Short Research Article

Antibiotic resistance profile and molecular characterization of *Escherichia coli* Extended-spectrum beta-lactamase producing isolated from Sylvanus Olympio Teaching Hospital in Lomé, Togo

ABSTRACT

Aims: describe antibiotic profile and identify molecular type of beta-lactamase-producing Escherichia coli strains isolated from Sylvanus Olympio teaching hospital in Lom**é.**
Study design: it was a cross sectional study on *E. coli* bacterial strains stored at -80°C.
Place and Duration of Study: the study was undertaken at Sylvanus Olympio Teaching Hospital in Lomé from 1st March 2009 to 31th December 2010.
Methodology: we collected fifty-three strains of *Escherichia coli*, identified by api20E®. Antibiotic susceptibility test was performed using disk diffusion method in agar plate and PCR for molecular characterization.
Results: during 2 years of surveillance, 1156 *Enterobacteria were isolated*. Of them, 300 (25.95%) were beta-lactamase producing. Amongst these 300 strains, 53 (17.67%) were *E. coli* ESBL-producer and they were all (100%) sensitive to imipénème; 33.96% for cefoxitin, 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 *blaTEM* gene, 1/53 (1.89%) carry neither TEM gene nor SHV gene**.**
Conclusion: 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

Keyword s: 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 (enterobacteria, *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 to spectrum cephalosporins expanded (SHV-2, 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). Data indicate that the infection caused by an ESBL-producing organism is an emerging problem in outpatient settings in various parts of the

world, including Canada, France, Israel, Spain, Italy and UK[5]. The geographical distribution is not homogeneous and prevalence varies considerably from one region to another. In Egypt in 2015, 65.09% enterobacteria out of MDR strains have been isolated as ESBL-producers [6], 64.3% have been reported in Sierra Leone [7] while only 13.2% reported in Uganda [8] and 37.96% in Ghana [9]. The ESBL genes are coded by plasmids, which facilitates the exchange between different bacteria strains. The aim of this study is to characterised the antimicrobial susceptibility profile of *Escherichia coli* isolated from clinical specimen and to investigate genes associated by PCR

2. MATERIAL AND METHODS

2.1Study design and samples collection

Enterobacteria strains were retrospectively collected from Sylvanus Olympio Teaching Hospital (CHU Sylvanus Olympio) in Lomé from March 2009 to December 2010. They were isolated from various biological specimens as pus, urines, vaginal samples, 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/CASFM2013vjuin.pdf) ».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 (AM), 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

2.3.1 Extraction of DNA from Escherichia coli strains

Strains stored in glycerol at -70 ° C were regenerated on Luria-Bertani agar (LB). A colony is 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 were 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.

2.3.2 Gene amplification

The amplification reaction was performed in a thermocycler (MJ Research PTC 200, Peltier Thermal Cycler) type. The total reaction volume of 30 µL and 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. For gene amplification, the samples 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 generate a fragment 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 EPI7 software was used for the statistical analysis of the data. Chi2 values were calculated by the method of comparison of the proportions. A *p-value <0.05* was considered as statistical significant.

3. RESULTS

3.1 Age and sex

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

3.2 Sample and frequency of the ESBL

Total percentage of 23.72% and 28.24% of enterobacteria BLSEs-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).

Table1: Distribution of Enterobacteria *ESBL*-producer from 2009 to 2010

Years	Enterobacteria	Enterobacteria ESBL-Producer	*Escherichia coli* BLSE-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%)

In average, 17.67% are *E. coli ESBL*-producers were detected over the total number Enterobacteria ESBL-producer. Fifty-three strains were identified as E. coli ESBL-producer including 23 (43.40%) from pus samples, 20 (37,74%) from urines, 2 (3.77%) from blood samples, 2 (3.77%) from vaginal samples, 1 (1.89%) from CSF and 5 (9.43%) from other types of specimens. (Table 2)

