



Original Research Article

Prevalence and resistance profile of *Enterobacteriaceae* producing extended spectrum Beta-Lactamases isolated from the laboratory of bacteriology and parasitology in National Public Health Laboratory of Ouagadougou

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The resistance of *Enterobacteriaceae* to certain classes of antibiotics is mostly related to the production of extended spectrum beta-lactamases (ESBLs). It represents a real public health problem, especially in developing countries. This study aims of this study was to determine the prevalence of ESBLs-producing *Enterobacteriaceae* isolated from pathologic products. The susceptibility to antibiotics was analysed according to the recommendations of CA-SFM-2013 using the double synergy method. The results showed that 5.55% (6/108) of *Enterobacteriaceae* produced E-SBL. *Enterobacteriaceae* producing ESBLs showed the highest rates of resistance to Amoxicillin (100%), Amoxicillin + Clavulanic acid (100%), Ceftriaxone (100%), Cefotaxime (100%), Co-trimoxazole (100%), Cefoxitin (100%), Nalidixic acid (83.33%) and Ciprofloxacin (83.33%). The rate of resistance to Aminoglycosides (Gentamicin and Tobramycin) was 33.33%. All strains were sensitive to Imipenem. ESBL-producing *Enterobacteriaceae* are frequently (5.55%) present in the community environment in Burkina Faso, hence the need for a national policy to monitor these bacteria.

Keywords: Antibiotic, profile of resistance, *Enterobacteriaceae*, ESBLs, Ouagadougou.

INTRODUCTION

The emergence and spread of multi-resistant bacterial strains pose enormous public health problems (Elhani, 2011; Stryko et al., 2016) that led the (World Health Organization, 2011) to dedicate the World Day against multi-resistant bacteria. Bacteria have developed enormous mechanisms to inhibit antibiotics action, thus making the treatment of infections with these bacteria very challenging complex (Arcilla et al., 2017; Ouédraogo et al., 2017). The expansion of this multi-resistance can be explained on several levels including: self-medication, the exchange of materials, generics between bacterial strains (Gilbert et al., 2010), modification of antibiotic targets, and production of inhibitory enzymes of antibiotics action (Ouédraogo et al.,

2017).

Nowadays, *Enterobacteriaceae* producing ESBLs are less sensitive or even resistant to the majority of beta-lactams (Fursova et al., 2015). This family of antibiotics predominates in the therapy of human infections (Anago et al., 2015). According to the European Antibacterial Resistance Surveillance Network (EARS), in Europe, the resistance of *Escherichia coli* strains to third generation cephalosporin due to ESBLs production has increased from 85% to 100% in 2009 (Elhani, 2011).

In Burkina Faso, only a few studies regarding ESBLs-producing *Enterobacteria* (ESBLs) have been conducted in health centres (Dabiré et al., 2013). For example, (Dabiré,

Table 1. Isolated and identified *Enterobacteriaceae* species by origin and frequency

Germ	Stools	Urine	Pus	CVS	CSF	Total	Frequency(%)
<i>Escherichia coli</i>	01	72	00	12	00	85	78.70
<i>Klebsiella. pneumoniae</i>	00	08	01	03	00	12	11.11
<i>Klebsiella oxytoca</i>	00	02	00	00	00	02	01.85
<i>Klebsiella ornithinolytica</i>	00	00	00	01	00	01	00.92
<i>Proteus vulgaris</i>	00	01	00	00	00	01	00.92
<i>Proteus mirabilis</i>	00	02	01	00	00	03	02.77
<i>Enteobacter cloacae</i>	00	03	00	00	00	03	02.77
<i>Provideniastuarti</i>	00	00	01	00	00	01	00.92

Legend: CVS: Cervico-Vaginal Secretions; CSF: Cerebro-Spinal Fluid

2014; Ouédraogo et al., 2016) were reported respectively 67% of ESBLs hospitalized patients in Charles De-Gaulle Pediatric among those 58% in hospitalized patients in Universities centres hospitals (Yalgado Ouédraogo, Sourou Sanou, Charles De-Gaulle Pediatric). To the best of our knowledge, no studies have been conducted yet on non-hospitalized patients in Burkina Faso. The objective of this study was to determine the prevalence of *Enterobacteriaceae* producing ESBLs isolated from National Public Health Laboratory (NPHL) of Ouagadougou and their antibiotic resistance profile to antibiotics usually used to treat of Gram-negative bacilli infections.

