Original Research Article

Prevalence and Antibiogram Characteristics of Bacteriuria and Cadidiuria among Indigenes of Selected Parts of Akure North, Akure

ABSTRACT

Aims: The aim of this study was to evaluate the prevalence and antibiogram characteristics of bacteria and fungi in urine samples of some selected towns in Ondo State.

Study design: Experimental design.

Study location: Urine samples were collected from patients visiting University of Medical Science Teaching Hospital Akure and indigenes of Ogbese, Ipogun-Ayo and Ita-oniyan community in Ondo North, Nigeria.

Methodology: Pre-survey of the study participants was carried out using randomly distributed questionnaires. Two hundred and forty-three (243) urine samples were randomly collected from apparently healthy male and female participants cutting across different age groups. Presumptive identification of isolated bacteria and fungi were cultured on general and differential media cysteine lactose electrolyte deficient agar, blood agar, nutrient agar, and Potato dextrose agar, for fungal isolates) were done using standard methods. The clinical and relative typed isolates were collected from Federal Institute of Industrial Research Oshodi (FIIRO) and were subjected to antibiotic sensitivity pattern using array of ten (10) conventional antibiotics according to Clinical Laboratory Standard Instruction.

Results: *Stapylococcus aureus* was the most frequently occurred (27.2%) bacteria isolated from the urine samples while, *Candida albicans* had the least (5.6%) occurrence. These findings revealed that bacteriuria and candidiuria were prevalent among the indigenes of Ipogun-Ayo, Ondo State, with antibiotic sensitivity to few conventional antibiotics. This is an indication of UTI among the indigenes and urgent need for medical attention.

KEY WORDS: Urinary Tract Infections, Bacteriuria, Candidiuria, Colony forming unit, Antibiotics.

1. INTRODUCTION

Urinary Tract Infection (UTIs) is categorized as uncomplicated or complicated. Uncomplicated UTIs normally affect persons who are otherwise healthy and have no physical or nervous urinary tract abnormalities [1] while, complicated UTIs is accompanying with factors increasing colonization, catheters and immunocompromised state of an individual [2]. UTIs are commonly encountered in both the community and hospital environment [3]. The infections have been reported among people of different ages, but have been found to be more prominent with women [3, 4]. UTI is found to be predominant in

women at age 20, one third of women are known to visit health facilities treating and retreating urinary tract infections. This infection is usually not common in males, but, when present could lead to renal damage and chronic renal failure [5]. Bacteriuria is significant when supposed sterile mid-stream urine exceeds 10^5 cfu/ ml. Many microorganisms are known to cause urinary tract infections, but the most common causative agents are bacteria namely: *Escherichia coli, Enterobacter aerogenes, Proteus mirabilis*, and *Staphylococcus* spp [6]. Candiuria on like bacteriuria is not usually found in healthy persons. Candiduria is mostly found in immunosuppressed and immunocompromised patients [7]. Candida albicans is most frequent fungi causing UTI in patients. [7, 8] defined candiduria to be the presence of candida species when more than 10^4 CFU/mL in urine. Antibiogram susceptibity pattern is limited among people in Ondo state UTI survey particularly in the study area. This study was aimed to determine the bacterial and profile, antibiogram pattern and the infections in the studied area. The increase in the antimicrobial resistance poses a challenge in treating and controlling UTIs [9].

2. MATERIALS AND METHODS

2.1 Ethics Approval

Permission for the collection of urine samples for this research was obtained from the Ethics Committee Ministry of Health, Akure, Nigeria. This allowed the collection of urine samples. The areas of collection were among patients of University of Medical Sciences Teaching Hospital, Akure and indigenes of Ogbese, Ipogun-Ayo and Ita-oniyan community.

2.2 Collection of Urine Samples

Two hundred and forty-three (243) urine samples were collected with clear instructions to the participants. Clean-catch midstream urine sample was obtained from each participant into sterile universal bottles.

2.3 Isolation

The samples were immediately transported to Microbiology Laboratory, Federal University of Technology Akure, using a sample box containing ice packs within 1-2 hours. Four growth media (Potato Dextrose Agar, Nutrient agar, CLED agar and blood agar) were prepared according to manufacturers' manual and sterilized accordingly. Fifteen milliliters of each growth medium was aseptically poured into sterilized petri dishes and allowed to gel. A loopful of collected urine sample was aseptically transferred on the agar plates and spread evenly. The labeled inoculated plates were incubated at 37 ˚C aerobically for 24 hours and 25 ˚C colony forming units for 72 hours for bacteria and fungi respectively. Significant growth was taken at $2x10^6$ and $2x10^5$ spore mL^{-1} [10]. Colonies were observed and counted in cfu/ml using colony counter. Distinct colonies were sub cultured on nutrient agar and pure cultures were stored in sterile slant bottles and kept in 4 ˚C for further studies.

