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Full Length Research Paper

Epidemiology and resistance patterns of ESBL-producing Enterobacteriaceae in a major city in Burkina Faso

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Increasing bacterial resistance to antimicrobial agents has become an issue of concern. A major problem of the treatment of infections caused by Enterobacteriaceae using antibiotics is the emergence of Extended-spectrum β-lactamases (ESBL)-producing Enterobacteriaceae. This study aims to determine the prevalence of ESBL-producing Enterobacteriaceae strains in Ouagadougou, Burkina Faso, and describe their resistance profile to other antibiotics commonly used in the infections treatment. 486 clinical strains of Enterobacteriaceae were obtained from patients attending three health centers in Ouagadougou (Burkina Faso) from November 2014 to October 2015. Biochemical identification was performed and antibiotics susceptibility test was performed using the disk diffusion method. Data was analyzed with the Excel and ANOVA one-way software GraphPad Prism version 5.01 software. Results revealed occurrence of *Escherichia coli* (60.9%, 194) predominated followed by *Klebsiella* spp. (22.4%, 109). Antibiotics susceptibility test revealed that 86.8% strains were resistant to amoxicillin, 81.3% to trimethoprim-sulfamethoxazole, 61.9% to ceftriaxone, 58.6% to cefotaxime and 58.4% to cefepime. It was observed that 99.8% were susceptible to imipenem while 16.6% were resistant to fosfomycin and 12.3% to amikacin. However, 38.5% (187/486) of the strains were ESBL-producing, 67.9% (127/187) of which came from Yalgado Ouedraogo University Hospital Center, 23.5% (44/187) from Charles De Gaulle Paediatric University Hospital Center and 8.6% (16/187) from Saint Camille Hospital. This study thus showed a high prevalence of Extended-Spectrum B-Lactamases producing Enterobacteriaceae strains in Ouagadougou (38.5%). It underlined the need for routine detection and systematic reporting of ESBL strains in different health facilities in Burkina Faso, so that measures could be taken to prevent their spread and treatment failures.

Key words: Enterobacteriaceae, Extended-Spectrum Beta-lactamases (ESBL), Burkina Faso.

INTRODUCTION

Bacterial resistance to antibiotics is on the raise worldwide in healthcare setting and in community which tend to be posing a lot of challenges to the effective treatment of infections. Resistance of pathogenic bacteria to β-lactam antibiotics, a group of antibiotic mostly used for the treatment of bacterial infections because of their broad antibacterial spectrum and excellent safety profile has taken a great threatening dimension with the emergence Extended-Spectrum B-Lactamase (ESBL) producing *Enterobacteriaceae* (Abdallah et al., 2015). The ESBLs first described in 1983 in Germany arose from a single nucleotide polymorphism in the bla_{SHV} genes that altered specificity to oxyimino-cephalosporins. Overtime there has been a wide spread of ESBLs with an ever evolving ability to hydrolyze penicillins, first, second and third generation cephalosporins and monobactams but not carbapenems (Lukac et al., 2015; Tekiner and Ozpinar, 2016). In Africa, there has been various reports of ESBL producing *Enterobacteriaceae* (ESBL-E) implicated in causing infections across all ages. Sangare et al., (2017) noted the very high and increasing frequency of ESBL-E in their report on the prevalence of ESBL-E in teaching hospitals in Mali. In a similar study Oduro-Mensah et al., (2016) reported an overall 37.96% of 137 *Enterobacteriaceae* clinical isolates exhibiting ESBL phenotype in Ghana with *Klebsiella* spp. and *Escherichia* coli taking a lead. To further substantiate this, Farra et al. (2016) identified the high rate of faecal carriage of ESBL-E in healthy children in Bangui Central African Republic which portend a high risk of continuous dissemination of multi-drug resistant pathogen with grave consequences to the general health of the public. However there is paucity of information and extended study of ESBL producing *Enterobacteriaceae* (ESBL-E) in Ouagadougou of which study conducted has been restricted to single health centers (Zeba et al., 2007; Métuor-Dabiré et al., 2014; Ouedraogo et al., 2016). Hence this study aimed to determine the prevalence of ESBL producing *Enterobacteriaceae* in three of the major health centers (Yalgado Ouedraogo Teaching Hospital (CHU-YO), Charles De Gaulle Paediatric Teaching Hospital (CHUP-CDG) and Saint Camille Hospital (HOSCO)) in Ouagadougou and to describe their resistance to antibiotics commonly used in the treatment of Gram negative bacterial infections.

