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DOI: 10.46903/gjms/21.01.1183

ORIGINAL ARTICLE

PREVALENCE OF UROPATHOGENS AND ITS ANTIMICROBIAL SUSCEPTIBILITY IN THREE MISSION HOSPITALS IN ABAKALIKI, EBONYI STATE OF NIGERIA

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ABSTRACT

Background: Uropathogens are group of micro-organisms capable of causing urinary tract infections (UTIs). Treatment of infections due to uropathogens has become difficult because of indiscriminate use of antibiotics and ability to easily acquire resistance mechanisms by these pathogens. The objective of this study was to determine the prevalence and antimicrobial susceptibility profile of bacterial uropathogens implicated in UTI among patients attending the three Mission hospitals in Abakaliki, Ebonyi State of Nigeria.

Materials & Methods: This was a descriptive study done from 1st January 2022 to 30th June 2022, in three Mission Hospitals of Nigeria 1-Rural Improvement Mission hospital, Ikwo (Hospital A); 2-Mater Misrecordea Hospital, Afikpo (Hospital B) and 3- Saint Vincent hospital, Ndubia (Hospital C) and all are located in semi-urban areas of the Ebonyi State. Inclusion criteria were urine samples of both out-patient and admit hospital patients. Both genders of all age groups patients were included. The bacterial isolates were detected using standard microbiological methods and re-confirmed using colonial morphology. Antimicrobial susceptibility profile was performed using Kirby Bauer disc diffusion.

Results: Total 650 bacterial uropathogens isolates were collected from the laboratory units of the three mission hospitals and among them 529 Gram negative bacterial uropathogens were identified and re-confirmed following standard microbiological methods. Overall prevalence of uropathogens in these mission hospitals were 164 (80%), 222 (86%) and 143 (77%) from Hospital A, B and C respectively. *E. coli* with 58 (35%), 76 (34%) and 50 (35%) prevalence in hospital A, B and C respectively was the most prevalent and frequently isolated uropathogens. Imipenem and meropenem are the two most potent antibiotics against uropathogens in these Mission hospitals. In contrast, the isolated uropathogens developed 100% resistance to tigecycline, ertapenem, aztreonam and ampicillin/sulbactam.

Conclusion: Gram negative uropathogens are predominantly the most cause of UTIs and determination of antimicrobial susceptibility profiles of bacterial uropathogens has become crucial in the treatment of UTIs.

KEY WORDS: Bacterial; UTI; Prevalence; Antimicrobial Susceptibility.

Cite as: Egwu IH, Egwu-Ikechukwu MM, Nnabugwu CC, Mustapha JK, Ali CM. Prevalence of uropathogens and its antimicrobial susceptibility in three mission hospitals in Abakaliki, Ebonyi state of Nigeria. Gomal J Med Sci 2023 Jan-Mar;21(1):8-15. https://doi.org/1046903/gjms/21.01.1183

1. INTRODUCTION

1.1 Background: Uropathogens are group of microorganisms that has the ability to cause various degree of illness in the urinary tracts of humans. The growth and multiplication of these organisms are usually within and around the genito-urinary tract system (GUTS), where they are implicated in urinary tract infections.[1] UTI has become an important human microbial diseases and affects all age groups in both communities and hospitals with high morbidity.[1,2] A community acquired (CA) or nosocomial (N) UTIs occurs within community less than 48h of hospitalization or within 48h after admission into a health facility, or within three days after discharging from hospital[3,4,5]. Worldwide, the occurrence of both CA- UTIs and N-UTIs each year is estimated at about 150 million with high health cost.[6] With *Staph-*

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Date Submitted:	05-07-2022
Date Revised:	20-12-2022
Date Accepted:	05-01-2023

ylococcus saprophyticus, *Staphylococcus aureus*, *Escherichia coli*, *Proteussp*., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococci* - the commonest pathogenic microorganisms associated with UTIs.[7,8]UTI normally affects the urinary tract the urethra, prostate, kidneys and bladder.[4,9] Globally, the major cause of UTIs is bacteria but viral and fungal organisms can also be responsible, and their occurrence varies from one geographical location to another.[10] Its occurrence is highly dependent but not limited to age, sex, duration/length of hospital stay, catheterization, postrate, antimicrobials exposure, sexual activities, diabetes and UTI history.[7,11]

