



## Original Article

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## Faecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in healthy volunteers and hospitalized patients in Ouagadougou, Burkina Faso: prevalence, resistance profile, and associated risk factors

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### Abstract:

**Background:** Extended spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) are a serious challenge to patients' treatment. The aim of this study is to determine the prevalence of ESBL-PE, investigate the associated resistance, and analyze the associated risk factors for acquisition of ESBL-PE.

**Methodology:** A cross-sectional study was conducted on healthy volunteers and inpatients. After obtaining informed consent, rectal swabs were collected from each participant for isolation of Enterobacteriaceae on Hektoen enteric agar containing 4µg/L cefotaxime. The Enterobacteriaceae isolates were identified using biochemical tests and ESBL production was confirmed by the double-disc synergy test of amoxicillin and clavulanic acid. Antibiotic susceptibility test of each isolate was done by the disc diffusion method and interpreted using the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints version 5.0.

**Results:** During the study period, prevalence of faecal ESBL-PE among the study participants was 54.5% (103/189); 53.5% among healthy volunteers and 55.7% among inpatients ( $p=0.87$ ). The major ESBL-PE isolates was *Escherichia coli* (71%) followed by *Klebsiella pneumoniae* (16%). The isolates in hospitalized patients were resistant to norfloxacin (84.2%), cotrimoxazole (89.5%), and gentamicin (7.0%). The isolates from healthy volunteers were resistant to norfloxacin (86.2%), cotrimoxazole (82.8%), and gentamicin (1.7%). Gender, age, and previous antibiotic use were not significantly associated with carriage of ESBL-PE ( $p=0.51$ ).

**Conclusion:** The high prevalence of ESBL-PE in this study is worrying. There is an urgent need to develop measures to monitor and limit the spread of these multidrug-resistant organisms in healthcare facilities and the community in Burkina Faso.

**Keywords:** faecal carriage, ESBL-PE, healthy volunteers, inpatients, Burkina Faso

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## Portage fécal d'entérobactéries productrices de bêta-lactamases à spectre étendu chez des volontaires sains et des patients hospitalisés à Ouagadougou, Burkina Faso: prévalence, profil de résistance et facteurs de risque associés

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## Résumé:

**Contexte:** Les entérobactéries productrices de bêta-lactamases à spectre étendu (EP-BLSE) représentent un défi majeur pour le traitement des patients. Le but de cette étude est de déterminer la prévalence de EP-BLSE, d'étudier la résistance associée et d'analyser les facteurs de risque associés pour l'acquisition de EP-BLSE.

**Méthodologie:** Une étude transversale a été menée sur des volontaires sains et des patients hospitalisés. Après avoir obtenu le consentement éclairé, des écouvillons rectaux ont été prélevés sur chaque participant pour isoler les entérobactéries sur gélose entérique Hektoen contenant 4µg/L de céfotaxime. Les isolats d'Enterobacteriaceae ont été identifiés à l'aide de tests biochimiques et la production de BLSE a été confirmée par le test de synergie double disque de l'amoxicilline et de l'acide clavulanique. Le test de sensibilité aux antibiotiques de chaque isolat a été réalisé par la méthode de diffusion sur disque et interprété en utilisant les recommandations de la version 5.0 des seuils cliniques du Comité européen sur les tests de sensibilité aux antimicrobiens (EUCAST).

**Résultats:** Au cours de la période d'étude, la prévalence de EP-BLSE fécale parmi les participants à l'étude était de 54,5% (103/189); 53,5% parmi les volontaires sains et 55,7% parmi les patients hospitalisés ( $p=0,87$ ). Les principaux isolats de EP-BLSE étaient *Escherichia coli* (71%) suivis de *Klebsiella pneumoniae* (16%). Les isolats des patients hospitalisés étaient résistants à la norfloxacine (84,2%), au cotrimoxazole (89,5%) et à la gentamicine (7,0%). Les isolats de volontaires sains étaient résistants à la norfloxacine (86,2%), au cotrimoxazole (82,8%) et à la gentamicine (1,7%). Le sexe, l'âge et l'utilisation antérieure d'antibiotiques n'étaient pas significativement associés au portage de EP-BLSE ( $p=0,51$ ).

**Conclusion:** La forte prévalence de EP-BLSE dans cette étude est préoccupante. Il est urgent de développer des mesures pour surveiller et limiter la propagation de ces organismes multirésistants dans les établissements de santé et la communauté au Burkina Faso.