MATERIALS AND METHODS

Study site

This cross sectional study was conducted between November 2014 to October 2016 to determine the prevalence and susceptibility of *Enterobacteriaceae* to β-lactams, aminoglycosides and quinolones in Ouagadougou. Three major health centers Yalgado Ouedraogo Teaching Hospital (CHU-YO), Charles De Gaulle Paediatric Teaching Hospital (CHUP-CDG) and Saint Camille Hospital (HOSCO) in Ouagadougou were chosen for the study because they received the highest number of patients and cases in the city. Ouagadougou is the capital city of Burkina Faso with a population of about 2 million people. CHU-YO is the largest medical institution located in Ouagadougou. Over 150,000 patients are annually attended to in these three health care facilities.

Sample collection, isolation and identification of bacteria

Four hundred and eighty-six samples were collected from 486 patients (Male: 246; Female: 240; Children < 15 years: 121) from the three study locations in the following order CHU-YO 312 patients, CHUP-CDG 94 patients and HOSCO 80 patients. Samples collected included 325 urine samples, 109 pus samples, 8 blood samples, 17 stool samples, 17 vaginal swab samples and 10 pleural fluid samples. Urine and stool samples were collected in sterile universal bottles, pus, pleural fluid and vaginal swab samples were collected with sterile swab sticks and blood samples were collected in EDTA bottles. Samples were immediately transported to the laboratory in a thermo-box container at 4°C after collection for processing. All samples were cultured on Eosin Methylene Blue (EMB) and Cystine Lactose Electrolyte Deficient (CLED) agar using standard microbiological procedure and incubated at 37°C for 24 h. Presumptive colonies were subcultured on nutrient agar to obtain pure colonies. Isolates were identified using Gram staining, biochemical testing and the API 20 E gallery (BioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing

Susceptibility to antimicrobial agents was determined by the Kirby Bauer disc diffusion method on Muller-Hinton agar as described by the Clinical and Laboratory Standard Institute (CLSI, 2005). The antibiotic discs were obtained from BioMérieux, France (BioMérieux, Marcy l'Etoile, France). Antibiotics used were: gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg), amoxicillin (20 µg), amoxicillin + clavulanic acid (20 + 10 µg), cefepime (30 µg), cefotaxime (5 µg), ceftriaxone (30 µg), cefoxitin (30 µg), imipenem (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25 - 23.75 µg) and fosfomycin (200 µg). *E. coli* ATCC 25922 was used as control for susceptibility testing.

Detection of ESBL strains

The screening and phenotypic tests for ESBL strains were performed in line with CLSI guidelines on Muller-Hinton agar. In this test, a disc of amoxycillin+clavulanic acid (20 + 10 µg) was placed at the centre of the Petri dish already inoculated with the test strain while cefepime (30 µg), cefotaxime (5 µg) and ceftriaxone (30 µg) discs were placed at a distance of 20 - 25 mm (centre to centre) from the amoxycillin+ clavulanic acid disc on the same dish. Zones

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Table 1. Distribution of strains according their origin and the clinical samples.

