink to reddish colour in chromagar orientation.

***Streptococcus* sp**
Morphology: These were gram positive cocci occurring singly, in pairs, in short chains and also in long chains. They are non- motile. Some species are capsulated.

Culture: Blood agar: produced beta haemolytic colonies on sheep blood agar. Colonies were small (0.5-1mm), colourless, dry, shiny or mucoid some species are alpha haemolytic or gamma haemolytic.

Biochemical tests: catalase negative, litmus milk decoloration negative, ascending hydrolysis negative and no growth at 6.5% sodium chloride concentration, PYR (pyrrodonyl test) positive.

***Enterococcus* sp**
Morphology: gram positive cocci occurring in short chains, singly or arranged in pairs. They are non-capsulate and non-motile.

Culture: Enterococci isolated were aerobic organisms and grew optimally at 37^0C.

Blood agar: enterococci isolates were non haemolytic, colonies appeared grey and mucoid about 2mm in diameter.

CLED agar: enterococcal isolates appeared as small yellow colonies on cysteine lactose electrolyte deficient agar. They grew in the presence of 6.5% sodium chloride.

Bile Aesculin agar: black colonies were produced after 4 hours of incubation at 37^0C. Aesculin was hydrolysed to aesculatein.

Biochemical tests: lactose fermentation positive aesculin hydrolysis positive, litmus milk decoloration positive.

Chromagar orientation: turquoise blue colonies grew on this plate. This confirms the identity of *Enterococcus* sp.

Staphylococcus aureus
Morphology: gram positive cocci occurring mostly in clusters, non-motile and non-capsulate.

Culture: *Staph aureus* isolated grew well aerbically and microaerophilically. Blood agar: yellow to cream and occasionally white colonies of diameter 1-2mm size produced after overnight incubation at 37^oc. Thay are non-haemoltic when grown aerobically and microaerophilically. Colonies were slightly raised and easily emulsified. CLED agar: deep yellow colonies were produced. Biochemical test: catalase positive, coagulase positive.

***Pseudomonas* sp**
Morphology: these were gram negative, non-sporing motile rods. Some strains were capsulate.

Culture: these grew aerobically and produced blue-green pigments (pyocyanin) at the temperature of 37^0c.

Blood agar: the isolate yielded large, flat, spreading colonies with serrated edges, haemolysis and dark greenish blue pigment.

CLED agar: green colonies were produced on CLED medium with less pigmentation than in blood agar.

Biochemical reactions: isolates of this organism were oxidase positive. Produced acid only with glucose (no gas).

***Proteus* sp**
Morphology: Isolates were actively motile, non-capsulate, gram negative, pleomorphic rods.

Culture: Isolates were aerobic and grew well at 37^oc overnight.

Blood agar: Swarming (no discrete colonies) was observed with characteristics 'fishy' smell.

CLED agar: Blue-grey translucent colonies were observed. No swarming.

Biochemical test: Lactose fermentation negative, urea positive, β-galactosidase (ONPG) negative, indole negative.

***Klebsiella* sp**
Morphology: These isolates were Gram negative, non-motile and capsulated rods.

Culture: These isolates were aerobic and grew well at 37^oc.

Blood agar: Colonies of *Klebsiella* sp. were large, grey-white and mucoid.

CLED agar: Isolates appeared as large, yellow (lactose fermenting) and mucoid colonies after 24 hours incubation at 37^oc.

Biochemical reactions: Lactose fermentation positive, indole negative. Dark pink to reddish colour on chromagar orientation.

***Candida* sp**
Morphology: Isolates were small, oval, often with budding cells and gram positive.

Culture: Isolates were aerobic and grew well at 37^oc.

Blood agar: cream coloured pasty colonies were observed after 24 hours of incubation at 37^oc. The colonies had distinctive yeast smelling and budding cells were easily seen by direct microscopy in stained or unstained preparations.

Coagulase negative staph
Morphology: gram positive cocci occurring mostly in clusters, non-motile, non-capsulate.

Culture: coagulase negative staph isolated grew well aerobically and microaerophilically.