**Mots clés:** portage fécal, EP-BLSE, volontaires sains, patients hospitalisés, Burkina Faso

## Introduction:

Antibiotic resistance (AMR) is a threat to global health and has tremendous impact on human development. It is associated with prolonged patients' hospitalization, increase health expenditure, morbidity and mortality (1). According to a study in Senegal, the cost of treatment of infection due to extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) may be up to 100 € (2). The multi-drug resistance (MDR) issue according to the World Health Organization (WHO) involves several families of bacteria including the family Enterobacteriaceae (3). Acquisition of MDR is favored by several factors such unfavorable socio-demographic conditions, inappropriate use of antibiotics, absence of regulations for acquisition of antibiotics, and varying quality of antibiotics among others (4).

Carriage of ESBL-PE is associated with high risk of developing ESBL-PE infection (5), and also constitutes an important reservoir for MDR diffusion. This is a serious public health problem because treatment of infections caused by ESBL-PE entails the use of high ceiling antibiotics which are often unavailable in low-income-countries. Thus, the emergence of ESBL-PE has become a daily concern for treatment of infections in these countries. In Burkina Faso, the first study on carriage of ESBL-PE reported an overall ESBL-PE prevalence rate of 32% in western Bobo Dioulasso (4). This present study focuses on ESBL-PE carriage among healthy volunteers and hospitalized patients

in Ouagadougou, the capital city of Burkina Faso.

## Materials and method:

### Study setting

The study was conducted in the Tengandogo University Hospital, which has 600 beds, distributed across different specialized acute care units; medicine, surgery, gynaecology, obstetrics, and paediatrics.

### Study design and period of study

This was a cross-sectional study conducted during the period July 1 to November 30, 2017.

### Subject participants

The study subjects were 88 patients who were hospitalized at the Tengandogo University Hospital for more than 48 hours (people with digestive pathologies were excluded from the study), and 101 healthy volunteers (healthy personnel and accompanying persons).

### Ethical considerations

The study was approved by the Tengandogo University Hospital Board (Authorization No. MS/SG/CHUBC/DG/DSM 2017-569, October 27, 2017). Written informed consent was obtained from all subjects and at least one parent for each child before enrollment. Participants were enrolled voluntarily. The confidentiality of the obtained information from the subjects was respected, as the participants and their samples were codified.

### Samples and data collection

Each participant was interviewed by a healthcare professional using a questionnaire to obtain information on age, gender and antibiotic use/treatment during the previous 3 months before the study. Rectal samples were taken by swabbing the rectum of each subject, using sterile swab soaked in sterile physiological saline, which was inserted into the rectum to about 2 cm and rotated 2 to 3 times. The swab was then put back into the swab container, and transported immediately to laboratory for microbiological analysis (6).

### Microbiological culture and isolate identification

All rectal samples were immediately seeded on Brain Heart Infusion (BHI) broth and incubated for 5 hours at 37°C to improve bacteriological yield (7). After this enrichment phase, 100 µL of the broth was transferred to Hektoen enteric agar (Oxoid, UK) supplemented with 4 µg/L cefotaxime and incubated at 37°C for 24 hours. Predominant colonies of different morphotypes were identified to species level by using in-house biochemical test panels including Triple Sugar Iron (TSI) agar, Sulfur-Indole-Motility test, Simmons' citrate agar, and urease test.

### Detection of ESBL-PE

The detection of ESBL production was routinely performed by the disc diffusion synergy method using third generation (3GC) discs of cefotaxime (30µg), ceftriaxone (30µg), and ceftazidime (30µg) (HiMedia, Ltd, India) placed at a distance of 20-30 mm apart around a disc of amoxicillin+clavulanic acid (20+10µg) as recommended by the Antibiogram Committee of the French Microbiology Society (8,9). ESBL production was detected by the presence of synergy between the third generation cephalosporins and the inhibitor (clavulanic acid) (8-10). In case of high-level cephalosporinase production, the combined double-disc synergy test was performed using cloxacillin-supplemented agar medium. *Klebsiella pneumoniae* ATCC 700 603 (ESBL-positive) and *Escherichia coli* ATCC 25922 (ESBL-negative) were used as control strains.