Strain	Health Center n(%)				Clinical Samples n(%)						
	CHU-YO	CHUP-CDG	HOSCO	Total	Urine	Pus	Blood	VS	stools	PF	Total
E. coli	194	53	49	296	211	57	1	13	5	9	296 (60.9)
Enterobacter spp.	14	03	7	24	19	3	2	0	0	0	24 (4.9)
Citrobacter spp.	6	0	0	6	5	1	0	0	0	0	6 (1.2)
Klebsiella spp.	63	32	14	109	75	26	5	3	0	0	109 (22.5)
Proteus spp.	21	0	8	29	11	17	0	1	0	0	29 (6)
Providencia sp.	1	0	0	1	1	0	0	0	0	0	1 (0.2)
Salmonella spp.	8	5	1	14	0	4	0	0	9	1	14 (2.9)
Serratia spp.	1	1	1	03	3	0	0	0	0	0	3 (0.6)
Shigella boydii	1	0	0	01	0	0	0	0	1	0	1 (0,2)
Shigella flexxneri	3	0	0	03	0	0	0	0	3	0	3 (0,6)
Total	**312 (64.2)**	**94 (19.3)**	**80(16.5)**	486 (100)	**325(66.9)**	**108 (22.2)**	**08 (1.6)**	**17(3.5)**	**18 (3.7)**	**10 (2.1)**	486 (100)

VS = Vaginal swab, PF = Pleural fluid.

of inhibition between the third generation cephalosporin discs and amoxicillin+clavulanic acid were observed after 18-24 h incubation at 37°C. Extension of inhibition zone around one or more cephalosporin discs nearest to the amoxycillin+clavulanic acid, was considered ESBL positive (CLSI, 2005).

Statistical analysis

Data was analyzed using ANOVA one-way. Chi-square test ($\chi 2$) was used to establish statistically difference in proportions for categorical data and statistical significance was set as P values of < 0.05. The statistical analysis was performed using GraphPad Prism version 5.01 (GraphPad Software, Inc.).

RESULTS

Distribution of strains according to origin and clinical samples

Four hundred and eighty-six isolates were obtained from all three collection centers. Three hundred and twelve (64.2%) was isolated from CHU-YO, 94 (19.3%) from CHUP-CDG and 80 (16.5%) from HOSCO. Urine yielded 325 (66.9%) of Enterobacteriaceae isolates making it the highest while 108 (22.2%) was isolated from pus. Two bacterial species were predominant in the three collection sites. *E. coli* had an occurrence of 296 (60.9%) and *Klebsiella* spp. had an occurrence of 109 (22.5%) as shown in Table 1.

Antibiotic susceptibility testing

Isolates displayed a resistance rate of 86.8% to amoxicillin and 35.2% to amoxicillin + calvulanic acid. Resistance rate to ceftriaxone and cefotaxime were 61.9% (301) and 58.6 (285), respectively. Furthermore, it was revealed that 0.2% (1) of the isolate was resistant to imipenem. Resistance rate of isolates to aminoglycosides was 12.3% (60) to amikacin and 51.0% (248) to gentamicin. Quinolones resistance rate was 68.3% (332) to nalidixic acid and 61.1% (297) to ciprofloxacin.

ESBL-producing strains

Out of 486 isolates tested, 187 (38.5%) were ESBL-producing, 127 (67.9%) from CHU-YO, 44 (23.5%) from CHUP-CDG and 16 (8.6) from HOSCO (Table 3). Plate 1 shows double disc synergy and a key hole phenomenon that was exhibited by *Klebsiella pneumonia*, and Table 4 shows the ESBL species distribution according to the sample. Difference between the proportions of ESBL isolates from the 3 sites was statistically significant ($p < 0.0001$). Furthermore, the difference between the ESBL bacteria isolated was not statistically significant ($p=0.1260$) with respect to age. In addition, 81 (43.3%) ESBL-E isolates were obtained from patients on antibiotic treatment, of which 16.7% (31/187) of antibiotics used were β-lactams.

Resistance profile of ESBL isolates to other antibiotics

The rate of resistance of ESBL isolates to other antibiotics is shown in Table 5. Resistance rates to tobramycin, nalidixic acid and ciprofloxacin in this study was 81.3% (152), 89.8% (168) and 83.4% (154), respectively. The susceptibility test of ESBL-E to aminoglycosides resulted in 3 antibiotypic profiles: the wild-type susceptible to all aminoglycosides 28 (14.9%), those that had cross-resistance to kanamycin, tobramycin gentamycin 128 (68.4%) and those that were resistance to all amino-glycosides 15 (8.0%).

