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Research Artcle

Prevalence of Extend Spectrum Beta Lactamases Producing Enterobacteriaceae and their Antibiotic Susceptibility in Lomé, Togo

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Abstract

Objective: The aim of this study was to determine the prevalence of *Enterobacteriaceae* producing Extended Spectrum Beta-Lactamases (ESBL) and to assess their antibiotic susceptibility.

Materials and Methods: The study included 1377 bacterial strains isolated at the University hospital Sylvanus Olympio and the Polyclinic Wossinu-Gbogbo both in Lomé between 2009 and 2011. The antibiotic susceptibility test was performed agar disk diffusion assay as recommended by the Antimicrobial susceptibility Committee of the French Society for Microbiology. The production of Extended Spectrum Beta-Lactamases was assayed by double-disk synergy technique.

Results: A total of 309 strains produced the ESBLs, a global prevalence of 22.44%. The strain distribution was 35.34% for *Enterobacter sp*., 27.84% *Klebsiella sp*., 23.76% for *E.coli*, 16.30% for *E.coli* Alkalescens- Dispar, 14.18% for *Citrobacter sp*. and 7.56% for *Proteus sp*. These strains were more frequently isolated from pus (47.90%) followed by urine (40.78%). The resistance rates observed with *E.coli* were respectively 0.68%, 1.38% and 4% for imipenem, colistin and cefoxitin. Regarding *Klebsiella sp*., these rates were 3.70%, 1.27% and 4%, respectively.

Conclusion: This study showed that the prevalence of ESBL has reached an alarming level in Lomé. *E. coli* and *Klebsiella sp*. are the most implicated microorganisms. Nationwide studies are planned to investigate the problem.

Keywords: Bacterial strains; Multidrug resistance; Extended Spectrum Beta-Lactamase; Antibiotics.

Introduction

The emergence and the dissemination of antibiotic resistance constitute a major public health problem [1,2,3]. This phenomenon results in an increasing morbidity and mortality from infectious diseases. One approach in the fight against antimicrobial resistance involves the prevalence studies of multi-resistant strains circulating in hospitals and their susceptibility to antibiotics. Thus the studies conducted around the world have mainly complained the Gram-positive cocci [4] and Gram negative bacteria, including *Enterobacteriaceae* [2,5]. The *Enterobacteriaceae* have been found to express a high acquired resistance to the main beta-lactam antibiotics through the production of enzymes called Extended spectrum Beta-Lactamases (ESBLs). These enzymes inactivate cephalosporins of the first, second and third generations [6,7]. The genes encoding for these enzymes are carried by plasmids and coexist with the genes for resistance to other antibiotics [7]. *Escherichia coli* and *Klebsiella pneumoniae* were found to be the most implicated bacteria in the expression of ESBLs [8,9]. Hence, from a study conducted in Cameroon, *Enterobacteriaceae* secreting

ESBLs were around 12%, of which 14.3% *E. coli* and 18.8% *K. pneumoniae* [10]. In Benin, in the department of Zou and Collines, 22% strains of *E. coli* were ESBL producing [11]. In Togo, some authors reported the resistance of bacterial strains to some antibiotics, but until now there is a lack of studies addressing exclusively the Extend Spectrum Beta-Lactamases producing bacteria [12,13]. This study therefore aimed to determine the prevalence of *Enterobacteriaceae* producing Extended Spectrum Beta-Lactamases in two health facilities in Lomé, and to test their susceptibility to several antibiotics.

Materials and methods

Isolation and identification of bacterial strains

The bacterial strains were isolated at the University Hospital Sylvanus Olympio and the Polyclinic Wossinu-Gbogbo in Lomé, following the methods in force in the two centers [14,20,21]. The samples were pathologic specimens routinely analyzed in the two centers. These included vaginal swabs, urines, pus and bloods received during the period time from January 2009 to December 2011. For the processing, vaginal swabs, urine and pus samples were directly seeded on the Eosin ethylene Bleu (EMB) agar. The blood samples were used to inoculate vials of blood culture hemoline (Bio Mérieux, France) for at least seven days to detect the bacterial growth. Afterwards, the Gram staining was performed on the subcultures prior to the identification with the API 20E System (Bio Mérieux, France) [15,16,17]. Duplicated strains were discarded.