The diagnosis of UTIs is mainly urine samples of both out- and in- patients, and cases may be asymptomatic or symptomatic.[3]Gram negative organisms are mainly responsible for UTIs accounting for about 90% of UTI infections while Gram positives responsible for about 10%.[2]The treatment of UTI is mostly commenced with empirical treatment by physicians and therapy is often based on results obtained from the antimicrobial susceptibility profile of the uropathogens.[12,13]Development of resistance by these uropathogens in the management and treatment of UTIs poses an enormous public health challenge especially in resource-limited country like Nigeria.[14,15,16] The antimicrobial resistance profile of bacterial isolates from urinary tracts varies from one geographical location to another and from institution to institution.[11,17]

1.2 Research Problems (RPs), Knowledge Gaps (KGs) & Rationale: The increasing trend of antimicrobial resistance globally especially in Gram negative organisms such as *E. coli*, *Klebsiella* spp, *Pseudomonas* spp, *Proteus mirabilis* and *Acinetobacter baumannii* is a major public health concern[18,19,20,21] and since there is no available record of uropathogens among patients attending these three Mission hospitals in Abakaliki, Ebonyi State, this study therefore becomes important. Hence, determination of the common causative agent of UTIs and the result of their antibiotic susceptibility profile from this research is expected to provide physicians alternative antibiotics for empirical therapy of UTIs prior to laboratory investigations.

1.3 Research Objectives:

RO 1: This study is designed to determine the most common causative agents of UTIs in three mission hospitals.

RO 2: To determine the antibiotic susceptibility profiles of Gram negative organisms implicated in UTIs.

2. MATERIALS AND METHODS

2.1. Study Area: Three Mission hospitals in Ebonyi State, Nigeria were used as study areas and they includes; Rural Improvement Mission hospital, Ikwo (Hospital A); Mater Misrecordea Hospital, Afikpo (Hospital B) and Saint Vincent hospital (Hospital C), Ndubia and are located in semi-urban areas of the State. They are the major Mission hospitals among the six Mission hospitals in the state which provides the basic health requirement for both semi- urban and rural dwellers in their respective zones. Each of the three Mission hospitals was selected as a representative of the three senatorial zones in Ebonyi State thus; Ebonyi Central zone: Rural Improvement Mission hospital, Ikwo; Ebonyi South zone: Mater Misrecordea Hospital, Afikpo and Ebonyi North zone: Saint Vincent hospital, Ndubia.

2.2. Study Design and Sources of Isolates: This was a descriptive study, 650 cases selected randomly. All the clinical isolates were collected from laboratory units of the three Mission hospitals and transported in sterile screw-cap bijoux bottle containing 5ml of nutrient broth, to the Laboratory Unit of Applied Microbiology Department, Ebonyi State University, Abakaliki for further re-investigation, analysis and confirmation.

2.3. Inclusion and Exclusion criteria: All bacterial isolates were recovered from urine samples of in- and out-patients attending these Mission hospitals with signs and symptoms suggestive of UTIs. Those that do not meet up to these criteria were excluded.

2.5 Isolates Confirmation/Culture and Identification: The isolates were confirmed using standard microbiological methods. Briefly, each of the bacterial isolate was further aseptically inoculated into MacConkey agarplates, Cystine lysine electrolyte deficient (CLED) agar, and blood agar. The plates were then incubated aerobically at 37°C for 24 h. The bacterial isolates were further identified and re-confirmed using colonial morphology, Gram stain reaction and standard biochemical tests such as indole, oxidase, H_2S production, catalase, coagulase, urease and citrate utilization tests as described by Cheesbrough.[22]

