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Incidence of Nosocomial Urinary Tract Infections and Antibiotic Susceptibility Pattern of Uropathogenic Bacterial Isolates of Patients from Selected Hospitals in Nsukka, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Urinary Tract Infection (UTI) is one of the significant healthcare and hygienic-based diseases ravaging millions of people, especially in third world countries with poor hospital settings. This cross-sectional and laboratory investigation was done to determine the incidence of nosocomial urinary tract infections (NUTIs) and antibiotic susceptibility pattern of the uropathogenic bacterial isolates from hospital patients in Nsukka, Southeastern Nigeria. Urine specimens of fifty patients were collected and analyzed between May and June 2018. Kirby-Bauer disk diffusion method was used for detection of antibiotic susceptibility pattern for all isolates. Bacteriuria was observed in urine specimens of 31 (62%) out of the 50 participants, with the specimens of 13 yielding significant growth at 42% rate of nosocomial urinary tract infections (NUTIs). The most predominant bacterial isolate of urinary tract infection was *Staphylococcus* spp. (31%); followed by *Escherichia*, *Klebsiella* and *Proteus* with (23%) each. Isolates were mostly susceptible to Ciprofloxacin (87.1%), while other isolates were mainly resistant to Zinnacef (937%). Pertinent recommendations were made with respect to treatment, multidrug resistance mitigation and aseptic and optimal health services delivery practices.

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1. INTRODUCTION

Urinary tract infection is said to exist when a significant number of microorganisms (bacteriuria), are detected in properly collected mid-stream clean-catch urine in cultures with and greater than (>) 10^5 colony forming units (cfu) of bacteria/ml of urine. It is a common bacterial infection known to affect the different parts of the urinary tract (upper and lower) and the occurrence is found in both males and females, although high in females due to their reproductive anatomy [1].

Nosocomial urinary tract infection is an infection acquired during hospital care which is not present or incubating at admission but occurring more than 48 hours after admission [2]. Urinary tract infection is regarded as one of the commonest hospital acquired infections accounting for more than 30 % of infections reported by acute care hospitals [3]. Virtually all healthcare-associated UTIs are caused by instrumentation of the urinary tract as well as inadequate cleaning procedures concerning uniforms, washing, sterilization of equipment and other preventive measures that may be ignored thus providing a route for pathogens to spread [4]. Other risk factors include host's susceptibility, substandard patient care practices and the hospital environment. Also, worrisome is the catalogue of reports including the WHO, in [5], which revealed that the frequent use of antibiotics in hospitals has created selective pressure, which has caused most microorganisms to develop antibiotics resistance.

Urinary tract infections are primarily caused by Gram-negative bacteria, but Gram-positive pathogens may also be involved. More than 95 % of uncomplicated UTIs are monobacterial. The most common pathogen for uncomplicated UTIs is *Escherichia coli* (75% – 95%), followed by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococci*, and *Proteus mirabilis*. Distribution of these uropathogens may also differ by type of infection or patient population [6].
This study was designed to determine the incidence of urinary tract infections of healthcare origin and antibiotic susceptibility pattern of such uropathogenic bacterial isolates from among patients admitted in hospital settings within the target area.

2. MATERIALS AND METHODS

2.1 Study Population

A cross-sectional study was carried out with two urine specimens taken from fifty (50) consenting inpatients in different hospitals.

2.2 Sample Collection and Processing

First and second urine specimens were taken within the first twelve (12) hours and seventy-two (72) hours of a patient's admission respectively using well-labeled universal containers which were initially distributed to the patients. Necessary demographic data from the subjects were obtained using semi-structured questionnaire.

The specimen samples were analyzed within two hours after collection at Microbiology Department Laboratory. Ten millilitres of each urine specimen was transferred into a clean dry test tube. The urine specimen was centrifuged at 4,000 rpm for 5 minutes. Urinalysis was carried out using Medi-Test Combi 9 Urine test strip manufactured by Macherey-Nagel GmbH & Co. (Germany) and the colour change compared to a standard chart to determine the findings. The urine sample was examined microscopically as a wet preparation to detect significant pyuria, that is, white blood cells in excess of 10 cells/microlitre (10^6/litre) of urine, red cells, casts, epithelial cells, crystals, bacteria, yeast cells, *Trichomonas vaginalis* motile trophozoites, and *Schistosomiasis haematobium* eggs.

