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Research Article

Extended-Spectrum Beta-Lactamase (ESBL) - Producing *Enterobacteriaceae* Isolated in Cotonou: Characteristics and Risk Factors

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Abstract

Background: Infections with Extended-Spectrum Beta Lactamases Producing-*Enterobacteriaceae* (ESBL-E) constitute a global public health problem particularly in sub-Saharan Africa.

Objective: To determine prevalence and resistance patterns of ESBL-E and to assess the risk factors associated with infections caused by these bacteria in Cotonou.

Methods: From February to August 2015, this cross-sectional study was conducted on patients from whom *Enterobacteriaceae* were isolated at the Microbiology Laboratory of the University Teaching Hospital in Cotonou. For each patient, demographical and clinical data were recorded and samples were processed using routine procedures. Isolates obtained were identified and antimicrobial susceptibility testing performed using standard laboratory methods.

Results: A total of 374 strains were collected among which, 210 (56.2%) were ESBL-E. A significant percentage of the isolated ESBL-E species were *Escherichia coli* (40.5%) while *Klebsiella pneumoniae* and *Enterobacter cloacae* accounted for 37.1% and 21.4% respectively. Most active antibiotics on ESBL-E were imipenem, amikacin and fosfomycin with susceptibility rates of 98.9%, 97.1% and 95.1% respectively. Antimicrobial use, current hospitalization in any unit and particularly being hospitalized in the newborn unit were more significantly associated with infections with ESBL-E than no previous history of antimicrobial use, not hospitalized in any unit and no admission in a newborn unit (Odd ratios; ORs = 2.15, 3.72 and 30.15 respectively).

Conclusion: The prevalence of ESBL-E was high in Cotonou and infections with ESBL-E was associated with antimicrobial use, current hospitalization and being a patient from the newborn unit.

Keywords: *ESBL; Risk Factors; Resistance; Cotonou*

Introduction

Extended-spectrum beta-lactamase (ESBL) was first reported in *Enterobacteriaceae* in Germany in 1983 but has rapidly spread to other gram-negative bacteria [1]. These enzymes confer bacterial resistance to various classes of antimicrobials including penicillins, first-, second-, and third-generation cephalosporins and aztreonam [2,3]. ESBL-encoding genes are generally located in plasmids that frequently bear resistance genes for additional antibiotics such as aminoglycosides and fluoroquinolones [4-6]. This therefore limits options for treating infections due to ESBL-producing bacteria.

Infections due to ESBL-producing *Enterobacteriaceae* (ESBL-E) represent a major public health problem in sub-Saharan African countries with prevalence rates as high as 62.3% in bacteremia in Mali and 64.3% in urinary tract infections in Sierra Leone [7,8]. In Benin, information on ESBL-E is scarce. Available data showed a prevalence of 22% in 2005 in the center of the country while a higher rate of 35.5% was documented eight years later in Cotonou, the biggest city in Benin, located at the southern part of the country [9,10]. In these two studies, it is noteworthy that the sample size was small and risk factors associated with ESBL-E infections were not assessed.

This present study was undertaken to determine the prevalence and resistance patterns of ESBL-E and assess risk factors associated with infections caused by these bacteria in Cotonou.

Materials and Methods

Setting: Benin is a country with a size of 114,763 square kilometers and an estimated population of 10.9 million [11]. Cotonou is the biggest city in the country with approximately 673,000 populations in 2013 [12]. The University Teaching Hospital Hubert Koutoukou Maga, located in Cotonou, is a 617-bed hospital and the biggest in the country.

Study design and patients: We conducted a cross-sectional study from February to August 2015. All patients including in-and outpatients, from whom *Enterobacteriaceae* were isolated at the Microbiology Laboratory were enrolled into the study. For each of them, demographical and clinical data were recorded.

Laboratory procedures: All samples were subjected to routine laboratory procedures and *Enterobacteriaceae* isolates were identified using API 20E (bioMérieux, France). Antibiotic susceptibility testing (AST) was performed following recommendations of The French Society of Microbiology while ESBL-E were identified using the double disc synergy test [13]. *Escherichia coli* ATCC 25922 was used for quality control and results were interpreted according to the criteria recommended by The French Society of Microbiology [13]. Intermediate susceptibility results were considered as resistant.