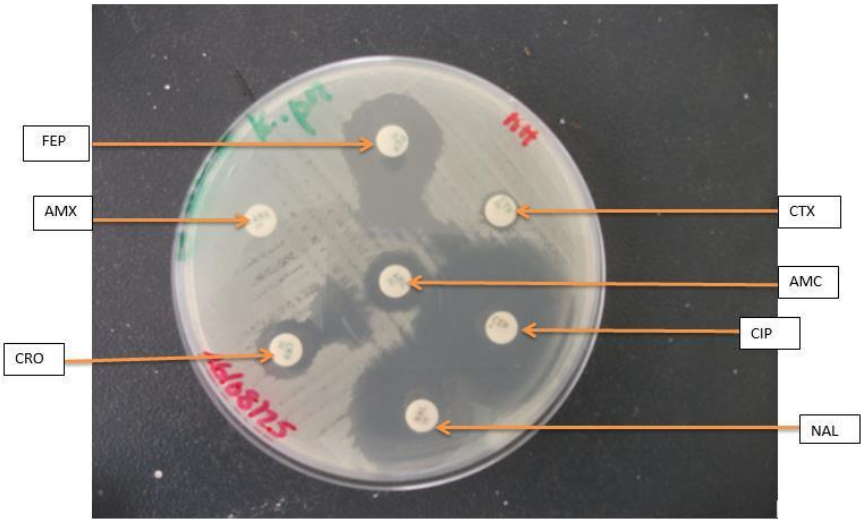

Plate 1. Representative image (*Klebsiella* spp.) of Double Disc synergy Test (DDS). Synergy between cefotaxime (CTX), ceftriaxone (CRO), cefepime (FEP) and amoxicillin + clavulanic acid (AMC) disc in center showing the keyhole phenomenon.

DISCUSSION

In this study, we determined the prevalence of ESBL producing Enterobacteriaceae (ESBL-E) and their resistance to antibiotics commonly used in the treatment of Gram negative bacterial infections in three major health care facilities in Ouagadougou. Enterobacteriaceae remains the major pathogens causing community-acquired and hospital-acquired infections including infections of the gastrointestinal tracts, urinary tract, sepsis, meningitis and medical device-associated infections (Mathlouthi et al., 2016). Urine, of all 6 clinical sample types analyzed gave the highest number of Enterobecteriaceae of which 50.1% were from Males. One hundred and eighty-seven (38.5%) of the 486 isolates obtained were ESBL producing with 21.0% from male. This is in line with the reports of Siraj et al. (2014) in Ethiopia and Ouedraogo et al. (2016) in Burkina Faso in which urine yielded a higher number of bacterial isolates. Hijazi et al. (2016) also reported a similar finding in Lebanon with male children having a higher colonization frequency (33.9%) of ESBL-E in contrast to their female counterparts that had a frequency of (15.9%). However this observation is a deviation from the normal trend of having more bacterial isolates from female urine samples since they were more at risk of acquiring urinary tract infection compared to their male counterparts (Ameri et al., 2014). *E. coli* and *Klebsiella* species has been identified as members of Entero-bacteriaceae that paly a lead role in hospital/community acquired infections, which is not different from our findings with *E. coli* and *Klebsiella* spp. being the most prevalent. *E. coli* had an occurrence frequency of 296 (60.9%) while 109 (22.5%) *Klebsiella* spp. were recorded. In a related study, Manjula et al. (2013) reported a high prevalence of *E. coli* (56.79%) and *Klebsiella* spp. (19.9%) isolated from patients in Karnataka region India having urinary tract infection. Similarly, in Dakar Senegal, *Klebsiella* spp. was reported to be the major ESBL-E isolated from patients (Ndir et al., 2016). Antibiotic resistance is a global problem which varies across countries as a result of hygiene levels in hospital and antibiotic management policies. As shown in this study, resistance of Enterobacteriaceae to regularly used antibiotics is unflinching and ever evolving. There was 86.8% resistance to amoxicillin, 35.2% resistance to amoxicillin + clavulanic acid. This rate of resistance could be attributed to selective pressure since these antibiotics has overtime been a first line drug in the treatment of bacterial infections. Isolates also displayed a remarkable resistance to cephalosporins tested. There was an average resistance of 58.5% to cefotaxime and cefepime, while resistance to ceftriaxone and cefoxitine was 61.9 and 26.1% respectively (Table 2). Mathlouthi et al. (2016) affirm this finding in their report of isolates from Tunisian and Libyan hospitals with 80% resistance to ceftazidime, cefotaxime, amoxicillin + clavulanic acid, amoxicillin and ciprofloxacin. Our report of 38.5% prevalence of ESBL-E in this study is relatively high; however a similar study by Ouedraogo et al. (2016) in Burkina Faso recorded a higher prevalence of 58%. This variation in findings could be explained by the size, duration and area where the two studies were conducted. The clinical impact of ESBL-producing pathogens on morbidity and mortality in infectious diseases in both children and adults as well as their economic burden are well documented (Lukac et al., 2015). Thus, ESBL-E is a threat that should be tackled head on. Resistance of ESBL-E to aminoglycoside was