Susceptibility testing and detection of beta lactamases production

The susceptibility to antibiotics was performed by agar disk diffusion and the results were interpreted following the recommendations of the Antimicrobial susceptibility Committee of the French Society for Microbiology (CA-SFM) [19]. The inhibition zone around each disk was measured and compared to diameters corresponding to inferior critical concentration and superior critical concentration. The categorization criteria are defined as sensitive (S), intermediate (I) and resistant (R) for each antibiotic used [14,19, 23]. However, all intermediate strains were considered as resistant in this study. A total of 24 antibiotics including amoxicillin(20µg), amoxicillin+clavulanic acid (20-10µg), carbenicillin (100µg), Imipenem (10µg), cephalothin (30µg), cefoxitin (30µg), cefotaxime (5µg), ceftazidime (10µg), ceftriaxone (30µg), gentamicin (10µg) amikacin (30µg), netilmicin (10µg), kanamycin (10µg), chloramphenicol (30µg), colistin(10µg), nalidixic acid (30µg), ciprofloxacin (5µg), pefloxacin (5µg), levofloxacin (5µg), ofloxacin(5µg), norfloxacin (10µg), doxycycline (15µg), tetracycline (15µg) and trimethoprim+sulphamethoxazole (1.25-23.75µg) were tested. The production of ESBL was assayed by the double disk synergy technique described by Jarlier et al. [18]. The Amoxicillin+clavulanic acid disk (AMC) was deposited between third generation cephalosporins (C3G) disks namely ceftazidime, cefotaxime or ceftriaxone at a distance of 2 to 3 cm on Muller Hinton agar plate. After 18 to 24 hours incubation, the production of ESBL was revealed by the appearance of a characteristically inhibition zone between the AMC disk and those of C3G referred to as a "champagne-cork".

Statistical analysis

The percentages of resistance were compared among species on the Epi-info software version 6 by Fisher's test with the statistical significance set at $P < 0.05$.

Results

Prevalence of ESBL producing strains of *Enterobacteriaceae*

A total of 1377 strains belonging to several genera and species were isolated in the present study. The main genera, contributing with more than 20 strains each, were *Escherichia*, *Klebsiella, Enterobacter*, *Proteus* and *Citrobacter*. The species from the following genera *Shigella*, *Salmonella*, *Morganella* and *Yersinia* contributed with less than 20 strains each, this was referred to as other *Enterobacteriaceae* in (Table 1). At the species level, *E. coli* and *K. pneumoniae* were the leading agents, accounting for 48.29% and 23.60% of the total isolates, respectively. *Enterobacter gergoviae* and *Proteus mirabilis* represented 8.13% and 6.32%, respectively. The other species contributed with less than 5% (Table 1). The ESBL production was not detected in some species

such as *Enterobacter cloacae*, *Citrobacter freundii* and in the genera of *Shigella*, *Salmonella*, *Yersinia* and *Morganella* referred to us other bacteria in (Table 1). The double disk synergy test showed that 309 of the 1377 strains produced the ESBL, a global rate of 22.44%. These ESBL producing strains were classified by order of importance as follows: *Enterobacter gergoviae* (36.61%), *K.pneumoniae* (28.31%), *E. coli* (23.73%), *E. coli* Alkalescens-Dispar (16.13%), *Citrobacter diversus* (15.00%), *Klebsiella oxytoca* (11.11%), *Proteus mirablis* (8.05%) and *Proteus vulgaris* (7.41%) (Table 1).

