

Antimicrobial Resistance in Uropathogens Associated with Community Acquired Urinary Tracts Infections in Port Harcourt, Nigeria

Abstract

Urinary tract infections (UTI) remain a major public health challenge and foremost cause of morbidity and economic and manpower losses worldwide. This is a retrospective study aimed at ascertaining the prevalence of the bacterial uropathogens and the antimicrobial resistance patterns of the dominant strains. It involved a review of laboratory records of the over 1426 males and females of various age brackets between January 1, 2021 and December 31, 2022. Out of 1426 urine culture samples 884 (62%) were from females and 542 (38%) males, with 834 (58.5%) negative cultures while 592 (41.5%) yielded bacterial growths. A total of 403 uropathogens comprising 11 species were obtained. Gram negative bacteria constitute 73.9% of the isolates while gram positive bacteria were 26.1%. *Escherichia coli* was dominant with 347 strains (54.7%), followed by *Staphylococcus aureus* 65 (10.3%), *Enterococcus faecalis* 60 (9.5%). Over half of the uropathogens were resistant to more than 50% of the tested antimicrobials, giving a cumulative resistance of 50.5%. The most resistant uropathogen was *Pseudomonas aeruginosa* with a resistant profile of 64.1%, followed by *Klebsiella pneumoniae* (63.7%), *Staphylococci* (55.7%), *Streptococcus pneumoniae* (55.2%), *Acinetobacter baumannii* (51.7%), *Proteus mirabilis* (51.4%), *Escherichia coli* (48.0%), *Enterobacter cloacae* (47.1%), *Enterococcus faecalis* (46.5%) and *Serratia marcescens* (43.3%). This study will benefit clinicians and healthcare practitioners in the empirical choice of antibiotics against UTI; however the high prevalence of AMR necessitates conduct of urine cultures to identify particular uropathogens and the appropriate antimicrobials in order to curtail increase in AMR among uropathogens.

Keywords: *Antimicrobial Resistance, Uropathogens, Escherichia coli, Community Acquired Urinary Tract Infections,*

Introduction

Urinary tract infections (UTI) remain one of the major public health challenges and a foremost cause of morbidity and attendant costs in terms of economic and manpower losses worldwide.^{1,2,3,4} (after 3.) UTI may be community acquired or nosocomial. The community acquired infection occurs when a person gets infected with any of the etiological agents prior to being admitted in a hospital as contrasted with nosocomial or hospital acquired urinary tract infections which as the name suggests is contracted during stay in the hospital. Community acquired UTI is more prevalent in developing or resource challenged countries.^{5,6} UTI may also be classified as uncomplicated and complicated; most community acquired infections are uncomplicated especially in healthy adults including non-pregnant women; while complicated UTI is more prevalent with nosocomial infections especially catheterized and surgical patients or those who are otherwise immuno-compromised. (Muhammad *et al* 2020) Another classification is based on the site of infection along the urinary tract such as pyelonephritis, cystitis, prostatitis, urethritis etc.²

Like most infections, UTI may be symptomatic or asymptomatic. Symptoms may include frequent micturition, urgency, dysuria, chills, fever, supra-pubic tenderness (tenderness is sign / pain), nausea, vomiting etc.⁷. Bacterial uropathogens constitute up to 95% of the etiologic agents of UTI with a vast majority being Gram negative rods; *Escherichia coli* remains the foremost and predominant uropathogen, with between 45 and 95% (45% to 95%) of the

infections attributable to it. Other notable culprits include *Klebsiella*, *Proteus*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, and *Citrobacter*. Notable Gram-positive bacterial culprits include *Staphylococci* and *Enterococci*.^{2,8}

The gold standard in diagnosis of UTI is by urine analysis, culture and sensitivity testing to identify the causative organisms and the most appropriate antimicrobial agents for treatment. However, the high prevalence and the need to commence treatment immediately as well as the absence of adequate laboratory facilities in some areas, often necessitates the commencement of treatment empirically while awaiting culture results where it is available. This makes imperative the possession of information on the resistance/ susceptibility patterns of the etiologic agents of UTI, in order to make appropriate choice of effective.^{9,10}