2.4 Collection of Typed Cultures

Typed cultures relative to bacterial and fungal isolates from urine (*Escherichia coli* ATCC 25922 and *Pseudomonas aeurginosa* ATCC 10145) were collected from Obafemi Awolowo

University Research laboratory while, *Staphylococcus aureus* NCTC 5571, , *Proteus vulgaris* ATCC 29905, *Enterococcus faecalis* ATCC 23241 and *Klebsiella pneumonia* ATCC 13883 were collected from Federal Institute of Industrial Research Oshodi (FIIRO), Lagos. All these isolates were subjected to confirmation in the Microbiology laboratory of Federal University of Technology Akure. The isolates were sub-cultured and assayed appropriately for colonial, morphological and biochemical tests.

2.5 Presumptive Identification of Bacterial Isolates from Urine

Colonial, morphological and biochemical characteristics of the isolates were determined accordingly [11]. These colonial features include the opacity, increase and decrease in their colony forming ability on different growth media, colour, shape, edge, elevation and surface. Gram staining reaction was assayed for each bacterial and fungal isolates. The biochemical tests carried out were sugar fermentation test, oxidase, urease, catalase, coagulase, citrate, sulpfide, indole and motility tests.

2.5.1 Gram Staining Reactions

Small inoculum of the bacterial and fungal colony was emulsified in sterile distilled water and used to make thin preparation on glass slide. Then slide was kept safe to air-dry. The smear was fixed to preserve microorganisms and to prevent smear from being washed away from slides during staining. The slide was flooded with crystal violent stain for 60 seconds and then quickly washed off under a slow running clean tap water, the water on the slide was removed and smear covered with lugol's iodine for 60 seconds and washed off with clean running tap water. Decolourizer (ethanol) was added for about 20 seconds in other to decolourize purple dye-iodine complexes from the isolates and then washed under slow flowing tap water; secondary stain (safranin) was added for 60 seconds, then was washed off under slow running tap water and left to air dry. The slides were first examined microscopically, with the 40x objective lens to check the staining and to see distribution of material. A drop of oil immersion was placed on the stained smear then examined under with a light microscope using oil immersion objective (x100) lens [12,4].

2.5.2 Fermentation of Sugars

Arabinose, Glucose, Fructose, Galactose, Lactose Sorbitol, Sucrose, Maltose and L-manitol were used for the sugar fermentation test. 1.0 g of each sugar was weighed and dispended into different conical flasks and labeled appropriately, 2.5 g of peptone water was added up to 100 ml of distilled water and 0.01 g of phenol red was added as the indicator 5 ml of each sugar was dispensed into 20 mls test tubes with Durham's tube introduced in upturned position into each test tube. Each test tube was corked with and labeled appropriately; and sterilized 15 minutes at 121℃. After sterilization the test tubes were allowed to cool. Bacterial isolates were now aseptically inoculated into the sugar solution and incubated at 37 ℃ to 24-72 hours. Changes in the colour from red to yellow specifies production of acid, which implies that there was sugar utilization by the microorganism and appearance of bubbles in the Durham's tube shows gas production but if there is no colour change, acid nor gas is not produced which implies a negative reaction [14].

2.5.3 Oxidase Test

A piece of filter paper was soaked with 2-3 drops of oxidase reagent. Using a sterile piece of stick, a colony of the test organism was picked and smeared on the filter paper reactions were observed for 10 seconds. Positive oxidase indicators the prescience of blue – purple colour while a negative reaction shows no colour change within the stipulated time [14].

2.5.4 Urease Test

The test organism was cultured by streaking over a agar surface. A urea free inoculated medium send as the control. Release of ammonia brings about colour change from yellow to pink or red which designated a positive result and no change show a negative result [14].

2.5.5 Catalase Test

This test distinguished enzyme producing baacteria by the breakdown of hydrogen peroxide to oxygen and water. 3% hydrogen peroxide was prepared and a drop was placed on a glass slide. Using a sterile wooden stick, a colony of the test organism of 24 hours old culture was placed in the hydrogen peroxide and mixed together gently. Effervescence caused by the organism by the liberation of oxygen by the organism designates a positive result while, a negative result shows the absence of catalase production [14].

$$2H_2O_2\,(l) \longrightarrow 2H_2O\,(l) + O_2\,(g)$$

2.5.6 Coagulase Test

A 24hrs old culture was emulsified in normal saline on clear grease free slide containing a drop of distilled water on both end of the slide to make two thick suspensions. Then a loopful of plasma was added to one of the suspensions and mixed gently. The second suspensions contain no plasma. This was used to differentiate any granular appearance of the organism from true coagulase clumping. Clumps or precipitates in 10 seconds indicates a positive result while, no clumping within 10 seconds indicates a negative test [14].