Blood agar: the colonies appeared white and non haemolytic.

CLED medium: some colonies appeared yellow white others appeared white.

Biochemical tests: catalase positive, coagulase negative.

Table 4: Specimens and number of organisms isolated (% in brackets).

Clinical samples	TOTAL SAMPLE NO (n)	*E.coli*	*Strept*	*Entero*	*Staph. Aureus*	*Pseud*	*Proteus*sp.	*Kleb.*sp.	*Candida*sp.	CONS
Urine	139	46(33.1)	13(9.4)	24(17.3)	10(7.2)	11(7.9)	12(8.6)	16(11.5)	0(0)	7(5)
HVS	133	30(22.6)	9(6.8)	12(9.0)	10(7.5)	6(4.5)	6(4.5)	9(6.8)	45(33.9)	6(4.5)
Ear swab	69	20(29.0)	1(1.5)	7(10.1)	6(8.7)	12(17.4)	10(14.5)	6(8.7)	0(0)	7(10.1)
Wound swab	74	10(13.5)	10(13.5)	7(9.5)	8(10.8)	14(18.9)	15(20.3)	5(6.8)	0(0)	5(6.8)
U/S	81	30(37)	10(12.3)	4(4.9)	10(12.3)	11(13.6)	11(13.6)	2(2.5)	0(0)	3(3.7)
Blood	19	5(26.3)	5(26.3)	4(21.1)	3(15.9)	2(10.5)	0(0)	0(0)	0(0)	0(0)
Aspirates	45	3(6.7)	15(33.3)	4(8.9)	10(22.2)	2(4.4%)	3(6.7)	4(8.9)	0(0)	4(8.9)
CSF	22	2(9.1)	10(45.5)	4(18.2)	3(13.6)	1(4.5)	0(0)	2(9.1)	0(0)	0(0)
Sputum	50	6(12)	22(44)	2(4)	4(8)	4(8)	6(12)	6(12)	0(0)	0(0)
Total	632	152	95	68	64	63	63	50	45	32
Prevalence(%)	100	24.1	15.0	10.8	10.1	10.0	10.0	7.9	7.1	5.0

Key:
HVS= High vaginal swab. U/S= Urethral swab. CSF= Cerebrospinal fluid.
E. coli = Escherichia coli. Sptrept= *Sptreptoccocus sp. Enterococcus sp.* Pseud= *Pseudomonas sp.*
CONS= Coagulase Negative Staphyloccoci sp. Kleb= *Klebsiella sp.*

Speciation using API 20 strept identification system by Biomereux
API 20 strept result displayed on the Table 5 and interpreted using the reading table provided by Biomereux and control ATCC 29212 showed that three species of *Enterococcus* were isolated in this study. These are *Enterococcus faecalis*, *E. faecium* and *E. avium.*

Enterococcus faecalis
The 21 parameters that were used in the table of identification and their results for *E. faecalis* were as follows: Voges proskauer positive, Hippurate positive. aesculin hydrolysis positive, pyrrodonyl arylamidase positive, α-galactosidase negative, β-glucuronidase negative, β- galactosidase positive, alkaline phosphatase positive, leucine amino peptidase positive, arginine dehydrogenase positive, D-ribose positive, L-arabinose negative, D-mannose positive, D-sorbose positive, D-lactose, D-trehalose positive, inulin negative, D-raffinose negative, starch (amidon) positive, glycogen negative, β-haemolysis negative. These parameters were separated into groups of three according to manufacturer's label and a value 1, 2 or 4 was indicated for each positive reaction. By adding together the values corresponding to positive reactions within each group, a seven digit profile number 7173711 was obtained. The identification was done using database (V.7.0) with APIWEB identification software. The 7 digit numerical profile was manually entered via the keyboard. The name of the specie *Enterococcus faecalis* appeared on the screen as the specie identification. This identification was supported by conventional biochemical characteristics of the isolates as displayed in Table 4 as follows: mannitol positive, sorbose negative, arginine positive, arabinose negative, sorbitol positive, raffinose negative, motility negative, pigment negative, sucrose positive.