Probably more worrisome than the global public health challenge posed by the widespread prevalence of uropathogens is the pervasive and increasing menace of antimicrobial resistance which has been constricting the choice of antibiotics used in the treatment of UTI, given particularly that the acute infections are mostly treated by empirical choice of antibiotics.⁵ Inappropriate use, misuse and abuse of antibiotics particularly in resource poor countries where these drugs are available across the counter, coupled with minimal or absence of regulatory controls has continued to conspire with other factors such as absence laboratory facilities and healthcare personnel, scarcity of quality antimicrobial agents, increase in fake and adulterated drugs to complicate the problem of multidrug resistance.^{5,9}

This study was thus aimed at ascertaining the prevalence of the bacterial uropathogens and determining the antimicrobial resistance patterns of the dominant strains. It sought to provide empirical evidence for personnel involved in prescription and administration of antibiotics to make a better informed decision in the selection and administration of antimicrobial agents.

Methodology

Design and Setting

This is a retrospective, cross sectional, observational research carried out between January 1, 2021 and December 31, 2022 at Diagnostix and Scientifique Medical and Research Laboratories in Port Harcourt. It was essentially a review of the laboratory records of the over 1426 patients including males and females of various age brackets who were referred from public and private healthcare facilities as well as walk-in patients, whose urine samples were received, recorded and analyzed for microscopy, culture and sensitivity. All those whose records completely captured the details of their names, age, sex, bacteria isolated, resistant and susceptible drugs were included in the study while those with incomplete records were excluded.

Ethical Approval

The study does not involve any physical contact with persons or clinical specimens; however written consent of the management of the healthcare facility was sought for and obtained before commencement of the study. The research was conducted according to the principles of the Declaration of Helsinki,

Collection and Analysis of Patients Data

Using a data collection checklist, information about the sex, age, bacterial isolate and antibiogram of the isolates were obtained from the laboratory records.

Collection of samples, Isolation and Identification of Uropathogens

As stated in the standard operational procedure (SOP) midstream clean catch urine samples were collected in sterile, wide mouthed universal bottles and analyzed within two hours of collection. They were inoculated on sterile culture plates of Cystine–Lactose–Electrolyte-Deficient (CLED) agar, Blood Agar and MacConkey Agar. Standard wire loops of 1 µL in diameter were used in the inoculation, followed by incubation at 37°C for 18 to 24-hour period. The number of colonies was counted to determine the significance of the number of colony forming units (CFU). The cultures with significant colony counts, i.e. counts up to 10⁵ CFU/ml were carefully examined and the relevant colonial characteristics recorded. Gram staining and relevant biochemical test were then carried out for the identification of the uropathogens.¹¹

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) were carried out using the Kirby-Bauer disk-diffusion method. Muller-Hinton agar was prepared by following the manufacturers' guidelines and poured into sterile petri dishes; and were seeded with the relevant susceptibility disks. The antimicrobial agents tested were: Amoxicillin/cloxacillin(20µg), Azithromycin (30 µg), Ceftriaxone(30 µg), Chloramphenicol(30 µg), Ciprofloxacin(10 µg), Levofloxacin(20µg), Gentamicin (10 µg), Norfloxacin(10 µg), Rifampicin(20 µg), Streptomycin(30 µg) (Oxoid, England). Resistance data were interpreted according to National Committee for Clinical Laboratory Standards (NCCLS).¹²

Data Analysis

Descriptive statistical analysis was carried out using the Microsoft Excel spreadsheet software 2007 (older version any reason?)