2.5.7 Citrate Test

Simon citrate agar was prepared as slopes of medium in bijou bottles according to the instruction of the manufacturer, poured aseptically and allowed to gel. Overnight both culture of the bacteria was inoculate on the medium and incubated at 37 ℃ for 3-5days colour change from green to bright blue colour indicates a positive citrate test while, no colour change indicates a negative citrate test of medium [13, 15].

2.5.8 SIM Test (Sulfide, Indole, Motility)

SIM agar was prepared according to the manufactures specification in test tubes and sterilized in the autoclave for 15 minutes at 121℃. After the agar is cooled and became a semi-solid-medium, 24hrs cultures of the tested organisms were inoculated in the SIM medium by the medium by making a stab on the center of the medium to a depth of ½ inch, and then incubated aerobically at 37 ℃ for 24hours. Then the following results were recorded. A H_2S positive test was denoted by blackening of the medium along the inoculation line. H_2S negative result means there is no blackening. Positive motility test indicate by a diffused zone of growth from inoculation line while, a negative result means

otherwise [15]. Indole positive result was affirmed by pink to red colour band at the top of the medium once Kavacs Reagent has been added. A green/yellow colour denotes indole negative result [13].

2.6 Identification of fungal isolates

The fungal colonies were sub-cultured on Potato Dextrose Agar. The isolates were identified based on their morphological and microscopic features. Two drops of cotton-blue-in-lactophenol were placed on clean glass slide and small piece of mycelium free of medium was removed with sterile inoculating needle and transferred on to the stain. The mycelium was teased (picked) out with the needles and covered with clean cover slip carefully avoiding air bubbles and observed under the microscope for vegetative and reproductive structures [16].

2.7 Antibiotic Susceptibility Test

Antibiotic susceptibility patterns of the bacterial isolates were evaluated using disc diffusion assay [17]. Antibiotic disc containing the following antibiotics was used: Gentamicin (10 μg), Amoxicillin (30 μg), Chloramphenicol (30 μg), Erythromycin (10 μg), Streptomycin (10 μg), Augmentin (30 μg), Septrin (30 μg), Ampiclox (30 μg), Zinnacet (30 μg), Pefloxacin (10 μg), Rocephin (30 μg), Sparfloxacin (30 μg), Tarivid (30 μg) and Ciprofloxacin (5 μg). Standardized culture of each isolate was used to seed Mueller-Hinton agar aseptically. These were allowed to solidify and the antibiotic discs were aseptically placed on the surface of the culture media in a sterile condition. The plates were incubated at 37°C for 24 hr. Zones of inhibition were recorded and compared with Committee for Clinical Laboratory Standards Interpretative Chart, [18].

2.8 Statistical Analysis

All data odtained were subjected to one way analysis of variance (ANOVA) using SPSS 20.0v. Difference between means was determined by Duncan's New Multiple Range Test at ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

Table 1 shows the frequency of occurrence of uropathogen in respect to study location. Ita-oniyan had the highest (43.80%) of *S. saprophyticous* and least (7.14%) with *K. pneumonia.* Ogbese had the highest (37.50%) of *P. vulgaris* and lowest (11.11%) of *E. coli*. Ipogun-Ayo had the highest microbial distribution of (37.04%) with *E. coli* and lowest (2.50%) with *P. vulgaris.* University of Medical Sciences Teaching Hospital, Akure. (UNIMED) had the highest (36.36%) of *C. albicans* and least (9.39%) of *S. saprophyticous.* The total microbial distribution of uropathogens across study location showed Ipogun-Ayo to have the highest (28.72%) while; Ita-oniyan had the least (23.07%) among others. It was revealed that Ipogun-Ayo had the highest percentage of bacteriuria among the study site. The result of this work is in corroboration with the discoveries of [19, 20] who in their study on the assessment of edemicity of prazinquatel where Ipogun-Ayo had the highest prevalence of 18.0%. This present study also implicated Ipogun-Ayo to have the highest prevalence of (28.72%).

