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Isolation and Phenotypic Characterization of Extended Spectrum Beta-lactamase Producing Bacteria from Urinary Tract Infection Patients in Adamawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author UA designed the study, wrote the protocol and performed the statistical analysis. Author JM wrote the first draft of the manuscript and managed the analysis of the study. Both authors managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: The irrational and inappropriate use of beta-lactam antimicrobial drugs has led to the advent of resistant strains worldwide. Beta-lactam resistance is mainly attributed to acquiring beta-lactamase genes, the consequence has been preventable treatment failures (sometimes fatal) in patients.
Aims: To Identify the Incidence of Extended-spectrum Beta-lactamae Producing Bacteria In Urinary Tract Infection (U.T.I). Patients (outpatients and inpatients) of Specialist Hospital Jimeta-Yola Adamawa State, North-east Nigeria.
Methodology: It was a Cross-sectional study carried out in Microbiology Unit of the Department of Science Laboratory Technology, School of Life Sciences, Modibbo Adama University of Technology Yola, Adamawa State, Nigeria. Between April and June 2016, The Strains Were Isolated, Identified And Screened For E.S.B.L By Double Disc Diffusion Synergy Tests (DDDST)

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Using Augmentin and Third-Generation Cephalosporin Cefuroxime-CRX, Cefixime-CXM and Ceftazidime-CAZ Purchased from Rapid labs. U.K.

Results: Of the 150 isolates, 49%(74/150) were Gramm positive and 51%(76/150) were Gramm negative, *S. aureus* 29% (44/150) was found to be the most prevalent organism followed by E*. coli* 21%(31/150) while *P. aeruginosa* has the lowest 2%(4/150), of the 150 isolates,17.4%(26/150) were E.S.B.L producers with female patients accounting for the 84.6%(22/26), out of 31 *E. coli* isolates 25%(8/31) were ESBL producers, out of the 21 *K. pneumonia* isolates 19%(4/21) is ESBL producers, out of the 16 *S. feacalis* isolates 6%(1/16) is ESBL producers, out of the 14 *P. mirabilis* isolates 21%(3/14) is ESBL producers, out of the 4 isolates 50%(2/4) are ESBL producers and none of the isolates of *S. epidermis* and *E. aeruginosa* was ESBL producer.

Conclusion: Periodic Study Of E.S.B.L Should Be Employed To Monitor Local Resistance Pattern And Comparative Study Of Both The Phenotypic And Genotypic Detection Should Be Employed To Authenticate The Reliability Of The Phenotypic Detection Method(S).

Keywords: Urinary tract infection; β-lactamase; β-lactam antibiotics; double disc diffusion synergy test (DDST); Adamawa State; North-East Nigeria.

1. INTRODUCTION

Urinary tract infections (UTI), also known as acute cystitis or bladder infection are a serious threat to human health affecting millions of people annually resulting in high morbidity and mortality, UTI is common in both males and females, females are more susceptible than males, with three-quarters of UTI occurring in pregnant women and one quarter in non-pregnant women. It is the most common form of bacterial infection in women with more than 10% developing UTI every year [1].

Beta-lactams are the most common drugs for the treatment of bacterial infections and account for more than 50% of global antibiotic consumption [2].

The irrational and inappropriate use of beta-lactam drugs has led to the advent of resistant strains worldwide. Beta-lactam resistance is mainly attributed to acquiring beta-lactamase genes localized on mobile circular genetic elements such as plasmids or transposons [1,3].

ESBL impart resistance to extended-spectrum cephalosporin, generally used to treat infections brought about by Gram-negative bacteria [3].

A study from Muhammad Abdullahi Wase Specialist Hospital, Kano showed that 37 (9.25%) isolates were ESBL producers based on DDST, four species identified as ESBLs producers include *Citrobacter spp*., *Enterobacter spp., Escherichia coli* and *Salmonella spp*. [2].

In Nigeria, there have been reports of the reoccurring cases of antimicrobial resistance by most pathogenic organisms against many antibiotics. Moreover, several studies establishing the presence of ESBL Producing clinical bacterial isolates from specific localities within the western and eastern part of the country have also been reported, ESBL-producing Gram-negative bacteria are emerging and impacting significantly on the management of patients and treatment costs [4].

Many clinical laboratories including the wider medical community may not be fully aware of the importance of ESBLs and how to detect them. The laboratories may also lack the resources to detect and to play role in the control of the spread of these resistance mechanisms, this is responsible for a continuing failure to respond appropriately and prevent the rapid worldwide dissemination of pathogens possessing these beta-lactamases. The consequence has been preventable treatment failures (sometimes fatal) in patients who received inappropriate antibiotics and outbreaks of multidrug-resistant bacteria that required expensive control efforts [5].

