Original Article

Extended-Spectrum Beta-lactamase Production and Antimicrobial Susceptibility Pattern of Uropathogens in a Tertiary Hospital in Northwestern Nigeria

Fatima Jummai Giwa, Oluwafemi Temidayo Ige[1], Daniel Musa Haruna[2], Yahaya Yaqub[3], Tanko Zainab Lamido[3], Shuaibu Yahaya Usman[3]

Department of Medical Microbiology, Faculty of Basic Clinical Sciences, College of Health Sciences, Ahmadu Bello University, [3]Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, [1]Department of Medical Microbiology, Faculty of Clinical Sciences, College of Medicine, Kaduna State University, Kaduna, [2]Cluster Coordinator, WHO FCT Office, Abuja, Nigeria

Abstract

Background: Globally, there is a changing trend in the antibiotic susceptibility pattern of Gram-negative uropathogens to the conventional drugs used in the treatment of urinary tract infections due to the production of extended-spectrum beta-lactamases (ESBLs). **Aim:** This study aimed to determine ESBL production and antimicrobial susceptibility pattern in uropathogens. **Materials and Methods:** Five hundred urine samples submitted to the Medical Microbiology Department of Ahmadu Bello University Teaching Hospital from January to June 2012 were analyzed by conventional methods. Modified standardized Kirby-Bauer disc diffusion method was used for antimicrobial susceptibility testing. ESBL production by *Escherichia coli* and *Klebsiella pneumoniae* isolates was screened for using the Clinical and Laboratory Standards Institute guidelines 2012 and confirmed by the double-disc synergy tests. **Results:** Five hundred samples were analyzed. Of these, a total of 175 Gram-negative isolates were obtained. Isolation rates were *E. coli* – 56%, *K. pneumoniae* – 20%, *Proteus mirabilis* – 16%, and *Pseudomonas aeruginosa* – 4%. ESBL production was observed in 34.3% of all the isolates. Fifty percent (50%) of *E. coli* and 40% of *K. pneumoniae* were identified as ESBL producers and were found to be resistant to multiple antimicrobial agents. Imipenem and nitrofurantoin had sensitivity of 100% and 70%, respectively, while susceptibility to ciprofloxacin and gentamicin was low at 35% and 30%, respectively, although 96% sensitivity was observed with amikacin. ESBL producers and nonproducers showed a high level of resistance of over 95% to ampicillin, amoxycillin, and trimethoprim-sulfamethoxazole. **Conclusion:** This study found a high rate of ESBL production (34.4%) among uropathogens with multidrug resistance. Clinical microbiology laboratories should routinely incorporate ESBL detection methods in their laboratory procedures for continuous surveillance of multidrug-resistant isolates and antibiograms to guide empirical therapy.

Keywords: Antimicrobial susceptibility pattern, extended-spectrum beta-lactamases, northwestern Nigeria, tertiary hospital, uropathogens

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections leading patients to seek medical care[1] and are the most common hospital-acquired infections accounting for 40% of nosocomial infections.[2] More than 80% of these infections are attributable to the use of indwelling urethral catheters.[3] The hospital environment plays an important role in determining the organisms involved in UTIs. Hospitalized patients are more likely to be infected with *Escherichia, Klebsiella, Proteus, Staphylococci, Pseudomonas, Enterococci,* and *Candida* spp.[4] These strains are more drug resistant and carry a higher morbidity and mortality index, especially for multidrug-resistant Gram-negative bacteria which produce extended-spectrum beta-lactamases (ESBLs).

Originally, ESBL-producing strains were confined to hospital settings, but lately, these organisms are becoming prevalent in the community,[5] leading to high resistance rates of antimicrobials used in the treatment of UTIs worldwide and the spread of ESBLs.[6,7]

Address for correspondence: Fatima Jummai Giwa, Department of Medical Microbiology, Faculty of Basic Clinical Sciences, College of Health Sciences, Ahmadu Bello University, Samaru Zaria, Kaduna State, Nigeria.
E-mail: drfatimagiwa70@gmail.com

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ESBLs are primarily produced by the *Enterobacteriaceae* family of Gram-negative organisms with particular reference to *Klebsiella pneumoniae*, *Klebsiella oxytoca, Escherichia coli,* and *Proteus* spp.[6,8] ESBLs are also found in nonfermentative Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[9]

