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Research Article

Antibiotics Resistant Profile of Multi Drug Resistance Uropathogenic *Escherichia coli* in Southeastern Nigeria

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Abstract

Objective: To determine the prevalence and antimicrobial resistance pattern of uropathogenic *Escherichia coli (E. coli)* from patients in a tertiary hospital, in southeastern Nigeria.

Method: This was a cross sectional study carried out at the Medical Microbiology Department of University of Nigeria Teaching Hospital (UNTH) Enugu, in southeastern Nigeria from July 2013 to June 2016. Ethical Clearance was obtained from Health research ethical committee of University of Nigeria Teaching Hospital Enugu. A total of 6553 urine samples were examined and cultured in 5% sheep blood agar and MacConkey agar plate. The plates were incubated aerobically at 37°C for 18 hours. After which E. coli isolates were identified using standard biochemical tests. Antimicrobial resistant testing were done on Muller Hinton plates using modified Kirby-Bauer method and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Analytical and descriptive statistics was done using statistical package for the social sciences (SPSS) version.

Result: Out of 6553 urine samples, 2062 showed significant growth with E. coli (65.6%) being the most predominant followed by Klebsiella spp. (19.3%), Proteus spp (6.2%), Pseudomonas aeruginosa (4.7%) and Staphylococcus aureus (3.5%). The age group 30-39 years (27.3%) had the highest prevalence. (χ^2 =572.1, p= 0.001). Females constituted 81.4 % of the study (χ^2 =469.43, p< 0.000). The antibiotics that showed yearly increase in resistance were ciprofloxacin, augmentin, amoxicillin, cotrimoxazole, nitrofurantoin, ceftriaxone, gentamicin and ofloxacin. All the isolates had a multiple antibiotics resistance (MAR) index greater than 0.2.

Conclusion: *E. coli* isolates showed multiple drug resistance to commonly used antibiotics in the treatment of urinary tract infections. Antibiotic guidelines and good practice of antibiotic stewardship is of paramount importance for the optimum care of the patient.

ABBREVIATIONS

ATCC: American Type Culture Collection; CLSI: Clinical and Laboratory Standards Institute; *E. coli Escherichia coli*; MAR: Multiple Antibiotics Resistance Spp: Species; UNTH: University of Nigeria Teaching Hospital

INTRODUCTION

Infection of the urinary tract system is the second commonest site of infections after the respiratory tract system [1-4]. It is estimated that 10% of people will experience it during their lifetime, with women more affected than men [5,6]. *E. coli* is the commonest bacterium seen, causing 85% of community-acquired and 50% of hospital-acquired UTIs [7]. There are reports of increasing number of isolates resistance to antimicrobials [8-10]. Resistant uropathogenic *E. coli* in the United States increased from 9% in 2001 to 17% in 2010 [11]. Also in Nigeria, a study done in Shika-Zaria showed 95% resistance to routinely used drugs for the treatment of urinary tract infections [12]. The Knowledge of the resistant pattern will help to advice the clinicians on antibiotic guidelines and stewardship to optimize patients care.

MATERIALS AND METHODS

Study design

A cross- sectional study was done covering a period of 3 years (from July 2013 to June 2016) in UNTH Enugu. The biodata namely, gender, age, clinics and wards were obtained from the request forms of the patients sent to the microbiology laboratory for UTI investigations. All the urine samples were routinely examined macroscopically and microscopically, and then cultured with calibrated loop technique delivering 0.002ml on 5% sheep blood and MacConkey agar plates. The plates were incubated aerobically at 35-37^{0}C for 18-24 hours. The suspected colonies of *E. coli* which showed significant growth (≥ 10^5cfu/ml) were Gram stained and identified with the following tests; motility, indole production, methyl red, Voges Proskeur, triple iron sugar utilization, urea test and citrate utilization tests as described in standard bacteriological methods [13].

Susceptibility testing of confirmed isolates was done by disc diffusion method using modified Kirby-Bauer on Mueller-Hinton agar. The sensitivity was interpreted according to CLSI guideline [14]. *E coli* (ATCC 25922) was used as control. Data analysis was done using SPSS version [15]. Hence, the results were summarized and presented in tables displaying frequencies and percentages (descriptive statistics). Inferential statistics was conducted using chi square test to determine the association between independent variables and dependent variables. Statistical significance was set at $p<0.05$. Multiple antibiotics index was also determined.

