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Extended-spectrum β-lactamase Occurrence among Gram-negative Bacilli Isolated from Urine of Apparently Healthy Individuals at Obafemi Awolowo University, Ile-Ife, Nigeria

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aims: This study determines the carriage of extended-spectrum β-lactamase (ESBL) in Gram-negative bacilli (GNB) isolated from the urine of apparently healthy individuals at Obafemi Awolowo University, Ile-Ife, Nigeria.
Study Design: The study was designed to evaluate ESBL occurrence among GNB isolated from urine samples of recruited subjects at Obafemi Awolowo University, Ile-Ife, Nigeria.
Methodology: Mid-stream urine samples were collected from 70 apparently healthy and consenting participants. The samples were inoculated onto MacConkey agar and incubated at 37°C; the isolates were identified using standard methods. The antibiotic susceptibility profile and ESBL test were performed using standard methods.
Results: A total of 47 (67.1%) monomicrobial GNB were isolated. *Escherichia coli* (15, 31.9%) was the most predominant isolate identified. All isolates were multi-drug resistant; β-lactamase was produced in 17 (36.2%) isolates and ESBL, in only two isolates. ESBL-producing isolates showed resistance to non-β-lactam antibiotics.

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Conclusion: The isolates were multi-drug resistant, and the occurrence of ESBL-producing GNB isolated from the urine was observed. This study shows the complexity of antibiotic resistance and highlights the need for surveillance to address the serious issue of multi-drug resistance.

Keywords: Gram-negative bacilli; urine; antibiotics; resistance; extended-spectrum β-lactamase; community.

1. INTRODUCTION

The urinary tract in the human body is responsible for the production, storage, and excretion of urine and it consists of a variety of organs. Urine secreted by the normal kidney is sterile and remains so while it travels to the bladder. However, the voided urine in a normal person may contact thousands of bacteria per millilitre of microbial flora in the urethra and in cases of obstruction or reflux bacteria may ascend from the bladder to give rise to kidney infection [1]. Acute cystitis, poor personal hygiene and cultural habit have been attributed as predisposing factors to urinary tract infection [2]. Enteric bacteria have been shown to be the most frequently isolated uropathogens [3], which may be as a result of exposure to faecal organisms because of proximity to the urinary tract especially in women.

Urinary tract infections (UTIs) are typically not life-threatening, and they only cause reversible damage. However, when the kidney is involved, the risk of irreparable tissue damage and bacteremia increases [4]. *Escherichia coli* is the predominant causative organism responsible for UTIs. It is the causative agent of uncomplicated UTIs in 75%–90% of cases [5] because some strains possess specific uropathogenic determinants [1]. UTIs can result from mono- or polymicrobial infection. A comparison between mono- and polymicrobial infectious episodes showed that the latter was more often hospital-acquired and more frequently associated with urinary catheters. *Pseudomonas aeruginosa* is more commonly associated with polymicrobial infections, whereas *E. coli* is more common associated with monomicrobial infections [6].

In clinical practice, β-lactams are among the most important and frequently used antibiotics for treating bacterial infections. However, Gram-negative bacterial isolates commonly show resistance to β-lactam antibiotics [7]. The most prevalent mechanism of resistance is the production of β-lactamase which inactivates β-lactams by hydrolysing β-lactam rings [8-9]; these are either plasmid or chromosomally mediated. The gene encoding these enzymes has been observed to coexist with other antimicrobial resistance determinants [10].

Antibiotic abuse is a risk factor for antibiotic resistance and extended-spectrum β-lactamase (ESBL) acquisition [11-12]. The persistent exposure of bacterial strains to β-lactams has induced mutation and continuous production of ESBLs; these, in turn, have increased their activity against newly developed β-lactam antibiotics [13-14]. Antibiotic-resistant uropathogens cause both community- and hospital-acquired UTIs [15]. ESBL-producing bacterial isolates have been found in both hospital and community settings [6,16]. Furthermore, ESBL-producing enterobacteriaceae may acquire resistance to non-β-lactam antibiotics in the community [17-18]. The present study determines the occurrence of ESBL among Gram-negative bacilli (GNB) isolated from the urine of apparently heathy individuals at the Obafemi Awolowo University.

