

Studies on *Escherichia coli* associated with diabetic wounds; multi drug resistance and the occurrence of beta lactamase production in south-western Nigeria

Abstract

The prevalence of patients presenting with infections due to multi-drug resistant organism brought about the pharmacokinetics of antimicrobial agents and the discovery of drug resistant strains. This study focuses on the occurrence of these resistant *Escherichia coli* producing ESBL against the Cephalosporins in our tertiary health care centers in South-Western Nigeria. A total of 100 specimens collected from patients with one form of diabetic ulcer, were microbiologically analyzed, processed and identified using standard microbiological methods. Sixty (60) *Escherichia coli* isolates were recovered from the clinical samples. The highest and lowest recoveries to study area were (28) and (6) respectively. The susceptibility pattern was determined using the Agar-diffusion method (Kirby bauer). There was a remarkable (100) susceptibility to Cabapenam, and a high resistance for Ampicillin and Tetracycline (41.6%), the organism showed resistant to one or all of the third generation cephalosporin in use (Ceftazidime, Ceftriaxone, Cefotaxime and Amoxicillin) with 11(18.3%), 20(33.3%). The ESBL phenotypic identification was carried out on strains that showed relative susceptibility or resistance to one or more of the Cephalosporins. Using the Double disk synergy test (DDST), a total of 7 (11.6%) positive ESBL strains was identified. The University College Hospital Ibadan presented a 5(27.7%) while the University of Benin Teaching Hospital had 2 (6.6%). There was no classical and significant ESBL producer from Ladoko Akintola University of Technology Teaching Hospital and Obafemi Awolowo University Teaching hospital.¹

Keywords: prevalence, ESBL-extended spectrum beta lactamase, cephalosporins, penicillins, gram-negative bacteria, enzymes, microbiology, characteristics, and structure, epidemiology, penicillin-binding proteins, first isolation, β -lactam antibiotics

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Introduction

The β -Lactam agents such as penicillins,² cephalosporins, monobactams and carbapenems, are among the most frequently prescribed antibiotics worldwide. In Gram-negative pathogens, β -lactamases remain the most important contributing factor to β -lactam resistance,³ and their increasing prevalence as well as their alarming evolution seem to be directly linked to the clinical use of novel sub-classes of β -lactams.⁴ β -Lactamases are bacterial enzymes that inactivate β -lactam antibiotics by hydrolysis, which result in ineffective compounds.⁵ At least 400 different types of β -lactamases, originating from clinical isolates, have been described and a website has been created to monitor the latest developments among the newer types of β -lactamases.⁶ Several excellent reviews have recently been published describing the microbiology, characteristics, and structure, epidemiology and treatment options of organisms producing newer types of β -lactamases. This report does not aim to be comprehensive, but rather to illustrate that extended-spectrum β -lactamase-(ESBL) producing bacteria are emerging pathogens in the community, and that clinical laboratories play a critical role for their detection and control. Many genera of gram-negative bacteria possess a naturally occurring, chromosomally mediated β -lactamase. These enzymes are thought to have evolved from penicillin-binding proteins, with which they show some sequence homology. This development was likely due to the selective pressure exerted by β -lactam-producing soil organisms found in the environment.⁷ The first plasmid-mediated β -lactamase in gram-negatives, TEM-1, was described in the

early 1960s.⁵ The TEM-1 enzyme was originally found in a single strain of *E. coli* isolated from a blood culture from a patient named *Temoniera* in Greece, hence the designation TEM.^{8,9} Being plasmid and transposon mediated has facilitated the spread of TEM-1 to other species of bacteria. Within a few years after its first isolation, the TEM-1 β -lactamase spread worldwide and is now found in many different species of members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. Another common plasmid-mediated β -lactamase found in *Klebsiella pneumoniae* and *E. coli* is SHV-1 (for sulphurhydryl variable). The SHV-1 β -lactamase is chromosomally encoded in the majority of isolates of *K. pneumoniae* but is usually plasmid mediated in *E. coli*.¹⁰