200 **Table 1:** Frequency of occurrence of uropathogens in respect to study locations

201

Isolate	**Ita-oniyan**		**Ogbese**		**Ipogun-Ayo**		**UNIMED**		**Total (per organis %)**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	
Staphylococcus aureus	7	**13.2**	15	**22.30**	17	**32.08**	14	**26.42**	53
Escherichia coli	5	**18.51**	3	**11.11**	10	**37.04**	9	**33.33**	27
Proteus vulgaris	6	**25.00**	9	**37.50**	6	**2.50**	3	**12.5**	24
Staphylococcus sapropyiticus	14	**43.80**	5	**15.63**	10	**31.25**	3	**9.38**	32
Enterococcus feacalis	4	**21.05**	4	**21.05**	6	**31.58**	5	**26.32**	19
Klebsiella pneumoniae	1	**7.14**	5	**33.71**	3	**21.48**	5	**35.71**	14
Pseudomonas aeruginosa	5	**33.33**	4	**26.67**	3	**20.00**	3	**20.00**	15
Candida albicans	3	**27.27**	3	**27.27**	1	**9.09**	4	**36.36**	11
Total (per location %)	**45**	**23.07 %**	**48**	**24.61 %**	**56**	**28.72%**	**46**	**23.59%**	**195**

Table 2 shows the percentage occurrence of microorganisms isolated from urine samples. The presumptive isolates were *S. saprophyticous, K. pneumonia, P. vulgaris, C. albicans, E. coli, S. aureus, E. feacalis, P. aeruginosa.* This result shows *S. aureus* to be most frequently occurred with (27.2%) while *C. albicans* had the lowest (5.6%) among others. The microorganisms isolated from the urine samples were; *Proteus vulgaris, Staphylococcus aureus, Escherichia coli, Staphylococcus saprophyticus, Enterococcus feacalis, Klebsiella pneumonia, Pseudomonas aureginosa* and *Candida albicans.* The presence of these organisms in urine samples was however, substantial to the findings of [21, 22]. *Staphylococcus aureus* to be the most frequently occurred uropathogen with 27.2%. The presence of *S. aureus* at higher percentage is however, supported by [22] that *Staphylococcus* spp. is common to cause urinary tract infections in younger persons. Odoki *et al.,* [21] also recorded *S. aureus* to be more prominent to cause UTIs among pregnant women in Uganda.

However, probable cause for increase in the percentage of *Staphylococcus aureus* in urine samples from patients and participants could be as a result of contamination during collection of urine samples. Secondly, since this infection was found to be prominent with persons that are in their sexually active stage and age, it could also be as result of oral sexual intercourse, thereby causing bacteuria [22]. Another factor could be over use of antibiotics making *Staphylococcus* species to grow resistance to the effectiveness of the drugs. This will make it possible for the organisms by producing enzymes that destroys the active drugs [23]. *Staphylococci* are known to be resistant to penicillin G by producing a β −Lactamase that destroys the drug. *Staphylococcus* species could have also been able to gain resistance to the administered conventional drugs by failure to activate autolytic enzymes in the cell wall, which then resulted in the inhibition without killing the bacteria. This could also be as a result of survival of the bacteria in the bladder through progression in the intracellular bacteria communities (IBCs) [22].

Table 2: Percentage occurrence of microorganisms isolated from urine samples

Isolate	Total	Percentage
Staphylococcus aureus	53	27.2
Escherichia coli	27	13.8
Proteus vulgaris	24	12.3
Staphylococcus sapropyiticus	32	16.4
Enterococcus feacalis	19	9.7
Klebsiella pneumonia	14	7.2
Pseudomonas aeruginosa	15	7.7
Candida albicans	11	5.6
	195	**100%**

3.3 Occurrence of uropathogens in relation to age and sex distribution

Figure 1 shows the distribution of bacterial and fungal isolates across different age groups; ages 5-15 ages had 6.58% male and 4.93% female, ages16-26 had 8.64% male and 13.6% female, ages 27-37 had 9.42% male and 18.93% female; ages 38-48 had 3.29% male and 11.11% female and ages from 49 and above had 9.88% male and 13.6% female. [24] reported that, UTI when complicated may be life threatening thereby involving the lower urinary tract and/or the upper urinary tracts. The age of the participants and patients that featured in this study, which had the highest occurrence of the infection were people within the age ranges of 27-37 years. This result is corresponding to that of [22] who reported that people within this age range and those that are at ages lesser than fifty years of age are mostly susceptible to the infection. Findings from this study also revealed that female have higher (18.93%) of the uropathogens compared to male folks. [25, 26], reported in that urinary tract infections occurs four times more frequently in female than in male which is usually because of their anatomical makeup. The female anatomy allows easy passage of uropathogens from the rectum to the genitourinary part. The importance of the ascending route of infection is supported by the shortness of length of the urethra (4cm) and also the proximity to the warm moist vulva (which encourages the growth of uropathogens). According to [22] the perirectal areas are also prone to been colonized by uropathogens. Aydin *et al.,* [27] also reported that fluctuation in the female hormone; progesterone stimulates the growth of uropathogens.

