



Antibiotic Resistance Profile and Molecular Characterization of *Escherichia coli* Extended-Spectrum Beta-Lactamase-Producing Isolated from Sylvanus Olympio Teaching Hospital in Lomé, Togo

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AYD and SA designed the study, authors KDM and SD performed the statistical analysis, authors KDM and MS wrote the protocol, MS and KDM wrote the first draft of the manuscript. Authors KDM, SDT, SD and AEA managed the biological analyses of the study. Authors KDM and MS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study is to describe the antibiotic profile and identify the molecular type of beta-lactamase-producing *Escherichia coli* isolated from clinical specimens at Sylvanus Olympio teaching hospital in Lomé. The study span from 1st March 2009 to 31st December 2010. We collected fifty-three isolates of *Escherichia coli*, identified by api20E®. Antibiotic susceptibility test was performed

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using the disk diffusion method in an agar plate and PCR for molecular characterization. During the two years surveillance periods, 1156 isolates of *Enterobacteriaceae* were collected from which, 300 (25.95%) were beta-lactamase producing bacteria. Amongst these 300 strains, 53 (17.67%) were *E. coli* ESBL-producer and they were all (100%) sensitive to imipenem; 33.96% for ceftazidime, 7.55% and 5.66% for ceftazidime and ceftriaxone, respectively, despite the production of beta-lactamase. The resistance to quinolones associated with resistance to beta-lactams exceeds 90% while moderate in aminoglycosides from 16.98% to 75%. Among 53 bacterial strains of *E. coli* producing beta-lactamase, 52/53 (98.11%) carried the *bla*TEM gene, 1/53 (1.89%) carry neither TEM gene nor SHV gene. Our findings suggest an emergence of multi-resistance *E. coli* ESBL-producer strains, probably clonal, in Togo. A better knowledge of the epidemiology of resistance will improve the therapeutic management of patients while reducing the prescription of large spectrum antibiotics.

Keywords: ESBL; *Escherichia coli*; beta-lactamase; TEM; SHV.

1. INTRODUCTION

The β -lactam are a family of antibiotics widely used in clinic against bacterial infections [1,2]. However, their intensive use is followed by the early onset of resistance. The first β -lactamases encoded by plasmids TEM-1/2, SHV-1 were initially described in the 1960s in *Escherichia coli* and *Klebsiella pneumoniae* and was then disseminated in other species (other *Enterobacteriaceae*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*) [3]. Before the emergence of these enzymes, new β -lactam stable (including cephalosporins with extended spectrum) have been developed in the years 70-80. Thus, the first β -lactamase able to hydrolyze cephalosporins expanded (SHV-2, a mutant of SHV-1) was described in 1983 in a strain of *Klebsiella pneumoniae* in Germany [4]. Because of their spectrum of activity expansion, these enzymes have been called "Extended-Spectrum-Beta-Lactamase" (ESBL). There are several groups of ESBLs. The largest groups are the mutants of TEM and SHV β -lactamases, with over 150 members. The second largest group of ESBLs is the CTX-M enzymes. Based on sequence homology, these are divided into five subgroups with 40 members. Most of these subgroups have evolved as a result of the chromosomal β -lactamase genes escaping from *Kluyvera spp.*, an enterobacterial genus of little clinical importance. Having migrated to mobile DNA, the CTX-M β -lactamases may evolve further with a rapid dissemination [5]. Data indicate that the infections caused by ESBL-producing organisms are an emerging problem in outpatient healthcare settings in various parts of the world, including Canada, France, Israel, Spain, Italy and UK [6]. The geographical distribution is not homogeneous, and prevalence varies considerably from one region to another. In Egypt in 2015, 65.09%

Enterobacteriaceae out of MDR strains have been isolated as ESBL-producers [7], 64.3% have been reported in Sierra Leone [8] while only 13.2% reported in Uganda [9] and 37.96% in Ghana [10]. In Togo, only a few studies have been conducted. There were no related data before 2010. In a recent research data published by Toudji et al., with a total of 309 ESBL-producing strains, *Escherichia coli* was more common (51,13%), followed by *Klebsiella spp.* (30, 10%), ($P < 0.01$). These bacteria were isolated from pus (47,90%) in majority and urine samples (40, 78%). [11]. Being plasmid and transposon mediated, these enzymes easily spread worldwide and can now be found in many other species of the family *Enterobacteriaceae*, [5]. The aim of this study was to characterize the antimicrobial susceptibility profile of *Escherichia coli* isolated from clinical specimens and to investigate genes associated using Polymerase Chain Reaction (PCR) molecular method.