MATERIALS AND METHODS

Samples, isolation and identification of *Enterobacteriaceae*

A total of 773 samples from non-hospitalized patients (267 stools, 326 urines, 70 cervico-vaginal secretions, 11 pus and 99 cerebrospinal fluid) were collected in LNPH (a reference centre of Burkina Faso in several areas such as biomedical analyses and food quality control) for isolation of *Enterobacteriaceae* on the agar Eosine Methylene Blue agar plates. The API 20E was used for the identification of *Enterobacteriaceae* isolated according to the manufacturer's recommendation (Biomérieux, France).

Antibiotic susceptibility and ESBLs production

Antibiotic resistance was tested Muller Hunton (MH) by using an inoculum of Co MacFarland 0.5. Thus, twelve antibiotics including Amoxicillin (25 µg), Amoxicillin + Clavulanic acid (20/10 µg), Imipenem (10 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefoxitin (30 µg), Gentamicin (15 µg), Tobramycin (10 µg), Ciprofloxacin (5 µg), Cotrimoxazole (25 µg), Nalidixic acid (30 µg), and Cefamandole (30 µg) were tested. The double synergy test according the recommendation of Company Antibiotic Committee French Microbiology (CA-SFM) 2013 using a combined action of Amoxicillin + Clavulanic and a third-generation cephalosporin was used for the detection of the

phenotypic expression of ESBLs. The tests were carried out in Petri dishes containing six discs each of which Amoxicillin + Clavulanic was placed in the centre of one petri dishes. Two petri dishes were used for each inoculum and incubated at 37 °C for 24 hours. Inhibition diameter of the antibiotics was interpreted according to the CA-SFM (2013).

References strains used for double synergy test

Three reference strains (*E. coli* ATCC 25922, *E. coli* ATCC 35218, and *K. pneumoniae* ATCC 700603) were used as a control for the antibiotic susceptibility test sensitive and the production of E-SBL.

Statistical analysis

The data were analysed using Excel 2013.

RESULTS

Enterobacteriaceae identification

The cultures of 18 to 24 hours on specific agar were allowed to identify with API 20E kit 108 *Enterobacteriaceae* from 145 bacterial strains, i.e. the rate of infection was 74.48%. These results were showed a clear predominance of *Enterobacteriaceae* in bacterial infections among pathological products received from LNPH. Table 1 shows the level of different strains in pathological products. Of the five types of pathological samples used in this study, *Enterobacteriaceae* pathogens had been isolated and identified from the stool, urine, pus and vaginal sampling.

Prevalence of *Enterobacteriaceae* producing beta-lactamases

Detection of ESBLs

After incubation during 18 to 24 hours, the antibiograms were carried out, the ESBLs phenotype was in the form of a