Over the last 20 years, many new β -lactam antibiotics have been developed that were specifically designed to be resistant to the hydrolytic action of β -lactamases. However, with each new class that has been used to treat patients, new β -lactamases emerged that caused resistance to that class of drug. Presumably, the selective pressure of the use and overuse of new antibiotics in the treatment of patients has selected for new variants of β -lactamase. One of these new classes was the Oxyimino-Cephalosporins, which became widely used for the treatment of serious infections due to gram-negative bacteria in the 1980s. Not surprisingly, resistance to these expanded-spectrum β -lactam antibiotics due to β -lactamases emerged quickly. The first of these enzymes capable of hydrolyzing the newer β -lactams, SHV-2, was found in a single strain of *Klebsiella ozaenae* isolated in Germany.⁸ Because of their increased spectrum of activity, especially

against the oxyimino-cephalosporins, these enzymes were called extended-spectrum β -lactamases (ESBLs). Today, over 150 different ESBLs have been described. These β -lactamases have been found worldwide in many different genera of *Enterobacteriaceae* and *P. aeruginosa*. This review will focus on the characterization of ESBLs, the importance of detection of these enzymes, and their epidemiology.

Extended-spectrum b-lactamases (ESBLs)

ESBLs were first described in 1983, and have the ability to hydrolyse oxyimino-cephalosporins, and monobactams, but not cephamycins or carbapenems.¹ Although ESBLs have been described in a range of *Enterobacteriaceae* and *Pseudomonadaceae* from different parts of the world; they are most often identified in *Klebsiella pneumoniae* and *Escherichia coli*. These enzymes belong to the Ambler class A and D β -lactamases.^{1,2} The activity of Class A enzymes is inhibited in vitro by β -lactamase inhibitors such as Clavulanic acid, Sulbactam and Tazobactam but those belonging to class D are not. The majority of ESBLs identified in clinical isolates to date, have been SHV or TEM types, which have evolved from narrow-spectrum β -lactamases such as TEM-1, -2 and SHV-1.¹¹

Susceptibility and biochemical characteristics

ESBLs contain a number of mutations that allow them to hydrolyse expanded-spectrum β -lactam antibiotics. While TEM- and SHV-type ESBLs retain their ability to hydrolyse penicillins, they are not catalytically as efficient as the parent enzymes.⁴ In addition, the expansion of the active site that allows the increased activity against expanded-spectrum cephalosporins may also result in the increased susceptibility of ESBLs to β -lactamase inhibitors.⁷ ESBLs are not active against cephamycins, and most strains expressing ESBLs are susceptible to ceftioxin and cefotetan. However, it has been reported that ESBL-producing strains can become resistant to cephamycins due to the loss of an outer membrane porin protein.

Materials and methods

Study design

One Hundred and Fifteen *E. coli* isolates which were resistant to Ceftazidime were collected from four University Teaching Hospitals located in different Cities throughout the south western states of Nigeria (University College Hospital, Ibadan, Oyo State, Obafemi Awolowo University Teaching Hospital, Ife, Osun state, Ladoké Akintola University of Technology Teaching hospital, Idiseke, Osun State, and University of Benin Teaching Hospital, Benin city, Edo state). The clinical microbiology laboratories of the hospitals performed identification of the species of the isolated strains and preliminary determinations of their susceptibility patterns.¹²

Microbiological methods

The bacterial isolates potentially harbouring ESBLs were those with a positive phenotypic confirmatory test for ESBLs according to current National Committee for Clinical Laboratory Standards (NCCLS) criteria (National Committee for Clinical Laboratory Standards (NCCLS) 2002).

Antibiotic susceptibility testing

All strains were re-identified before the study. The strains were inoculated into MacConkey's agar and initially identified by glucose

and lactose fermentation and oxidation, citrate utilization, urea hydrolysis, indole and oxidase production and motility tests. Selected isolates were further identified by the VITEK identification system (bioMérieux, Lyon, France). Antibiotic susceptibility tests were performed on Mueller-Hinton (MH) agar (Oxoid). Antibiotic disks were obtained from Oxoid. The susceptibility and MIC values of the bacteria were tested against ceftazidime, ceftazidime-clavulanate,¹³ cefotaxime, aztreonam, imipenem-cilastatin, meropenem, ceftioxin, amikacin, gentamicin and cefotaxime-clavulanate. MICs were determined by replicating approximately 10^4 CFU of bacteria per spot by the aid of multipoint inoculators onto freshly prepared MH agar plates containing serial twofold dilutions of the related antibiotics. Agar plates were evaluated after 18 hours of incubation at 37 °C.

