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First detection of the BES-type Extended-Spectrum β-Lactamase produced by Enterobacteria at Saint Camille Hospital of Ouagadougou (Burkina Faso)

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Several studies have been reported on the *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$ genes in Extended-spectrum β-lactamase (ESBL) producing Enterobacteria, however very few studies reported in the literature are related to *bla*$_{BES}$ in ESBL producing Enterobacteria. This study concerns the molecular epidemiology of the *bla*$_{BES}$ gene in Enterobacteria identified from in-patients and out-patients at Saint Camille hospital of Ouagadougou (Burkina Faso). The study was first involved microbiological identification of Enterobacteria that are implicated in antibiotic resistance using API 20 E system; the antibiotics susceptibility test was secondly performed by the diffusion method and the molecular characterization was finally made by PCR to detect the *bla*$_{BES}$ gene. Data were entered and analyzed using Excel 2013 and EPI Info version 6.0 software. A p-value < 0.05 was considered as significant. A total of 60 isolates of ESBL-producing Enterobacteria were found: 21 (35%) *Escherichia coli*; 18 (30%) *Klebsiella pneumoniae*; 6 (10%) *Enterobacter cloacae*; 4 (7%) *Proteus mirabilis*; 4 (7%) *Serratia marcescens*; 3 (5%) *Citrobacter freundii*; 1 (1.6%) *Enterobacter aerogenes*; 1 (1.6%) *Citrobacter brakii*; 1 (1.6%) *Citrobacter youngae* and 1 (1.6%) *Salmonella arizonae*. Molecular characterization revealed the presence of the *bla*$_{BES}$ gene in 38 (63.3%) of bacterial isolates carried by patients. The presence of *bla*$_{BES}$ gene in ESBL producing Enterobacteria at Saint Camille Hospital in Ouagadougou was therefore established in this study for the first time in Burkina faso.

Key words: Enterobacteria, Extended-spectrum β-lactamase (ESBL), *bla*$_{BES}$, gene, hospital, resistance, Ouagadougou.

INTRODUCTION

Antimicrobial resistance constitutes an increasingly human health hazard worldwide, but the hospital has always been known to be the greatest risk (Bradford, 2001). Thereby, the first antimicrobial resistance surveillance data published by the World Health Organization (WHO, 2018) showed high levels of resistance to several serious bacterial infections in both high and low income countries. Antimicrobial resistance is responsible for about 700,000 deaths a year worldwide and has huge implications for the cost of healthcare

(Jasovsky et al., 2016). The production of Extended-Spectrum β-lactamases (ESBLs) by Enterobacteria is the main mechanism of the antimicrobial resistance. Several studies have been conducted on the major genes involved in the production of ESBLs. The most common ESBLs are the Temoneira (TEM), Variable sulfhydryl (SHV) and Cefotaximase-Munich (CTX-M) types (Sadeeq et al., 2018).

The first plasmid TEM-1-type β-lactamase was isolated in 1965 in Greece from a strain of *Escherichia coli* isolated in a patient named Temoneira hence the name (Zubair et al., 2015). The SHV-types ESBLs are derived by punctual mutations from the original SHV-1 enzyme. The origin of these enzymes is probably a variant of the *Klebsiella pneumoniae* chromosomal penicillinase bla_{SHV} gene (Brisse and Verhoef, Haeggman et al., 2004). Currently, more than 180 SHV-types ESBLs variants have been described (Liakopoulos et al., 2016). CTX-M ESBLs were initially described in 1986 in Japan, Germany and France in 1989 (CTX-M-1) and have since spread widely around the world (Thomson and Moland, 2000). CTX-M is the most prevalent ESBLs worldwide (Anna et al., 2014).

Besides the so-called major ESBLs there are minor types ESBLs such as BES-type (*Brazilian extended spectrum)* which are less studied (Cattoir, 2008).

It has less wide distribution and is characterized by a high level of resistance to Ceftazidime and sometimes to Aztreonam rather than Cefotaxime (Arlet and Philippon 2003; Bradford, 2001). The prevalence of the bla_{BES} gene in ESBL-producing Enterobacteria is rare. Investigations on ESBL-producing Enterobacteria in Burkina faso is relatively recent and the bla_{BES} gene is not concerned yet.

To fill the gap on the molecular epidemiology of the bla_{BES} gene, this study was undertaken to detect the bla_{BES} gene in ESBLs-producing Enterobacteria at Saint Camille Hospital of Ouagadougou (Burkina Faso).