Species	Isolated strains N(%)	ESBL producing strains N(%)
Escherichia coli	665(48.29)	158 (23.76)
Escherichia coli A –D	31(2.25)	5 (16.13)
Klebsiella pneumoniae	325(23.60)	92 (28.31)
Klebsiella oxytoca	9(0.65)	1 (11.11)
Enterobacter gergoviae	112(8.13)	41 (36.61)
Enterobacter cloacae	4(0.29)	0(0.00)
Proteus mirabilis	87(6.32)	7 (8.05)
Proteus vulgaris	27(1.96)	2 (7.41)
Citrobacter diversus	20(1.45)	3 (15.00)
Citrobacter freundii	1(0.07)	0(0.00)
Other Enterobacteriaceae	96(6.97)	0 (0.00)

Table 1: Distribution of isolated ESLM producing Enterobacteriaceae.

Escherichai coli A-D for *Escherichia coli* Alkalescens-Dispar, The other *Enterobacteriaceae* for the species of the genera *Shigella, Salmonella, Yersinia and Morganella*

Main sources of isolation of ESBL producing strains

Four pathologic products namely the pus, urine, blood and the vaginal swabs were the source of isolation of these ESBL producing *Enterobacteriaceae*. In the ascending order of contribution, the pathologic products were ranked as follows: vaginal swabs 3.56%(11/309), blood 7.77%(24/309), urine 40.78%(126/309) and pus 47.90%(148/309) (Figure 1).

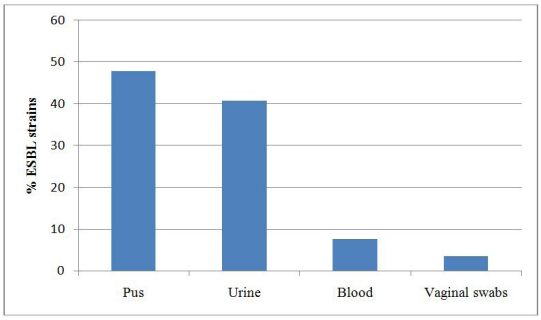

Figure 1: Distribution of ESBL strains among the analyzed samples.

The distribution of bacterial species in the treated samples is presented in (Figure 2).

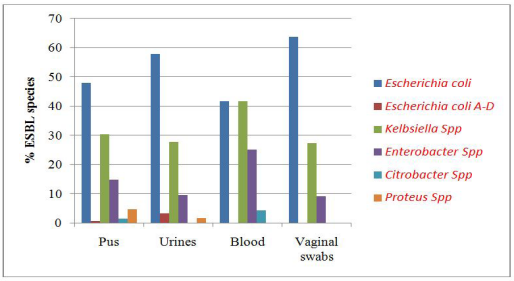

Figure 2: Distribution of bacterial strains among pathologic samples

Globally, *E. coli* was the most frequently isolated species, by occurring in all the analyzed samples at the following rates 63.64%, 57.94%, 47.97% and 41.67% from vaginal swabs, urine, pus and blood respectively. On the other hand, *E. coli* A-D was not found in blood and vaginal swabs. This bacterium was found to occur preferentially in urine samples. The species from the genera *Klebsiella* and *Enterobacter* were also found in all the analyzed samples. For these microorganisms, blood and pus were the main isolation sources. *Citrobacter* species were only found in blood and pus while *Proteus* species were found in pus and urine. Pus samples were the mains source of bacterial isolation by leading to the isolation of all incriminated microorganisms.