Screening for ESBL producers

Isolates inhibited by ≥ 2 mg/L of at least one of the oxyimino- β -lactam Ceftazidime, Cefotaxime and Aztreonam, Ceftriazone were considered as putative ESBL producers. ESBL production was confirmed by double-disc synergy test (DDST). DDST was done to determine synergy between a disc of Amoxicillin/Clavulanic acid (20 μ g/10 μ g) and 30 mg disc of each 3GC antibiotics earlier mentioned. MH agar plates were prepared and inoculated with standardized inoculum (0.5 McFarland tube) to form a pure culture. Disc (30 μ g) of each 3GC antibiotics was placed on the agar at a distance of 15 mm centre to centre from amoxicillin/clavulanic acid disc. ESBL production was interpreted if the inhibition zone around the test antibiotic disc increased towards the Amoxicillin/Clavulanic acid disc or if neither discs were inhibitory alone but bacterial growth was inhibited where the two antibiotics diffuse together.¹⁴

Results and discussions

A total of 60 *Escherichia coli*, isolated from four Teaching Hospital in the South Western Nigeria, where studied for the presence of the ESBL (Extended spectrum beta-lactamase) production, none of the isolates were found to be Carbapenem resistant (Table 1), the susceptibility pattern of the isolates amongst the various clinical samples, Ampicillin and Tetracyclin showed a (78.5%) resistance for stool samples, (44.4% and 77.7%) for swab and (94.7%, and 78.9%) for urine samples (100%) susceptibility for Carbapenem was recorded across the various clinical samples. The distribution of ESBL positive isolates in the following health care centers in Nigeria, shows a total of (7) ESBL positive isolates was recorded, the University college hospital with a (5) occurrence and the University of Benin teaching hospital with (2) ESBL positive *E. coli*. Table 2 Shows the antimicrobial profile of *Escherichia coli* to the Cephalosporins, this showed that across the clinical samples, 52(86.6%) (52/60) isolates showed complete resistance to the third generation Cephalosporins, a total of 23(38.3%) (23/60) showed a partial resistance to one of the Cephalosporins. Table 3 shows the gender and age distribution of ESBL producing *Escherichia coli* isolated from the tertiary health care centers in south western Nigeria. The highest Occurrence of ESBL in respect to age and gender shows that the patients of ages 31-60 had a high occurrence of (85.7%) and the ages of 21-30 with (14.2%). The gender distribution showed that females had a (57.1%) high occurrence than in males (42.8%) occurrence. Shows the number and percentage distribution of ESBL in various Hospitals in south western Nigeria. Out of the seven ESBL positive isolates the University college hospital had a 5(27.7%) occurrence and the University of Benin teaching hospital with a 2(6.6%) occurrence, Ladoké Akintola

University of Technology teaching hospital and Obafemi Awolowo University teaching hospital had no occurrence. Figure A and B, Shows the positive phenotypic presence of an ESBL using the DDST method of ESBL presumptive identification.¹⁵

Table 1 Distribution of Isolates from samples

Sample	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Klebseilla specie</i>	<i>Enterococcu specie</i>	<i>Streptococcus pneuemonia</i>
SWAB	60	20	4	4	12

Table 2 Antimicrobial susceptibility pattern of isolates

Organism/ No. of isolates	GEN	AMP	TET	CAB	OFL	CPX	PEF	CAZ	CRO	AMT
<i>Escherichia coli</i>										
60 R	12	48	47	O	4	4	4	20	26	27
S	48	12	13	60	56	56	56	40	34	33
<i>Staphylococcus aureus</i>										
20 R	0	12	0	7	-	-	0	-	-	-
S	20	8	20	13	-	-	20	-	-	-
<i>Klebseilla sp</i>										
4 R	0	4	2	0	0	0	0	-	-	-
S	4	0	2	4	4	4	4	-	-	-
<i>Enterococcus sp</i>										
4 R	0	2	0	0	0	0	0	-	-	-
S	4	2	4	4	4	4	4	-	-	-
<i>Streptococcus pneumonia</i>										
12 R	0	2	0	-	0	0	0	-	-	-
S	12	10	12	-	12	12	12	-	-	-

Index: GEN, gentamycin; CAB, cabapenam; TET, tetracycline; OFL, ofloxacin; CPX, ciprofloxacin; PEF, pefloxacin; CAZ, ceftazidime (30µg); CRO, ceftriaxone (30µg); AMT, aztreonam (30µg); AMP, ampiclox

Table 3 Gender and age distribution of ESBL *Escherichia coli* isolated from patients in the four tertiary health care centers in south western Nigeria

