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PREVALENCE AND CONTROL OF URINARY TRACT INFECTIONS IN AKURE METROPOLIS USING THE EXTRACT FROM *Periplaneta americana*

Ayomide Ebenezer Akinlalu and Muftau Kolawole Oladunmoye*

Address (es): Ayomide Ebenezer Akinlalu,
Federal University of Technology Akure, School of Science, Microbiology department, P.M.B 704, Akure, Ondo state, Nigeria. +2348165189869.

*Corresponding author: ayobishopmide@gmail.com

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ABSTRACT

Urinary tract infections (UTIs) are one of the most common microbial infections of human. It occurs in all ages and gender. This study was aimed at determining the prevalence and control of UTIs in Akure metropolis using the extract from *P. americana* (American cockroach). In this study, 128 urine samples were collected from in-patients at Ondo State University of Medical Science Teaching Hospital Complex Akure and were analyzed for the presence of uropathogens. Isolates were identified using biochemical characteristics. The isolates were subjected to antibiotic sensitivity test using disc diffusion method. A total of 120 American cockroaches were dissected into head, thorax and abdomen and extracts were prepared from the three different parts. Extracts were tested against bacterial isolates *in-vitro* using agar well diffusion method. The isolates include *Escherichia coli* (44.79%), *Klebsiella pneumoniae* (21.87%), *Staphylococcus aureus* (15.63%), *Proteus miriabilis* (9.38%), *Pseudomonas aeruginosa* (6.25%), and *Streptococcus pyogens.* (2.08%). Females between the ages of 20-39 years showed the highest frequency of occurrence of the disease. Only extracts from the head of *P. americana* showed activities against some typed and clinical isolates. The highest and the least zone of inhibition was observed in *E. coli* (ATCC 29822) (17.67±0.10mm) and *S. aureus* (NCTC 6571) (12.58±0.88mm) respectively. This research revealed that extracts from the head of *P. americana* possess antimicrobial efficacy against some pathogenic bacteria implicated in UTIs.

Keywords: *Periplaneta americana*, Urinary tract infections, *in-vitro*

INTRODUCTION

Urinary tract infections (UTIs) are serious health problems which can cause a high morbidity rate in males and females **(Wasmy *et al.*, 2018).** This problem occurs more often in women (69%) than in men (31%) because a woman's urethra is shorter. The short urethra makes it easier for bacteria from the anus or genital area to reach the bladder. Patient with catheter or patients suffering from complaints of prostatitis are also prone to UTIs **(Priyadharsini *et al.*, 2014).** Forty percent of women in the United States develop UTI during their lifetime, making it one of the most common infections in women. UTI is uncommon in circumcised males, and by definition, any male UTI is considered complicated. Many cases of uncomplicated UTI will resolve spontaneously, without treatment, but many patients seek treatment after observing symptoms. Treatment is aimed at preventing spread to the kidneys or developing into upper tract disease/pyelonephritis, which can cause the destruction of the delicate structures in the nephrons and lead to hypertension **(Long *et al.*, 2018; Tang *et al.*, 2019)**
Urinary Tract Infections can be categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities **(Hooton, 2012).**
About 75 – 80% of UTIs is mostly dominated by *E. coli* followed by *S. saprophyticus* 10-15%. While, anatomy or physiological factors cause abnormality of urinary tract and lead to localize infectious bacteria, such as different species of Klebsiella, Proteus, Enterobacter, Enterococcus, Staphylococcus and *Pseudomonas aeruginosa.* Those bacteria are more common in most of the cases and infrequently cause to uncomplicated cystitis and pyelonephritis **(Mohammed *et al.*, 2017).** The mainstay of treatment of urinary tract infections is antibiotics. However, increasing antibiotic resistance is causing concern about the future of treating those with complicated and recurrent UTI **(Shepherd and Pottinger, 2013).**
P. americana (American cockroach) belongs to the family Blattidae. They are nocturnal omnivore's insects that live in damp places throughout the world. They have a ventrally positioned chewing mouth parts made up of a collection of appendages, a long segmented antennae and a pair of membranous wings arising from the mesothorax that are thick and leathery. Also the body of *P. americana* is externally covered by a hard chitinious exoskeleton secreted by underlying cells which provides surfaces for attachment of muscles and also protects the body **(Billah *et al.*, 2015**). It has been discovered that the body parts have been used as herbal mixtures in boiled, fried and powdery form to treat epilepsy, earache, boils, diffusing urination, whooping cough, menstrual regulation, asthma, lactation stimulation and real colic **(Costa-Neto, 2009).**