ESBLs are enzymes capable of hydrolyzing the penicillins, first-, second-, and third-generation cephalosporins and aztreonam but not the cephamycins or carbapenems and are inhibited by beta-lactamase inhibitors such as clavulanic acid.[7] ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species.[10]

The prevalence of ESBLs is increasingly being reported worldwide, and it varies according to geographic location and is directly linked to the use and misuse of antibiotics.[11] In Africa, ESBL-producing organisms have been reported in Egypt, Morocco, Tunisia, Senegal, and South Africa.[12] In Nigeria, prevalence rates range from 5% to 44.3% as shown in several studies by Olowe and Aboderin, Yusha'u *et al*., Akujobi and Ewuru, Mohammed *et al*., Olonitola *et al*., and Ogefere *et al*.[13-18] in Ogun, Kano, Nnewi, Maiduguri, Zaria, and Benin, respectively. In many parts of the world, 10%–40% of strains of *E. coli* and *K. pneumoniae* express ESBLs.[10] The Study for Monitoring Antimicrobial Resistance Trends (SMART) study, conducted in the Asian Pacific in 2007, reported the prevalence of ESBL production in *Enterobacteriaceae* to be highest in India. ESBL production among *E. coli* was 79.0%.[19]

Numerous outbreaks of infection due to ESBL-producing organisms have been described on every continent of the globe and pose challenging infection control issues. Some initial outbreaks of infection have been supplanted by endemicity leading to increased patient morbidity and mortality.[20,21] Incidentally, the laboratory detection of ESBLs can be complex and is not routinely performed in most laboratories.

The presence of ESBLs gives limited therapeutic options for treatment since plasmids responsible for ESBL production simultaneously carry multiple resistant genes to other antimicrobial classes such as aminoglycosides, fluoroquinolones, trimethoprim, chloramphenicol, tetracyclines, and cotrimoxazole giving rise to the development of multidrug resistance.[21,22]

Currently, the drugs of choice for the treatment of infections caused by ESBL-producing organisms are the carbapenems. The use of carbapenems, however, has also been associated with the emergence of carbapenem-resistant organisms.[10]

Colistin, polymyxin B, tigecycline, and fosfomycin have been shown to have substantial antimicrobial activity against ESBL-producing *Enterobacteriaceae* and merit further evaluation.[8] Temocillin also showed very promising effects.[23,24] These drugs are, however, not available in most developing countries.

This study aimed to determine ESBL production in uropathogens and their antibiotic susceptibility pattern in a tertiary health facility in northwestern Nigeria.

Materials and Methods

This was a prospective study conducted on 500 nonrepetitive urine samples submitted to the Medical Microbiology Department of Ahmadu Bello University Teaching Hospital, Shika-Zaria, from January to June 2012 from in-and out-patients with suspected UTI. These samples were processed within 1 h of collection. The ethical committee of the institution approved the study.

Urine microscopy was done using a drop of uncentrifuged urine to determine significant pyuria. The urine sediment was also examined microscopically.[25]

The samples were inoculated on Cysteine Lactose Electrolyte Deficient and Blood Agars and incubated at 37°C for 18–24 h under aerobic conditions. A significant bacteriuria count was also done using a calibrated wire loop on a blood agar plate. Discrete colonies were picked from the plate and a secondary Gram-staining was done. Further identification was done by using standard biochemical tests such as oxidase, motility, triple sugar iron, urease, citrate, and indole tests.[25]

Antimicrobial susceptibility testing

This test was done using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar as described by the Clinical and Laboratory Standards Institute (CLSI 2012) guidelines.[26]