Data analysis: Comparison between ESBL-E and non- ESBL-producing-*Enterobacteriaceae* was done using the chi-squared test and Fisher's exact test where appropriate. Univariate and multivariate analysis were used to examine the associations of various risk factors with ESBL-E. Multivariate analysis using a stepwise method was performed including only variables with $p < 0.05$ in univariate analysis. All analyses were conducted using SPSS version 20.0 with significance set at 5% level. The odds ratio (OR) was calculated with a confidence interval (CI) of 95%.

Ethical considerations: The study was approved by the institutional review board.

Results

A total of 374 *Enterobacteriaceae* strains isolated from 351 patients were collected during the study period. They were made up of 201 (53.7%) from urines, 116 (31.0%) from blood, 41 (11.0%) from pus while 9 (2.4%) and 7 (1.9%) were from cerebro-spinal fluid and other specimens respectively. The majority, 188 (50.2%) were *Escherichia coli* while *Klebsiella pneumoniae* and *Enterobacter sp* accounted for 108 (28.8%) and 59 (15.8%) respectively. Other bacterial isolates were *Proteus mirabilis* 7 (1.9%), *Citrobacter freundii* 4 (1.1%), *Morganella morganii* and *Serratia* sp each 2 (0.5%) while each of *Citrobacter koseri*, *Providencia rettgeri*, *Providencia stuartii* and *Salmonella enterica* accounted for 1 (0.3%).

Of total isolates investigated, 210 (56.2%) were ESBL-E. They were *E. coli* 85 (40.5%), *K. pneumoniae,* 78 (37.1%) and *E. cloacae,* 45 (21.4%) while two (1.0%) were *C. freundii* and *Serratia* sp. As shown by figure 1, most active antibiotics on ESBL-E strains were imipenem, amikacin and fosfomycin with 99.0%, 97.1% and 95.2% susceptibility rates respectively.

Sex factor associated with infections with ESBL-E in univariate analysis (Table 1) was not significantly associated in multivariate analysis ($p = 0.197$) (Table 2). Antimicrobial use, current hospitalization in any unit and particularly being hospitalized in the newborn

unit were more significantly associated with infections with ESBL-E than no previous history of antimicrobial use, not hospitalized in any unit and no admission in a newborn unit (OR = 2.15, 95% CI = 1.05 - 4.40, OR = 3.72, 95% CI = 1.48 - 8.43 and OR = 30.15, 95% CI = 3.07 - 225.90 respectively).