MATERIALS AND METHODS

Sterilization of materials

All materials were sterilized before and after use. Glass wares were thoroughly washed with detergents, rinsed with distilled water and sterilized in an autoclave at 121ºC for 15 minutes. Sterile plastic petri dishes were used all through. Inoculating loop was heated in a naked Bunsen burner to redness and allowed to cool before use. Work bench was properly disinfected using cotton wool soaked in 70% alcohol.

Sample collections

A total number of 128 Urine samples were collected in sterile urine bottles from patients at the Ondo State University of Medical Science Teaching Hospital Complex Akure and Mother and Child hospital Akure and was transported at a temperature of 4-8◦C with coolant pack to the laboratory within 24 hours for microbiological analysis.

Isolation of bacteria from urine samples

Urine samples were immediately transferred aseptically using sterile and flamed inoculating loop by streaking unto MacConkey agar, Blood agar, and Cysteine Lactose Electrolyte Deficient Agar (CLED) which are routine media for urine culture. They considered as selective and differential medium for the isolation, purification and identification of uropathogens **(Chaturvedi *et al.*, 2017).** All plates were incubated at 35-37ºC for 24 hours.

Identification of bacteria from urine samples

Colonies on the MacConkey agar, CLED agar and blood agar were identified following their colonial characteristics. Single pure cultures were then transferred onto a nutrient agar medium for preservation. The final identification of the isolates was done using biochemical identification method.

Maintenance of clinical isolates

Stock cultures were maintained on nutrient agar slants and stored at 4 C for further study.

Collection of *P. americana*

A total of 120 cockroaches were collected from households, storerooms, refuse dumps, pit toilets using cockroach traps into sterile jars with small hole on top to provide aeration and transported to the laboratory for processing.

Preparation and purification of *P. americana* extract

The collected American cockroaches (*P. americana)* were maintained in an insectary at 25±2 °C temperature with a 12 hours light/dark ratio, and fed on dried bred, dates and water. The cockroaches were killed by placing them in a freezer for about 10 to 20 minutes until they became immobilized. The surface of cockroaches were sterilized with 70% ethanol to remove the microfloras present on their surfaces. The cockroaches were then dissected into the head, thorax and abdomen using a sterile surgical blade. Each group of cockroaches were processed by macerating them separately in a clean dry mortar and pestle. 5grams of each groups were then dissolved into 10ml of 20% Dimethyl sufoxide (DMSO) and kept in the refrigerator for 24 hours according to the method of **Vidhu and Evans, (2015).** After 24 hours, they were then centrifuged and purified by passing them through Millipore filter paper and used for *in-vitro* antibacterial evaluation.

Standardization of bacterial inoculum

A loop full of test bacterial isolates were inoculated on nutrient broth and incubated for 24 hours. 0.2ml from the 24 hours' broth culture of the bacteria was dispensed into 20ml sterile nutrient broth and incubated for 3 to 5 hours to standardize the culture to 0.5 McFarland standards (10^6 CFU/ml) before use according to method described by Clinical Laboratory Standards Institute **(CLSI) (2014).**