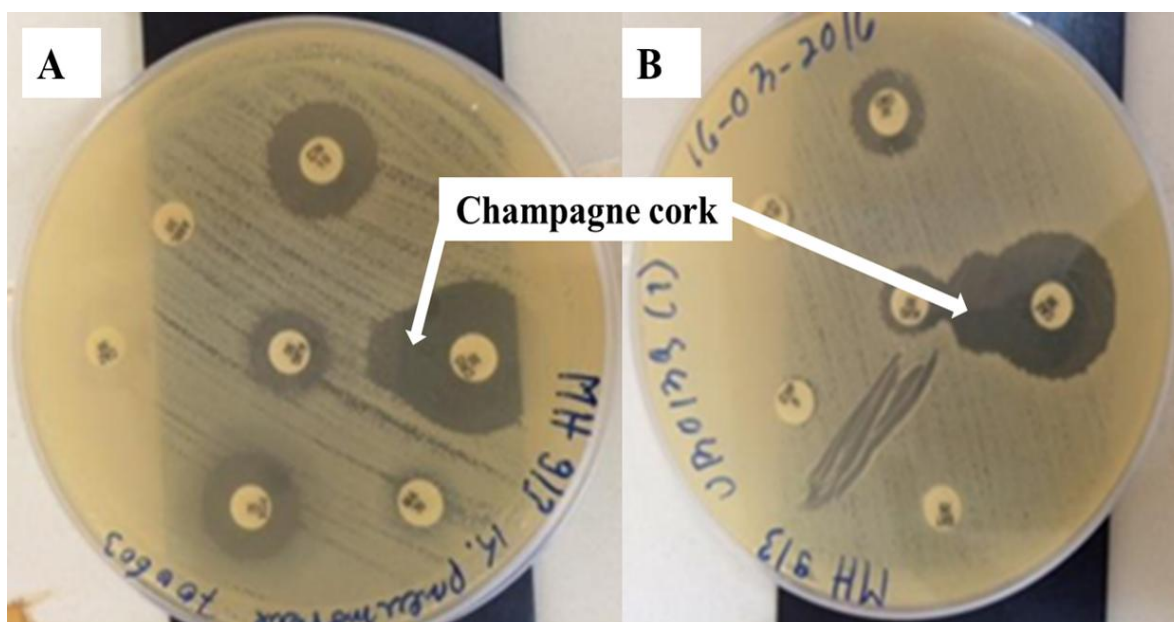


Figure 1. Phenotypic expression of ESBLs

Legend: A: Phenotypic expression of ESBLs by a strain of *Klebsiella pneumoniae*; B: Phenotypic expression of ESBLs by a strain of *Escherichia coli*

Table 2. Resistance profile of each type of antibiotic *Enterobacteriaceae* strain

	NA	IMI	GM	TOB	FOX	MA	SXT	CAZ	CRO	AMC	AUG	CIP
<i>E. coli</i> (n=85)	54.11%	0%	28.23%	31.76%	21.17%	47.05%	84.70%	23.52%	40%	96.47%	89.41%	56.47%
<i>K. pneu</i> (n= 12)	16.66%	0%	0%	16.66%	16.66%	16.66%	83.33%	16.66%	16.66%	100%	83.33%	8.33%
<i>K. oxyt</i> (n= 02)	50%	0%	0%	50%	50%	50%	100%	50%	50%	100%	100%	50%
<i>K. orni</i> (n=01)	0%	0%	0%	0%	0%	0%	100%	0%	0%	100%	100%	0%
<i>P. mira</i> (n= 01)	33.33%	0%	33.33%	33.33%	33.33%	33.33%	66.66%	33.33%	33.33%	100%	100%	33.33%
<i>P. vulg</i> (n= 03)	0%	0%	0%	0%	100%	100%	100%	100%	100%	100%	100%	0%
<i>E. cloa</i> (n= 03)	33.33%	0%	0%	0%	100%	67%	66.66%	33.33%	33.33%	100%	100%	0%
<i>Pr. stu</i> (n= 01)	100%	0%	0%	0%	100%	100%	100%	0%	100%	100%	100%	0%
Set of isolates	48.14%	00%	23.14%	28.70%	25%	44.44%	83.33%	24.07%	37.96%	97.22%	89.81%	47.22%

Legend: *E. coli*: *Escherichia coli*; *K. pneu*: *Klebsiella pneumoniae*; *K. oxyt*: *Klebsiella oxytoca*; *K. orni*: *Klebsiella ornithinolytia*; *P. mira*: *Proteus mirabilis*; *P. vulg*: *Proteus vulgaris*; *E. cloa*: *Enterobacter cloacae*; *Pr. stu*: *Providencia stuartii*; NA : Nalidixic Acid; IMI : Imipenem; GM : Gentamicin; TOB : Tobramycin; FOX : Cefoxitin; MA : Cefamandol; SXT : Cotrimoxazol; CAZ : Ceftazidime; CRO : Ceftriaxone; AMC : Amoxicillin; AUG : Augmentin (Amoxicillin + Clavulanic Acid) ; CIP : Ciprofloxacin

champagne cork between Amoxicillin + Clavulanic acid and one of the third-generation cephalosporins (Figure 1). In the present study, 6 of 108 *Enterobacteriaceae* or 5.55% isolates produced an ESBL. Among the ESBL-producing *Enterobacteriaceae* species source were from three types of samples including, three in the urine, one in the pus and two in the cervico-vaginal secretions. ESBLs positive isolates 4.7% represented by the *E. coli* and 13.33% of *Klebsiella* spp. The other isolated *Enterobacteriaceae* were not ESBL producers.