RESULTS AND DISCUSSIONS

A total of 6553 urine samples (mid stream urine 5786, urine collected from catheter 546 and supra pubic aspirates 221) were sent to the Medical Microbiology Department for urine microscopy, culture and sensitivity. Two thousand and sixty-two (2062) samples yielded significant growth of microbial colonies. Of the 2062 significant growth, 1353(65.6 %) samples yielded *E. coli*. *Klebsiella* species 398 (19.3 %), *Proteus* species 128 (6.2%), *Pseudomonas aeruginosa* 97 (4.7%), *Staphylococcus aureus* 72 (3.5 %), *Enterococcus* species 49 (2.4 %), *Candida* species 34 (1.7 %) as shown in (Table 1). The age group 30-39 years (27.3 %) had the highest prevalence, with the least being 0-9 years (2.1 %) (Table 2). There was significant difference in the distribution of *E. coli* among the age groups ($\chi^2 = 572.1$, $p = 0.001$). The sex distribution revealed that 81.4% were females, and this was significant ($\chi^2 = 469.43$, $p<0.000$) as shown in (Table 2). The antibiotics that showed yearly significant increase in resistance to the *E. coli* isolates were ciprofloxacin, augmentin, amoxicillin, cotrimoxazole, nitrofurantoin, ceftriaxone, gentamicin and ofloxacin (Table 3). All the isolates had multiple antibiotics resistance (MAR) index greater than 0.2 as shown in (Table 4).

Urinary tract infections (UTIs) are among the most commonly encountered infectious diseases affecting people of all age groups worldwide [1]. Multidrug resistant uropathogenic *E. coli* is causing serious public health problem and is associated with poor patient outcomes, increased length of hospital stay and increased costs. This study provides the prevalence and current antibiotic resistant pattern of uropathogenic *E. coli* in UNTH Enugu.

In this study, *E. coli* was found to be the most common cause of urinary tract infections with a rate of 65.6%. The occurrence of *E. coli* as the most common cause of UTI has been reported in previous studies though with variations in their rates. In Abuja [8], Okada, [16], Ethiopia [17] and India [18], rates of 37%, 41.16%, 63.6% and 38.06% were reported respectively. These variations in the rates of *E. coli* isolated from different studies may be because of differences in sample size and the populations studied. Also difference in identification methods may also influence the rates reported from different studies. However, *Klebsiella* spp *and Staphylococcus aureus were* reported in studies done in Rivers [19]. And Edo states [16]. Respectively as the most predominant pathogen. Bacteria causes of UTI can show geographic variations and may even vary over time within a population [20].

Table 1: Percentage frequency of isolates.

Isolates	Total number (%)
Escherichia coli	1353 (65.6)
Klebsiella species	398 (19.3)
Proteus species	128 (6.2)
Pseudomonas aeruginosa	97 (4.7)
Staphylococcus aureus	72 (3.5)
Others	14 (0.7)
Total	2062 (100)

Abbreviations: % = percentage

Table 2: Age and sex distribution of *Escherichia coli.*

Age groups (years)	Male	Female	Number of patients
0-9	12	17	29 (2.1%)
10-19	34	53	87 (6.4%)
20-29	52	243	295 (21.8%)
30-39	72	297	369 (27.3%)
40-49	12	169	181 (13.4%)
50-59	31	189	220 (16.3%)
60-69	19	74	93 (6.9%)
70-79	9	28	37 (2.7%)
80 -90	11	31	42 (3.1%)
Total	252	1101	1353(100%)

Abbreviations: % = percentage

The incidence of UTI was highest among the age groups 30-39 year old followed by 20-29. This is in keeping with previous studies which demonstrated high prevalence of UTI in ages 20-39 years [21,22]. However, this is not in agreement with the study done in Cameroon [22], that they reported high prevalence in older age group. The age group reported in this study is the most sexually active group of the population and sexual activities predispose them to UTI. Most women of child-bearing age fall within this group.

Our study showed that the frequency of UTI is greater in females as compared to males [15,23,24]. This finding may be attributed to anatomy of the females urogenital system that has shorter urethra and the close proximity of their urethral opening to the anus [18], hence making it easier for enteric normal flora to colonize the urinary system. Also, prostatic fluids are known to have antibacterial properties and this may contribute to lower rate of infection seen in males [25].