2. MATERIALS AND METHODS

2.1 Study Design and Samples Collection

The *Enterobacteriaceae* strains were collected from Sylvanus Olympio Teaching Hospital (CHU Sylvanus Olympio) in Lomé from March 2009 to December 2010. They were isolated from various biological specimens including pus, urines, vaginal swabs, lumbar punctures and blood from in and outdoor patients.

2.2 Identification and Susceptibility Test

Biochemical identification test was performed using Api20E biochemical test kit (bioMérieux Marcy-l'Etoile-France). Susceptibility to antibiotics was determined by disk diffusion on Muller-Hinton agar plate (Sanofi Diagnostics Pasteur, Marnes la Coquettes, France) as recommended by the « Comité

de l'Antibiogramme de la Société Française de Microbiologie (http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM2_013vjuin.pdf) ».the ESBL pattern was scanned using a classic method based on detection of the synergy between a disc of amoxicillin + clavulanic acid and third generation cephalosporin discs: cefotaxime and ceftriaxone. The presence of *ESBL* is noted by the appearance of a "champagne Cork" picture after 18 to 24 hours of incubation at 37 ° C. twenty-one antibiotics were tested in this study: amoxicillin + acid clavulanic (AMC), Ceftriaxone (CRO) Cefotaxime (CTX), Cefoxitin (FOX), Ceftazidime (CAZ), ceftriaxone (CRO) Netilmicin (NET), tobramycin (TM) gentamicin (GM), Amikacin (AN), sulfamethoxazole-trimethoprim (SXT), acid Nalidixic (NA), norfloxacin (NOR), ciprofloxacin (CIP), Chloramphenicol (C), Pefloxacin (PEF), Tetracycline (TE), levofloxacin (LVX), kanamycin (K), Ofloxacin (OFX), Imipenem (IMP).

2.3 Molecular Characterization

The strains previously stored in glycerol at -70 ° C were regenerated on Luria-Bertani agar (LB). A colony was transferred into 5 mL of Luria-Bertani broth. After overnight incubation at 37 ° C, 1.5 mL of the bacterial suspension was centrifuged at 11,000 rpm for 10 minutes. The bacterial cells were washed twice with sterile distilled water. 500 µL was transferred to a new tube for nucleic acid extraction. This suspension is incubated for 10 minutes at 95°C to lyse the cells. The mixture was centrifuged for 10 minutes at 11000 rpm and the supernatant containing the total DNA was used for gene amplification.

The amplification reaction was performed in a thermocycler (MJ Research PTC 200, Peltier Thermal Cycler). The total reaction volume of 30 µL was composed of amplification buffer, 1.5 mM MgCl₂, 0.2 mM of di-deoxyribonucleotide, 0.5 µM of specific primers, 1.5U of Taq polymerase and 5µl of DNA suspension. The products of the gene amplification were analysed on a 1.5% agarose gel. The electrophoresis conditions were 100 volts, 48 mA for 1 hour. For *blaTEM* genes, the primers used are OT-1-F (5'-TTGGGTGCACGAGTGGGTTA-3') and OT-2-R (5'-TAATTGTTGCCGGGAAGCTA-3') it generates 465 bases pair fragment. During the amplification, the PCR preparations were denatured at 94°C for 5 minutes followed by 30 cycles each composed of denaturation at 94°C. for 30 seconds, hybridization at 52°C. for 30

seconds, Elongation at 72°C. for 60 seconds. The final elongation was carried out at 72°C. and lasted for 10 minutes for *blaSHV* genes, the primers used SHV-A(5'-CACTCAAGGATGTATTGTG-3') and VHS- B (5'-TTAGCGTTGCCAGTGCTCG-3') which generated a fragment length of 885 base pairs. The conditions of the amplification are as follows: denaturation of 15 seconds at 96°C. followed by 30 cycles including denaturation of 25 seconds at 96°C., hybridization for 15 seconds at 50°C. and elongation for 15 seconds at 72°C. The final elongation lasts 2 minutes at 72°C.

2.4 Analysis of the Data

The Epi Info version 7 software was used for the statistical analysis of the data. Chi-square values were calculated using the method of comparison of the proportions. A *p-value* <0.05 was considered as statistically significant.

3. RESULTS

3.1 Age and Sex

The age of patients ranged between 0 and 93 years with an average of 42,58 years. The modal class is comprised between 20 to 50 years and includes 20 patients (37,74%). Most of the patients are male 32/53 (60%) compared to 27% of the female with a sex ratio of 2.85.