2.6 Antibiotic Susceptibility Studies: Antibiotic susceptibility profiles of the bacterial isolates were determined using disk diffusion method on Mueller-Hinton agar following CSLI criteria.[23] Up to five discreet colonies of bacterial pathogens were inoculated in 5ml of sterile nutrient broth, to give fine bacterial suspension which is equivalent to 0.5 McFarland turbidity standards. Using sterile swab stick, the surface of a well labeled Mueller-Hinton agar (MHA) plate was streaked with the suspension of the bacterial isolates. The antibiotic disc comprising of the following antibiotics and concentrations; Amikacin (AMK) -30 μg, Tigecycline (TGC) -15 μg, Gentamicin (CN) -10 μg, Ciprofloxacin (CIP) -5 μg, Ertapenem (ETA) -10 μg, Aztreonam (ATM) -30 μg, Meropenem (MEM) - 10 μg, Imipenem (IPM) -10 μg, Ceftazidime (CAZ) - 30 μg; Cefotaxime (CTX) -30 μg, Cefoxitine (FOX) -30μg, Cefepime (FEB) -30μg, Cefuroxime (CXM) -30 μg, Ampicillin/Sulbactam (SAM) -20 μg and Amoxacillin/Clavulanic acid (AMC) -30 μg was aseptically placed on the surfaces of the solidified Mueller- Hinton agar (MHA) plates using forceps. The MHA plates containing antibiotic discs were then inverted and incubated at 37°C for 24 h. Following overnight incubation, zones of inhibition diameter were measured using meter rule and interpreted according to CLSI recommendations.

3. RESULTS

3.1 Clinical Bacterial Isolates: Of the 650 bacterial isolates comprising of 206, 259 and 185 from hospitals A, B and C, Ndubia respectively. A total of 529 bacterial isolates were re-confirmed as uropathogens, comprising of 164 (80%), 222 (86%) and 143 (77%) from hospitals A, B and C, Ndubia respectively.

3.2 Prevalence of Bacterial Uropathogens: Of 164 (80%), 222 (86%) and 143 (77%) confirmed uropathogens recovered from hospitals A, B and C, Ndubia respectively, *Escherichia coli* with 58 (35%), *E. coli* 76(34%) and 50 (35%) were the most prevalent and

Table 1: Prevalence of uropathogens isolates in the three Mission hospitals

Bacterial isolates	RIM			Mater Misrecordea Hospital, Afikpo			St. Vincent Hospital		
	TNIC	TNCI (%)	% PIH	TNIC	TNCI (%)	% PIH	TNIC	TNCI (%)	% PIH
E. coli	73	58 (79)	35	90	76 (84)	34	60	50 (83)	35
Pseudomonas spp	49	39 (80)	24	57	49 (86)	22	38	29 (76)	20
Klebsiella spp	48	40 (83)	24	67	60 (90)	27	45	33 (73)	23
Proteus spp	36	27 (75)	16	45	37 (82)	17	42	31(74)	21
Total	206	164 (80)		259	222 (86)		185	143 (77)	

Keys: RIM= Rural Improvement Mission hospital, Ikwo; TNIC=Total Number of Isolates Collected; TNCI (%)= Total Number of Confirmed Isolates , % PIH= percentage prevalence of each Isolate in the Hospital.

Table 2: Antimicrobial susceptibility and resistance profiles of bacterial isolates in RIM

Antibiotics used (µg)	E. coli (n=58)		Pseudomonas spp (n=39)		Klebsiella spp (n=40)		Proteus spp (n=27)	
	N(%R)	N(%S)	N(%R)	N(%S)	N(%R)	N(%S)	N(%R)	N(%S)
AMK-30	51(88)	7(12)	36(92)	3(8)	29(73)	11(27)	6(22)	21(78)
TGC-15	57(98)	1(2)	34(87)	5(13)	40(100)	0(0)	27(100)	0(0)
CN-10	55(95)	3(5)	3(8)	36(92)	20(50)	20(50)	26(96)	1(4)
CIP-5	27(47)	31(53)	11(28)	28(72)	22(55)	18(45)	3(11)	24(89)
ETA-10	40(69)	18(31)	34(87)	5(13)	40(100)	0(0)	27(100)	0(0)
ATM-30	52(90)	6(10)	37(95)	2(5)	39(98)	1(2)	24(89)	3(11)
MEM-10	9(16)	49(84)	7(18)	32(82)	1(2)	39(98)	1(4)	26(96)
IPM-10	2(34)	56(97)	3(8)	36(92)	0(0)	40(100)	0(0)	27(100)
CAZ-30	27(47)	31(53)	17(44)	22(56)	20(50)	20(50)	18(67)	9(33)
CTX-30	35(60)	23(40)	14(36)	25(64)	27(68)	13(32)	11(41)	16(59)
FOX-30	19(33)	39(67)	20(51)	19(49)	21(53)	19(47)	21(78)	6(22)
FEB-30	42(72)	16(28)	28(72)	11(28)	17(43)	23(57)	24(89)	3(11)
CXM-30	17(29)	41(71)	31(79)	8(21)	19(47)	21(53)	13(48)	14(52)
SAM-20	56(97)	2(3)	18(46)	10(26)	40(100)	0(0)	27(100)	0(0)
AMC-30	45(78)	13(22)	18(46)	21(54)	3(7)	37(93)	5(19)	22(81)