Resistance profile of *Enterobacteriaceae*

Enterobacteriaceae showed high resistance to Amoxicillin (97.22%), Amoxicillin + Clavulanic acid (89.22%), and

Cotrimoxazole (83.33%) low resistance to amino glycosides (Gentamicin and Tobramycin) and to third-generation cephalosporin and no resistance to Imipenem according to Table 2.

Resistance profile of ESBLs producing *Enterobacteriaceae*

All E-ESBLs had higher levels of resistance to penicillin and cephalosporin than all *Enterobacteriaceae*. Thus, the resistance profile of the E-ESBLs was identified as the following 100% to Amoxicillin, Ceftazidime, Amoxicillin + Clavulanic acid, Cotrimoxazole, Ceftriaxone, Cefoxitin; 83.33% to Ciprofloxacin, Nalidixic Acid; and 33.33% to Tobramycin and Gentamicin but 100% of sensibility to

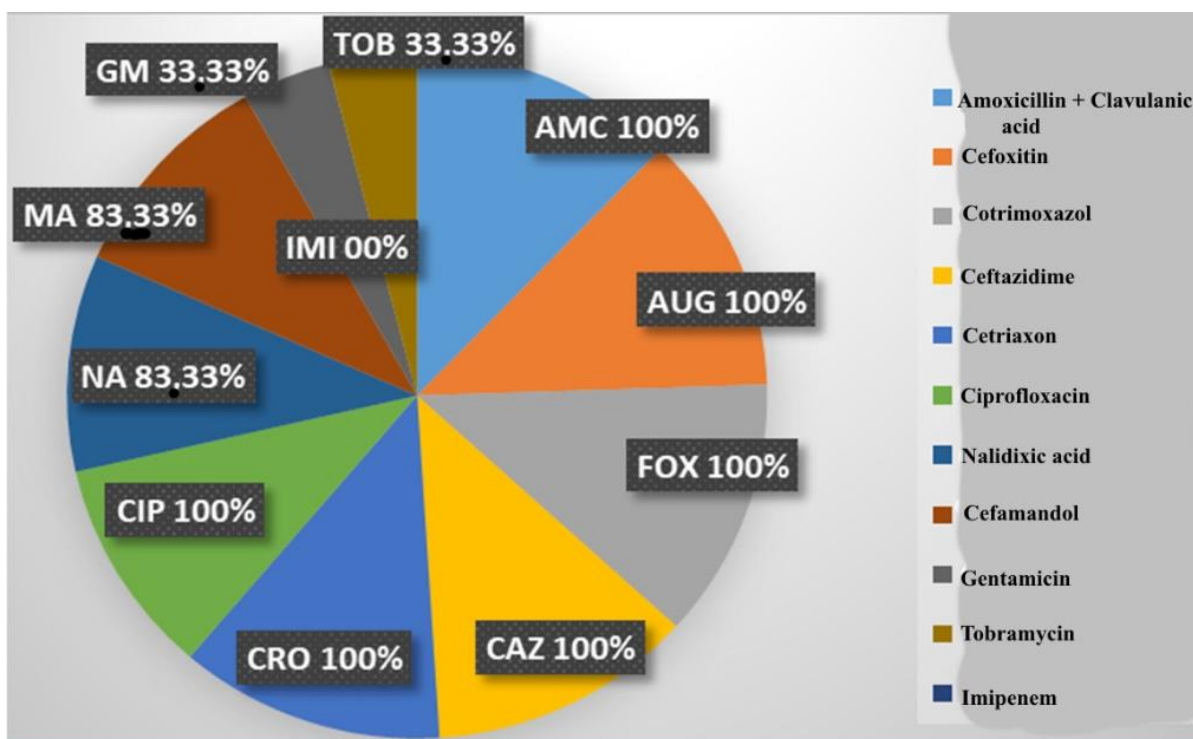


Figure 2: ESBLs *Enterobacteriaceae* resistance rate to antibiotics

Legend: NA : Nalidixic Acid; IMI : Imipenem; GM : Gentamicin; TOB : Tobramycin; FOX : Cefoxitin; MA : Cefamandol; SXT : Cotrimoxazol; CAZ : Ceftazidime; CRO : Ceftriaxone; AMC : Amoxicillin; AUG : Augmentin (Amoxicillin + Clavulanic Acid); CIP : Ciprofloxacin.

Imipenem (Figure 2).