Impaired and immunodeficiency state of the host could be a possible reason for the increase in the microbial population thereby casing urinary tract infections. When a person is immunodeficient, this could lead to frequent, severe and recurrent urinary tract infections [27]. In accordance with the findings of [28]who highlighted some predisposing factors to UTIs as (immunodeficiency) diabetes mellitus, organ transplants, (urinary irregularities) urinary calculi, voiding dysfunction, reduced urine flow; (behavioural effects) sexual intercourse, spermicide use and estrogen deficiency. High volume of retention of urine could be responsible for recurrent UTIs in male patients, and also those that with lower UTIs symptoms [28].

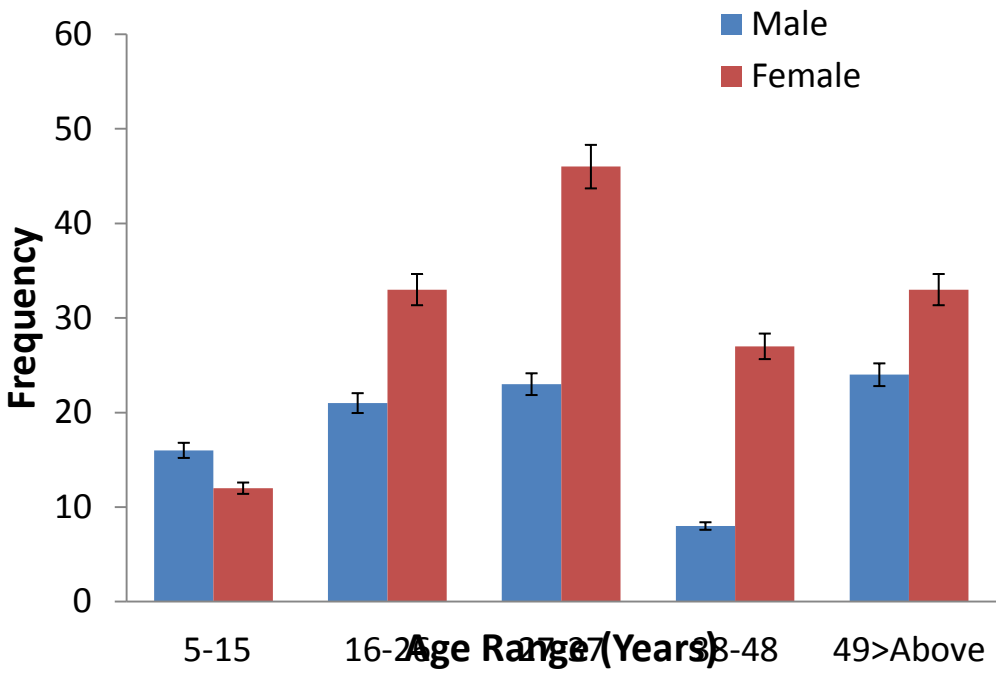

Figure 1: Frequency occurrence of uropathogens in relation to age and sex distribution

3.4 Variation in the Colony Counts on Different Culture Media

Table 3 shows the variation in the colony counts on different culture media. CLED, BA, NA, and PDA were used for bacterial and fungal isolates respectively. NA had the highest (greater than the colony forming unit for bacterial isolates) being a general purpose media. CLED agar had higher cfu for bacteria that had affinity for cysteine and lactose. Blood agar also exhibited greater cfu for hemolytic bacteria distinguishing the α, β and gamma hemolysis bacteria among others while, PDA on supported the growth of fungal isolates after the introduction of chloramphenicol before the 40˚C cool agar was poured into petri-dishes and allowed to set.