2. MATERIALS AND METHODS

2.1 Sample Collection and Microbial Analysis

This study follows institutional protocols, and informed consent was obtained from study participants. Urine samples were collected from apparently healthy students at the Obafemi Awolowo University, Ile-Ife, Nigeria. Questionnaires asking about a history of hospitalisation, antibiotic use, and demographic details such as age and sex were administered. Only volunteers who had a history of hospitalisation >48 h or had not been hospitalised within the last year were included in the study [19]. Mid-stream urine was collected once from 70 volunteers irrespective of age (18–30 years) and sex in sterile universal bottles. The urine samples were inoculated onto MacConkey agar for 1–2 h after collection using a sterile inoculating loop. The samples were incubated at 37°C for 24 h. Colonies of the bacterial isolates were examined and Gram-stained. Various biochemical tests were conducted as described

in a previous study [20], and the isolates were identified by referring to Bergey's Manual of Determinative Bacteriology [21].

2.2 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed on all isolates using the Kirby-Bauer disc diffusion method according to Clinical Laboratory Standards Institute guidelines [22]. The bacterial isolates were inoculated onto 5 mL sterile normal saline, and their turbidity was adjusted to match 0.5 McFarland standard to give 10^8 CFU/mL. *E. coli* ATCC 25922 was used as the control strain. The antibiotics tested included amoxicillin (10 µg), augmentin (30 µg), ceftriaxone (30 µg), cloxacillin (10 µg), cotrimoxazole (25 µg), gentamicin (10 µg), imipenem (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), ofloxacin (5 µg), penicillin (10 units), and tetracycline (30 µg). The susceptibility of each isolate was determined by measuring the zone of inhibition. Multi-antibiotic resistance was recorded as resistance to more than two classes of antibiotics. All intermediate resistant isolates were grouped as resistant for convenience.

2.3 Beta-lactamase Assay

The isolates were tested for their ability to produce β-lactamase by using the acidometric agar plate assay, as described in a previous study [23]. Penicillin G was added to 100 mL of distilled water containing 1.5 g of agar and 0.2 mL of 0.5% phenol red solution to yield a concentration of 5000 µg /mL at pH 9.0. The isolates were spot-inoculated on the media plate and incubated at 35°C for 1 h. Strains that produced β-lactamase appeared as a bright yellow zone around the colonies.

2.4 Extended Spectrum Beta-lactamase Test

The double-disc synergy protocol was used as described in a previous study [24]. The isolates were swabbed on the entire plate of the nutrient agar plate. A susceptibility disc containing amoxicillin-clavulanate (20/10 µg) was placed at the centre of the plate, and discs containing ceftriaxone (30 µg) antibiotics were placed 30 mm (centre-to-centre) from the amoxicillin-clavulanate disc. The enhancement of the zone of inhibition of ceftriaxone caused by the synergy of clavulanate in the amoxicillin-clavulanate disc was an indication of a positive result.

A simple percentage/mean was used to present the frequency of the bacterial isolates and the antibiotic susceptibility profile.

3. RESULTS

In the 70 urine samples processed, GNB were recorded in 47 (67.14%). *E. coli* was the dominant isolate, and *Citrobacter* sp. was the least common one (Table 1).

The antibiotic susceptibility profile of the isolates showed that virtually all isolates were resistant to penicillin and 38 (80.85%) were susceptible to imipenem (Table 2).

All isolates (100%) were resistant to more than three classes of antibiotics (Table 3). β-lactamase was detected in 17 (36.17%) of the isolates, and ESBL was seen in only two of the isolates (Table 4).

ESBL co-resistance with non-β-lactam antibiotics showed that the two isolates that expressed ESBL were resistant to ofloxacin, cotrimoxazole, gentamicin, and tetracycline (Table 5).

Table 1. Frequency of GNB isolated from urine of apparently healthy individuals

Isolates	Frequency (%)
E. coli	15 (31.9)
Proteus sp.	11 (23.4)
Enterobacter sp.	8 (17)
Klebsiella sp.	6 (12.8)
Pseudomonas sp.	5 (10.6)
Citrobacter sp.	2 (4.3)
Total	47 (100)

4. DISCUSSION

The study reports the ESBL carriage and antibiotic susceptibility profile of Gram-negative bacteria isolated from urine samples obtained from apparently healthy individuals. The study focused on monomicrobial cultures of GNB from the urine of 47 participants. UTIs can result from mono- or polymicrobial infection. Polymicrobial infections are more often hospital-acquired and are more frequently associated with urinary catheters. *E. coli* has been identified to be more common in monomicrobial UTIs [6]. In this study, *E. coli* was the most predominant organism isolated. The isolation of faecal coliforms from urine may be indicative of poor hygiene [2].