2.3 Bacteriological Assays

Four different media including nutrient agar, MacConkay agar (MA), Kilgler iron agar and eosin methylene blue agar were used in the study. All media were prepared according to the manufacturer's instructions. A loopful (0.001 ml) of well mixed un-centrifuged urine was inoculated onto the surface of MacConkey and blood agar media using a standard wire loop. All plates were then inoculated at 37°C aerobically for 24 hours. The plates were then examined macroscopically for bacterial growth.

McFarland turbidity standard (0.5) was prepared by adding 0.5 ml of a 1.175% (wt/vol) barium chloride dehydrate ($BaCl_2$. H2O) solution to 99.5 ml of 1% (vol/vol) sulphuric acid (H_2SO_4). The turbidity standard was then aliquoted into test tubes identical to those used to prepare the inoculum suspension [7].

A significant growth was considered if the number of colony is $>10^5$ colony forming unit (cfu)/ml. Colonial appearance and morphological characteristics of isolated bacteria were noted and isolated colonies were subjected to preliminary test like Gram staining. These preliminary tests were followed by biochemical reactions for identification of the isolated organism. And the isolated organisms were subjected to antibiotic susceptibility testing.

2.4 Antibiotic Sensitivity Testing and Interpretation of Zones of Inhibition

The following drugs in an antibiotic disc (ABTEK, India) were used for antibiotic sensitivity test:

Amoxacillin (30 µg), Ampiclox (30 µg), Augmentin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (10 µg), Erythromycin (10 µg), Gentamycin (10 µg), Pefloxacin (30 µg), Recephin (25 µg), Septrin (30 µg), Sparfloxacin (10 µg), Streptomycin (30 µg), Tarivid (10 µg), and Zinnacef (20 µg).

Using the interpretative chart provided by Hardy Diagnostics (1430 West McCoy Lane, Santa Maria, CA 93455, USA) as recommended by the consensus standard by the Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS) M100 document, the zone sizes of each organism were interpreted as either resistant (R), intermediate (I) or sensitive (S).

2.5 Statistical Analysis

The results were analysed statistically using Multiple Antibiotic Resistance (MAR) Index. The MAR index was determined by following the procedure described by Osundiya et al. [8,7].

> MAR index for an isolate = Number of antibiotics to which isolate is resistant / Total number of antibiotics to which isolate was tested

The percentage frequencies of occurrence of the bacteria isolates from clinical specimens positive for urinary tract infection among patients of various age groups and sexes were calculated using

Frequency (%) = (n / N) x (100/ 1)

Where

> n = Number of occurrence of bacterial species,
> N = Total number of bacteria isolated.

Also,

> n = Number of susceptible or resistant species,
> N = Total number of bacterial isolates for percentage frequencies of susceptible/resistant bacterial species.

3. RESULTS

3.1 Urine Analysis and Microscopy

In Table 1, the pH of the urine samples examined ranged from 5.0-8.0. The distribution of samples positive for other parameters were: blood, 30%; protein, 32%; urobilinogen, 20%; bilirubin, 10%; ascorbic acid, 18%; nitrite, 6%; and glucose, 10%.

Light microscopy of urine samples of the study participants gave the following results: presence of red blood cells, 24%; pus cells, 88%; epithelial cells, 32% and bacterial cells, 62%.

3.2 Urine Sample Culture and Associated Demographic Factors of the Study Population

Out of the 100 urine samples from 50 patients examined in the study population, 31 or 62% of samples yielded bacterial growth (bacteriuria) with 26 samples yielding significant growth at 42 % rate for nosocomial urinary tract infections (NUTIs) among the 13 infected patients who were identified to have acquired it on admission in the various hospitals. The most predominant bacterial isolate of this urinary tract infection was *Staphylococcus spp.* (31 %), followed by *Escherichia*, *Klebsiella* and *Proteus* with (23 %) each (Fig. 1).

The result in Fig. 2 indicates the bacterial organisms were isolated from more males than females, while age distribution pattern of patients associated with NUTIs revealed patients above

50 years of age suffered more from the infections (Fig. 3).