The susceptibility pattern of uropathogenic *E. coli* varies in different geographical locations and empirical treatment by the clinician is based on the knowledge of the local susceptibility patterns. The present study shows increasing resistance of the uropathogenic *E. coli* to different classes of antimicrobials. All the uropathogenic *E. coli* were resistant to three or more of the antibiotics tested, with multiple antibiotic resistance indexes greater than 0.2.This suggests that the organisms may have been previously exposed to the different antibiotics used in this study. Multiple antibiotics resistance (MAR) index is a tool that reveals the spread of bacterial resistance in a given population [26]. MAR index of a bacterial species greater than 0.2 signifies that the strain

Table 3: Yearly antimicrobial susceptibility pattern of *E. coli.*

Antibiotics	2013 – 2014		2014 – 2015		2015 – 2016		Statistical difference		
	Sensitivity (%)	Resistance (%)	Sensitivity (%)	Resistance (%)	Sensitivity (%)	Resistance (%)	X^2-stat	df	Sig
Ciprofloxacin	127 (32)	270 (68)	103 (24.2)	323 (75.8)	110 (20.8)	420 (79.2)	15.5	2	<0.001*
Augmentin	115 (29.0)	282 (71.0)	108 (25.4)	318 (74.6)	112 (21.1)	418 (78.9)	7.6	2	0.022*
Cotrimoxazole	138 (34.8)	259 (65.2)	104 (24.4)	322 (75.6)	104 (19.6)	426 (80.4)	27.8	2	<0.001*
Amoxicillin	122 (30.7)	275 (69.3)	116 (27.2)	310 (72.8)	100 (18.9)	430 (81.1)	18.7	2	<0.001*
Levofloxacin	185 (46.6)	212 (53.4)	206 (48.4)	220 (51.6)	253 (47.7)	277 (52.3)	0.26	2	0.878
Nitrofurantoin	297 (74.8)	100 (25.2)	288 (67.6)	138 (32.4)	417 (78.7)	113 (21.3)	15.2	2	<0.001*
Ceftriaxone	218 (54.9)	179 (45.1)	231 (54.2)	195 (45.8)	327 (61.7)	203 (38.3)	6.8	2	0.034*
Gentamicin	183 (46.1)	214 (53.9)	191 (44.8)	235 (55.2)	201 (37.9)	329 (62.1)	7.6	2	0.022*
Ofloxacin	127 (32.0)	270 (68.0)	129 (30.3)	297 (69.7)	132 (24.9)	398 (75.1)	6.4	2	0.042*
Imipenem	199 (50.2)	198 (49.8)	219 (51.4)	207 (48.6)	286 (54.0)	244 (46.0)	1.4	2	0.488
Meropenem	206 (51.9)	191 (48.1)	215 (50.5)	211 (49.5)	266 (50.2)	264 (49.8)	0.3	2	0.867
Cefotaxime	197 (49.6)	200 (50.4)	211 (49.5)	215 (50.5)	250 (47.2)	280 (52.8)	0.7	2	0.688

Key: % = percentage; *= statistical significant

Table 4: Multiple antibiotics resistance indices for *E. coli.*

No of resistant antibiotics	Frequency	MAR index	Percentage (%)
3	17	0.25	1.3
4	24	0.333	1.8
5	36	0.417	2.7
6	97	0.500	7.2
7	129	0.583	9.5
8	179	0.667	13.2
9	299	0.750	22.1
10	394	0.833	29.2
11	178	0.917	13.2
Total	1353	5.25	100.0

Key: % = Percentage

originated from an environment where the antibiotics are used [26,27], so this high rate of resistance to the antibiotics in this study maybe due to the common use of these drugs in treatment of UTI in our environment. Inappropriate exposure to antibiotics is the main driving force of development of drug resistance. This is in agreement with studies done in Abuja [8], Zaria [27], Iran [23] and Pakistan [28]. That also reported resistance of the isolates to commonly used drugs in treating UTIs.

The international guideline for treatment of UTI suggests cotrimoxazole and amoxicillin-clavulanic acid as the drugs of choice for treatment of urinary tract infections [28]. These drugs that were recommended for UTI treatment showed high resistance in this study, so their continual usage will always lead to treatment failure with resultant poor patient outcome. The yearly resistant profile showed imipenem followed by cefotaxime, meropenem and levofloxacin to be least resistant. This may be because these drugs are rarely used in the treatment of uncomplicated UTI so they are less likely to be abused. The resistant rates vary in different countries, regions and institutions over time. This shows the superiority of the knowledge of local resistance patterns for proper management of the patients. Hence, there is need for mandatory urine culture and susceptibility tests for all patients suspected of having UTIs so as to guide for proper treatment. This empirical system should be incorporated in the national and regional antibiotic stewardship policy which should drive the search for new effective antibiotics probiotics, and research in microbiome for future use.

CONCLUSION

There is increase in multiple drug resistant uropathogenic *E .coli* in this study. Hence, the need for urgent development of guideline for the effective treatment of UTIs in our hospital. Active Implementation of this guideline and practice of antibiotic stewardship will surely reduce the multi-drug resistance pattern in the coming years.

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