Results

Frequencies of Distribution of Samples and Growths of Uropathogens Associated with Community Acquired Urinary Tract Infections in Port Harcourt, Nigeria

The laboratory records of a total of 1426 urine culture samples were reviewed in the course of this study; including 884 (62%) females and 542 (38%) males. A total of 834 (58.5%) were negative cultures while 592 (41.5%) yielded bacterial growths; among the negative cultures, 558 (66.9%) were females and 276 (33.1%) were males, 374 (63.2%) of the positive cultures were females while 218 (36.8%) samples were produced by males. With regards to the age brackets of the subjects, preponderant of the samples came from the 31 to 40 and 21 to 30 age brackets with 356 (25%) and 328 (23%) of the samples respectively. The proportion of samples collected

from the other age brackets were as follows: 41 – 50 (199; 14%), 11 – 20 (186; 13%), 51- 60 (156; 10.9), above 60 (102; 7.2%) and ages 10 years or less (99;6.9%) The highest number of isolates (111; 18.8%) were obtained from the 21 to 30 years age bracket, followed by 110 (18.6%) from the 31 to 40 years bracket; the least number of isolates (37; 6.3%) were recovered from the ages of 10 and below.

Table 1: Frequency of Distribution of Samples and Growths of Uropathogens Associated with Community Acquired Urinary Tract Infections in Port Harcourt, Nigeria

Age (years)	Number of samples						Absence of Growth						Presence of Growth					
	F	%	M	%	T	%	F	%	M	%	T	%	F	%	M	%	T	%
≤10	56	6.3	43	7.9	99	6.9	41	8.0	21	5.9	62	7.4	25	6.2	12	7.6	37	6.3
11 - 10	13	15.			18	13.					10	12.			16.			14.
21 - 30	4	2	52	9.6	6	0	71	9	31	8.8	2	2	58	6	26	5	84	2
31 - 40	24	27.		15.	32	23.	15	29.		18.	21	26.		17.		25.	11	18.
41 - 50	3	5	85	7	8	0	2	7	65	4	7	0	71	6	40	3	1	8
51- 60	17	19.	18	33.	35	25.	14	29.		27.	24	29.		18.		23.	11	18.
> 60	3	6	3	8	6	0	9	2	97	4	6	5	73	1	37	4	0	6
Tot	12	14.		13.	19	14.		16.	10.	12	14.		11.		18.			12.
al	4	0	75	8	9	0	85	6	38	7	3	7	47	7	29	5	76	8
	85	9.6	71	1	6	9	43	8.4	15	4.2	58	7.0	59	6	39	7	98	6
	69	7.8	33	6.1	2	7.2	17	3.3	9	2.5	26	3.1	41	1	35	2	76	8
	88	62.	54	38.	14	10	51	57.	35	65.	83	58.	40	45.	15	29.	59	41.
	4	0	2	0	26	0	1	8	4	3	4	5	3	6	8	2	2	5

Legend

M: Males; **F:** Females; **T:** Total

Frequencies of Uropathogenic Species Associated with Community Acquired Urinary Tract Infections in Port Harcourt, Nigeria

A total of 403 bacterial uropathogens made up of 11 species were obtained from the subjects within the sample period; gram negative bacteria constitute 73.9% of the isolates while gram positive bacteria were 26.1%. *Escherichia coli* was dominant isolate with 347 strains (54.7%), followed by *Staphylococcus aureus* 65 (10.3%), *Enterococcus faecalis* 60 (9.5%), *Proteus mirabilis* 49 (7.7%), *Staphylococcus saprophyticus* 36 (5.7%), *Streptococcus pneumoniae* 25

(3.9%), *Klebsiella pneumoniae* 17 (3.0%), *Pseudomonas aeruginosa* 17 (2.7%), *Enterobacter cloacae* 7 (1.1%), *Acinetobacter baumannii* 7 (0.9%) and *Serratia marcescens* 3 (0.5%). The number of strains obtained from female subjects was 403 (63.6%), while the males contributed 321 strains (36.4%).