3.3 Antibiotic Sensitivity and Multiple Antibiotic Resistance Indices of Bacteria Isolates

Ciprofloxacin (83. 3%, 60% and 100%) appeared as the most effective drug both against Gram positive and Gram negative pathogenic isolates, followed by Streptomycin, Gentamycin, and Pefloxacin, while Erythromycin (100%) is effective against Gram-positive pathogenic isolates. Isolates showed high resistance to Zinnacef (100%) followed by Ampiclox (75%) which are of the Gram-positive and Amoxacillin (100% and 91.6%) of both Gram-positive and Gram-negative (Table 3). Multi-drug resistance capacity was detected in most of the bacterial isolates with *Staphylococcus* and *Klebsiella* spp. having the highest multiple antibiotic resistance index (MARI) of 0.714 each, while *Bacillus* and *Escherichia* spp. were associated with the least MARI of 0.357 and 0.429 respectively (Table 4).

Table 1. Microscopic examination and analysis of urine samples of participants

	Microscopy				Appearance							pH										
	RBCs	PCs	BCs	ECs	Clear/Amber	Turbid/Amber	Clear/Pale yellow	Pale yellow	Amber	Turbid	Colourless/clear	5.0	6.0	7.0	8.0	Blood	Protein	Urobilinogen	Bilirubin	Ascorbic acid	Nitrite	Glucose
Number of positive samples	12	44	31	16	13	7	1	24	1	1	3	18	16	15	1	15	16	10	5	9	3	5	1
Percentage (%) frequency	24	88	62	32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	30	32	20	10	18	6	10	2

RBCs = Red blood cells, PCs = Pus cells, BCs = Bacterial cells, ECs = Epithelial cells, NA = Not available.

Table 2. Characteristics of bacterial isolates identified using MA culture media, microscopy and biochemical tests

Colony appearance				Microscopy				Biochemical tests						
								Sugar ferm test						
Colour	Edge	Elevation	Texture	Size (mm)	Gram reaction	Shape	Arrangement	Catalase	Coagulase	H_2S	Gas	Glucose	Lactose	Tentative organism
Pale pink	Punctiform	Raised	Smooth	3.00	+	cocci	clusters	+	+	-	+	+	+	*Staphylococcus* spp
Pink	Round	Raised	Smooth	2.00	-	rod	chains	+	-	-	+	+	+	*Klebsiella* spp
-	-	-	-	-	+	rod	clusters, chains	+	-	-	-	+	+	*Bacillus* spp
Cream	Round	Raised	Smooth	3.00	-	rod	clusters	+	-	+	+	+	-	*Proteus* spp
Pink	Round	Flat	Smooth	2.00	-	rod	chains	+	-	-	+	+	+	*Escherichia* spp
Pink	Round	Flat	Smooth	1.00	+	cocci	chains	+	-	-	-	+	+	*Streptococcus* spp