ESBLs were discovered in 1980, nursing homes and hospitals are found as main reservoirs with patients as the reservoir for these resistant organisms [6].

Although, in new CLSI interpretive criteria, routine ESBL testing is no longer necessary but can be done for epidemiological and infection control purposes, European Union Committee on Antimicrobial Sensitivity Testing (EUCAST) recommends that laboratories continue to screen and confirm ESBL production and to change the cephalosporin report from susceptible or intermediate to resistant if the isolate tests positive for E.S.B.L production [7].

ESBLs detection is an essential interest for infection control and epidemiological program [5], Hence this study was to determine the E.S.B.L producing isolates from the urine of U.T.I suspected patients attending Specialist Hospital Jimeta-Yola, Adamawa State, North-East Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area/Study Population

This work Was Carried Out In The Microbiology Unit of the Department of Science Laboratory Technology, School of Life Sciences, Modibbo Adama University of Technology Yola, Adamawa State, Nigeria, while the samples were obtained from patients (both in and outpatients) from Specialist Hospital Jimeta-Yola, Adamawa State, through the laboratory unit of the hospital.

2.2 Sample Collection

A total of 210 Samples of urine were collected (between April 2016 and June 2016) from patients, in sterile urine containers labelled.

2.2.1 Inclusion criteria

All inward and Outpatient (OPD) samples from UTI symptomatic patients were included irrespective of age and sex.

2.2.2 Exclusion criteria

All clinical urine samples for routine Examination other than UTI suspected patients.

Clean-catch midstream urine were collected.

The samples were processed without any delay. The samples were inoculated Manitol salt agar (MSA) and CLED agar and incubated at 37°C for 24 hrs. Isolates were further characterized using conventional/standard microbiology techniques such as colony morphology, Gram-staining and other biochemical tests [8].

2.3 Double Disc Diffusion Test (DDST)

0.5 McFarland standardized inoculum was swabbed onto Mueller-Hinton agar plate. An Augumentin disc (Amoxycillin 20 µg and clavulanic acid 10 µg), and 30 µg disk each of third-generation cephalosporin ($3GC_s$; Ceforuxime-CRX, Cefixime-CXM and Ceptazidime-CAZ) purchased from Rapid labs. U.K. was used.

The $3GC_S$ were placed 20 mm (centre to center). From the centrally placed Augmentin on Mueller-Hinton agar plates pre-swabbed with the test culture and incubate at 37°c for 24 hours and results were read. An increase in the zone of inhibition of Augmentin ≥5 mm compared to any of the 3GCs was inferred as synergy and the strain considered as ESBL producer.

2.3.1 Disc strength

Third-generation Cephalosporins (30 µg) and Augumentin (20/10 µg).

3. RESULTS AND DISCUSSION

From Fig. 1, of the total 210 samples collected (82 males and 128 female patients), 57.2%(121) showed significant bacterial growth while 42.8% (89) showed either insignificant growth or non-bacterial growth, out of the 121 positive urine samples 29 shows multiple bacterial growths yielding 150 bacterial isolates of which 49%(74) were gram-positive while 51%(76) were gram-negative as seen in Fig. 2, organisms isolated were *Staphylococcus aureus* 44(29%), *Escherichia coli* 31(21%), *Klebsiella pneumaniae* 21(14%), *Streptococcus faecalis* 16(11%), *Proteus mirabilis* 14(9%) *Staphylococcus epidermis* 14(9%), *Enterobacter aeruginosa* 6(4%) and *Pseudomonas aeruginosa* 4(3%) as seen Fig. 3, out of the 150 isolates, 26(17.4%) were extended-spectrum beta-lactamase producers while 124(82.6%) were non-extended spectrum beta-lactamase producer (Fig. 4) with males accounting for 4(15.4%) and females accounting for 22(84.6%) as represented in Fig. 6.