DISCUSSION

E. coli (78.7%) and *Klebsiella* spp (13.9%) were the predominant strains isolated from stool, urine, pus and vaginal samples. Similar findings had been highlighted in a study conducted in Morocco on hospitalized patients suffering from chronic renal failure (Chemlal et al., 2015). They incriminated *E. coli* and *Klebsiella* spp. in 58.3% and 29.2% of the cases (Chemlal et al., 2015). *Enterobacteriaceae* are part of human commensal flora. However, some species, including *E. coli* and *Klebsiella* spp. can evolve in opportunistic pathogens in case of weak immunity and poor lifestyle, including hygiene practices, dietary habits and self-medication (Oudraogo et al., 2017; Harris et al., 2015; Sansonetti, 1987).

Appreciable number of *Enterobacteriaceae* (5.55%) were ESBLs producing bacteria. In contrast, Dabiré (2014) and Ouédraogo et al. (2016) reported 58% and 67.22%, respectively, of ESBLs from hospitalized patient in Burkina Faso. The differences could be explained by the category of subjects in these studies and their exposure to antibiotic-resistant strains. However, their presence in the community would be due to a spread of strains from hospitals. The problem of ESBLs bacteria in Africa health centres has been

addressed by several authors and the situation seems to be generalized because several studies have also mentioned the presence of ESBLs (Oduro-Mensah et al., 2016; Anago et al., 2015; Beyene et al., 2014; Newire et al., 2013). The level of resistance of *Enterobacteriaceae* becoming more worrying. This has been highlighted in many investigations in hospitals (Anago et al., 2015, 35.5%; Oduro-Mensah et al., 2016, 37.96%; Tshilumbu, 2006, 17.8%; and Andrianarivelo et al., 2010, 50%). However, the spread of ESBLs among the populations could be explained by a deplorable level of health security hygiene. Indeed, the phenomenon of resistance in *Enterobacteriaceae* could be explained firstly by the production of inhibitory enzymes of antibiotics, secondly by changing the targets of antibiotics and finally by natural resistance (Muylaert et al., 2012; Lawrence et al., 2013; Newire et al., 2013). Although the resistance profile of all *Enterobacteriaceae* is increasingly worrying, however, that specific to ESBLs remains to be desired.

All *Enterobacteriaceae* in this study were resistant to most antibiotics used. Anago et al. (2015) found also that their strains of *E. coli* showed resistance phenotypes amoxicillin (92.8%), ampicillin (94%), trimethoprim /sulfamethoxazole (85.7%), and imipenem (03.6%). The high levels of resistance to beta-lactam are due to the ability of the bacteria to produce the enzymes (ESBLs) to hydrolyse the antibiotics (Day et al., 2019). This

hypothesis has been shared by several other authors (Dabiré, 2014; Ma et al., 2013; Lyimo et al., 2016; Mnif et al., 2013; Shi et al., 2015; Mshana et al., 2016; Oduro-Mensah et al., 2016).

Moreover, strains producers of ESBLs were more resistance to the same antibiotics. Indeed, ESBLs is one of the main factors of resistance to *Enterobacteriaceae* (Paterson et al., 2005; Fukuchi et al., 2016). This had been also highlighted in France where the prevalence of 38% of third-generation cephalosporin-resistant *Enterobacteriaceae* had been reported (Thiolet et al., 2016). In addition, the increasing prevalence of the resistance of ESBLs producing *Enterobacteriaceae* to third-generation of cephalosporins has been reported in several hospitals (Dabiré et al., 2013; Harris et al., 2015; Durrmeyer et al., 2012). For the first time, we reported the variation of prevalence in antibiotic resistance of *Enterobacteriaceae* and their ESBLs production at a non-hospital centre (LNSP) in Burkina Faso.

CONCLUSION

The study showed a predominance of *Enterobacteriaceae* in urinary infections, vaginal infections and pus. Isolated bacteria showed very high levels of resistance to all antibiotics used, except imipenem. However, some strains showed resistance to aminoglycosides (Gentamicin and Tobramycin). The use of the technique called “double synergy test” has allowed better detection of ESBLs. The prevalence of *Enterobacteriaceae* producers of ESBLs (5.55%) among patients from the community indicated the resistance spreading from hospital to general population. In light of the present investigations, there is no doubt that ESBLs play a crucial role in bacterial resistance.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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