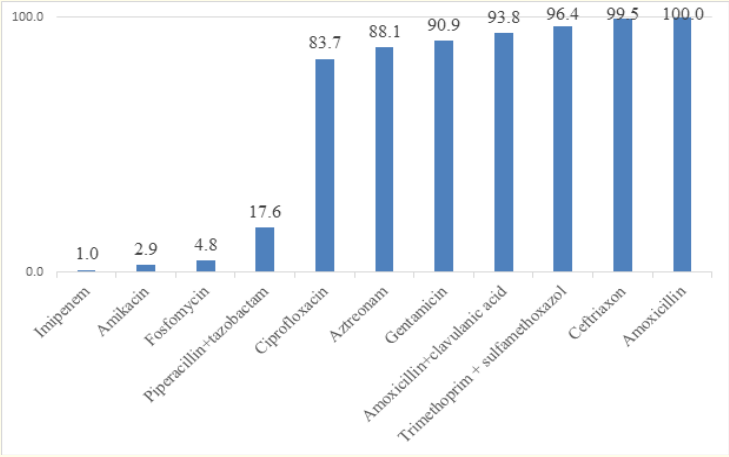

Figure 1: *Prevalence of resistance on ESBL-E strains.*

Variable		ESBL-E		
		No n (%)	**Yes n (%)**	**P**
Gender	Male	61 (34.1)	118 (65.9)	< 0.001
	Female	103 (52.8)	92 (47.2)	
Median age (years)		43.0	30.0	0.05
Current hospitalization	Yes	78 (31.6)	169 (68.4)	< 0.001
	No	86 (67.7)	41 (32.3)	
Hospitalization unit	Newborn	1 (1.4%)	56 (33.1%)	< 0.001
	Pediatric	3 (4.1%)	19 (11.2%)	
	Adult Medicine	30 (40.5%)	36 (21.3%)	
	Surgery	32 (43.2%)	41 (24.3%)	
	Admission and Intensive care	12 (16.2%)	17 (10.1%)	
Hospitalization history	Yes	45 (33.3)	90 (66.7)	< 0.001
	No	116 (59.2)	80 (40.8)	
Antimicrobial use	Yes	82 (42.0)	113 (58.0)	< 0.001
	No	73 (66.4)	37 (33.6)	

Table 1: *Univariate analysis of risk factors for ESBL-E.*

Variable	OR [95% CI]	p-value
Sex		
Male	1	
Female	0.62 [0.3 - 1.3]	0.197
Antimicrobial use		
No	1	0.035
Yes	2.15 [1.05 - 4.40]	
Current hospitalization		
No	1	0.002
Yes	3.72 [1.48 - 8.43]	
Hospitalization unit		
Surgery	1	
Newborn	30.15 [3.07 - 225.90]	< 0.001

Table 2: *Multivariate analysis of risk factors for ESBL-E.*

Discussion

Infections due to ESBL-E are increasingly a major public health concern worldwide. The situation may be worse in developing countries especially in sub-Saharan African countries where adequate public health measures are not in place. However, there is a large heterogeneity on the magnitude of the disease within regions, stressing the need to assess the actual burden in each setting.

In this study, the prevalence of ESBL-E was 56.2 %. This rate was higher than 22% reported by Ahoyo., *et al*. in 2005 in Benin and 35.5% documented by Anago., *et al.* some years later [9,10]. Although those two previous studies were carried out on a relatively small number of patients, our findings indicate a trend of prevalence increase from year to year in Benin. Unlike in developed countries, high prevalence rates of ESBL-E have been reported in Africa, Asia and South America [14-19]. This high prevalence rates may be ascribed to lack of measures to limit the development of antibiotic resistance. These measures are complex, multi sectorial, and involve collaborations with agriculture, health, trade and social sciences [20].

Concerning bacterial susceptibility to antibiotics, imipenem showed the least resistance rate of 1.0% (Figure 1). Indeed, carbapenems are either only recently available in Benin and/or too expensive to be widely used. However, resistance to carbapenems have already emerged since two (1.0%) out of the 210 ESBL-E strains were resistant to these drugs.

Findings from our study showed 83.7% resistance rate for fluoroquinolones. This group of antibiotics are available in oral form making them to be easily abused especially in environment like ours where antibiotics use is not regulated. For aminoglycosides, resistance rate observed depends on the drug tested. While the resistance rate to gentamicin was very high (90.9%), that of amikacin was 2.9%. The low resistance rate of amikacin may be explained by the fact that this drug is not readily available in Benin thus, risk of selecting strains resistant to it by its inappropriate use might be low. Nonetheless, uncontrolled widely introduction of active drugs in the country may lead to dramatic development of resistant pathogens.

Risk factors for ESBL-E infections found in this study include current hospitalization, antimicrobial use particularly beta-lactams, and being patients from the newborn unit. These findings were in keeping with what was previously documented [21,22]. It is well known that newborns are at risk of infections but the risk observed in our study is probably linked with nosocomial infection thus, stressing the need for strengthening infection control policies in our environment [23,24].

The main limitation of this study was that laboratory data were prospectively collected while clinical data were collected retrospectively therefore, patients eligible to laboratory tests who did not provide samples to the laboratory were not included in the study.

Conclusion

In conclusion, the prevalence of ESBL-E was high in both out- and in-patients in Cotonou and risk factors associated with infections to these bacteria were antimicrobial use, current hospitalization and being a patient at the newborn unit. Urgent implementation of antimicrobial stewardship program may limit development and spread of resistant pathogens in our community.

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