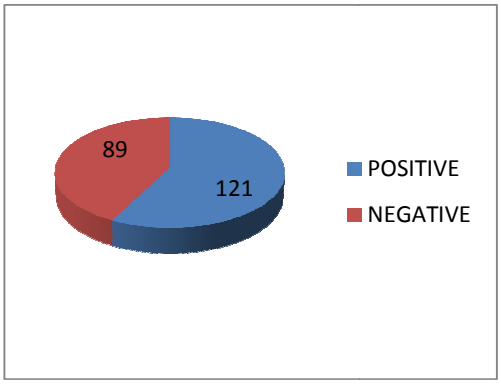

Fig. 1. Infection among patients

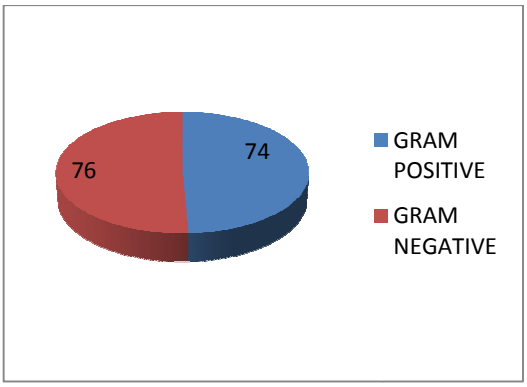

Fig. 2. Distribution of the isolates based on gram reaction

ESBLs producing microbes are one of the global public health concerns. The spread of ESBL producing bacteria compromises the activity of broad-spectrum antibiotics and affecting therapeutic success with a significant impact on treatment outcome. This necessitates for the utilization of simple, rapid, cost-effective and reliable techniques for the identification of ESBL. The continued emergence of ESBLs presents diagnostic challenges to the clinical bacteriology laboratories [5].

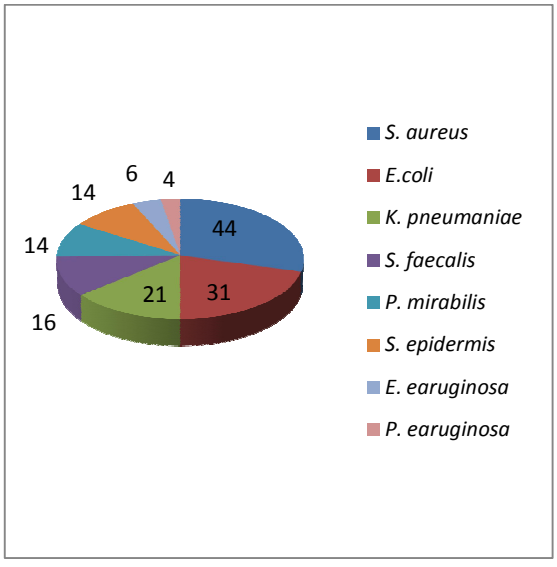

Fig. 3. Distribution of the isolates

From this study, out of the 150 isolates, 49%(74) were gram-positive while 51%(76) were gram-negative (Fig. 1) The high occurrence of the gram-negative may be due to poor hygienic practices among the patients which were reported as important factors leading the spread of ESBLs due to non-ESBLs producing strains acquiring plasmids responsible for ESBLs production [2].

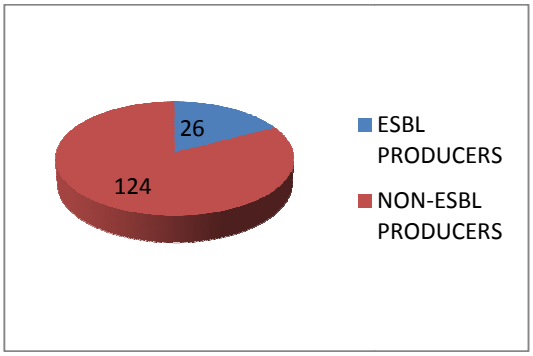

Fig. 4. Distribution according to ESBL production

From this study, out of the 150 isolates 26(17.4%) were ESBL producers which is higher than 8% reported [9], roughly relates with 12.9% reported by [6] and significantly lower than 56.8% reported [5], and 43% by [10,11], Although Previous studies from have reported the prevalence of the ESBL producers to be 6.6 to 68%. [12] but the obvious lower value compared to the report by [5] may be attributed to the fact that recently the co-existence of both AmpC and ESBLs in some gram-negative organisms have been reported, the AmpC beta-lactamases are the cephalosporinase that are poorly inhibited by clavulanic acid and they can be differentiated from ESBLs by their ability to hydrolyze the cephamycins, such strains with co-existing AmpC-lactamases may give negative test for the detection of ESBLs, because Clavulanic acid which is used in the standard DDST test for the ESBL detection acts as an inducer of the high-level AmpC production and it leads to the resistance 3GCs as well as 3GCs and Clavulanic acid, so even if ESBL is present, it will not be detected and it may result to false-negative test [13].