Enterococcus faecium
The 21 parameters that were used in the table of identification and their results for *Enterococcus faecium* were as follows (see Table 5): voges proskauer positive, hyppurate hydrolysis positive aesculin hydrolysis positive, pyrrodonyl Aryalamidase positive, α-galactosidase positive, β-glucuronidase negative, β-galactosidase positive, alkaline phosphatase positive, leucine amino peptidase positive, arginine dehydrogenase positive, D-ribose positive, L-arabinose positive, D-mannose positive, D- sorbose positive, D-lactose positive, D-trehalose positive, inulin negative, D-raffinose negative, starch (amidon) positive, glycogen negative, β-haemolysis negative.

The numerical profile is 7377511. This was keyed into the APIWED software and the identification was *Enterococcus faecium.*

This identification was supported by conventional biochemical characteristics of the isolates as displayed in table 4 as follows: mannitol positive, sorbose negative,

arginine negative, arabinose positive, sorbitol positive, raffinose negative, motility negative, pigment negative, sucrose positive.

Enterococcus avium

The 21 parameters that were used in the table of identification and their results were as follows (see Table 5) voges proskauer positive, hyppurate hydrolysis negative, aesculin hydrolysis positive, pyrrodonyl arylamidase positive, α-galactosidase negative, β-glucuronidase negative, β-galactosidase negative, alkaline phosphatase positive, leucine amino peptidase positive, arginine dehydrogenase negative, D-ribose positive, L-arabinose positive, D-mannose positive, D-sorbose positive, D-lactose positive, D-trehalose positive, inulin negative, D-raffinose negative, starch (amidon) positive, glycogen negative, β-haemolysis negative.

The numerical profile is 5166710. This was keyed into the APIWEB software and the identification was *Enterococcus avium.*

This identification was supported by conventional biochemical characteristics of the isolates displayed in Table 6 as follows: mannitol positive, sorbose positive, arginine negative, arabinose positive, sorbitol positive, raffinose negative, motility negative, pigment negative, sucrose positive.

Table 5: API 20 Strep results of three of the species isolated.

SUBSTRATES REACTION	ATCC 29212	PL 1	PL 4	PL 5
VOGES PROSKAUER (VP)	+	+	+	+
HIPPURATE HYDROLYSIS (HIP)	+	+	+	-
ESCULIN HYDROLYSIS (ESC)	+	+	+	+
PYRRODONYL ARYLAMIDASE (PYRA)	+	+	+	+
α- GALACTOSIDASE (α- GAL)	-	+	-	-
β- GLUCURONIDASE (β-GUR)	-	-	-	-
β- GALACTOSIDASE (β- GAL)	+	+	+	-
ALKALINE PHOSPHATASE (PAL)	+	+	+	+
LEUCINE AMINO PEPTIDASE (LAP)	+	+	+	+
AGININE DEHYDROGENASE (ADH)	+	+	+	-
D- RIBOSE (RIB)	+	+	+	+
L- ARABINOSE (ARA)	-	+	-	+
D- MANOSE (MAN)	+	+	+	+
D- SORBOSE (SOR)	+	-	+	+
D- LACTOSE (LACT)	+	+	+	+
D- TREHALOSE (TRE)	+	+	+	+
INULIN (INU)	-	-	-	-
D- RAFFINOSE (RAF)	-	-	-	-
STARCH (AMIDON) (AMD)	+	+	+	-
GYCOGEN (GLY)	-	-	-	-
β-HEMOLYSIS (β-HEM)	-	-	-	-
NUMERICAL PROFILE	7173711	7377511	7173711	5166710
IDENTIFICATION	E. faecalis	E. faecium	E. faecalis	E. avium

Table 6: Conventional biochemical characteristics of the isolates.