Ferm= fermentation, spp. = species

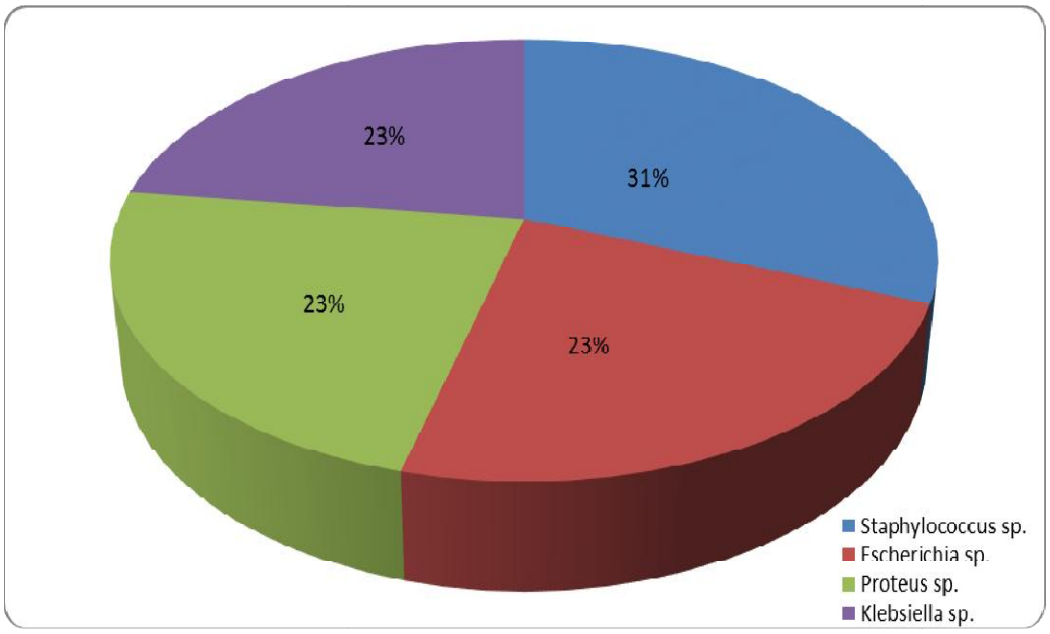

Fig. 1. Percentage of the bacterial isolates from clinical specimens positive for NUTI

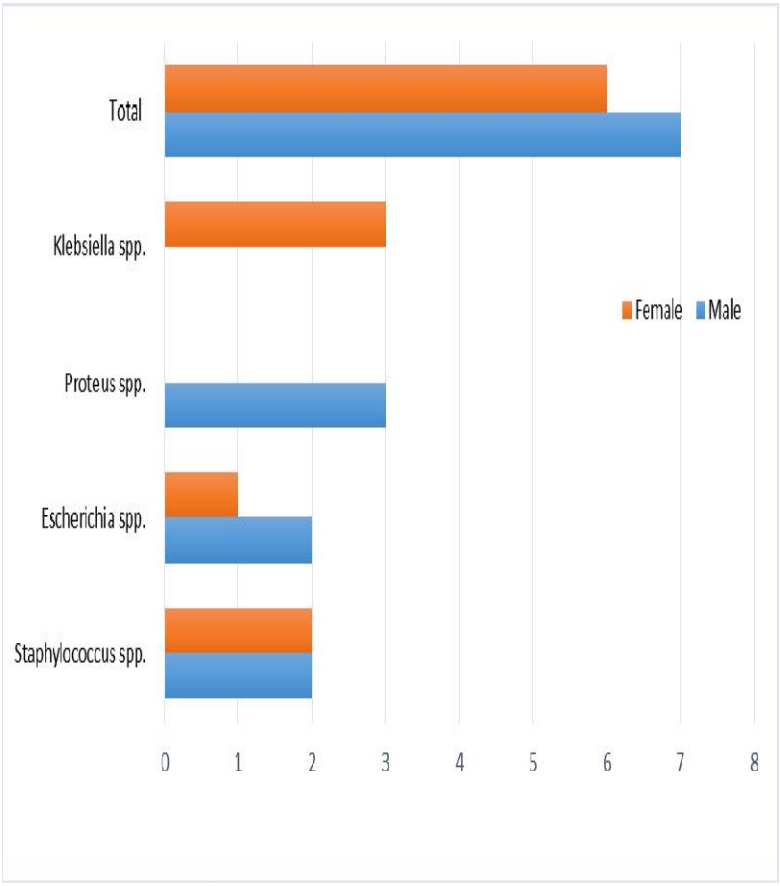

Fig 2. Distribution of the causative agents of NUTI among male and female study participants