Antibiotic susceptibility of the main ESBL producing strains

(Table. 2) shows the resistance profile of the most represented *Enterobacteriaceae* namely *E. coli*, *Klebsiella sp*., *Enterobacter sp*., *Proteus sp*. and *E. coli* A-D. All strains were highly resistant to beta-lactams with a maximum rate of 100%. Imipenem, however completely inhibited all the strains of *Enterobacter sp*, *E. coli* A-D and *Proteus sp*., while the strains of *E. coli* and *Klebsiella sp*. showed a fair resistance with rates of 0.68% and 3.7% respectively. These resistance rate were found to be statistically different according to p-values <0.05 calculated by the Fisher's test. The *E. coli* A-D strains did not expressed resistance to aminoglycosides tested in this study i.e. amikacin and netilmicin. To these same antibiotics, *Proteus sp* showed respective resistance rates of 30% and 40%, in addition to totally resist to gentamicin and kanamycin (100%). In *E. coli, Klebsiella sp* and *Enterobacter sp*, the resistance seemed stratified. It was low against amikacin and high with gentamicin. *Enterobacter sp*. and *Proteus sp*. totally resisted to chloramphenicol, while colistin strongly inhibited all strains accepted *Proteus sp*. All the tested microorganisms highly resisted to cyclines and sulfonamides (resistance rates above 90%).

Antibiotic classes	Antibiotics	*E. coli* (*N*=158)	*Klebsiella spp* (*N*=81)	*E. spp* (*N*=41)	E. coli *A-D* (*N*=5)	Proteus Spp. (*N*=9)	p value
Beta-lactams	Amoxicillin	100	100	100	100	100	-
		87.76	100	100	100	100	<0.001
	Carbenicillin	99.2	100	100	100	100	<0.001
	Imipenem	0.68	3.7	0	0	0	0.016
	Cefalotin	100	98.77	100	100	100	0.4
	Cefotaxim	100	100	100	100	100	-
	Ceftazidim	97.28	100	100	100	100	0.028
	Ceftriazone	97.16	100	100	100	100	0.028
Aminosides	Gentamicin	82.2	94.64	96.3	-	100	<0.001
	Amikacin	13.43	4.23	2.86	0	30	<0.001
	Netilmicin	24.82	37.33	35.14	0	40	<0.001
	Kanamycin	88.89	69.23	80	-	100	<0.001
Phenicols	Chloramphenicol	55.56	56.86	100	33.33	100	<0.001
Polymyxins	Colistin	1.38	1.27	0	0	100	<0.001
Quinolone andFluoro-	Nalidixic acid	89.61	95.83	-	-	100	0.03
	Quinolones	85.11	78.95	63.89	100	70	<0.001
	Pefloxacin	86.86	78.95	65.71	100	70	<0.001
	Levoxacin	85.04	73.02	60	100	70	<0.001
	Ofloxacin	83.94	76.47	63.64	100	62.5	<0.001
	Norfloxacin	86.67	75	69.23	100	62.5	<0.001
Cyclines	Doxycycline	97.47	89.83	96.3	-	100	0.004
	Tetracycline	95.53	91.11	92.59	100	100	0.001
Sulfonamides and related	Trimethoprim+ sulfamethoxazole	98.15	98.31	94.44	100	100	0.015

Table 2: Percentage of resistant strains to several antibiotics.

Discussion

A fact of increasing concern in the recent decades in clinical bacteriology is the emergence of multidrug resistance among *Enterobacteriaceae* strains isolated from patients. Addressing this problem should include the detection of resistant phenotypes. This has been initiated in many ways throughout the world. However, in Togo, there is a lack of published studies concerning the antibiotic resistance pattern. For this reason, our aim in this study was to evaluate the prevalence of Extended Spectrum Beta-Lactamases producing *Enterobacteriaceae* (ESBLE) and their susceptibility to several antibiotics. The identified strains included *E. coli*, *K. pneumoniae*, *Enterobacter sp*, *Citrobacter diversus*, *C.freundii*, *P.mirabilis*, *P.vulgaris*, *K.oxytoca*, *E. coli* A-D and *Morganella morganii*. Our results indicated that the global prevalence of ESBL producing strains was 22.44%. This prevalence seemed to be very low according to the reported data from the studies conducted in some African countries such as Senegal (34%) [24] and Egypt (75.8%) [25]. On the other hand the current value is higher than the value recorded in Cameroon (12%) [10]. Analyzing the results by bacterial species, high prevalence was recorded for *Enterobacter sp*., followed by *Klebsiella sp.* and *E. coli*. Among ESBL-producing strains, the frequency of isolation of *Enterobacter sp*. was13.27% versus 51.13% for *E. coli* strains, and 30.10% for strains of *Klebsiella sp*. The two last microorganisms occupied 81.23% of total ESBL-producing strains. Several studies have also highlighted the increasing prevalence of these two strains [25-27]. The abscesses and urinary tract infections were the main sources of ESBLE; an opposite result was obtained in a study in Saudi Arabia in 2005 where the urine contained more ESBL than suppurations [28]. Our results also showed that the blood samples contained 7.77% of ESBLE, a value similar to those reported by Wani et al. [29]. This study also revealed an unexpected antibiotic resistance pattern of the isolated strains. Thus, despite the high resistance to beta-lactam antibiotics, there was resistance to other families of antibiotics like aminoglycosides, tetracyclins and quinolones. Beta-lactamases