Phenotypic characteristics	ATCC 29212	PL 1	PL 4	PL 5
Manitol	+	+	+	+
Sorbose	-	-	-	+
Arginine	+	+	+	-
Arabinose	-	+	-	+
Sorbitol	+	+	+	+
Sucrose	+	+	+	+
Motility	-	-	-	-
Pigment	-	-	-	-
Identification	*E.faecalis* (control)	*E. faecium*	*E. faecalis*	*E. avium*

Key: PL1… Parklane sample 1
PL4… Parklane Sample 4
PL5… Parklane sample 5
ATCC 29212 …American Type Culture Collection number 29212

Table 7 showed that 68 isolates of *Enterococcus* were speciated into 3 species using the API 20 Strept Identification system.

1. *E. faecium* with numerical profile of 7377511 had 39 isolates and percentage of 57.35%.
2. *E. faecalis* with numerical profile of 7173711 had 25 isolates and percentage 36.76%
3. *E. avium*with numerical profile of 5166710 had 4 isolates and 5.88%.

These three isolates were confirmed, using conventional biochemical methods of characterization (table 6).

Table 7: Summary of the API 20 strep identification of the isolates.

NUMERICAL PROFILE	IDENTIFICATION	NO OF ISOLATES	PERCENTAGES
7377511	*E. faecium*	39	57.35
7173711	*E. faecalis*	25	36.76
5166710	*E. avium*	4	5.88
TOTAL		68	100

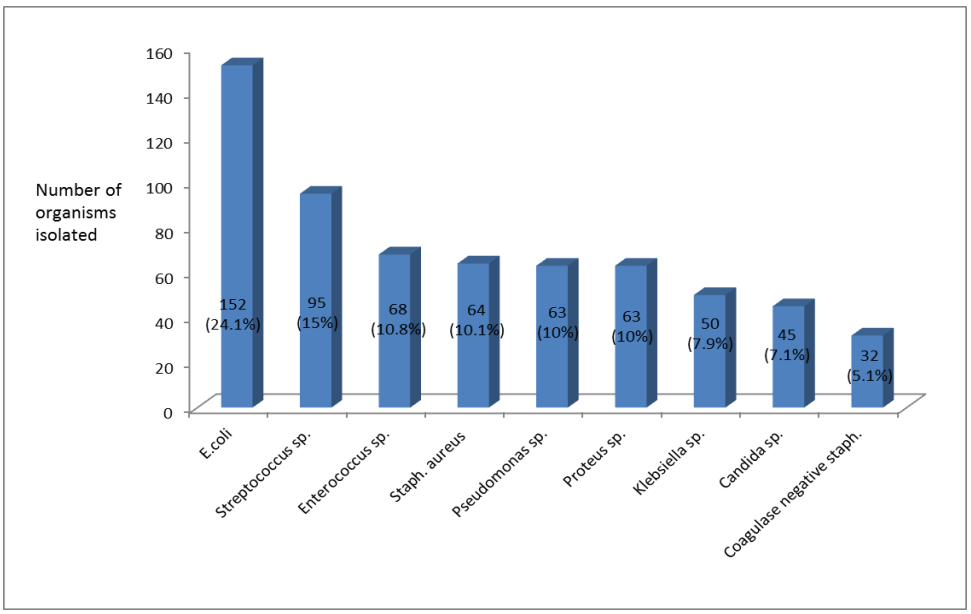

Figure 1: Shows the number and prevalence of organisms isolated from the study.

DISCUSSION

Enterococci have evolved from normal commensal to leading pathogens that cause infections in humans.[17] Infections caused by enterococci are endocarditis, bacteremia, urinary tract, hospital, infants and central nervous system infections.[16, 18]

The presence of pus cells in 125 (89.4%) urine samples out of 139 urine samples that yielded bacterial growth is typical of urinary tract infections. Al-Saimary *et al.* (2006)[19] reported a higher percentage of 93.3% in a cytological examination of 105 urine specimens from patients with urinary tract infections. These reports agree with the report of Fowler (1986)[20] which states that the clinical features of urinary tract infections include bacteriuria, pyuria, haematuria, burning sensation on urination frequent and urgent urination. Haematuria was also noted in 21 (15.1%) urine samples out of the 139 urine samples that yielded bacteria. Al- Saimary *et al.*(2006)[19] reported a higher percentage of 39% urine samples with red blood cells.