Table 3: Variation in colony counts in culture media used for urine samples

Organisms	**CLED**	**BA**	**NA**
Bacteria (10^5 cfu/ml)			
A	+	+	+
B	+	-	+
C	-	-	+
D	+	+	+
E	+	-	+
F	+	-	+
G	-	-	+
Fungi (10^6 cfu/ml)	**PDA**		
H	+		

KEY: A = *Staphylococcus aureus*, B **=** *Escherichia coli*, **C=** *Proteus vulgaris,* D= *Staphylococcus sapropyiticus*, E**=** *Enterococcus feacalis,* F**=** *Klebsiella pneumonia,* G**=** *Pseudomonas aeruginosa*, H=*Candida albicans*, + = indicates a higher colony count observed for a particular microbiological media, - = indicates a less colony count observed for a particular microbiological media, CLED = cysteine lactose electrolyte deficient agar, BA = Blood agar, NA = Nutrient agar, PDA = potato dextrose agar

3.5 Colonial Characteristics of Bacterial and Fungal isolates

Table 4 shows the colonial characteristics of bacterial and fungal isolates considering the following features; opacity, colour of the colonies with respect to different culture media, shape, edge, elevation, surface.

Table 4: Colonial characteristics of isolated bacteria

Isolates	Opacity	Colour	Shape	Edge	Elevation	Surface
A	Opaque	Deep yellow on CLED	Cicular	Undular	Raised	Smooth
B	Opaque	Pink on MacConkey	Circular	Entire	Raised/convex	Smooth
C	Opaque	Colourless on MacConkey	Circular	Entire	Convex	Smooth
D	Opaque	White to pink on MacConkey	Circular	Entire	Convex	Smooth
E	Opaque	White on MacConkey	Circular	Entire	Convex	Smooth
F	Opaque	Grey on CLED	Circular	Small mucoid	Convex	Smooth
G	Opaque	Greenish on nutrient agar	Round	Entire	Flat	Rough
H	Opaque	Cream to white	Round	Entire	Raised	Smooth

KEY: A = *Staphylococcus aureus*, B **=** *Escherichia coli*, **C=** *Proteus vulgaris,* D= *Staphylococcus sapropyiticus*, E**=** *Enterococcus feacalis,* F**=** *Klebsiella pneumonia,* G**=** *Pseudomonas aeruginosa*, H=*Candida albicans*,

3.6 Biochemical Characteristics of the Bacterial Isolates

Table 5 shows the biochemical characteristics of the bacterial isolates. The probable isolates were subjected to Simon citrate test, Urease test, oxidase test, motility (to confirm it an organism is motile or not), indole test, catalase, coagulase, sugar fermentation test, grams reaction, among the others.

Table 5: Biochemical and morphological characteristics of bacteria isolate.

Isolates	Gram stain	Cell shape	urease	oxidase	Citrate	Motility	Indo	Catalase	Coagulase	gas	H_2S	Mannitol	Lactose	Sucrose	Glucose	Galactose	Maltose	**Presumptive Isolates**
A	+	Cocci	-	-	-	-	-	+	+	-	-	+	+	+	+	+	+	*Staphylococcus aureus*
B	-	Rod	-	-	-	+	+	+	-	+	-	+	+	-	+	+	+	*Escherichia coli*
C	-	Rod	+	-	-	+	-	+	-	-	+	-	-	+	+	-	-	*Proteus vulgaris*
D	+	Cocci	+	-	-	-	-	+	+	-	-	+	+	+	+	+	+	*Staphylococcus sapropyiticus*
E	+	Cocci	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	*Enterococcus feacalis*
F	-	Rod	+	-	+	-	-	+	+	+	-	-	+	+	+	-	+	*Klebsiella pneumoniae*
G	-	Rod	-	+	+	+	-	+	+	-	-	+	-	+	-	-	-	*Pseudomonas aeruginosa*

KEY: KEY: A = *Staphylococcus aureus*, B **=** *Escherichia coli*, **C=** *Proteus vulgaris,* D= *Staphylococcus sapropyiticus*, E**=** *Enterococcus feacalis,* F**=** *Klebsiella pneumonia,* G**=** *Pseudomonas aeruginosa*, **+ = Positive, - = negative**

3.7 Antibiogram Susceptibility Pattern of Bacterial and Fungal Isolates in respect to the Tested Conventional Antibiotics

Table 6 shows the antifungal activities of convention antifungal agents against test clinical and typed fungal isolates. *C. albican* ATCC 10231 had the highest (21.00±0.58 mm) zone of inhibition compared to clinical fungal isolates.

Table 7 and 8 showed the antibiogram susceptibility pattern of Gram-positive and Gram-negative in respect to the tested conventional antibiotics. Ciprofloxacin was most effective while septrin was least effective (1.33±0.33 mm) zone of inhibition. Typed bacterial and fungal isolates were more susceptible to the conventional antibiotics than clinical isolates. *P. aeruginosa* ATCC 10145 had the highest (22.00±1.15 mm) zone of inhibition while, *K. pneumonia,*had the least (16.33±0.67 mm) zone of inhibition to ciprofloxacin at (30*µ*g).