Despite all the methods developed during the last two decades, the identification of ESBLs by conventional phenotypic methods remains sometimes difficult in practice and becomes more so as ESBLs are more and more frequently associated with overproduced cephalosporinases, broad spectrum Beta-lactamases (TEM-1, TEM-2, SHV 1, SHV-11, etc.) or MBLs. Enzymic mechanisms of resistance, including ESBLs can also be associated with an outer-membrane permeability

defect or an efflux mechanism, complicating further the interpretation of susceptibility data. Consequently, molecular detection and identification of B-lactamases would be essential for a reliable epidemiological investigation of antimicrobial resistance. Molecular detection is usually based on amplification by PCR, followed by sequencing, which relies on the use of specific primers, allowing the amplification of family or group members. Because Beta-lactamases are characterized by a wide genetic diversity, an exhaustive enzyme Characterization using a PCR approach can be time-consuming, and a microarray approach would be of great help in detecting simultaneously several enzyme-encoding genes from a single strain. Two approaches have already been described: the first for detection at the family level and the second for the identification of the ESBL mutants of TEM-1 [14].

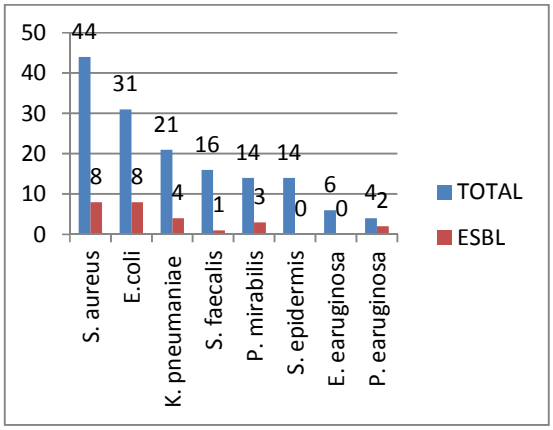

Fig. 5. ESBL Production among the various isolates

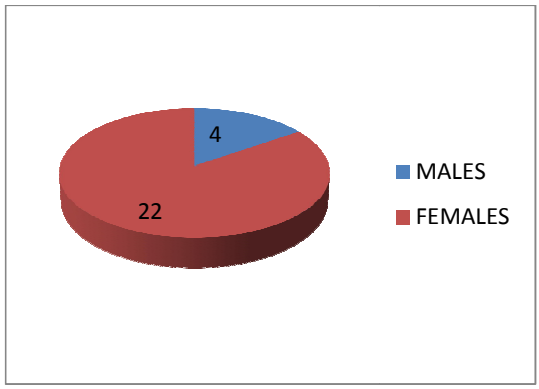

Fig. 6. Distribution of ESBL Producers with Patient's Gender

From Table 1, the study revealed that a higher percentage of females (71%) were suffered from UTI as compared to males (29%), which is also comparable with other studies [9-12]. well, the known fact is that UTI occurs more commonly in women due to their anatomy (short urethra and proximity to anal opening). Men are usually less prone to UTI as compared to females, due to the longer course of the urethra and bacteriostatic properties of prostate secretions [13].

From this study, S. *aureus* (29%) is the most prevalent U.T.I with women accounting for 33 of the 44 isolates, followed by *E. coli* which is the most prevalent gram-negative bacteria accounting for the 21% (31/150) of the U.T.I isolated, this in agreement with most of the recent studies of U.T.Is and could be attributed to differences in geographical locations and standards of hygiene [4-7,15].

Table 1. Distribution of the isolates according to the patient's gender

Bacteria	Proportion					
	Number		Males		Females	
	N	%	N	%	N	%
S. aureus	44	29	14	33	30	28
E. coli	31	21	8	19	23	22
K. pneumonia	21	14	3	7	18	17
S. faecalis	16	11	8	19	8	8
P. mirabilis	14	9	3	7	11	10
S. epidermis	14	9	3	7	11	10
E. aeruginosa	6	4	3	7	3	3
P. aeruginosa	4	3	1	2	3	2
Total	150	100	43	100	107	100

4. CONCLUSION

In this study, 17.2% of the isolates were ESBL producers, ESBL production is a common phenomenon in UTI patients and screening by DDST for these enzymes is a good epidemiological tool to assess the overall situation in a defined geographic location.

Using other β-lactam inhibitors like sulbactam and tazobactam, modified tests enhances the effectiveness of E.S.B.L detection, the periodic study of E.S.B.L should be employed to monitor local resistance pattern and comparative study of both the phenotypic and genotypic detection should be employed to authenticate the reliability of the phenotypic detection method(s) for successful treatment of infection relating to resistance in the hospital especially in resource-poor places.

The knowledge of the resistance pattern of bacterial strains in a geographical area will help to guide the appropriate antibiotic use and such institutional studies will help to formulate an empirical antibiotic policy for treatment.

CONSENT

Written consent was obtained from the patients for publication of this research work.

ETHICAL APPROVAL

Approval was obtained from the management of the hospital before the commencement of this research work.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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