Keys: AMK-Amikacin-30µg; TGC-Tigecycline-15µg); CN-Gentamicin-10µg; CIP-Ciprofloxacin-5µg; ETA-Ertapenem-10µg; ATM-Aztreonam-30µg; MEM-Meropenem-10µg; IPM-Imipenem-10µg; CAZ-Ceftazidime-30µg; CTX-Cefotaxime-30µg; FOX- Cefoxitine-30µg; FEB-Cefepime-30µg; CXM-Cefuroxime-30µg; SAM-Ampicillin/Sulbactam-20µg; AMC-Amoxacillin/Clavulanic acid-30µg.

frequently isolated uropathogens in the three Mission hospitals respectively as shown in table-1.

3.3 Antimicrobial Susceptibility and Resistance Profiles of Bacterial Isolates from RIM: The result of antimicrobial susceptibility and resistance profiles of bacterial isolates from RIM is shown in table 2.

3.4 Antimicrobial Susceptibility and Resistance Profiles of Bacterial Isolates from Mater Misrecordea Hospital, Afikpo: The result of antimicrobial susceptibility and resistance profiles of bacterial isolates from Mater Misrecordea Hospital, Afikpo is shown in table 3.

Table 3: Antimicrobial susceptibility and resistance profiles of bacterial isolates in Misrecordea Hospital, Afikpo

Antibiotics used (µg)	E. coli (n=76)		Pseudomonas spp (n=49)		Klebsiella spp (n=60)		Proteus spp (n=37)	
	N (%R)	N (%S)	N (%R)	N (%S)	N (%R)	N (%S)	N (%R)	N (%S)
AMK-30	5 (7)	71 (93)	42(86)	7(14)	60(100)	0(0)	29(78)	8(22)
TGC-15	76 (100)	0 (0)	44(90)	5(10)	60(100)	0(0)	26(70)	11(30)
CN-10	5 (7)	71 (93)	46(94)	3(6)	58(97)	2(3)	8(22)	29(78)
CIP-5	42 (55)	34 (45)	31(63)	18(37)	28(47)	32(53)	14(38)	23(62)
ETA-10	76 (100)	0 (0)	47(96)	2(4)	60(100)	0(0)	35(95)	2(5)
ATM-30	76(100)	0(0)	49(100)	0(0)	60(100)	0(0)	32(86)	5(14)
MEM-10	1(1)	75(99)	0(0)	49(100)	1(2)	59(98)	0(0)	37(100)
IPM-10	0(0)	76(100)	3(6)	46(94)	1(2)	59(98)	0(0)	37(100)
CAZ-30	34(45)	42(55)	22(45)	27(55)	33(55)	27(45)	8(22)	29(78)
CTX-30	30(39)	46(61)	10(20)	39(80)	15(25)	45(75)	16(43)	21(57)
FOX-30	24(32)	52(68)	28(57)	21(43)	19(32)	41(68)	13(35)	24(65)
FEB-30	67(88)	9(12)	36(73)	13(27)	11(18)	49(82)	33(89)	4(11)
CXM-30	35(46)	41(54)	21(43)	28(57)	30(50)	30(50)	19(51)	18(49)
SAM-20	75(99)	1(1)	38(78)	11(22)	60(100)	0(0)	31(84)	6(16)
AMC-30	70(92)	6(8)	28(57)	21(43)	13(22)	47(78)	17(46)	20(54)

Keys: AMK-Amikacin-30µg; TGC-Tigecycline-15µg); CN-Gentamicin-10µg; CIP-Ciprofloxacin-5µg; ETA-Ertapenem-10µg; ATM-Aztreonam-30µg; MEM-Meropenem-10µg; IPM-Imipenem-10µg; CAZ-Ceftazidime-30µg; CTX-Cefotaxime-30µg; FOX- Cefoxitine-30µg; FEB-Cefepime-30µg; CXM-Cefuroxime-30µg; SAM-Ampicillin/Sulbactam-20µg; AMC-Amoxacillin/Clavulanic acid-30µg.