### Antibiotic susceptibility test

Antimicrobial susceptibility test (AST)

was performed on each isolate by the disc diffusion (Kirby-Bauer) method. A suspension in saline solution (0.9% NaCl) equivalent to 0.5 McFarland standards (or to an optical density OD of 0.08-0.10 read at 625 nm) was used. The incubation was carried out at 37°C aerobically for 18 to 24 hours. Reading and interpretation were carried out according to the recommendations of the Antibiogram Committee of the French Microbiology Society (8).

The following single discs were used for the AST assay; amoxicillin+clavulanic acid (30µg), ceftriaxone (30µg), ceftazidime (30 µg), cefotaxime (30µg), meropenem (10µg), gentamicin (30µg), norfloxacin (10µg), and sulfamethoxazole-trimethoprim (25µg). The inhibition zone diameters were used to categorize isolates as sensitive (S), intermediate (I) or resistant (R). *Escherichia coli* ATCC 25922 was used as the control strain in the AST assay.

### Statistical analysis

Data entry was performed on Excel 2013 and statistical analysis of the data was done using XLSTAT 2017 version 19.5. The distributions of the variables were compared by the  $\chi^2$  independence test. The significance level was set at 5%.

## Results:

### Prevalence of faecal carriage of ESBL-PE

A total of 103 subjects were faecal carriers of ESBL-PE, giving an overall prevalence rate of 54.5% (103/189). Forty-nine of the 88 (55.7%) inpatients and 54 of 101 (52.4%) healthy volunteers were carriers of ESBL-PE. The prevalence of ESBL-PE carriage was slightly higher among hospitalized patients than among healthy volunteers but the difference was not statistically significant ( $p=0.87$ ).

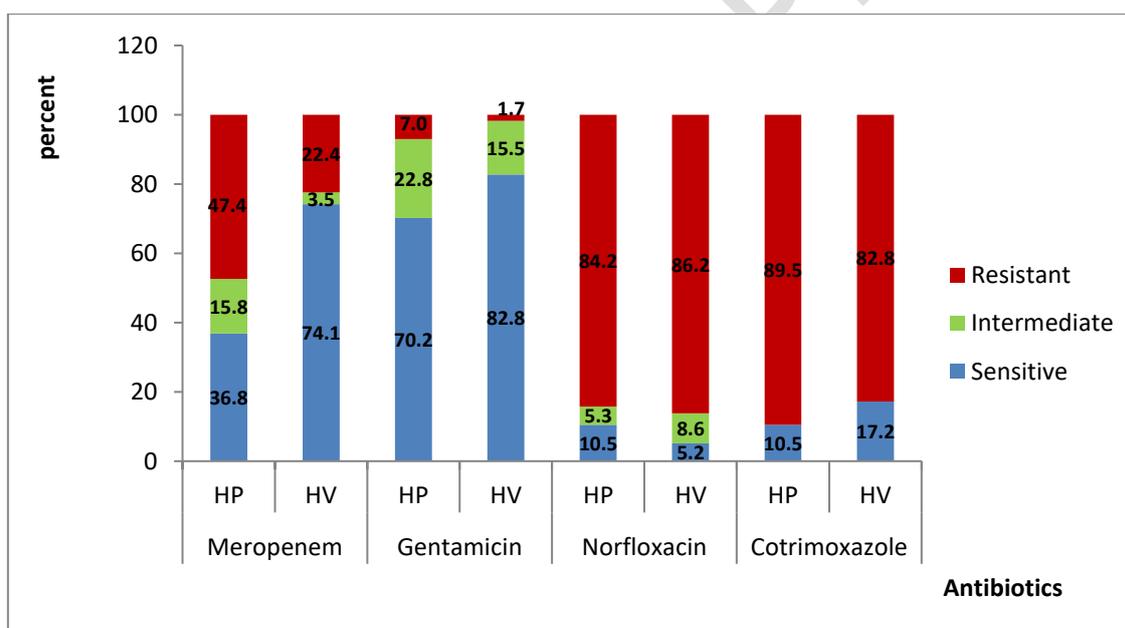
### Distribution of ESBL-PE in the study population

ESBL-PE isolates were identified as *Escherichia coli* (71.3%), *Klebsiella pneumoniae* (15.7%), *Klebsiella oxytoca*, *Enterobacter cloacae*, *Enterobacter agglomerans*, *Citrobacter freundii* and *Proteus mirabilis* (Table 1).

