1 ***Original Research Article***
2
3 # Prevalence and Antibiogram Characteristics of Bacteriuria
4 # and Cadidiuria among Indigenes of Selected Parts of Akure
5 # North, Akure

6 **ABSTRACT**

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

9 **Study design:** Experimental design.

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

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

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

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

29 ## 1. INTRODUCTION

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

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

51 ## 2. MATERIALS AND METHODS

52 ### 2.1 Collection of Urine Samples

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

56 ### 2.3 Isolation

57 The samples were immediately transported to Microbiology Laboratory, Federal University of
58 Technology Akure, using a sample box containing ice packs within 1-2 hours. Four growth
59 media (Potato Dextrose Agar, Nutrient agar, CLED agar and blood agar) were prepared
60 according to manufacturers' manual and sterilized accordingly. Fifteen milliliters of each
61 growth medium was aseptically poured into sterilized petri dishes and allowed to gel. A
62 loopful of collected urine sample was aseptically transferred on the agar plates and spread
63 evenly. The labeled inoculated plates were incubated at 37 ˚C aerobically for 24 hours and
64 25 ˚C colony forming units for 72 hours for bacteria and fungi respectively. Significant
65 growth was taken at $2x10^6$ and $2x10^5$ spore mL^{-1} [10]. Colonies were observed and counted
66 in cfu/ml using colony counter. Distinct colonies were sub cultured on nutrient agar and pure
67 cultures were stored in sterile slant bottles and kept in 4 ˚C for further studies.

68 ### 2.3 Collection of Typed Cultures

69 Typed cultures relative to bacterial and fungal isolates from urine (*Escherichia coli* ATCC
70 25922 and *Pseudomonas aeurginosa* ATCC 10145) were collected from Obafemi Awolowo
71 University Research laboratory while, *Staphylococcus aureus* NCTC 5571, , *Proteus vulgaris*
72 ATCC 29905, *Enterococcus faecalis* ATCC 23241 and *Klebsiella pneumonia* ATCC 13883
73 were collected from Federal Institute of Industrial Research Oshodi (FIIRO), Lagos. All these
74 isolates were subjected to confirmation in the Microbiology laboratory of Federal University
75 of Technology Akure. The isolates were sub-cultured and assayed appropriately for colonial,
76 morphological and biochemical tests.

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77 ## 2.4 Presumptive Identification of Bacterial Isolates from Urine

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

84 ### 2.4.1 Gram Staining Reactions

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

98 ### 2.4.2 Fermentation of Sugars

99 Arabinose, Glucose, Fructose, Galactose, Lactose Sorbitol, Sucrose, Maltose and L-manitol
100 were used for the sugar fermentation test. 1.0 g of each sugar was weighed and dispended
101 into different conical flasks and labeled appropriately, 2.5 g of peptone water was added up
102 to 100 ml of distilled water and 0.01 g of phenol red was added as the indicator 5 ml of each
103 sugar was dispensed into 20 mls test tubes with Durham's tube introduced in upturned
104 position into each test tube. Each test tube was corked with and labeled appropriately; and
105 sterilized 15 minutes at 121℃. After sterilization the test tubes were allowed to cool.
106 Bacterial isolates were now aseptically inoculated into the sugar solution and incubated at
107 37 ℃ to 24-72 hours. Changes in the colour from red to yellow specifies production of acid,
108 which implies that there was sugar utilization by the microorganism and appearance of
109 bubbles in the Durham's tube shows gas production but if there is no colour change, acid nor
110 gas is not produced which implies a negative reaction [14].

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113 ### 2.4.3 Oxidase Test

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

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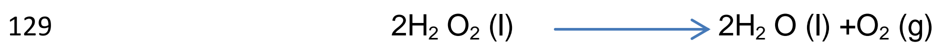
2.4.4 Urease Test

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].

2.4.5 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].



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

2.4.6 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].

2.4.7 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].

2.4.8 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].

2.5 Identification of fungal isolates

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

163 **2.6 Antibiotic Susceptibility Test**

164 Antibiotic susceptibility patterns of the bacterial isolates were evaluated using disc diffusion
165 assay [17]. Antibiotic disc containing the following antibiotics was used: Gentamicin (10 µg),
166 Amoxicillin (30 µg), Chloramphenicol (30 µg), Erythromycin (10 µg), Streptomycin (10 µg),
167 Augmentin (30 µg), Septrin (30 µg), Ampiclox (30 µg), Zinnacet (30 µg), Pefloxacin (10 µg),
168 Rocephin (30 µg), Sparfloxacin (30 µg), Tarivid (30 µg) and Ciprofloxacin (5 µg).
169 Standardized culture of each isolate was used to seed Mueller-Hinton agar aseptically.
170 These were allowed to solidify and the antibiotic discs were aseptically placed on the surface
171 of the culture media in a sterile condition. The plates were incubated at 37°C for 24 hr.
172 Zones of inhibition were recorded and compared with Committee for Clinical Laboratory
173 Standards Interpretative Chart, [18].
174

175 **2.7 Statistical Analysis**

176 All data odtained were subjected to one way analysis of variance (ANOVA) using SPSS
177 20.0v. Difference between means was determined by Duncan's New Multiple Range Test at
178 ($p \leq 0.05$).
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180

181 **3. RESULTS AND DISCUSSION**
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183 Table 1 shows the frequency of occurrence of uropathogen in respect to study location. Ita-
184 oniyan had the highest (43.80%) of *S. saprophyticous* and least (7.14%) with *K. pneumonia.*
185 Ogbese had the highest (37.50%) of *P. vulgaris* and lowest (11.11%) of *E. coli*. Ipogun-Ayo
186 had the highest microbial distribution of (37.04%) with *E. coli* and lowest (2.50%) with *P.*
187 *vulgaris.* University of Medical Sciences Teaching Hospital, Akure. (UNIMED) had the
188 highest (36.36%) of *C. albicans* and least (9.39%) of *S. saprophyticous.* The total microbial
189 distribution of uropathogens across study location showed Ipogun-Ayo to have the highest
190 (28.72%) while; Ita-oniyan had the least (23.07%) among others. It was revealed that
191 Ipogun-Ayo had the highest percentage of bacteriuria among the study site. The result of this
192 work is in corroboration with the discoveries of [19, 20] who in their study on the assessment
193 of edemicity of prazinquatel where Ipogun-Ayo had the highest prevalence of 18.0%. This
194 present study also implicated Ipogun-Ayo to have the highest prevalence of (28.72%).