Table 4: Antimicrobial susceptibility and resistance profiles of bacterial isolates in St. Vincent Hospital, Ndubia

Antibiotics used (µg)	E. coli (n=50)		Pseudomonas spp (n=29)		Klebsiella spp (n=33)		Proteus spp (n=31)	
	N (%R)	N (%S)	N (%R)	N (%S)	N (%R)	N (%S)	N (%R)	N (%S)
AMK-30	19(38)	31(62)	7(24)	22(76)	3(9)	30(91)	18(58)	13(42)
TGC-15	47(94)	3(6)	28(97)	1(3)	27(82)	6(8)	30(97)	1(3)
CN-10	10(20)	40(80)	29(100)	0(0)	25(76)	8(24)	22(71)	9(29)
CIP-5	21(22)	39(78)	11(38)	18(62)	15(45)	18(55)	13(42)	18(58)
ETA-10	43(86)	7(14)	29(100)	0(0)	33(100)	0(0)	31(100)	0(0)
ATM-30	48(96)	2(4)	29(100)	9(0)	30(91)	3(9)	29(94)	2(6)
MEM-10	0(24)	50(76)	0(0)	29(100)	2(6)	31(94)	0(0)	33(100)
IPM-10	1(16)	49(84)	0(0)	29(100)	1(3)	32(97)	3(10)	28(90)
CAZ-30	22(44)	28(56)	13(45)	16(55)	7(21)	26(79)	7(23)	24(77)
TX-30	19(38)	31(62)	10(34)	19(66)	15(45)	18(55)	17(55)	14(45)
FOX-30	10(20)	40(80)	12(41)	59(90)	17(52)	16(48)	25(81)	6(19)
FEB-30	41(82)	9(18)	20(69)	9(31)	21(64)	12(36)	4(13)	27(87)
CXM-30	18(36)	32(64)	12(41)	17(59)	18(55)	15(45)	12(39)	19(61)
SAM-20	39(78)	11(22)	23(79)	6(21)	29(88)	4(12)	26(84)	5(16)
AMC-30	29(58)	21(42)	19(66)	10(34)	27(50)	6(50)	10(32)	21(68)

Keys: AMK-Amikacin-30µg; TGC-Tigecycline-15µg); CN-Gentamicin-10µg; CIP-Ciprofloxacin-5µg; ETA-Ertapenem-10µg; ATM-Aztreonam-30µg; MEM-Meropenem-10µg; IPM-Imipenem-10µg; CAZ-Ceftazidime-30µg; CTX-Cefotaxime-30µg; FOX- Cefoxitine-30µg; FEB-Cefepime-30µg; CXM-Cefuroxime-30µg; SAM-Ampicillin/Sulbactam-20µg; AMC-Amoxacillin/Clavulanic acid-30µg.

3.5 Antimicrobial Susceptibility and Resistance Profiles of Bacterial Isolates from St. Vincent Hospital, Ndubia: The result of antimicrobial susceptibility and resistance profiles of bacterial isolates from St. Vincent Hospital, Ndubia is showed in table 4

4. DISCUSSION

Gram negative microorganisms are the major uropathogens implicated in urinary tract infection and UTIs are one of the most prevalent infections affecting human urinary system. Physicians usually employ empirical treatment in the therapy of UTI and the selection criteria of antimicrobial agents are based on the most likely pathogen involved and its expected resistance profile in a particular area.[24,25] Therefore, there is need for constant detection, monitoring and antimicrobial susceptibility testing of uropathogens in a particular locality.

4.1 Clinical Bacterial Isolates: In this study, *E. coli* with 58(35%), 76(34%) and 50(35%) was the most frequent and predominantly isolated uropathogen in the three mission hospitals. Similar studies conducted in Federal Teaching hospital, Abakaliki, South-Eastern Nigeria have also reported that *E. coli* 98(35.8%) was the most predominantly isolated uropathogen from patients with signs and symptoms of UTI (27). Alo, *et al*.[27] also reported *Klebsiella* sp with 20(7.3 %) as the second most prevalent Gram negative organism responsible for UTIs. The result of these studies are in agreement with the result obtained in our study where *Klebsiella* sp is the second most prevalent uropathogen responsible for UTIs in both Mater Misrecordea Hospital, Afikpo and St. Vincent Hospital Ndubia with 60(45%) and 33(23%) respectively.