Granular casts were noted in 34 (24.5%) urine samples out of 139 samples. Al- Saimary *et al.*(2006)[19] reported a close but higher percentage of 28.8% urine samples with granular casts out of 105 urine specimens of patients with urinary tract infections. This usually occurs when urinary tract infection has progressed from the urinary bladder through the urethras to the pelvis and calyces of the kidneys. This leads to a case of pyelonephritis which is associated with morbidity.[20] Complications of pyelonephritis include papillary necrosis with possible development of urethral obstructions, septic shock, perinephric abscess, scarring with diminished renal function or renal failure.[20]

Calcium oxalate crystals were also present in 48 (34.5%) urine samples out of 139 urine samples. This agrees roughly with 35.2% urine samples reported by Al-Saimary *et al.*(2006).[19] Calcium oxalate and calcium sulphates were found to be associated with kidney stones of both humans and rats (Kahn, 1997).[21]

Epithelial cells were present in 89 (64.0%) urine samples out of 139 samples. Al- Saimary *et al.*(2006)[19] reported a higer percentage of 81.9% urine specimens of patients with urinary tract infections. Of the 139 urine samples in this study, 13(9.4%) had epithelial cells in the range of 21 and above per high power field. Epithelial cells are cells that form thin surface coating on the outside of a body structure. The normal range of epithelial cells in urine 15-20 per high power field and if they are higher in urine they can signal a problem with the kidneys or an infection in the urinary system.[22] They established that the presence of epithelial cells has significance in relation to urethritis and *Chlamidia trachomatis*and *Neisseria gonorrhoeae*infections.

The wet mount microscopy of urine included a search for ova of *Schistosoma haematobium*because the study areas involved tertiary institutions that received patients from neighbouring Ebonyi State which is endemic for schistosomiasis. However, no ova of schistosomes were seen and recorded during the study. In contrast, prevalence rates of *S. haematobium*among school children in Alaukwu and Onicha communities of Ebonyi State, South East Nigeria was 47.9% and 11.0% respectively.[23]

The wet mount of high vaginal swab (HVS) showed a high level of pus cells signaling infection. Of the 133 HVS samples, 108 (81.2%) had pus cells and all the 133 (100%) had epithelial cells, 45 (33.8%) showed yeast cells and eventually yielded *Candida* sp. This is comparable to the work of Sevitha *et al*. (2012).[24] who recorded pus cells in 85% of cases of vaginal discharge, epithelial cells in 100% of cases of vaginal discharge and yeast cells in 35% of cases.

Clinical samples of urine and CSF were grouped according to the number of pus cells per high power field to establish significant values. Normal range of pus cells in urine is 5-8 in males and up to 10 in females.[20] The data showed that 64 (46%) of 139 urine samples had significant pyuria. Al-Saimary *et al.* (2006)[19] reported a higher percentage of 93.3% in a cytological examination of 105 urine specimens from patients with urinary tract infections.

Of the 22 samples of cerebrospinal fluid (CSF), 20 (90.9%) had pus cells; 5 (22.7%) had red cells which could be due to traumatic tapping during lumber puncture since the samples were not xanthochromatic. Normal range of pus cells in CSF is 0-1/hpf[25], meaning that 20 (90.9%) of the 22 CSF samples had significant number of pus cells/hpf. This agrees approximately with the work of Kulkarni (2016).[26] who established that 65 (80.2%) of 81 children who had bacterial meningitis had pus cells range of 2-25 per high power field.

Isolation and characterization

A total of 1008 samples were processed in this study and 632 (58.5%) yielded different species of bacteria and yeast cells. Using Gram staining and other biochemical methods these isolates were characterized into nine (9) genera of bacteria and yeast cells. These organisms and their prevalence are as follows: *E. coli* 152 (24.1%), *Streptococcus* sp. 95 (15%), *Enterococcus* sp. 68 (10.8%), *Staphylococcus aureus* 64 (10.1%), *Pseudomonas* sp. 63 (10.0%), *Proteus* sp. 63 (10.0%), *Klebsiella* sp. 50 (7.9%), *Candida* sp. 45 (7.1%) and coagulase negative staphylococci 32 (5.1%).