The Modified Kirby-Bauer standardized disc diffusion testing was done using the direct colony suspension method. A suspension was made from a 24 h growth of the organism in saline to match the 0.5 McFarland turbidity standard. This was seeded on the entire surface of a Mueller-Hinton agar plate while rotating the plate at an angle of 60° three times. The following antibiotic discs (Oxoid UK) with potencies were used: ceftazidime (30 μg), ceftriaxone (30 μg), ampicillin (30 μg), amoxicillin, nitrofurantoin (300 μg), Augmentin (20 mg amoxicillin and 10 mg clavulanic acid), trimethoprim-sulfamethoxazole(1.25/23.75μg),gentamicin(10μg), amikacin (30 μg), imipenem (10 μg), and ciprofloxacin (30 μg). The Mueller-Hinton agar plate was then incubated at (35°C–37°C) in an aerobic atmosphere for 18–24 h, after which the diameter of the zones of growth inhibition around the discs was measured with a ruler. A similar procedure was done using *E. coli* ATCC 25922 strain and *K. pneumoniae* ATCC 700603 as negative and positive controls. These results were further interpreted using the Performance Standards for Antimicrobial Susceptibility Testing, CLSI 2012.[26]

Extended-spectrum beta-lactamase screening test

All Gram-negative isolates were subjected to screening tests using ceftazidime (30 μg) and ceftriaxone (30 μg) discs. Those isolates with ceftazidime zone <22 mm and ceftriaxone zone <25 mm were then subjected to confirmatory tests.[26]

Double-disc synergy test

The double-disc synergy test as described by Jarlier *et al*.[11] was used to confirm ESBL production. Plates were inoculated for routine drug susceptibility using the modified Kirby-Bauer

standardized disc diffusion method. Ceftazidime (30 μg) and ceftriaxone (30 μg) discs were placed on either side of co-amoxiclav (20 + 10 μg) 15 mm apart. ESBL-positive strains showed an expansion of the zone of inhibition of either cephalosporin toward the clavulanate giving a dumbbell shape. This expansion occurred because the clavulanic acid present in the Augmentin disc inactivated the ESBL produced by the test organism.

Statistical analysis

Data analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 20 (Armonk, New York: IBM Corp). Results were presented as charts, tables, and figures as appropriate.

Results

During the study period, a total of 500 urine specimens from patients suspected with UTIs were processed. Most of the patients were females, i.e.,285 (57%), male: female ratio was 1:1.32, while age range was between 1 and 75 years. Majority were outpatients and isolation rates were higher in patients on admission in Intensive Care Unit (ICU) and surgical wards.

Out of the 500 samples, 175 (35%) were characterized as Gram-negatives, 265 (53%) had no growth, mixed growth was seen in 30 (6%) samples, while 40 (8%) were Gram-positives.

Isolation rates were found to be 56%, 20%, 16%, and 8% for *E. coli, K. pneumoniae, Proteus mirabilis,* and *P. aeruginosa,* respectively [Figure 1].

ESBL production was observed in 34.3% of all the isolates. Fifty percent (50%) of *E. coli* (46) and 40% (14) of *K. pneumoniae* were identified as ESBL producers and were found to be resistant to multiple antibiotics.

Antibiotic sensitivity to these ESBL isolates were 100% and 70%, respectively, for imipenem and nitrofurantoin while susceptibility to ciprofloxacin and gentamicin was low at 35% and 30%, respectively, while 96% sensitivity was observed with amikacin. ESBL producers and nonproducers showed a high level of resistance of over 95% to ampicillin, amoxycillin, and trimethoprim-sulfamethoxazole [Figure 2].

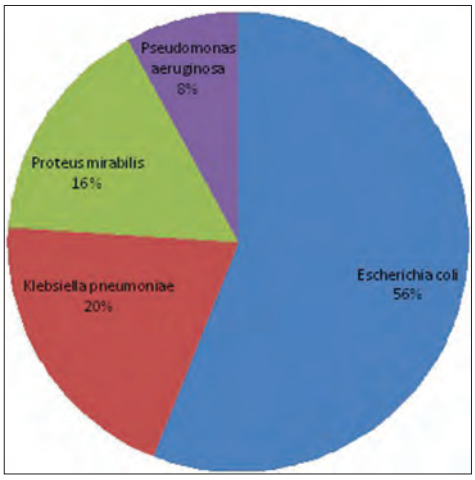

Figure 1: Distribution of Gram-negative uropathogens

Discussion

The findings in this study showed higher isolation rates in female inpatients compared with males and outpatients. Studies have shown that uncomplicated UTIs usually occur more in females than in males with an increase in age and sexual activity.[27-29] A study by Ben-Ami *et al*.[30] which examined risk factors for UTIs caused by ESBLs identified male sex, age >65 years, recent antibiotic use, recent hospitalization, and residence in a long-term care facility as independent predictors of risk of ESBL positivity by multivariate analysis. Similar findings were found in studies by Briongos-Figuero *et al*.[31] and Sammon *et al*.[32] which were in contrast to findings in this study.