Also, another study conducted among adult patients attending a tertiary hospital in Enugu, Nigeria, reported *E. coli* as the most dominant microorganisms amongst the Gram-negative pathogens, where *E. coli* 44(45.83%) has the highest prevalence followed by *Proteus* sp 11(11.46%).[16] However, a varied noticeable proportion of occurrence of *E. coli*, *Klebsiella* sp, *Pseudomonas* sp and *Proteus* sp were observed in these three mission hospitals and its similar to those previously reported by other studies in South East, Nigeria.[13,16,27] This variation in the occurrence of uropathogens implicated with UTIs further supports the fact that prevalence of uropathogens and their antibiogram varies from one health facility to another and from place to place.[28]

4.2Prevalence of Bacterial Uropathogens: The result of this study revealed that the overall prevalence of UTIs in these three hospitals were (164)80%, (222)86% and (143)77% in RIM, Mater Misrecordea Hospital Afikpo and St. Vincent hospital, Ndubia respectively. These high prevalence of UTI in these three hospitals as observed in this study is in accordance with 90.1% overall prevalence of UTI reported in Shashemene referral hospital, Ethiopia by Wubalem and Alemayehu.[2] The high prevalence of uropathogens in these hospitals may be as a result of the differences in age, sex, educational qualification, hygienic practices of the patients and previous exposure to antibiotics. These factors may probably be factors contributing to the growth of Gram negative uropathogens thus, leading to increased prevalence of UTI especially among the rural dwellers. Age, sex, poor hygiene and geographical location have also been reported as risk factors for high prevalence of UTI in other studies.[2, 26]

4.3 Antimicrobial Susceptibility and Resistance Profiles of Bacterial Isolates from RIM, Mater Misrecordea Hospital, Afikpo and St. Vincent Hospital Ndubia: The result of the antimicrobial susceptibility profiles of the isolated Gram negative uropathogens in these three mission hospitals revealed that carbapenems (imipenem and meropenem) are the two most potent and active antibiotics against uropathogens and therefore can serve for routine empirical treatment of UTI. The high susceptibility of imipenem and meropenem in this study may be because these antibiotics are not readily available in these hospitals. Therefore, they are not usually prescribed by physicians in these mission hospitals as a result of their unavailability. The unavailability of these life saving antibiotics (carbapenems) in these mission hospitals may be because of the high cost of these antibiotics. However, in absence of imipenem and meropenem, antibiotics such as cefuroxime, gentamicin, cefoxitin, amoxacillin/clavulanic acid, ciprofloxacin, amikacin, cefotaxime, cefepime and ceftazidime which are readily available in these rural hospitals can serve for empirical treatment of UTIs prior to laboratory investigation. Other studies have also reported imipenem, gentamicin, ofloxacin, amikacin, ciprofloxacin, ceftazidime and amoxacillin/clavulanic acid as the most active antibiotics against uropathogens thus validating the result of our study.[1,29,30,31] The result of the antibiogram of the isolated Gram negative uropathogens in the three mission hospitals showed that variation exists in their susceptibility and resistance profiles from one mission hospital to another. These variations support the fact that antimicrobial susceptibility testing should be carried out in order to determine the most potent antibiotics to be used in the treatment of UTIs in a particular health care system.[13]

CONCLUSION

E. coli was the most prevalent and most frequently isolated uropathogen in these three mission hospitals. There is high resistance to commonly used antibiotics as expressed by the isolated uropathogens in this study, as 100% resistance were observed in tigecycline, ertapenem, aztreonam and ampicillin/sulbactam antibiotics. Several reasons support this high resistance exhibited by these uropathogens and may include incorrect and inappropriate administration of antimicrobial agents in empiric treatment of UTIs by physicians, age, health hygiene, pregnancy etc.

We therefore recommend continuous monitoring and determination of antimicrobial susceptibility profiles of uropathogens implicated in UTI before administration of antibiotics. However, for empiric treatment of UTIs imipenem and meropenem have shown high susceptibility and are therefore recommended.

Acknowledgements: The authors are thankful to the Department of Applied Microbiology, Rural Improvement Mission hospital, Ikwo (RIM), Mater Misrecordea Hospital, Afikpo and Saint Vincent hospital, Ndubia for supporting and facilitating this work.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

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All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.