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

3.3 Susceptibility test distribution

We observed a significant number of resistance to antibiotics in *E. coli* ESBL-producers isolated of Pus23/53 (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 (Figure1). *E. coli* ESBL-producer has 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-value = 0.0002*). (Figure 1)

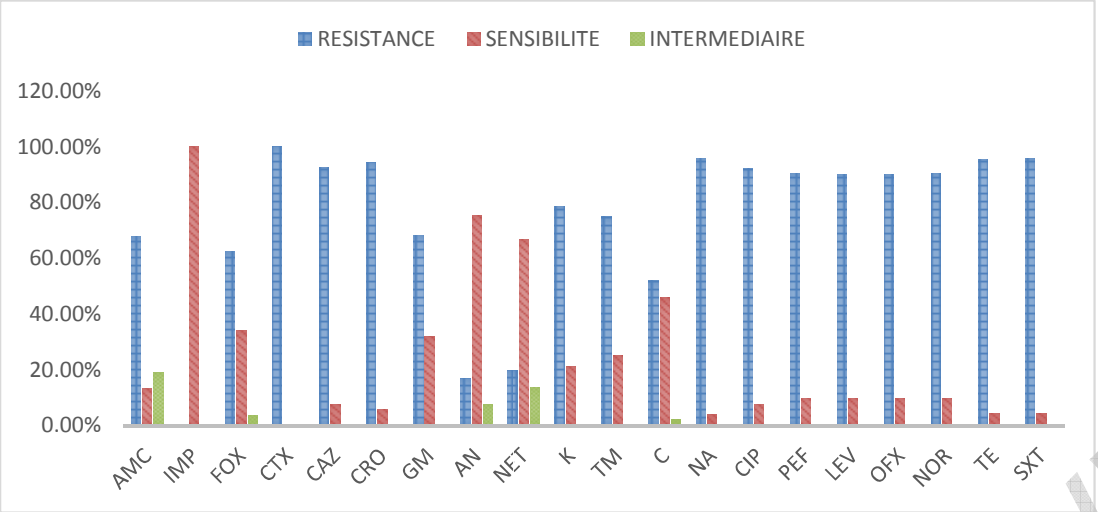

Figure 1: Distribution of resistance profile of *E. coli ESBL*-producer

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

3.4 Gene amplification

From the total of 53 strains of *E. coli* ESBL-producer, 52/53 (98.11%) are carriers of the gene *blaTEM*. none of them carries *blaSHV.* 1/53 (1.89%) carries neither the gene *blaTEM* nor *blaSHV* gene. No simultaneous genes association were detected within a single strain. (Table 3)

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

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

4. DISCUSSION

Massive use and misuse of antibiotics in the hospital and agriculture determine a selection pressure enabling the emergence of resistant bacteria [5]. 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 the similar incidence of ESBL enterobacteria (25.95%) and *E. coli* ESBL-producer (17.67%) to those found in some African countries with high rates. In Tanzania, 28.7% of the total number of *E. coli was* reported [10], 14.3% in Cameroon in 2005 [11]. Neighbour countries have also registered higher rate especially in Benin, where Abrams et al. have estimated the prevalence of *E. Coli* ESBL-producer to 22% in 2007 [12] and 35.5% in 2015 [13].In Ghana, it was estimated to 37.96% in 2016 [9]. 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 [12,13] because imipenem is to date the only treatment alternative of infections caused by Enterobacteriaceae producing of beta-lactamase. The association of quinolones resistance to the production of beta-lactamase in enterobacteria 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 [4,17]. 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 [13] and in Ghana [9], 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 *Klebsiella pneumoniae* and *Klebsiella 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 for proper management of the patient.

5.CONCLUSION

A better knowledge of the epidemiology of resistance will improve therapeutic management of patients and reduce the unnecessarily prescription of large spectrum antibiotics. This can be done only at the cost of a better monitoring. Our study showed that ESBL-producer enterobacteria 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 to look 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.

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