The predominant isolate in this study was *E. coli* followed by *K. pneumoniae*, *P. mirabilis,* and *P. aeruginosa* with isolation rates of 56%, 20%, 16%, and 8%, respectively. Literature and several studies on uropathogens showed *E. coli* as the most frequently isolated uropathogen followed by *K. pneumoniae*.[33-35]

ESBL production was observed in 34.3% of all the isolates. This was similar to findings of Bajpai *et al*., Aggarwal *et al*., and Babypadmin and Appalaraju[36-38] where ESBL production was found to be 36.8, 36%, and 39.9%, respectively. Other workers like Ogefere *et al*., Azekhueme *et al*., Tankhiwale *et al*., and Mathur *et al*.[18,33,39,40] in Calabar, Benin, Nagpur, and New Delhi in India found higher rates of ESBL production of 47.1%, 44.3%, 48.3%, and 58% among Gram-negative isolates. This was, however, in contrast to findings of Akujobi and Ewuru, Mohammed *et al*., and Khurana *et al*.[15,16,41] in Maiduguri, Nnewi, and India who found lower ESBL production rates of 23.6%, 16%, and 26.6%, respectively.

These observed variances may be attributed to differences in study design and patient selection and differing patterns of antibiotic stewardship in the various centers.[16,42] Moreover, geographical differences occur in clinical isolates which are also rapidly changing with time.[36]

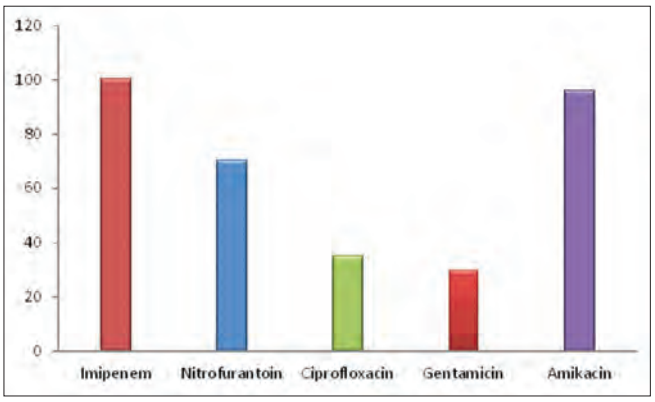

Figure 2: Antibiotic susceptibility pattern of ESBL-producing uropathogens

Fifty percent (46) of *E. coli* and 40% (14) of *K. pneumoniae* were identified as ESBL producers and were found to be resistant to multiple antibiotics. This trend has been observed in several studies where ESBL production was found to be highest in *E. coli* followed by *K. pneumoniae*.[15,33,36,43] The SMART study which was conducted in the Asian Pacific in 2007 found ESBL production in *Enterobacteriaceae* to be highest in India (79%) and in *E. coli*.[19]

The antimicrobial susceptibility pattern to ESBL isolates in this study were 100% and 70%, respectively, for imipenem and nitrofurantoin while susceptibility to ciprofloxacin and gentamicin was low at 35% and 30% respectively, while 96% sensitivity was observed with amikacin.

Carbapenems are regarded as the antibiotic of choice and mainstay of treatment used against infections caused by ESBLs.[7,8] This is consistent with findings in this study which showed a 100% susceptibility to imipenem which is similar to results from other studies[44,45] In contrast, resistance to carbapenems has been seen in some strains of *K. pneumoniae* and *E. coli* species, in the form of carbapenemases (*Klebsiella*-producing carbapenemases and New Delhi metallo-β-lactamases). A study by Bajpai *et al*.[36] showed a high resistance (52.1%) to meropenem, due to the presence of carbapenemase-producing isolates as a result of excessive use of carbapenems in ICUs. A similar study by Gupta *et al*.[45] also showed a resistance of 22.16% and 17.32% to meropenem and imipenem, respectively, mainly from isolates in ICUs. This is alarming and gives rise to an increasing concern over the judicious use of carbapenems in our health facilities.[46,47]