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

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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**

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

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

223

224 **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%**

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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].

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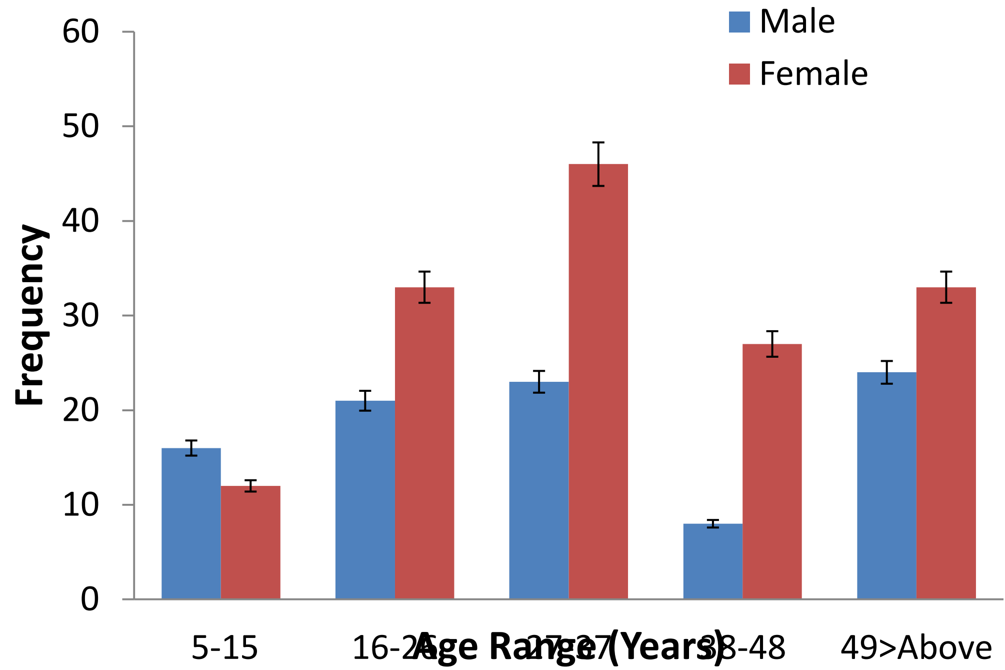

260 **Figure 1: Frequency occurrence of uropathogens in relation to age and sex**
261 **distribution**

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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

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300 **3.5 Colonial Characteristics of Bacterial and Fungal isolates**

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

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305 **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.

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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**

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309 **3.7 Antibiogram Susceptibility Pattern of Bacterial and Fungal Isolates in respect to**
310 **the Tested Conventional Antibiotics**
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312 Table 6 shows the antifungal activities of convention antifungal agents against test clinical
313 and typed fungal isolates. *C. albican* ATCC 10231 had the highest (21.00±0.58 mm) zone of
314 inhibition compared to clinical fungal isolates.
315 Table 7 and 8 showed the antibiogram susceptibility pattern of Gram-positive and Gram-
316 negative in respect to the tested conventional antibiotics. Ciprofloxacin was most effective
317 while septrin was least effective (1.33±0.33 mm) zone of inhibition. Typed bacterial and
318 fungal isolates were more susceptible to the conventional antibiotics than clinical isolates. *P.*
319 *aeruginosa* ATCC 10145 had the highest (22.00±1.15 mm) zone of inhibition while, *K.*
320 *pneumonia,*had the least (16.33±0.67 mm) zone of inhibition to ciprofloxacin at (30μg).
321 The pattern of conventional antibiotics in inhibiting the growth of both clinical and typed
322 organisms showed the varying abilities of each organism to resist the antimicrobial activity.
323 However, these disparities could be due to variation in the structure and components of the
324 microbial cell wall, because these attributes are the ultimate target of any antimicrobial agent
325 [29]. The result of the conventional drugs susceptibility test shows that Gram-negative
326 microorganisms were more susceptible to the antibiotics than Gram-positive organisms.
327 These differences may be due to the cell wall structural differences between the Gram-
328 negative and Gram-positive bacteria. The result is in corroboration with [23] who revealed
329 that the walls of Gram-positive that might be responsible for this resistance is the thick layer
330 of peptidoglycan which makes it resistance to osmotic pressure.

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

337
338 **Table 6: Antibiotics susceptibility pattern of conventional antifungi agents on fungal**
339 **isolates**
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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}

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

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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±0.58 cd	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 $p \leq 0.05$.
KEY: **PEF** = Pefloxacin, **CN** = Gentamycin, **APX** = Ampiclox, **Z** = Zinnacef, **AM** = Amoxacillin, **R** = Rocephin, **CPX** = Ciprofloxacin, **S** = Streptomycin, **SXT** = Septrin, **E** = Erythromycin

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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 $p \leq 0.05$.

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

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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.

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.

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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.

Dr. O.A.Durojaiye

Chariman, OSHREC

State Secretariat, Alagbaka, Akure, Ondo State. www.oshrec@ondostatemoh.gov.ng

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