The pattern of conventional antibiotics in inhibiting the growth of both clinical and typed organisms showed the varying abilities of each organism to resist the antimicrobial activity. However, these disparities could be due to variation in the structure and components of the microbial cell wall, because these attributes are the ultimate target of any antimicrobial agent [29]. The result of the conventional drugs susceptibility test shows that Gram-negative microorganisms were more susceptible to the antibiotics than Gram-positive organisms. These differences may be due to the cell wall structural differences between the Gram-negative and Gram-positive bacteria. The result is in corroboration with [23] who revealed that the walls of Gram-positive that might be responsible for this resistance is the thick layer of peptidoglycan which makes it resistance to osmotic pressure.

The wide spread use of conventional drugs both inside and outside medicine is important in the emergence and re-emergence of resistant bacteria [30]. The extensive and wrong use of antimicrobial agents has consistently leaded to the development of antibiotics resistance which has become a major problem globally [30]. [31] observed in recent discoveries that bacteria antibiotics resistance patterns may be due to the presence of large plasmid and the ability of the plasmids to undergo conjugation process.

Table 6: Antibiotics susceptibility pattern of conventional antifungi agents on fungal isolates

Isolate	Fluconazole	Itraconazole	Ketoconalzole
C. albican	11.00±0.58[a]	16.33±0.33[b]	26.00±0.58[c]
C. albican ATCC 10231	21.00±0.58[b]	16.00±0.58[a]	16.33±0.33[a]

Values represent means ± standard deviation of triplicate readings. Superscripts of the same letter in a row are not significantly different at P≤0.05.

Table 7: Antibiotics susceptibility pattern of conventional antibiotics on Gram- positive bacteria

Bacteria	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
S. aureus	10.00±0.58^{a}	12.00±0.58a^{b}	16.67±0.33^{d}	19.67±0.88^{e}	14.67±0.88^{cd}	15.67±0.88^{d}	19.67±0.88^{e}	11.00±0.58^{a}	13.33±0.33^{bc}	14.33±0.67^{cd}
S. aureus NCTC 6571	11.33±0.88^{a}	11.67±0.66^{a}	19.67±0.07^{c}	15.33±0.88b	14.00±0.58^{c}	14.33±0.67^{b}	20.67±0.88^{c}	14.00±0.58^{b}	16.33±0.58^{b}	14.33±0.67^{b}
S. saprophyticus	6.67±0.88^{a}	14.00±0.58^{bc}	19.00±0.58^{d}	13.67±0.88^{b}	16.33±0.67^{c}	14.33±0.33^{b}	18.67±1.20^{d}	14.33±0.33^{bc}	14.33±0.88^{bc}	14.33±0.67^{bc}
E. feacalis	0.00±00^{a}	14.67±0.88^{b}	14.33±0.66^{b}	15.00±0.58^{b}	13.00±0.58^{b}	13.00±1.15^{b}	18.33±0.58^{c}	14.000±0.58^{b}	15.00±0.57^{b}	13.67±0.33^{b}
E. feacalis ATCC 23241	1.00±0.58^{a}	14.00±0.58^{b}	16.33±1.52^{bc}	18.67±0.88^{ef}	15.00±0.58^{bc}	14.33±0.67^{b}	19.67±0.33^{e}	16.33±0.58cd	18.00±0.58^{def}	17.00±0.78^{de}

Values represent means ± standard error of triplicate readings. Superscripts of the same letter in a row are not significantly different at p≤0.05.
KEY: **PEF** = Pefloxacin, **CN** = Gentamycin, **APX** = Ampiclox, **Z** = Zinnacef, **AM** = Amoxacillin, **R** = Rocephin, **CPX** = Ciprofloxacin, **S** = Streptomycin, **SXT** = Septrin, **E** = Erythromycin

Table 8: Antibiotics susceptibility pattern of conventional antibiotics on Gram- negative bacteria