Antibiotic sensitivity test using commercial antibiotics

Antibiotic resistance of bacteria was determined by the disc diffusion method with the use of Mueller-Hinton agar, according to the Kirby-Bauer's method. The suspension of the test organism in nutrient broth was matched with 0.5 McFarland turbidity standards to give concentration of 1.5 × 108 CFU/ml, 0.5 ml of the suspension was transferred to prepared Mueller-Hinton agar and spread with a sterilized glass spreader. The surface of the agar was allowed to dry and antibiotic discs were aseptically picked and gently placed on the surface of agar media by the use of sterile forceps. The inoculated plates were incubated at 37ºC for 18 hours, after incubation, a clear zone of no growth in the immediate vicinity of an antibiotic disk was measured and recorded as zone of inhibition. The following antibiotics and their concentrations in parentheses were used; Tetracycline (30 µg), Ofloxacin (30 µg), Gentamicin (20 µg), Chloramphenicol (30 µg), Augmentin (30 µg), Ceftriaxone (30 µg), Nitrofurantoin (300 µg), Cotrimoxazole (25 µg), Ciprofloxacin (10 µg) and Amoxicillin (30 µg) (CLSI, 2014).

Antibacterial evaluation of *P. americana* extract *in-vitro*

The antibacterial screening was carried out using agar well diffusion method as described by **Billah *et al.*, (2015).** Sterile cotton wool swabs were used to pick the inocula for the streaking of the entire surfaces of Mueller-Hinton agar (MHA) plates rotating in 3 directions at approximately 60º for evenly distribution of inocula of the tests bacteria on the MHA plates. Then 6 mm diameter wells were created in five places on the inoculated MHA plates by the use of a sterile cork borer. Using a micropipette with sterile tips, 100 µl of extract stock solutions (500mg/ml) from the head of the cockroach was dispensed into one of the wells, thorax extract stock solution into another well and the abdomen extract stock solution into another well, chloramphenicol solution was dispensed into the fourth well to serve as the positive control and 30% DMSO in the fifth well in the MHA plate. The set-up was incubated aerobically at 37°C for 24 hrs. The inhibition zone diameters were measured using meter rule after 24 hrs incubation and recorded. The whole experiment was repeated for 2 more consecutive times and the mean diameters of zones of inhibition calculated for each bacteria.

RESULTS

Bacteria growths of urine samples

A total of 128 urine samples collected from UNIMEDTH and Mother and Child hospital, Akure were reviewed and 94 samples showed positive urine culture growth while 32 samples were sterile. Out of these 94 samples, 92 samples showed pure growths and 2 showed mixed growths which produced 96 bacteria isolates.

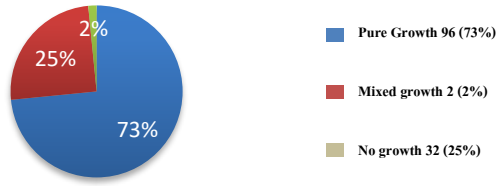

Figure 1 Percentage Growth of Bacterial Isolates from Collected Urine Samples

Occurrence of bacteria isolates from urine samples based on age and gender.

This study revealed that 74 (77.08%) bacteria isolates were detected in urine samples of female patients while 22 (22.92%) isolates were detected in urine samples of male patients. Table 1 shows the prevalence of UTIs in patients in relation to their age group and gender

Table 1 Prevalence of UTIs in patients in relation to their age group and gender

S/N	Age groups (years)	No. of Males	No. of females
1	0-20	4	6
2	21-40	6	26
3	41-69	8	20
4	61-80	4	16
5	80 above	0	6

Biochemical characterization of bacterial isolates from urine

The biochemical characterization of both the Gram positive and Gram negative bacteria isolated from urine samples is shown in Table 2 below

Table 2 Biochemical characterization of bacteria isolates in urine samples

BIOCHEMICAL TESTS	*E. coli*	*S. aureus*	*P. miriabils*	*K. pneumoniae*	*P. aeruginosa*	*S. pyogens*
Catalase	+	+	+	-	+	+
Coagulase	-	+	-	-	-	+
Motility	+	-	+	-	+	-
H_2S	+	-	+	-	-	-
Urease	-	-	+	-	-	-
Indole	+	-	-	-	-	-
Oxidase	-	-	-	-	+	-
Glucose	AG	+	+	AG	-	+
Sucrose	A	-	+	+	+	-
Lactose	+	+	-	+	-	+
Manitol	+	+	-	+	+	+
Citrate	-	-	+	+	+	-
Grams reaction	-ve rod	+ve cocci	-ve rod	-ve rod	-ve rod	+ve cocci

Legend: += Positive, -= negative, A = Acid production, AG= Gas production

Predominance of bacteria isolates from urine samples

The predominance of the various bacteria isolated from urine samples is shown in table 3 below.