produced by these strains allowed them to inactivate all penicillins at a rate between 87.76 and 100%. The results of this study susceptibility tests have confirmed this. All strains tested were sensitive to imipenem except the strains of *E.coli* and *K.pneumoniae* which displayed a very weak resistance to 0.68% and 3.6% respectively. Increasing resistance to this antibiotic expose the community to a lethal infection with ESBL-producing bacteria as carbapenems are the only subgroups of beta-lactams to be effective against these ESBLE producing strains. This observation was made on ESBL producing *E. coli* at Zou and Collines department in Benin (2007), where 5% resisted to imipenem. *E. coli* strains were susceptible at 83% to cefoxitin versus 81% for *K. pneumoniae* strains. This result is similar to the susceptibility studies of Spanu et al. in Italy in 2002, where approximately 80% of ESBL *Enterobacteriaceae* strains were susceptible to cefoxitin [30]. The molecule presents on the beta-lactam ring a methoxyl radical-0-CH3, that protects cefoxitin and imipenem against the action of the enzymes. According to Singleton et al., a molecule is protected from the action of enzymes by biochemical processes such as methylation which grafted a methyl on the beta-lactam ring. Hence, the beta-lactam ring was protected by methoxyl radicals who enabled these antibiotic molecules to escape from the action of beta-lactamases [31]. However, the low resistance observed is probably due to chromosomal over expression of cephalosporinase. This phenomenon is more observed in *Klebsiella* as outlined in the work of Stewards et al. [32]. ESBL producing *Enterobacteriaceae* resistance to aminoglycosides was lower than that observed with the beta-lactams. According to some authors, the misuse of antibiotics (in dosage and duration shortened treatment) results in the selection of resistant bacteria which have not been eliminated, to develop defense mechanisms of any kind including production enzymes [31]. These antibiotics are not often affordable in our regions like beta-lactams. Their toxicity is sometimes remarkable, and they are presented as injections thus limiting the self-medication. The strains of *E. coli* and *Klebsiella* species which do not produce beta-lactamases are naturally susceptible to aminoglycosides, quinolones. In this study, these strains exhibited a high resistance. For *E.coli* the resistance rates were 13.43% to 88.89% to aminoglycosides and 83.94% to 89.61% to quinolones. This quinolone resistance association was demonstrated in the work of Ahoyo et al. in Benin [11]. *Klebsiella sp* also displayed a resistance of 4.23% to 94.64% to aminoglycosides and 75% to 95.83% to quinolones. These observations support the work of Tolun et al. in 2004 [33]. According to some authors, the plasmid carrying the beta-lactam resistance gene also would bring the QnrAquinolone resistance

Conclusion

The results of this study have shown an effective presence of ESBL in Togo's health facilities. The main bacterial species involved are E. coli and Klesiella sp. These microorganisms have developed strong resistance to several antibiotics, thus complicating the empirical prescription for clinicians. Further studies are needed to determine at the molecular level the types of beta lactamases produced by these bacteria.

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