Bacteria	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
E. coli	8.00±0.58^{a}	19.67±0.88^{ef}	2.67±0.67^{a}	21.00±0.67^{f}	18.33±0.58^{e}	1.00±1.14^{a}	15.67±0.58^{cd}	14.00±0.83^{bc}	13.00±0.88^{cd}	12.00±0.67±0.58^{e}
E. coli ATCC 25922	9.00±0.58^{a}	21.00±0.58^{ef}	19.67±0.88^{de}	21.67±0.67^{f}	20.00±0.58^{def}	12.00±0.58^{a}	19.00±0.58^{d}	16.00±0.58^{c}	13.00±0.58^{a}	12.00±0.58^{cda}
P. vulgaris	9.0±0.58^{a}	0.33±0.33^{a}	16.00±0.58^{e}	19.00±0.58^{f}	18.00±0.58^{f}	1.00±0.58^{a}	15.67±0.33^{de}	13.33±0.88^{bc}	14.00±0.58^{cd}	20.67±0.33^{f}
P. vulgaris ATCC 29905	6.0±1.52^{a}	3.67±0.88^{a}	17.67±0.88^{d}	20.0±0.67^{e}	21.00±1.15^{f}	14.00±0.58^{b}	18.33±0.67^{de}	17.00±0.58^{cd}	14.67±0.33^{a}	21.33±0.33^{f}
K. pneumonia	00.0±0.00^{a}	00.00±0.00^{a}	18.33±0.58^{de}	16.33±0.67^{d}	15.33±0.33^{cd}	18.67±0.88^{e}	0.00±0.00^{a}	11.00±0.58^{b}	14.00±0.58^{c}	19.67±0.88^{ef}
K. pneumonia ATCC 13883	1.3±0.88^{a}	1.00±1.14^{a}	21.00±0.67^{f}	17.67±0.88^{cd}	18.33±1.20^{de}	18.00±0.58^{d}	2.00±0.58^{a}	13.67±0.88^{bc}	18.00±0.58^{d}	21.67±0.88^{ef}
P. aeurginosa	1.33±0.33^{a}	15.67±0.33^{cd}	15.33±0.33^{cd}	21.33±1.20^{e}	11.67±0.88^{a}	13.33±0.88^{bc}	18.33±0.67^{d}	16.00±0.58^{c}	19.00±0.58^{d}	17.00±1.00^{d}
P. aeurginosa ATCC 10145	3.0±0.58^{a}	17.36±0.88^{cd}	16.00±0.58^{bcd}	22.00±1.15^{e}	12.67±0.88^{a}	15.00±0.58^{b}	20.67±0.33^{e}	16.33±0.33^{bcd}	14.33±0.33^{ab}	18.00±0.58^{ef}

Values represent means ± standard error of triplicate readings. Superscripts of the same letter in a row are not significantly different at p≤0.05.

KEY: SXT = Septrin**, CH =** Chloranphnicol**, SP =** Sparfloxacin, **CPX =** Ciprofloxacin, **AM =** Amoxacillin, **AU =** Augumentin, **CN =** Gentamycin, **PEF =** Pefloxacin, **OFX = Tarivid, S =** Streptomycin

4. CONCLUSION

This study revealed *S. aureus* to be pfominent in causing UTIs in people at young ages and Ipogun-Ayo has high endemicity with bacteriuria compared to other study sites. Conventional antibiotics should be used appropriately as prescribe by the physician in other to avoid resistance.

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ONDO STATE GOVERNMENT
ONDO STATE HEALTH RESEARCH ETHICS COMMITTEE (OSHREC)
MINISTRY OF HEALTH

Email: oshrec@ondostatemoh.gov.ng Website: www.ondostatemoh.gov.ng
Health Research Ethics Committee Assigned Number: NHREC/18/08/2016
Protocol Number: OSHREC/12/11/2018/069.

RE: **Comparative Evaluation of Antimicrobial activity of Hibiscus sabdariffa and Honey on Microorganiosms Associated with Urinary Tract Infections.**

Name of Investigator: Bodunrinde Ruth Ebunoluwa
Address of Investigator: Department of Microbiology,
School of Sciences,
Federal University of Technology, Akure,
Ondo State, Nigeria.

Date of Receipt of valid application: 12/11/2018

Notice of FULL Approval After Full Committee Review

This is to inform you that upon your request for ethical approval and the submission of your research protocol, the consent form(s) and other participant information materials, the Health Research Ethics Committee has considered your protocol and found it to be in compliance with international standards and best practices.

Therefore, I am pleased to convey to you that the proposal under its reviewed State has been granted expedited/full approval in line with the contents of the protocol. This approval dates from 19/12/2018 to 18/12/2019. If there is delay in starting the research, please inform the OSHREC so that the dates can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside these dates. All informed consent forms used in this study must carry the OSHREC assigned number and duration of SHREC approval of the study. In multiyear research, endeavor to submit your annual report to SHREC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse effects are reported promptly to the OSHREC. No changes are permitted in the research without prior approval by the OSHREC except in circumstances outside the Code.

The OSHREC reserves the right to conduct compliance visit to your site without prior notification and to recall its approval if the conduct of the research deviates from the stated objectives, procedures and best practices.

Best Regards.

19/12/18

Dr. O.A.Durojaiye

Chariman, OSHREC

463