Table 2. Susceptibility rate of 486 strains of *Enterobacteriaceae* to antibiotics in Ouagadougou.

Antibiotic	Susceptibility rate	
	S(%)	I +R (%)
Gentamicin	238 (49.0)	248 (51.0)
Amikacin	426 (87.7)	60 (12.3)
Tobramicin	221 (45.5)	265 (54.5)
Amoxicillin	64 (13.2)	422 (86.8)
Amoxicillin/ clavulanic acid	315 (64.8)	171 (35.2)
Cefoxitine	359 (73.9)	127 (26.1)
Ceftriaxone	185 (38.1)	301 (61.9)
Cefotaxime	201 (41.4)	285 (58.6)
Cefepime	202 (41.6)	284 (58.4)
Imipenem	485 (99.8)	1 (0.2)
Nalidixic acid	154 (31.7)	332 (68.3)
Ciprofloxacin	189 (38.9)	297 (61.1)
Triméthoprim-sulfaméthoxazole	91 (18.7)	395 (81.3)
Fosfomycin	406 (83.5)	80 (16.5)

S = susceptible, R = resistant, I = Intermediate.

Table 3. Distribution of clinical isolates according the collection site, sex, age and the clinical samples.

Variable	Collection site						Total	
	CHU-YO (N=312)		CHUP-CDG (N=94)		HOSCO (N=80)		(N=486)	
	E-ESBL (N)	Not E-ESBL (n)	E-ESBL (n)	Not E-ESBL (n)	E-ESBL (n)	Not E-ESBL (n)	E-ESBL [n(%)]	Not E-ESBL [n(%)]
Sex								
F n(%)	59	86	23	26	3	43	85 (17.5)	155 (31.9)
M n(%)	68	99	21	24	13	21	102 (21.0)	144 (29.6)
Total n(%)	127	185	44	50	16	64	187 (38.5)	299 (61.5)
Age (year)								
[0-15]	10	16	41	45	3	6	54 (11.1)	67 (13.8)
[15-30]	27	38	2	1	2	18	31 (6.4)	57 (11.7)
[30-45]	33	52	0	3	8	17	41 (8.4)	72 (14.8)
[45-60]	28	29	0	1	0	9	28 (5.8)	39 (8.0)
>60	29	50	1	0	3	14	33 (6.8)	64 (13.2)
Total	127	185	44	50	16	64	187 (38.5)	299 (61.5)
Pathological products								
Urines	80	124	23	32	13	54	116 (23.9)	210 (43.2)
Pus	34	44	17	12	0	2	51 (10.5)	58 (11.9)
VS	3	7	0	0	0	6	3 (0.6)	13 (2.7)
Stools	4	5	0	3	3	2	7 (1.4)	10 (2.1)
Blood	1	0	4	3	0	0	5 (1.0)	3 (0.6)
Other	5	5	0	0	0	0	5 (1.0)	5 (1.0)
Total	127	185	44	50	16	64	187 (38.5)	299 (61.5)

F = female M = Male; VS = Vaginal Swab; Other = Pleural fluid; E-ESBL = *Enterobacteriaceae*-producing Extended Spectrum B-lactamases.

observed. There was 71.7% resistance to gentamycin and 81.3% to tobramycin. This observation is in consonant with the report of Obeng-Nkrumah et al., (2013) that reported 91.2% of ESBL-E resistance to gentamycin in Ghana. The resistance of ESBL-E isolates to quinolones was 89.8% for nalidixic acid and 83.4% for

Table 4. Distribution of BLSE-producing strains according to the pathological products in Ouagadougou.