3.2 Sample and Frequency of the ESBL

We collected 1156 isolates of Enterobacteriaceae in total from 2009 to 2010 using Api20e biochemical identification kit. A total 23.72% (139/586) and 28.24% (161/570) of Enterobacteriaceae ESBLs-producers were detected respectively in 2009 and 2010 with a non-significant difference. However, *E. coli* ESBL-producer's prevalence decreased from 23.74% to 12.42% with a statistically significant difference (*p-value*=0.046) (Table 1).

In average, 17.67% of *E. coli* ESBL-producers were detected out of the 300 Enterobacteriaceae ESBL-producer. Fifty-three strains were identified as *E. coli* ESBL-producer. the majority of *E. coli* was isolated from an outdoor patient (43.39%) follow by Intensive Care Unit patients (16.98%) and paediatric patients (11.32%). this included 23 (43.40%) isolated from pus samples, 20 (37,74%) from urines, 2 (3.77%) from blood samples, 2 (3.77%) from vaginal swabs, 1

(1.89%) from CSF and 5 (9.43%) from other types of specimens (Table 2).

3.3 Susceptibility Test Distribution

We observed a significant number of *E. coli* ESBL-producers resistant to antibiotics from Pus 23/53 (43.40%) and urine 20/53 (37.74%). Among beta-lactam antibiotics group, All the 53 isolates of *E. coli* ESBL-producer were sensitive to imipenem. However, high resistance was observed in the third generation Cephalosporine (100% to cefotaxime, 92.45% to ceftazidime and 94.34% to ceftriaxone). Among the Aminoglycosides, 76.25% were resistant to Gentamycin, 78.20% and 72.15% were respectively susceptible to Amikacin and Netilmicin. 52.20% were resistant to Colistin and 44.25% susceptible. *E. coli* ESBL-producer exhibited associated resistance to quinolones (95.83% for Nalidixic acid, 92.31% for ciprofloxacin, 90.20% for ofloxacin) as compared to aminoglycosides with a statistically significant difference ($Chi2 = 13.10$, $p\text{-value} = 0.0002$) (Fig. 1).

3.4 Gene Amplification

All the 53 isolates of *E. coli* were tested for *blaTEM* and *blaSHV* genes. From all the 53 strains of *E. coli* ESBL-producers, 52/53 (98.11%) were carriers of the gene *blaTEM*, none of them carries *blaSHV*. There was 1 out of the 53 (1.89%) carries neither the gene *blaTEM* nor *blaSHV* gene. No simultaneous genes association were detected among all the strains tested (Table 3).

4. DISCUSSION

Massive use and misuse of antibiotics in the hospital and farming determine a selection pressure enabling the emergence of resistant bacteria [6]. Resistance to antibiotics is a real public health concern worldwide. It has been shown that the production of β -lactamases is the most important mechanism of antibiotic resistance in Gram-negative bacteria. Our results showed a similar incidence of ESBL Enterobacteriaceae (25.95%) and *E. coli* ESBL-producer (17.67%) to those found in some African countries with high rates. In Tanzania, 28.70% of the total number of *E. coli* was reported [12], 14.3% in Cameroon in 2005 [13]. Neighbour countries have also registered higher rate especially in Benin, where the prevalence of *E. Coli* ESBL-producer was estimated to 22% in 2007 [14] and 35.5% in 2015