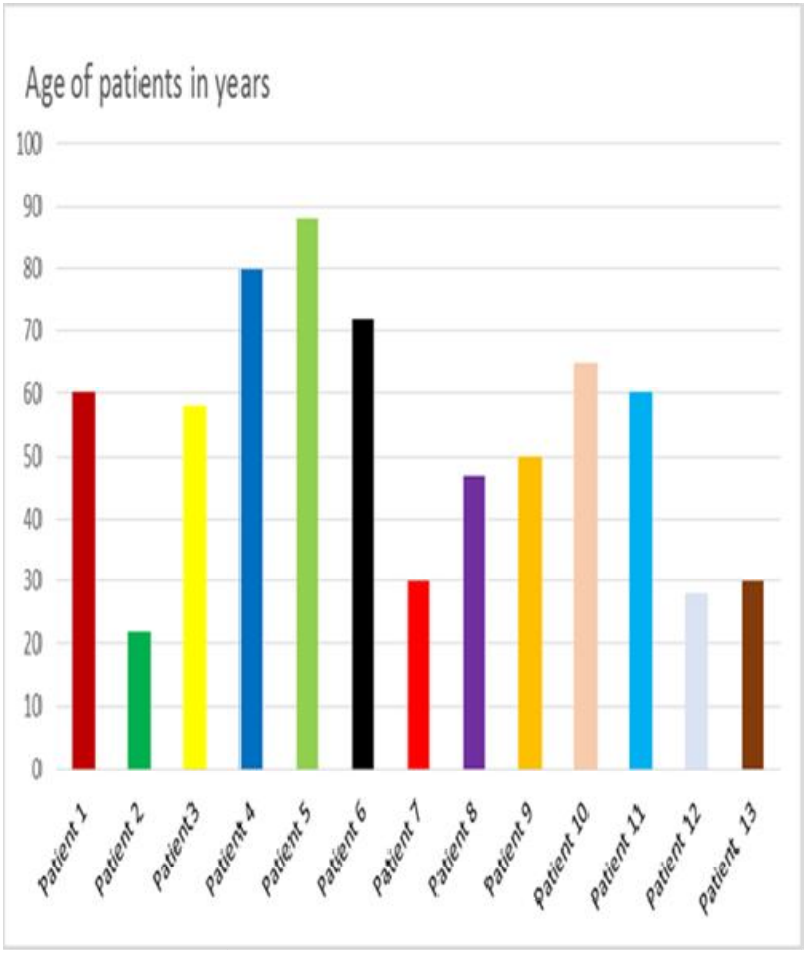

Fig. 3. Age distribution pattern of patients associated with NUTI

4. DISCUSSION

This paper describes a study undertaken to determine the incidence of UTIs in hospitals within Nsukka urban area as well as to evaluate the bacterial agents involved in this UTIs. It provides valuable laboratory and community-based data concerning urinary tract pathogens in the study location which enables its comparison with related works. Out of the 50 patients (27 males and 23 females) that participated in this study, 31 urine samples (62%) had confirmed bacteriuria cases, in which 26 samples from 13 patients showed significant growth for bacteria-causing urinary tract infections. These thirteen patients having the infection were diagnosed to have acquired it during their stay in the hospital, showing an increasing and incidence rate of 42% for the NUT infections. This major outcome corroborates increasing cases of UTIs originating in hospital settings and previous studies done and documented by Ethiopian researchers (9.2%) [8], and 24.7% that was reported in India [9].

In line with a similar and previous study, the findings in this study observed the prevalence of NUTI in males, 7 (14%) was higher than females 6, (12%) [10]. In contrast however, results of this present study was at variance with other findings which showed that the rate of UTI is greater in females as compared to males [11,12]. The possible reason is that the urinary tract system in male might be biased due to lengthier duration or usage of catheters in hospital and differences in the target setting as earlier reported. Also, it is shown that patients above 50 years of age are more associated with UTIs indicating that age could be a predisposing factor. However, this inference needs to be supported or confirmed by further studies with larger population.

Table 3. Antimicrobial sensitivity pattern of microorganisms by disk-agar diffusion method

Antibiotics	*Staphylococcus* spp. (n= 12)		*Klebsiella* spp. (n=5)		*Bacillus* spp. (n=3)		*Proteus* spp. (n= 4)		*Escherichia* spp. (n= 6)	
	S	R	S	R	S	R	S	R	S	R
Ampiclox	25% (3)	75% (9)	-	-	-	100% (3)	-	-	-	-
Gentamycin	66.7% (8)	33.3% (4)	20% (1)	80% (4)	66.7% (2)	33.3% (1)	75% (3)	25% (1)	100% (6)	-
Augmentin	-	-	-	100% (5)	-	-	50% (2)	50% (2)	100% (6)	-
Ciprofloxacin	83.3% (10)	16.7% (2)	60% (3)	40% (2)	100% (3)	-	100% (4)	-	100% (6)	-
Amoxacillin	8.3% (1)	91.6% (11)	20% (1)	80% (4)	-	100% (3)	75% (3)	25% (1)	66.7% (4)	33.3% (2)
Streptomycin	83.3% (10)	16.7% (2)	40% (2)	60% (3)	100% (3)	-	75% (3)	25% (1)	100% (6)	-
Septrin	50% (6)	50% (6)	20% (1)	80% (4)	100% (3)	-	50% (2)	50% (2)	66.7% (4)	33.3% (2)
Tarivid	-	-	20% (1)	80% (4)	-	-	75% (3)	25% (1)	83.3% (5)	16.7% (1)
Chloramphenicol	-	-	-	100% (5)	-	-	75% (3)	25% (1)	83.3% (5)	16.7% (1)
Zinnacef	-	100% (12)	-	-	-	100% (3)	-	-	-	-
Sparfloxacin	-	-	60% (3)	40% (2)	-	-	50% (2)	50% (2)	66.7% (4)	33.3% (2)
Pefloxacin	75% (9)	25% (3)	20% (1)	80% (4)	66.7% (2)	33.3% (1)	50% (2)	50% (2)	66.7% (4)	33.3% (2)
Erythromycin	66.7% (8)	33.3% (4)	-	-	100% (3)	-	-	-	-	-
Recephin	41.7% (5)	58.3% (7)	-	-	100% (3)	-	-	-	-	-