Table 2; Species and Frequencies of Uropathogens Associated with Community Acquired Urinary Tract Infections in Port Harcourt, Nigeria

Isolates	Females		Males		Total	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
<i>Escherichia coli</i>	235	58.3	112	48.5	347	54.7
<i>Staphylococcus aureus</i>	39	9.7	26	11.3	65	10.3
<i>Enterococcus faecalis</i>	33	8.2	27	11.7	60	9.5
<i>Proteus mirabilis</i>	32	7.9	17	7.4	49	7.7
<i>Staphylococcus saprophyticus</i>	20	5.0	16	6.9	36	5.7
<i>Streptococcus pneumonia</i>	13	3.2	12	5.2	25	3.9
<i>Klebsiella pneumonia</i>	11	2.7	8	3.5	19	3.0
<i>Pseudomonas aeruginosa</i>	7	1.7	10	4.3	17	2.7
<i>Enterobacter cloacae</i>	7	1.7	0	0	7	1.1
<i>Acinetobacter baumannii</i> (spelling)	4	1.0	2	0.9	6	0.9
<i>Serratia marsescens</i>	2	0.5	1	0.4	3	0.5
Total	403	63.57	231	36.43	634	100

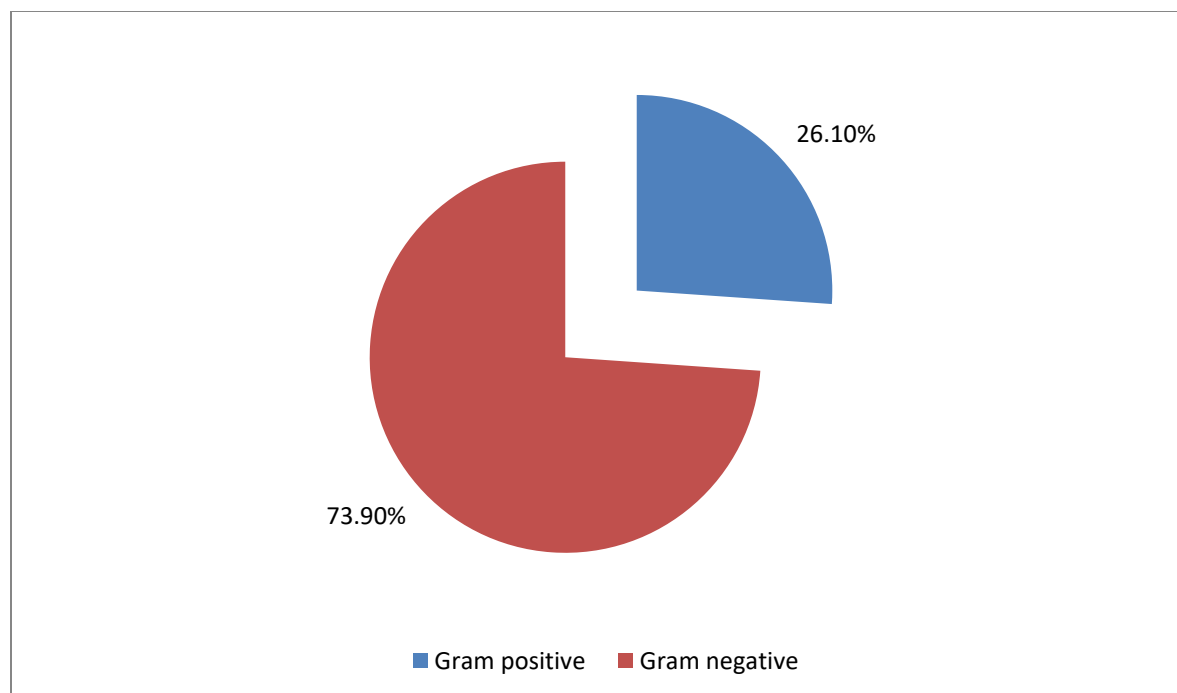


FIGURE 1 PREVALENCE OF GRAM POSITIVE AND GRAM NEGATIVE UROPATHOGENS ASSOCIATED WITH COMMUNITY ACQUIRED URINARY TRACT INFECTIONS IN PORT HARCOURT, NIGERIA