The 40 non-clinical samples collected from volunteers which included 20 urine samples, 10 urethral smear samples and 10 high vaginal swab samples did not yield any *Enterococcus* sp. However, *Streptococcus* sp., *Staph aureus*, coagulase negative staphylococci, *E. coli* and *Candida* sp. were isolated. The absence of *Enterococcus* sp. could be because these samples were not from the

sites where they exist as normal flora. The organism is known to be normal flora of the alimentary canal of man and animals.[27]

The isolates were grouped according the clinical samples from which they were isolated.

URINE: 139 clinical samples of urine yielded different bacteria as follows: *E. coli* 46 (38%), *Streptococcus* sp. 13 (9.4%), *Enterococcus* sp. 24 (17.3%), *Staphylococcus aureus* 10 (7.2%), *Pseudomonas* sp. 11 (7.9%), *Proteus* sp. 12 (8.6%), *Klebsiella* sp. 16 (11.5%), coagulase negative staphylococci 7 (5.0%) and no *Candida* sp. was isolated from urine. This result is in line with the work of Nwadioha *et al*., (2010)[28] who undertook a prevalence study in Nguru, Northern Nigeria of Uropathogenic microorganisms. The same set of organisms were isolated by them except that there were minor differences in their prevalence and that *Salmonella* was isolated in theirs but not isolated in this study. A similar study was carried out in India which also showed *E. coli* as predominant 50% among the isolates.[29] Another study that was carried out in Sagamu, Western Nigeria among pregnant and non-pregnant women recorded 26.6% and 5.6% prevalence respectively of significant bacteriuria (Olusanya *et al*., 1992).[30] Similar findings were also observed by Onyemelukwe *et al*. (2003)[31] in Enugu, Eastern Nigeria. From the above research works, it is obvious that urinary tract infection is a serious disease that deserves attention by researchers since it is encountered in virtually all levels of health care provision in Nigeria and beyond. It is interesting to note that *Enterococcus* sp. is the second leading cause of urinary tract infection in this study just as reported by Malani *et al*. (2002)[31] that *Enterococcus* sp. has become the second leading cause of urinary tract infection, wound infections and bacteremia in USA.

High vaginal swabs (HVS). 133 clinical samples of HVS yielded isolates as follows: 30 (22.6%) isolates of *E. coli*; 9 (6.8%) isolates of *Streptococcus* sp.; 12 (9.0%) isolates of *Enterococcus* sp., 10 (7.5%) isolates of *Staph aureus*; 6 (4.5%) isolates of *Pseudomonas* sp.; 6 (4.5%) isolates of *Proteus* sp.; 9 (6.8%) isolates of *Klebsiella* sp.; 45 (33.8%) isolates of *Candida* sp.; and 6 (4.5%) isolates of coagulase negative staphylococci. Candidiasis is the most predominant vaginal infection in this study. This agrees with the report of Corsello, (2003)[33] that in the second half of the twentieth century, the incidence of vaginal candidiasis increased dramatically and that it is estimated that 75% of women experience at least one episode of vulvovaginal candidiasis during their childbearing age, and approximately 40% experience a second attack. The second leadingcause of vaginal infection in this study is *E. coli* (22-.6%) followed by *Enterococcus* sp. (9.0%).

Ear swabs. Total number of isolates was 69 with 20 (29.0%) isolates of *E. coli*; 1 (1.4%) isolate of *Streptococcus* sp.; 7 (10.1%) isolates of *Enterococcus* sp.; 6 (8.7%) isolates of *Staph aureus*; 12 (17.4%) isolates of *Pseudomonas* sp.; 10 (14.5%) isolates of *Proteus* sp.; 6 (8.7%) isolates of *Klebsiella* sp.; no *Candida* sp. was isolated; 7 (10.1%) isolates of coagulase negative staphylococci. The above result is comparable to clinical studies conducted from 1998 to 2000, during which microbiology specimens were collected from 2039 subjects by 101 investigators throughout the United States. A total of 2838 bacteria, 32 yeast cells, and 17 molds were recovered from 2048 ear. Of the 202 bacterial species recovered, the species most frequently isolated was *Pseudomonas aeruginosa* (38%). The next 10 species most frequently isolated were: *Staphylococcus epidermidis*, 9.1%; *Staphylococcus aureus*, 7.8%; *Microbacterium otitidis*, 6.6%; *Microbacterium alconae*, 2.9%; *Staphylococcus caprae*, 2.6%; *Staphylococcus auricularis*, 2.0%; *Enterococcus faecalis*, 1.9%; *Enterobacter cloacae*, 1.6%; *Staphylococcus capitis*sub sp. *ureolyticus*, 1.4%; and *Staphylococcus haemolyticus*, 1.3%.[34]