Table 2. Antibiotic susceptibility profile of GNB isolated from urine of apparently healthy Individuals

Antimicrobial class	Antibiotics	n =47	Number of resistance (%)	Number susceptible (%)
	Amoxycillin		47 (100)	0.00
Penicillin	Cloxacillin		47 (100)	0.00
	Penicillin		46 (97.87)	1 (2.13)
Amoxicillin/Clavulanic	Augmentin		45 (95.75)	2 (4.25)
Cephalosporins	Ceftriaxone		36 (76.6)	11 (23.4)
Carbapenems	Imipenem		9 (19.15)	38 (80.85)
Nitrofuran	Nitrofurantoin		34 (72.34)	13 (27.66)
Aminoglycosides	Gentamicin		30 (63.83)	17 (36.17)
Quinolones	Nalidixic Acid		33 (70.21)	14 (29.8)
	Ofloxacin		28 (59.57)	19 (40.43)
Tetracycline	Tetracycline		34 (72.34)	13 (27.66)
Sulphonamide	Cotrimoxazole		45 (95.74)	2 (4.26)

This finding is in agreement with reports that showed that *E. coli* is the predominant isolate recovered from urine samples of apparently healthy individuals [25-26].

Table 3. Multiple antibiotic resistance of GNB isolated from urine of apparently healthy individuals

Number of antibiotics	Frequency of resistance	Percentage of resistance (%)
3	0	0.00
4	7	14.9
5	5	10.6
6	6	12.8
7	17	36.2
8	10	21.3
9	2	4.3
Total	47	100

In the present study, the antibiotic resistance profile showed that Gram-negative bacteria have high resistance to β-lactam and other groups of antibiotics; this is comparable to other findings on this issue [26-28], indicating the inadequacy of these antibiotics in treating UTIs. Studies have reported that isolates cultured from the urine samples of apparently healthy individuals are multi-drug resistant [27-28]. It is of serious concern to note that all isolates cultured in this study were multi-drug resistant, indicating that controlling the causes of multi-drug resistance remains challenging.

The ESBL-producing isolates observed in this study were *E. coli* and *Klebsiella* sp.; this is in agreement with other studies that reported that both isolates contain ESBL enzyme [13,18]. ESBL has been reported to confer resistance to all β-lactam antibiotics except cephamycin and carbapenems [29]. Carbapenems have been observed to be the best drugs for treating infections caused by ESBL-producing enterobacteriaceae [7,18].

Table 4. Frequency of β-lactamase and ESBL occurrence in bacterial isolates

Organism	Number of tested isolates	Frequency β-lactamase (%)	ESBL (%)
E. coli	15	8 (53.33)	1 (6.67)
Klebsiella sp.	6	3 (50)	1 (16.67)
Proteus sp.	11	5 (45.46)	0.00
Citrobacter sp.	2	0.00	0.00
Enterobacter sp.	8	1 (12.5)	0.00
Pseudomonas sp.	5	0.00	0.00
Total	47	17 (36.17)	2 (4.26)

Table 5. ESBL occurrence with co-resistance with non-β-lactam antibiotics

Isolates	Co-Resistance ESBL
E. coli	Ofloxacin, Gentamicin, Cotrimoxazole, tetracycline, nitrofurantoin
Klebsiella sp.	Ofloxacin, Gentamicin, Cotrimoxazole, tetracycline

In this study, ESBL-producing isolates were susceptible to imipenem, multi-drug resistant to β-lactam antibiotics, and co-resistant to non-β-lactam antibiotics. This is in agreement with reports that ESBL-producing organisms are resistant to both β-lactam and non-β-lactam antibiotics [13,17-18] This may be because genes encoding these enzymes have been observed to coexist with other antimicrobial resistance determinants [10].

5. CONCLUSION

In conclusion, this study revealed that the urine of apparently healthy individuals contains multi-drug resistant and ESBL-producing bacterial strains. Co-existence of ESBL and non-β-lactam resistance was also observed. This study suggests that clinicians as well as individuals must use antibiotics more judiciously to curtail the growing trend of multi-drug resistance.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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