Antimicrobial Resistance of Uropathogens Associated with Community Acquired Urinary Tract Infections in Port Harcourt, Nigeria

Over half of the uropathogens reviewed in this were resistant to more than 50% of the tested antimicrobial agents, giving a cumulative resistance of 50.5%. The most resistant uropathogen in this study was *Pseudomonas aeruginosa* with a resistant profile of 64.1%, followed by *Klebsiella pneumoniae* with a profile of 63.7%, *Staphylococci* (55.7%), *Streptococcus pneumoniae* (55.2%), *Acinetobacter baumannii* (51.7%), *Proteus mirabilis* (51.4%), *Escherichia coli* (48.0%), *Enterobacter cloacae* (47.1%), *Enterococcus faecalis* (46.5%) and *Serratia marcescens* (43.3%)

The most **resisted(resistant)** antimicrobial agent as observed in this study was nitrofurantoin which proved to be ineffective against 87.1% of the uropathogens. This was followed by the cephalosporin cephalexin (80.4%), the folate pathway inhibitor, Cotrimoxazole (75.1%), the penicillin Amoxicillin/clavulanate (64.2%), the fluoroquinolone Pefloxacin (46.5%), the cephalosporin Ceftriaxone (37.7%), the fluoroquinolone Ofloxacin (34.3%), the aminoglycoside gentamicin (27.8%), the fluoroquinolone ciprofloxacin (25.4%) and the aminoglycoside streptomycin

(22/1%)

Table 3: Antimicrobial Resistance of Uropathogens Associated with Community Acquired Urinary Tract Infections in Port Harcourt, Nigeria

Isolates	n	nx10	AC	CTX	CEP	CPX	SXT	CN	NIT	OFX	PFX	STR	CR	CR %
<i>Escherichia coli</i>	347	34	21	11	279	82	23	86	30	113	15	75	166	48.0
<i>Staphylococcus spp</i>	101	10	68	40	86	34	83	33	90	50	58	21	563	55.7
<i>Enterococcus faecalis</i>	600	60	34	18	39	13	50	14	49	20	31	11	279	46.5
<i>Proteus mirabilis</i>	490	49	31	26	41	12	41	10	40	23	16	12	252	51.4
<i>Streptococcus pneumoniae</i>	250	25	15	14	21	6	19	9	23	12	14	3	136	55.2
<i>Klebsiella pneumoniae</i>	190	19	16	9	20	4	19	12	19	7	10	5	121	63.7
<i>Pseudomonas aeruginosa</i>	170	17	15	7	15	7	14	7	17	11	10	6	109	64.1
<i>Enterobacter cloacae</i>	70	7	4	3	4	1	5	2	5	3	3	3	33	47.1
<i>Acinetobacter baumannii</i>	60	6	4	2	3	2	4	2	6	3	3	2	31	51.7
<i>Serratia marcescens</i>	30	3	2	1	2	0	2	1	2	1	0	2	13	43.3
Total	6340	634	407	239	510	161	476	176	552	243	295	140	3199	50.5
Percent	100	64.2	37.4	37.4	80.4	25.4	75.1	27.8	87.1	34.3	46.5	22.1	50.5	

AC: Amoxicillin/clavulanate; CTX: Ceftriaxone; CEP: Cephalexin; CPX: Ciprofloxacin; SXT: Cotrimoxazole; CN: Gentamicin; NIT: Nitrofurantoin; OFX: Ofloxacin; PFX: **Pefloxaacin (spell)**STR: Streptomycin; n: Number of isolates; CR: Cumulative resistance