Table 3 Predominance of bacteria isolates from urine samples

Isolates	Frequency	Percentage (%)
Escherichia coli	43	44.79
Klebsiella pneumoniae	21	21.87
Staphylococcus aureus	15	15.63
Proteus miriabilis	9	9.38
Pseudomonas aeruginosa	6	6.25
Streptococcus pyogens	2	2.08
Total	96	100

Antibiotic sensitivity pattern of urine bacterial isolates

Table 4 and 5 shows the broad spectrum conventional antibiotics subjected to Gram positive and Gram negative bacterial isolates in urine samples respectively.

Table 4 Antibiotic susceptibility pattern of urine bacterial isolates (gram negative) (mm)

ISOLATES	*E. coli*	*P. miriabilis*	*K. pneumoniae*	*P. aeruginosa*
CPX	20.00±0.00^{b}	20.00±1.15^{b}	8.37±0.12^{a}	17.00±0.58^{b}
AM	0.00±0.00^{a}	8.00±2.00^{b}	0.00±0.00^{a}	15.33±0.33^{c}
AU	9.33±1.76^{b}	4.00±4.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
CN	7.33±1.06^{ab}	9.33±4.80^{b}	0.00±0.00^{a}	10.67±0.33^{b}
PEF	6.67±3.53^{b}	18.00±2.30^{c}	0.00±0.00^{a}	13.33±0.33^{c}
OFX	14.00±0.00^{bc}	14.00±2.30^{bc}	0.00±0.00^{a}	12.67±0.67^{b}
S	8.00±1.22^{b}	9.33±2.67^{b}	0.00±0.00^{a}	0.00±0.00^{a}
SXT	8.00±1.22^{b}	13.33±2.40^{b}	0.00±0.00^{a}	0.00±0.00^{a}
CH	8.00±1.22^{b}	10.00±1.15^{b}	0.00±0.00^{a}	0.00±0.00^{a}
SP	17.33±2.91^{c}	14.67±1.33^{bc}	0.00±0.00^{a}	9.67±0.33^{b}

Legend: S- Streptomycin; CPX- Ciprofloxacin; AM- Amoxacillin; AU- Augmentin; CN- Chloramphenicol; PEF- Perfloxacin; OFX-Ofloxacin; S- Streptomycin; SXT-Septrin; CH- Chloramphenicol; SP- Sparfloxacin. Data are presented as mean ± standard error (where n=3). Values with the same superscripts in the same column are not significantly different at $p \leq 0.05$

Table 5 Antibiotic susceptibility pattern of urine bacterial isolates (gram positive) (mm)

ISOLATES	*S. aureus*	*S. pyogens*
CPX	14.67±1.33^{b}	16.00±2.31^{b}
AM	8.67±2.91^{b}	9.33±1.76^{b}
E	12.00±0.00^{b}	9.33±2.40^{b}
CN	0.00±0.00^{a}	0.00±0.00^{a}
PEF	9.33±1.76^{b}	12.67±0.00^{b}
R	13.00±1.76^{b}	12.67±0.67^{b}
S	0.00±0.00^{a}	12.00±1.15^{b}
SXT	0.00±0.00^{a}	2.67±2.67^{a}
Z	0.00±0.00^{a}	0.00±0.00^{a}
APX	0.00±0.00^{a}	2.00±0.00^{a}

Legend: CPX- Ciprofloxacin; AM- Amoxicillin; E- Erythromycin; CN- Chloramphenicol; PEF- Perfloxacin; R-Rinacef; S-Streptomycin; SXT-Septrin; Z-Zinnacef; APX-Ampicillin. Data are presented as mean ± standard error (where n=3). Values with the same superscripts in the same column are not significantly different at $p \leq 0.05$.