Strains	Clinical Samples [n (%)]						Total
	Urines	Pus	Blood	VS	Stools	PF	
E. coli	73	32	1	3	3	5	117 (62.6)
Klebsiella spp.	32	10	5	1	0	0	48 (25.7)
Proteus spp.	2	3	0	0	0	0	5 (2.7)
Enterobacter spp.	4	2	2	0	0	0	8 (4,3)
Citrobacter spp.	2	0	0	0	0	0	2 (1.1)
Salmonella sp.	1	1	0	0	3	0	5 (2.7)
Serratia spp.	1	0	0	0	0	0	1 (0.5)
Shigella flexneri	0	0	0	0	1	0	1 (0.5)
Total	115(61.5)	48(25.7)	8(4.3)	4(2.1)	7(3.7)	5 (2.7)	187 (100.0)

VS = Vaginal swab; PF = Pleural fluid.

Table 5. Susceptibility rate of 187 ESBL producing strains to antibiotics in Ouagadougou.

Antibiotics	Susceptibility rate		
	I (N, %)	R(%)	I+R(%)
Gentamicin	7(3.7)	127(67.9)	134(71.7)
Amikacin	2(1.1)	12(6.4)	14(7.5)
Tobramicin	4(2.1)	148(79.1)	152(81.3)
Amoxicillin	0	182(97.1)	182(97.1)
Amoxicillin/ clavulanic acid	7(3.7)	60(32.1)	67 (35.8)
Cefoxitine	1(0.5)	42(22.4)	43 (23.0)
Ceftriaxone	2(1.1)	177(94.6)	179(95.7)
Cefotaxime	2(1.1)	179(95.7)	181(96.8)
Cefepime	7(3.7)	166(88.8)	173(92.5)
Imipenem	0	0	0
Nalidixic acid	9(4.8)	159(85.0)	168(89.8)
Ciprofloxacin	10(5.3)	146(78.1)	156(83.4)
Triméthoprim-sulfaméthoxazole	0	163(87.2)	163(87.2)
Fosfomycin	5(2.7)	19(10.2)	24 (12.8)

S = susceptible, R = resistant, I = Intermediate.

ciprofloxacin. For other antibiotics, we observed a high rate of resistance to trimethoprim-sulfamethoxazole (87.2%). Both ESBL-E and non ESBL-E isolates were susceptible to imipenem and fosfomycin. Bourjilat et al. (2011) observed a similar trend in Morocco with all ESBL-producing isolates having total susceptibility to imipenem and fosfomycin. Also, Patwardhan and Singh (2017) in India reported 1,223 (96.5%) ESBL-producing Gram negative isolates that were susceptible to fosfomycin. This gives a glimpse of hope as this two antibiotics are still very much active against ESBL-E and can serve as a ready remedy when clinicians are confronted with multi-drug resistant ESBL-E. Conversely there should be caution in the use of these molecules as resistance to imipenem is beginning to emerge (Haidar et al., 2017). In Burkina Faso, as in many other African countries, the lack of antibiotic surveillance system, unfavorable hygiene conditions in hospitals, may be attributed to the spread of ESBL, as has been reflected in this study.

Conclusion

This study demonstrates the high prevalence of ESBL-producing Enterobacteriaceae in Ouagadougou. The spread of ESBL strains reduces the successful treatment ESBL bacterial infections. Nevertheless, ESBL bacteria remained susceptible to imipenem and fosfomycin, which are often drugs of choice for severe infections. This therefore highlights the need for routine detection and systematic reporting of ESBL bacteria in Burkina Faso to avoid therapeutic failures and the spread of these bacteria for effective management of bacterial infectious diseases. Clinicians must be cautious in the prescription

of antibiotics. Furthermore, antibiotic policy use is needed to limit the emergence and spread of ESBL strains.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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