Key: S = Sensitivity, R = Resistance

Table 4. Multiple antibiotic resistance indices (MARI) of bacteria isolated from patients having NUTI

Antibiotics to which isolate is resistant	Bacterial isolates	MARI
APX, CN, CPX, AM, SXT, S, Z, R, E, and PEF	*Staphylococcus* spp.	0.714
CN, AU, CPX, AM, SXT, S, OFX, CH, SP, and PEF	*Klebsiella* spp.	0.714
APX, CN, AM, Z, and PEF	*Bacillus* spp.	0.357
CN, AU, AM, SXT, S, CH, SP, and PEF	*Proteus* spp.	0.571
AM, SXT, OFX, CH, SP, and PEF	*Escherichia* spp.	0.429

Total number of antibiotics to which isolate was tested = 14

CN = Gentamycin, PEF = Pefloxacin, AU = Augmentin, AM = Amoxacillin, CH = Chloramphenicol, SXT = Septrin, S = Streptomycin, OFX = Tarivid, Z = Zinnacef, R = Recephin, CPX = Ciprofloxacin, E = Erythromycin, SP = Sparfloxacin, APX = Ampiclox

In this study, four genera that caused hospital-acquired UTI include *Staphylococcus* spp. which was the most common uropathogen of the Gram-positive organisms, followed equally in proportion by species of *Klebsiella*, *Proteus* and *Escherichia* of the Gram-negative bacilli. The prevalence of Gram-negative bacilli constituted 69.2 % of the total isolated bacterial cause of UTI whereas the rate of only Gram-positive cocci constituted (30.8 %) in consistence with findings from previous studies [13,14].

Also from the findings, Ciprofloxacin (83. 3%, 60% and 100%) appeared as the most effective drug both against Gram-positive and Gram-negative pathogenic isolates, followed by Streptomycin, Gentamycin, Pefloxacin while Erythromycin (100%) was effective against the Gram-positive uropathogens. High sensitivity seen in quinolones is in line with the findings in a previous study [10]. Based on observed results; fluroquinolones-, macrolides- and aminoglycosides-based antibiotics remain recommended for treatment of nosocomial urinary tract infections. However, most isolates showed a high level of resistance to Zinnacef (100 %) followed by Ampiclox (75 %) and Amoxacillin (100 % and 91.6 %) of both Gram-positive and Gram-negative organisms. MAR indices of the isolates especially *Staphylococcus* and *Klebsiella* spp. confirm the presence of multi-drug resistant strains and species of these organisms as previously reported in some parts of southeastern Nigeria [15,16]. These reports once more bring to fur the need for previously suggested and more proactive scientific actions of monitoring and surveillance of possible diseases caused by these microorganisms [17-22].

5. CONCLUSION

Urinary tract infection (UTI) is one of the most common bacterial infections and is a serious threat to public health. As a preventive measure, all healthcare associated centers should integrate proper monitoring of hospital data and surveillance of hospital acquired urinary tract infection in order to review epidemiological data and identify areas for intervention; assess and promote improved practice at all levels of health facility and ensure appropriate staff training in infection control and safety. This study therefore recommends the need for concerted personal hygiene, health education, hand washing and other standard infection control practices around hospital settings to be adhered to, judicious use of antibiotics to mitigate and possibly prevent multidrug resistant UTIs, so that nosocomial urinary tract infections can be controlled effectively.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained (University Medical Centre, Bishop Shanahan Hospital, Mount Araphat Hospital and St. Anthony Hospital) and within Nsukka District between May to June, 2018.

Before sample collection, verbal informed consent was obtained from all participants and they were educated on aseptic method of urine collection to avoid contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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