Table 1: Distribution of ESBL-PE in the study population

ESBL-PE	In-patients		Healthy volunteers		Total	
	n	%	n	%	n	%
<i>Escherichia coli</i>	38	66.7	44	75.9	82	71.3
<i>Klebsiella pneumoniae</i>	7	12.3	11	18.9	18	15.7
<i>Klebsiella oxytoca</i>	5	8.8	1	1.7	6	5.2
<i>Enterobacter cloacae</i>	2	3.5	1	1.7	3	2.6
<i>Enterobacter agglomerans</i>	3	5.3	1	1.7	4	3.5
<i>Citrobacter freundii</i>	1	1.8	0	0	1	0.9
<i>Proteus mirabilis</i>	1	1.8	0	0	1	0.9
Total	57	100	58	100	115	100

ESBL-PE = Extended Spectrum Beta-Lactamase-Producing Enterobacteriaceae; n = number



HP: Hospitalized Patients, HV: Healthy Volunteers

Fig 1: Susceptibility and resistance profile of ESBL-PE to selected antibiotics

#### Antibiotic resistance profile of ESBL-PE

Resistance rates of ESBL-PE to aminoglycosides, fluoroquinolones and sulfonamides were similar among inpatients and healthy volunteers, with 89.5% and 82.8% resistant to cotrimoxazole and 84.2% and 86.2% to norfloxacin respectively (Fig 1). However, resistance rate to meropenem of 57.4% among inpatients was significantly higher than 22.4% among healthy volunteers ( $p=0.007$ ).

#### Association of participant's characteristics and risk factors to ESBL-PE carriage

Analysis of participant's characteristics and risk factors by  $\chi^2$  test showed that none was associated with ESBL-PE carriage (Table 2). Antibiotics usage in the last 3 months ( $p=0.21$ ) and types of antibiotics used ( $p=0.51$ ) were not significantly associated with ESBL-PE carriage.

Table 2: characteristics and risk factors for ESBL-PE in the study population

Variables	Inpatients (n=88)		p value	Healthy volunteers (n=101)		p value
	ESBL positive (n=49)	ESBL negative (n=39)		ESBL positive (n=54)	ESBL negative (n=47)	
Mean age (±SD) (years)	45.91 (±23.02)	46.78 (±23.65)		39.75 (±8.94)	41.29 (±10.24)	
Gender n (%)						
Male	29 (59.2)	26 (66.7)	0.47	12 (22.2)	18 (38.3)	0.077
Female	20 (40.8)	13 (33.3)		42 (77.8)	29 (61.7)	
Age group (years)	n (%)					
≤1	6 (12.2)	7 (17.9)		0	0	NA
>1 – 20	4 (8.2)	3 (7.7)		1 (1.9)	0	
>20 – 40	16 (32.7)	12 (30.8)	0.078	33 (61.1)	23 (48.9)	
>40 – 60	9 (18.4)	5 (12.8)		18 (33.3)	21 (44.7)	
>60	14 (28.6)	12 (30.8)		2 (3.7)	3 (6.4)	
Antibiotic use/treatment in last 3 months, n (%)						
Yes	24 (48.9)	14 (35.9)	0.21	13 (24.1)	14 (29.8)	0.51
No	25 (51.0)	25 (64.1)		41 (75.9)	33 (70.2)	
Type of antibiotics used n (%)						
β-lactams	21 (87.5)	12 (85.7)	0.87	11 (84.6)	9 (64.3)	0.22
Others	3 (12.5)	2 (14.3)		2 (15.4)	5 (35.7)	

## Discussion:

In this study, β-lactams were the most frequently used antibiotics among the subjects, with a frequency of approximately 80%. The same observation was made by Ouédraogo et al., (4) in 2016, who reported 56.25% of β-lactams among antibiotics used by patients in the 3 months before detection of ESBL-PE carriage. Indeed β-lactams are available for patients use because they are less expensive, and easy to use by oral administration. This high rate could increase the pressure of the selection of resistant mutants in the country. Indeed, excessive consumption of penicillins and cephalosporins can cause mutations in bacterial populations, and genes encoding the synthesis of penicillinases, cephalosporinases or extended spectrum β-lactamases are transferable to other bacteria, thus increasing resistance to these antibiotics.