[15]. In Ghana, it was estimated at 37.96% in 2016 [10]. We have observed a lot more resistance of *E. coli* ESBL-producer isolated from pus and urines specimen, the majority were from outdoor patients, with statistical difference observed. This implies that multidrug resistance infections are a common community-acquired infection in our settings. High sensitivity to Imipenem seems encouraging than that found in Benin with 5% resistance [16]; [15] because imipenem is to date the only treatment alternative of infections caused by Enterobacteriaceae producer of beta-lactamase. The association of quinolones resistance to the production of beta-lactamase in Enterobacteriaceae has also been reported worldwide. It was demonstrated that the gene associated with resistance to quinolones, *qnrA*, *qnrB2*, *qnrB9*, *qnrB19* genes carried by a transferable plasmid is also the determinants of extended-spectrum beta-lactamase genes *blaTEM*, *blaSHV*, which leads to a simultaneous resistance to quinolones and the beta-lactams [17,4]. A high percentage of *E. coli* ESBL-producer resistant to chloramphenicol, sulfamethoxazole-trimethoprim and tetracycline may be due to their widely prescription, their availability and their affordable prices on the local market. Patients could get without a medical prescription, which could encourage the selection pressure. Gene amplification showed that 52 of the 53 (98.11%) strains of *E. coli* ESBL-producer carry gene *blaTEM*. The single remaining strain was isolated from urine specimen of a young woman of 29 years carry neither type *blaTEM* nor *blaSHV* genes. This suggests that this strain carries another gene coding for ESBL in Group A, probably *blaCTX-M* (not tested in this study), frequently encountered in hospitals in Africa. Unlike in Benin [15] and in Ghana [10], we have not detected simultaneous carriage of genes *blaTEM* and *blaSHV* within a single strain. However, a similar study conducted in Togo has shown the circulation of *blaCTX-M* in Togo within *K pneumoniae* and *K oxytoca*, but with lower prevalence [18]. Resistance profile and the ESBL genotypes found in *E. coli* in Togo showed that strains might belong to the same clone. The transmission could be done by multi-resistant clonal dissemination or transmission of a plasmid between several strains with clonal selection. This multi-resistance is related to the mode of circulation of the strain and transmission of plasmids between bacteria. It is possible to effectively control ESBL-producer emergence by accurate identification of pathogens and the resistance pattern, this will help the clinician to properly manage the patient.

Table 1. Distribution of enterobacteriaceae ESBL-producer from 2009 to 2010

Years	Enterobacteriaceae	Enterobacteria ESBL-Producer	<i>Escherichia coli</i> ESBL-producers
2009	586	139 (23.72%)	33 (23.74%)
2010	570	161 (28.24%)	20 (12.42%)
Total	1156	300 (25.95%)	53 (17.67%)

Table 2. Distribution of sources and department of isolation of *E. coli* ESBL-producer

Wards	Urine ^a	Pus ^b	Vaginal	Blood	CSF	Others	Total
Out patient	10	6	2	-	-	5	23 ^c (43.39%)
ICU	2	7	-	-	-	-	9 (16.98%)
Traumatology	-	4	-	-	-	-	4 (7.55%)
Gynecology	1	1	-	-	-	-	2 (3.77%)
MU	-	1	-	-	-	-	1 (1.89%)
Surgery	-	2	-	-	-	-	2 (3.77%)
Emergency	-	1	-	-	-	-	1 (1.89%)
Pediatrics.	2	1	-	2	1	-	6 (11.32%)
Neurology.	1	-	-	-	-	-	1 (1.89%)
Medicine	3	-	-	-	-	-	3 (5.66%)
Urology	1	-	-	-	-	-	1 (1.89%)
Total	20(37.74%)	23(43.40%)	2(3.77%)	2(3.77%)	1(1.89%)	5(9.43%)	53 (100%)

ICU: Intensive Care Unit, MU: Military Unit, CSF: Cerebrospinal fluids, (a) p-value= 0.0010, (b) p-value= 0.00031, (c) p-value=0.047

Table 3. Distribution of ESBL genotypes found in *E. coli*

Genotype size	TEM	SHV	TEM+SHV	Others	Total
	52(98.11%)	0	0	1 (1.89%)	53 (100%)



Fig. 1. Distribution of resistance profile of *E. coli* ESBL-producer

AMC: Amoxicillin and clavulanic acid, CRO: Ceftriaxone, CTX: Cefotaxime, FOX Cefoxitin CAZ: Ceftazidime, NET: Netilmicin, TM: Tobramycin, GM: Gentamicin, AN: Amikacin, SXT: sulfamethoxazole-trimethoprim, NA: Nalidixic acid, NOR: Norfloxacin, CIP: Ciprofloxacin, C: Chloramphenicol, PEF: Pefloxacin, TE: Tetracycline, LVX: Levofloxacin, K: Kanamycin, OFX: Ofloxacin

5. CONCLUSION

A better knowledge of the epidemiology of resistance will improve the therapeutic management of patients and reduce the unnecessarily prescription of broad-spectrum

antibiotics. This can be done only at the cost of better monitoring. Our study showed that ESBL-producer Enterobacteriaceae especially *E. coli* are emerging in Togo. This resistance profile is significantly associated with resistance to quinolones and majority of these *E. coli* ESBL-

producers carry genes *blaTEM*. They all exhibited full susceptibility to imipenem. This study will be carried on looking for other genotypes involved in the production of beta-lactams and expand to other ESBL producing Enterobacteriaceae capable of transmitting their plasmids to one another.

CONSENT

As per international standard or university standard written patient consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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