Antibacterial activity of crude extracts from *P. americana*

Extracts were prepared from the head, thorax and abdomen of *P. americana* and screened for its antibacterial efficacy. Typed isolates *E. coli* (ATCC 29822), *K. Pneumonia* (13888), *S. aureus* (NCTC 6571), *S. pneumonia* (ATCC 12384) and isolates from urine samples i.e *E. coli, S. aureus, P. aeruginosa, P. miriabilis, K. pneumonia and S. pyogens* were used as test bacteria. At the concentration of 500mg/ml, the extract from the head of *P. americana* showed activities against some typed and clinical bacterial strains. The highest zone of inhibition was observed in *E. coli* (ATCC 29822) (17.67±0.10mm) followed by *E. coli* (16.00±0.88mm), *S. aureus* (14.33±0.58mm) and least zone of inhibition against isolated *S. aureus* (NCTC 6571) (12.58±0.88mm). No effect was observed on other test organisms. Extracts from the thorax and abdomen showed no activity against any of the test organisms. Ciprofloxacin (positive control) displayed activity against all test organisms. It showed a maximum activity against *S. aureus* (38.33±1.20mm) and least against *E. coli* (31.67±0.28mm). DMSO (negative control) had no effect on all test isolates.

DISCUSSION

The percentage occurrence of Gram negative bacteria (82.29%) was higher than that of Gram positive bacteria (17.71%) and the high rate of bacterial growth that emerged in this study may return to the continuous use of antibiotics indiscriminately and without consulting a specialist doctor **(Shabab *et al.*, 2017).** This present study revealed 7 microorganisms as possible aetiological agents of UTI cases. These organisms were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus miriabilis, Staphylococcus aureus,* and *Streptococcus pyogens*. They are common causative agents of urinary tract infection as mentioned by **Priyadharsini *et al.* (2014).** The study revealed that gram negative bacteria are more predominant in UTIs than gram positive bacteria having *E. coli* as the most common organism and *K. pneumonia* as the second most common organism which is in accordance with the studies done by **Chaturvedi *et al.* (2017).** This higher prevalence of *E.coli* may be due to the faecal contamination from the use of toilets **(Bayode *et al.*, 2020).** Higher percentage of uropathogens were discovered in female patients compared to the male, this is in agreement with the research done by **Priyadharsini *et al.* (2014)** which stressed that the problem occurs more often in women than men because a woman's urethra is shorter. The short urethra makes it easier for bacteria from the anus or genital area to reach the bladder. Females between the age range of 21-40 were revealed to have the highest number of uropathogens in their urine which shows correlation with the result of **Bayode *et al.* (2020)** who deduced that females with the age group 21-30yrs and 31-40yrs demonstrated a high percentage of bacterial count for urine sampling. All tested bacterial isolates were susceptible to ciprofloxacin, *S. aureus* was resistant to multiple antibiotics and this is similar to the study reported by **Oseni *et al.* (2019).**

Extracts from the head of *P. americana* only had antibacterial effects against *E. coli* and *S. aureus*. This disagrees with **Billah *et al.* (2015)** who reported that the brain extracts *P. americana* were inactive against all the test bacteria and this may be due to the different extraction procedures used in both studies. This activity observed with the *P. americana* extracts may be due to the presence of antibacterial peptides produced by cockroaches against pathogenic bacteria as reported by **Dillon *et al.* (2005).** For it is known that cockroaches dwell in unsanitary places and therefore tends to harbor all sorts of microorganisms such as bacteria, fungi, and parasites on its body **(Tetteh-Quarco *et al.*, 2013).** Cockroaches use immunological responses similar to that of vertebrates to fight against pathogenic bacteria that may serve as a threat to it. These immunological responses signal complex glandular systems can secrete antimicrobial peptides against the pathogens **(Billah *et al.*, 2015).**

CONCLUSION

General inference from this study has revealed that Urinary tract infections are mostly found in females between the age ranges of 21-40. Extracts from the head of *P. americana* possess antimicrobial properties against *E. coli* and *S. aureus*. These findings therefore could be exploited in the treatment of Urinary tract infections caused by *E. coli* and *S. aureus*.

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