MATERIALS AND METHODS

Type of study

This is a cross-sectional study conducted at Saint Camille Hospital of Ouagadougou (Burkina faso) from September to November 2018. Samples collected consisted of stool samples, urine samples and vaginal swab samples from hospitalized patients or out-patients. 250 samples were collected from 250 patients. Samples are inoculated on ordinary media like Uri Select 4 medium Bio-Rad (Marnes-la-Coquette, France), Hektoen Enteric Agar medium (Taden, France) and BD Salmonella Shigella (SS) Agar medium (Heidelberg, Germany) to allow the growth of Enterobacteria and then incubated for 24 h at 37°C. Subsequently, Enterobacteria who grew on the previous media were subcultured on a BD Mueller-Hinton (MH) Agar medium (Heidelberg, Germany) and then incubated for 24 h at 37°C for antimicrobial assays.

Antimicrobial susceptibility test

Isolates were identified using Analytical Profile Index API 20 E (BioMérieux S. A., Marcy l'Etoile, France) identification method. Antibiotics susceptibility/resistance test was carried out on Mueller-Hinton (MH) medium with pure colonies of Enterobacteria according to the recommendations of the Committee of antibiogram of the French Society of Microbiology (CASFM/EUCAST, 2018). The antibiotic discs used were: Amoxicilline + Clavulanic acid (Augmentin) (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg) and Aztreonam (30 µg). All Enterobacteria resistant to Augmentin and at least one third generation cephalosporin were considered as ESBL-producing Enterobacteria for the study.

Molecular characterization of ESBLs

Extraction of bacterial DNAs

The boiling method was used to extract DNAs from bacteria (Ribeiro Junior et al., 2016). The strains were reactivated by culture on the MH medium for 18 to 24 h. An isolated colony is taken from the Petri dishes and suspended in 200 µl of distilled water previously aliquoted in labeled eppendorf tubes. Then immersion in a water bath at 100°C for 15 min was followed to release the genetic material. After immersion in a water bath, the suspension was centrifuged at 12,000×g for 10 min and the supernatant containing the DNA released is transferred to a new eppendorf tube. The concentration of the DNA was then determined using the Nanodrop type spectrophotometer (Biodrop®, Holliston, USA).

Molecular analysis

The reaction medium for PCR was constituted by a volume of 25 µl composed of the Master Mix, the DNA and the primers. PCR amplification of bla_{BES} gene was carried out with the following specific primers: BES-F 5' TAATAACCCTGACCAAGCCTA 3' and BES-R 5' CCCTTTCAAAAGTCATAAATC 3' were used for bla_{BES} with 879 bp as molecular weight (Bonnet et al., 2000) and using thermocycler Gene Amp 9700 PCR System (Applied Biosystems, Californie, États - Unis). PCR program consisted of an initial denaturation at 95°C for 5 min followed by 30 cycles (Denaturation 95°C/59 s, Annealing 50°C/59 s, Elongation 68°C/59 s) and a final Extension at 68°C for 5 min.

Electrophoresis on agarose gel

Agarose gel (1%) for electrophoresis was prepared with 1X TBE buffer with addition of Ethidium bromide (BET) which allowed visualization of the bands in the UV light. An electrophoretic migration at 110 millivolts for 30 min was performed on the PCR products using a molecular weight marker (1 kb). The fragments were visualized under UV light (Gene Flash) and the images were recorded.

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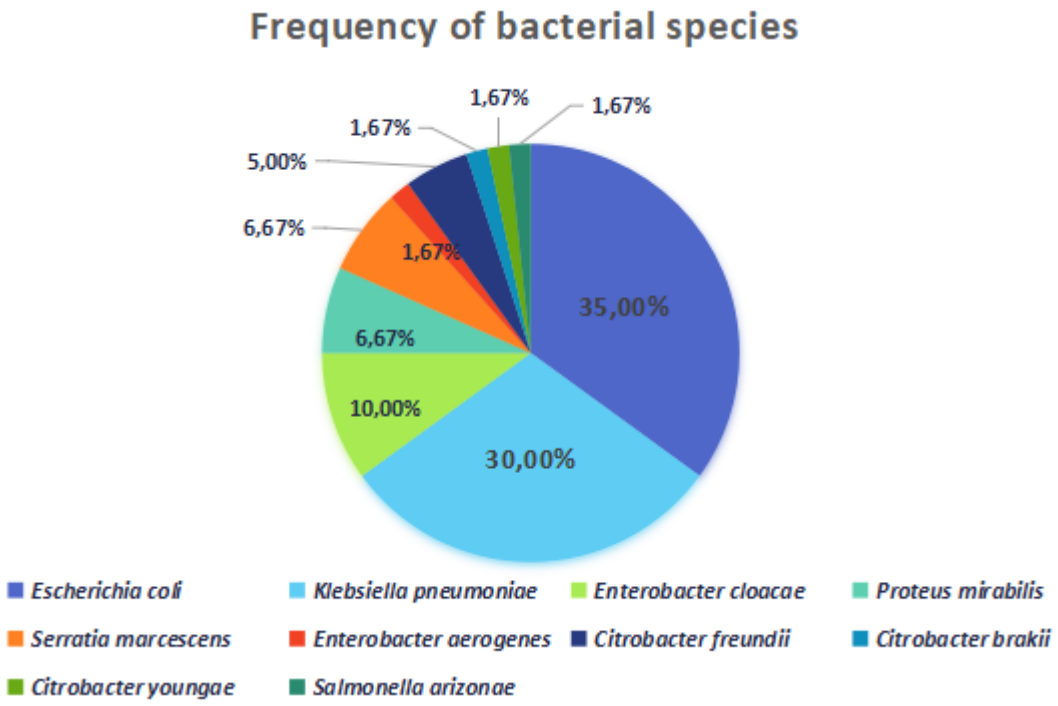