The prevalence of 54.5% of ESBL-PE carriage (55.7% among in-patients and 53.5% among volunteers) is high, and this could predispose individuals to increase risk of infections because these enterobacteria may be found in the urinary tract or on an operating wound, resulting in infections that are difficult to treat. This prevalence is higher

than those observed in the northern countries where the faecal carriage of ESBL-PE was in the range of 0.6% to 11.6% (11,12). However, our results are similar to those found in several countries in sub-Saharan Africa like Cameroon, where prevalence rate ranges between 16% and 55%. The prevalence of colonization by ESBL-PE in healthy subjects in West African countries varies between 10 and 100% (13). This high prevalence may be explained as consequences of excessive use of antibiotics, poor quality of antibiotics, poor hygiene, and absence of surveillance system networks for these multi-resistant bacteria. We did not find any significant difference between prevalence in hospitalized patients (55.7%) and healthy volunteers (53.5%), which is contrary to the study by Ouédraogo et al., (4) in Bobo Dioulasso, where there significant difference was reported. However, the prevalence rate both studies is high and indicates a need for urgent action by way of antimicrobial stewardship and infection prevention and control.

Among the ESBL-PE, the most frequent isolates were *E. coli* and *K. pneumoniae* both among hospitalized patients and healthy volunteers with frequencies of 71.3% and 15.7% respectively. Our results are similar to those reported by Ouédraogo et al.,

(4) in Burkina Faso and Tellevik et al., (14) in Tanzania, who both reported predominance of *E. coli* and *K. pneumoniae*. *Escherichia coli* is responsible for about 80% of urinary tract infections but is also implicated in suppurative infections while *K. pneumoniae* is frequently implicated in respiratory and post-operative infections. The high proportion of these two species among ESBL-PE is the cause of treatment failure for the infections that they may cause. Most studies on ESBL-PE carriage have shown a predominance of *E. coli* which may carry genes located on plasmids encoding the production of ESBLs, that could facilitate transfer to other enterobacteria (4,15).

The ESBL-PE showed resistance to meropenem of 47.4% and 22.4% respectively among hospitalized patients and healthy volunteers. This resistance to carbapenems is worrying because these are exclusive antibiotics used as last resort in the treatment of infections caused by ESBL-PE. This high resistance must have resulted from excessive prescription of carbapenems with the arrival of these antibiotics in generic forms. The ESBL-PE also showed high resistance to norfloxacin (86.2% among volunteers and 84.2% among inpatients) and sulfamethoxazole-trimethoprim (89.5% for inpatients and 82.8% for volunteers). The high resistance rate to fluoroquinolones and sulfamethoxazole-trimethoprim may have resulted from overuse due to easy access, and lack of control of these antibiotics in the markets. Plasmids carrying genes encoding ESBLs are known to also carry other genes conferring resistance to fluoroquinolones, aminoglycosides and cotrimoxazole (16). Our results are similar to those reported by Ouédraogo et al., (4) in Burkina Faso who showed that there were associated resistance of ESBL-PE to other families of antibiotics.

Analysis of the characteristics of the subjects and risk factors for faecal ESBL-PE carriage did not show any significant association with respect to age group, gender, previous use of antibiotics, and the types of antibiotics consumed by both the healthy volunteers and hospitalized patients. These findings are similar to those of Isendahl et al., (13) in 2012 in Guinea Bissau who did not observe any association between ESBL-PE carriage and previous consumption of antibiotics. However, our results contradicted those reported by Rodriguez-Bano et al., in Spain, Wu et al., in Hong Kong, and Ouédraogo et al., in Burkina Faso, who found association between ESBL-PE carriage and antibiotic use (4,17).

## Conclusion:

Our study revealed the prevalence of rectal carriage of ESBL-PE to be 54.5% which showed that rectal carriage of ESBL-PE among hospitalized (55.7%) and healthy volunteers (53.5%) is high in Burkina Faso. The ESBL-PE were mainly represented by *E. coli* and *K. pneumoniae* with high resistance to norfloxacin and cotrimoxazole in both hospitalized and healthy volunteers. However, none of the factors analysed was significantly associated with the carriage of ESBL-PE. In light of these findings, it is desirable to establish surveillance program for multi-resistant bacteria, and institute antimicrobial stewardship in the country.

## Authors' contributions:

SS, SI, OAS and IKS conceived and design of the study, SS and BS collected and analyse the samples, SS, SI, OAS, SaS, SY, PA., ZJ, DSNP, DMD and OB analyse and correct the results. All authors read and approved the final manuscript.

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