The susceptibility pattern of nitrofurantoin on ESBLs was found to be 70% in this study. Nitrofurantoin is a synthetic nitrofuran antimicrobial agent that has been in use for more than 50 years and continues to be effective for the treatment of uncomplicated UTIs in the ambulatory setting.[48] A persisting low prevalence of resistance to nitrofurantoin (1.9%–7.7%) was found among urinary *E. coli* isolates, including those resistant to trimethoprim-sulfamethoxazole or ciprofloxacin.[48] Surveys in the USA and Canada on *E. coli* urinary isolates found 1.1% resistance[49] which is similar to the resistance rate in France which was 1.8% among *E. coli* urinary isolates.[50] The low resistance to nitrofurantoin may be attributed to its ability to achieve very high urine concentrations.[51] Studies done have also found it effective *in vitro* against *E. coli* strains, including ESBL producers.[48,52] This corroborates the findings in this study and that of a study done in Europe where it was found that among 115 clinical isolates of *E. coli* ESBL producers, 71.3% were sensitive to nitrofurantoin.[53] In a similar vein, a study done in a tertiary care facility in Turkey showed resistance rates of 6.6% and 23.2% in ESBL-negative and ESBL-producing *E. coli*.[52] These results suggest that nitrofurantoin is a suitable, effective, and cheap alternative drug in the treatment of ESBL-producing *E. coli*-related lower UTI.[54]

ESBL producers and nonproducers in this study showed a high level of resistance of >95% to ampicillin, amoxycillin, and trimethoprim-sulfamethoxazole which are the routinely used drugs for the treatment of UTIs. This trend was also observed in studies conducted by Manjunath and Aboderin *et al*.,[55,56] in India and Nigeria, which suggests that these drugs may no longer be used routinely as empirical treatment ascribed to their widespread use, with resistance developing to such a level that using them would lead to treatment failure.[55,56]

Susceptibility results of ciprofloxacin and gentamicin seen in this study were low at 35% and 30%, respectively, while 96% sensitivity was observed with amikacin. The quinolones are increasingly becoming resistant due to their excessive use in the treatment of various infections resulting in high selective pressure, prevalent in an environment in which antibiotics are freely available without restrictions.[55,57] Moreover, resistance to third-generation cephalosporins as exhibited by ESBLs often coexists with resistance to other antibiotics. Such associated resistance was also seen with gentamicin and cotrimoxazole that showed low sensitivities. The sensitivity of amikacin in this study was quite high (96%). Similar studies done have suggested the use of amikacin in cases of drug-resistant *Enterobacteriaceae*[58,59] due to its high sensitivity, and it has been found to be generally more active against ESBL-producing and quinolone-resistant *E. coli* than other aminoglycosides.[59-61] Due to its property of being refractory to most aminoglycoside-modifying enzymes, amikacin has been successfully used to treat otherwise aminoglycoside-resistant infections, and it is the most widely used semisynthetic aminoglycoside.[62,63] Results from the SMART study carried out from 2009 to 2011 in the United States[61] indicate that the most effective drug against ESBL-producing uropathogens after the carbapenems is amikacin. A study by Sung-Yeon *et al*.,[64] which evaluated the outcome of amikacin used as outpatient parenteral antibiotic therapy for UTIs caused by ESBL-producing *E. coli*, found an 88.9% cure rate while a study by Al Zahrouni *et al*.[65] showed 100% susceptibility. The benefit derived by patients with the use of amikacin is the attainment of high urinary concentrations because 94%–98% of the unchanged drug is recovered in the urine at 24 h.[66] These findings suggest that amikacin may be a valuable treatment option for ESBL-producing uropathogens.

Conclusion

This study found a high rate of ESBL production (34.4%) in uropathogens with multidrug resistance. Clinical microbiology laboratories should routinely incorporate ESBL detection methods for continuous surveillance of multidrug-resistant isolates and antibiograms to guide empirical therapy. Effective hospital-based infection prevention and control and antibiotic stewardship programs aimed at limiting the spread and emergence of resistant isolates should be instituted in our health-care facilities.

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Conflicts of interest
There are no conflicts of interest.

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