Age Group (year)	Male	Female	Total (%)
10-Jan	-	-	0
20-Nov	-	-	0
21-30	-	1	1(14.2%)
31-40	-	2	2(28.5%)
41-50	2	-	2(28.5%)
51-60	1	1	2(28.5%)
61-70	-	-	-
71-80	-	-	-
Total	3(42.8)	4(57.1)	7(100)

The DDSTs (double disc synergy test) Showed that a total of 7 (11%) of the *Escherichia coli* isolated where positive and found to be a producer of the beta-lactamase, and it was found that this isolates where either resistant to either one or more of the third generation cephalosporins or a monobactam. Reports about the ESBL producing strains have been appearing for about 2-decades now. However laboratories in our environment have been slow to embrace ESBL detecting methods in part, because the clinical importance of identifying such strains remains elusive and perhaps because of dearth

of information on this new β-lactamase enzyme group now evolving worldwide. However there is now increasing clinical evidence that shows the importance of detecting these strains in our environment. The result of this study demonstrated that ESBL production was detected in *Escherichia coli* isolates with a (11.6%) occurrence and this is in line with a recent study on the occurrence of the ESBL production by Gram negative organism in eastern Nigeria, where prevalence of extended spectrum beta-lactamase (ESBL) *E. coli* 105(44.6%) (105 of 420) was reported. The fact that the present research recorded a prevalence rate of (11.6%) of ESBL *E. coli* clearly indicates the surge in the ESBL producing organism in the south western Nigeria. This underlines the urgent need for immediate intervention especially in area of enlightening the clinicians in the south-western Nigeria on the necessity for routine ESBL screening to avert increasing resistance to these antibiotics in the future. The occurrence of ESBL observed in this study is thus not out of place since sites of infections and their treatment application such as oral/device related antibiotic exposure and prolong stay in the hospital centers are potential risk factors for ESBL infections and colonization.

Consequently patients placed on these devices as a treatment measures are at a very high risk of acquiring ESBL enzyme infection and colonization. Of the four hospitals, University College Hospital Ibadan, had the greater number of ESBL occurrence from *E. coli* isolates with a 27.7%, the University of Benin Teaching Hospital

had a (6.6%) occurrence with no occurrence from Ladoko Akintola University of Teaching Hospital, Idiseke and Obafemi Awolowo University Teaching Hospital, Ile-Ife. The presence of ESBL *E. coli* in UCH showing the highest occurrence might be due to the hospital location and influx of patients into the health care center. The gender and age distribution showed that although women had a high occurrence of the ESBL *E. coli* isolates with 4(57.1%) than in males 3(42.8%). The age distribution showed that highest ESBL *E. coli* isolates were recovered from patients from the ages of 31-60years with a 6(85.7%) than in the other age group where the ages of 21-30 years of age had a 1(14.2%) occurrence. In Nigeria β -lactam antibiotics are the most frequently prescribed antibiotics against the aerobic Gram negative bacilli infections and selective pressure exerted by the extensive use of the β -lactam drugs especially in treating some life threatening infections most likely result in strains developing ESBL enzymes.⁵ The prevalence of ESBL-producing *Escherichia coli* in our environment appears to be quite high. This could be as a result of the misuse and abuse of antibiotics in this country. The indiscriminate and widespread use of antibiotics, particularly the beta lactam agent which are easily bought over the counter without a prescription from a doctor, or most commonly used by private practicing doctors who shy away from laboratory diagnosis, prescribe this beta-lactam agent as a frontline of treatment. The rate of the antibiotics resistance on the rise can be due to the emergence of ESBL enzymes which comprises the efficacy of the extended spectrum Cephalosporins in our environment. One of the findings in this study is the detection of some of the ESBL producing isolates appearing susceptible in-vitro, to the expanded spectrum Cephalosporins. This data is similar to those of other studies, which have reported that some ESBL-producing clinical isolates test susceptible *in-vitro* by using CLSI guidelines.⁵ This survey confirms and extends the above cited reports, showing a marked association between ESBL-production and resistance to the quinolones and the amino glycosides. Multidrug resistance is a clue that an isolate may be an ESBL-producer. The ESBL-producers were multiply resistant to most of the drugs tested for while the non ESBL-producers were highly susceptible to most of these drugs. The presence of this ESBL enzymes in our environment today reflects the overuse of the newer expanded-spectrum Cephalosporins by the medical community at large and there is no doubt that the widespread dissemination of organism producing ESBL and other beta lactamases will severely limit the therapeutic options of physicians facing these organism.

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Conflict of interest

The author declares there is no conflict of interest.

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