Discussion

To an appreciable extent, this study has contributed to ascertaining the prevalence of the bacterial uropathogens and the antimicrobial resistance patterns of the dominant strains associated with community acquired UTI in Port Harcourt. It has also presented empirical evidence for personnel involved in prescription and administration of antibiotics to make more informed decisions in the selection and administration of antimicrobial agents. Out of the 1426 urine samples reviewed in this study, a prevalence of 592 (41.5%) was recorded as yielding

bacterial growths. This result aligned very closely with the outcomes of a related study, which reported a UTI prevalence of 40% in Awka, Nigeria¹³ and 42.8% reported in Bangladesh⁷; but higher than 24.1% in Gondar, Ethiopia², 36.1% in Pakistan⁸, 27.4%, in Tanzania,⁶ 32.1% in Ethiopia¹. The prevalence is however less than the 64% reported by studies in Uganda¹⁴ and Karaikudi, India.¹⁵ The observed differences may be attributable to local peculiarities like status of antibiotic use and abuse, climatic factors and also the sample sizes. There were more females than males presenting for UTI investigations as well as contributing to the number of uropathogenic isolates with 374 (63.2%) from females while males accounted for 218 (36.8%); this is hardly surprising as females have been known to shoulder a larger chunk of the UTI burden as similar results were obtained elsewhere⁹. Age wise, the highest number of isolates (111; 18.8%) obtained from the 21 to 30 years age bracket, and 110 (18.6%) from the 31 to (to) 40 years bracket could be explained by the fact of being more incidental with most active reproductive ages.

Gram negative uropathogens constitutes a majority of the isolates with 298 (73.9%) while the Gram positive isolates were 105 (26.1%), this is not unexpected given that gram negative bacteria, especially rods have been widely reported as dominant causes of UTI.^{16,17}

The most prevalent uropathogen in this study is *Escherichia coli*, which has been extensively reported as the commonest etiologic agent, in addition to the other isolates found as regular isolates in UTI related cases.^{2,4,6} It is noteworthy that the isolates include members of the ESKAPE pathogens (*Enterococcus* sp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) causing much concerns over high rates of mortality, morbidity and economic losses across the world due to difficulties in treatment arising from their high prevalence of multidrug resistance.^{11,12,18} The overall antimicrobial resistance profile is indicative of high resistance rates among the uropathogens, which is a cause for concern. More than half of the isolates were resistant to more than 50% of the antimicrobial agents; while 40% of the antimicrobial agents have AMR profiles above 60%; these are clear indicators that the pathogens are getting increasingly resistant, while the antibiotics are becoming increasingly less effective in the battle against infections.¹⁹

Among the seven classes of antimicrobials tested, the fluoroquinolones, ciprofloxacin, levofloxacin, ofloxacin and pefloxacin; the aminoglycosides gentamicin and streptomycin and the third-generation Cephalosporin, Ceftriaxone that exhibited some appreciable potentials against the test organisms. This is consistent with a number of previous studies in Nigeria and elsewhere pointing to widespread use and misuse of such widely used antimicrobials like penicillins (amoxicillin/clavulanate), second generation cephalosporins (cephalexin), cotrimoxazole and nitrofurantoin, though there are conflicting results in few cases.^{18, 20, 21}

As a retrospective study, this research relied on previously generated records and so was subject to such limitations as having to adapt the obtained data to fit into the objectives of the study, such as the choice of antibiotics and absence of molecular identification. There is also the limitation of inadequacy of socio-demographics data because the tests were conducted for different objectives. As a cross sectional study, there was no determination of the effects of time and other variables.

Conclusion

The limitations notwithstanding this study has been able to isolate, identify, ascertain the prevalence and antimicrobial resistance patterns of bacterial uropathogens in Port Harcourt. This study will benefit clinician and other healthcare practitioners in the empirical choice of antibiotics in the treatment of UTI. The high prevalence of AMR makes it necessary to conduct urine cultures and susceptibility testing to identify particular uropathogens and the appropriate antimicrobials in order to curtail increase in AMR among uropathogens. It is suggested that multicenter cohort studies incorporating more antibiotics and be conducted to overcome some of the identified limitations.

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