Wound swabs. Total number of isolates was 74 with 10 (13.5%) isolates of *E. coli*; 10 (13.5%) isolates of *Streptococcus* sp; 7 (9.5%) isolates of *Enterococcus* sp.; 8 (10.8%) isolates of *Staph aureus*; 14(18.9%) isolates of *Pseudomonas* sp.; 15 (20.3%) isolates of *Proteus* sp.; 5 (6.8%) isolates of *Klebsiella* sp.; no *Candida* sp. was isolated; 5 (6.8%) isolates of coagulase negative staphylococci. Wound infection with enterococci is by direct contamination of wound with infected materials. The clinical features include inflammation and pus production.[35] In a 3 years study in Delhi, India, the prevalence of wound infections due to *Enterococcus* sp. was 8.6%[36] which is lower than the 10.8% (prevalence) of this study.

Urethral swabs (US). Total number of isolates was 81 with 30 (37.0%) isolates of *E. coli*; 10 (12.3%) isolates of *Streptococcus* sp; 4 (4.9%) isolates of *Enterococcus* sp; 10 (12.3%) isolates of *Staph aureus*; 11 (13.6%) isolates of *Pseudomonas* sp; 11 (13.6%) isolates of *Proteus* sp; 2 (2.5%) isolates of *Klebsiella* sp; no isolate of *Candida* sp; 3 (3.7%) isolates of coagulase negative staphylococci. Urethral swabs are used to collect samples from the urethra to investigate the cause of urethritis which is inflamation of the urethra. Enterococci have been implicated in 10% of all urinary tract infections and up to approximately 16% of nosocomial urinary tract infections.[32]

Blood. Total number of isolates was 19 with 5 (26.3%) isolates of *E. coli*; 5 (26.3%) isolates of *Streptococcus* sp.; 4 (21.1%) isolates of *Enterococcus* sp.; 3 (15.8%) isolates of *Staph aureus*; 2 (10.5%) isolates of *Pseudomonas* sp. Other organisms were not isolated. It is noteworthy that enterococci are the third leading cause of bacteraemia in this study and this is in line with the report that in 2005, there were 7066 reported cases of bacteraemia caused by *Enterococcus species* in the UK, an 8% increase from 2004 and twenty eight per cent of

all cases were antibiotic resistant.[37] The risk of death from vancomycin-resistant enterococci (VRE) is 75%, compared with 45% for those infected with a susceptible strain.[37] These figures were the same as in the USA. Over a 15 year period there was a 20-fold increase in VRE associated with nosocomial infections reported to CDC's National Nosocomial Infections Surveillance (NNIS).[38]

Aspirates. Total number of isolates was 45 with3 (6.7%) isolates of *E. coli*; 15 (33.3%) isolates of *Streptococcus*sp.; 4 (8.9%) isolates of *Enterococcus* sp.; 10 (22.2%) isolates of *Staph aureus*; 2 (4.4%) isolates of Pseudomonas sp.; 3 isolates of *Proteus* sp.; 4 (8.9%) isolates of *Klebsella* sp.;. no isolate of *Candida* sp.; 4 (4.9%) isolates of coagulase negative staphylococci. In a study of diabetic feet using needle aspiration, mixed aerobic and anaerobic organisms were isolated. The most frequently isolated organisms were enterococci (29.8%), anaerobic streptococci (25.6%) and species of *Proteus* (22.5%), *Clostridium* (9.8%) and *Bacteroides* (.2%).[39]

Cerebrospinal fluid (CSF). Total number of isolates was 22 with 2 (9.1%) isolates of *E. coli*; 10 (45.5%) isolates of *Streptococcus* sp.; 4 (18.2%) isolates of *Enterococcus* sp.; 3 (13.6%) isolates of *Pseudomonas* sp.; no isolate of *Proteus* sp.; 2 (9.1%) isolates of *Klebsiella* sp.; no isolate of *Candida* sp.; no isolate of coagulase negative staphylococci. Enterococci are unusual etiologic agents of bacterial meningitis. In a review of 151 cases of nosocomial meningitis, enterococci accounted for only 3% of the cases.[40] Enterococcal meningitis tends to occur in patients with chronic medical conditions that are often associated with the use of immunosuppressive therapy, underlying central nervous system disease (trauma, surgery, and epidural catheter), gastrointestinal pathology and *Strongyloides* species.[41] Also an association of *E. faecium* meningitis with *Strongyloides* hyperinfection has been reported.[41] The presumed pathogenesis is enterococcal bacteraemia originating from the gastrointestinal tract with secondary seeding of the meninges (Cappello and Hotez, 1993).[42]

Sputum. Total number of isolates was 50 with 6 (12%) isolates of *E. coli*; 22 (44%) isolates of *Streptococcus* sp.; 2 (4.0%) isolates of *Enterococcus* sp.; 4 (8.0%) isolates of *Staph aureus*; 4 (8.0%) isolates of *Pseudomonas* sp.; 6 (12.0%) isolates of *Proteus* sp.; 6 (12.0%) isolates of *Klebsiella* sp.; no isolates of *Candida* sp. and coagulase negative staphylococci. Respiratory tract infections rarely result from enterococci.[43] Enterococcal pneumonia has been reported as a nosocomial infection in severely debilitated patients receiving long-term antibiotic therapy.[44] Empyema due to *E. faecalis* is also uncommon. In three separate retrospective reviews of empyema.[45] and one prospective study of empyema in cirrhosis[46], 30 cases of enterococcal empyema were reported. A specific source of infection was found in two cases of *E. faecalis* empyema - one in a patient with endocarditis and splenic abscess[47] and one related to an esophagopleural fistula after pneumonectomy.[48]

Prevalence of infection caused by *Enterococcus* species

The prevalence of infection caused by *Enterococcus* species in this study was 10.8% and enterococcal infection was also the third leading cause of infection. Among the species identified, *E. faecium* showed the highest proportion of 39(57.4%) followed by *E. faecalis* 25(36.8%) and then *E. avium* 4(5.88). This is in line with the work of Murray, (1990)[14] reporting that *Enterococcus faecalis* and *Enterococcus faecium* are the most prevalent species cultured from humans; accounting for more than 90% clinical isolates. Hidron *et al*. (2008)[49] also reported that between 1978 and 2008, *Enterococcus faecium* has emerged as a leading cause of enterococcal infection in the United States. Because they produce bacteriocins, *Enterococcus* species have been used widely over the last decade in the food industry as probiotics or as starter cultures.[50] However, the difference between an enterococcal pathogen and an apparently safe food use strain is unclear and the potential for the latter to acquire virulent factors by gene transfer has been demonstrated.[51] Recent studies have pointed out that *E. faecium* and *E. faecalis* might be potential recipients of vancomycin resistance genes, and consequently, the FAO/WHO (2001)[52] have recommended that *E. faecium* should not be considered as probiotics for human use. Recently, enterococci have become one of the most common nosocomial pathogens, with patients having a high mortality rate of up to 61%.[53]

CONCLUSION

It was observed from this study that the incidence of *Enterococcus sp* was high. It is therefore, advised that more attention should be given to this organism. Laboratories should establish protocols for identification of these organisms. Public enlightenment should be put in place by public health departments.

ACKNOWLEDGEMENTS

Our unreserved gratitude goes Prof. A.O. Ekundayo, Dr. A.R Akpe and all those who contributed to the success of this research and presentation of this manuscript.

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