Figure 1. Distribution of enterobacterial isolates by size.

Data processing

The clinical data was entered on Excel 2013 and then analyzed with the Standard Statistical Package for Social Sciences (SPSS) version 17.0 for Windows and the EPI Info version 6.0 software. All tests of significance were considered statistically significant at P-value < 0.05.

RESULTS AND DISCUSSION

In this study, out of 250 strains isolated, 60 (24.09%) of Enterobacteriaceae were resistant to at least one third generation cephalosporin or azthreonam and Amoxicillin + Clavulanic acid. The distribution of strains involved in bacterial resistance by ESBL production is presented by Figure 1.

Results for the sensitivity/resistance of the 60 enterobacterial isolates to the different antibiotics tested showed that 46 strains (76.6%) were resistant to Cefotaxime, 45 strains (75%) were resistant to Ceftriaxone, 42 strains (70%) were resistant to Aztreonam and 35 strains (58.3%) were resistant to Ceftazidime. Resistance profile by bacterial strain were shown in Table 1. It was noticed that all strains were resistant to Amoxicillin + Clavulanic acid (Augmentin®).

Molecular characterization of the ESBLs by PCR revealed that 38 (63.3%) strains carried the *bla*$_{BES}$ gene as shown by the electrophoresis bands (Figure 2). The distribution of the *bla*$_{BES}$ gene according to the bacterial species is presented in Table 2.

These results are comparable to those found in some studies, particularly those found at the Laghouat Hospital (Algeria): 43% *Escherichia coli*, 30% *Klebsiella pneumoniae* and 20% *Enterobacter cloacae* (Lagha, 2015). Other studies that was done at Charles De Gaulle Paediatric Teaching Hospital (CHUP/CDG) of Ouagadougou (Burkina faso), showed 47.22% of *Escherichia coli*; 15.55% *Klebsiella pneumoniae* and 3.33% *Klebsiella oxytoca* (Metuor-Dabire, 2014). These prevalences of ESBLs produced by *Escherichia coli* and *Klebsiella pneumoniae* were also described in South America (45.4 to 51.9%) (Villegas et al., 2008) and Saudi Arabia (55%) (Al-Agamy et al., 2009).

These results confirm that the overall prevalence of ESBL production by Enterobacteria fluctuates considerably according to the geographical zones, to the countries and to different hospitals. However, the bacterial strains mainly concerned by antibiotic resistance were *E. coli* and *K. pneumoniae* with the high level of ESBL production.

The antibiotic susceptibility profile of the 60 strains tested showed resistance to most of β-lactams antibiotics (Table 1): 46 strains (76.6%) were resistant to Cefotaxime, 45 strains (75%) were resistant to Ceftriaxone, 42 strains (70%) were resistant to Aztreonam and 37 strains (58.3%) were resistant to Ceftazidime. These levels of antibiotic resistance in the study could be explained by the misuse of antibiotics in animal and human health or because of the poor living and hygiene conditions observed in developping

Table 1. Resistance profile of different isolates to antibiotics used.

Bacterial strain	ATM	CAZ	CTX	CTR	Synergy image
Escherichia coli	13	12	15	15	4
Klebsiella pneumoniae	12	8	11	12	0
Enterobacter cloacae	6	4	4	4	0
Proteus mirabilis	0	0	2	2	2
Serratia marcescens	3	3	3	3	0
Enterobacter aerogenes	0	1	0	0	0
Citrobacter freundii	1	2	3	1	0
Citrobacter brakii	0	0	0	1	0
Citrobacter youngae	0	0	0	0	1
Salmonella arizonae	1	0	1	0	0
Total	36	30	39	38	7

ATM = Aztreonam, CTX = Cefotaxime, CTR =Ceftriaxone, CAZ = Ceftazidime.

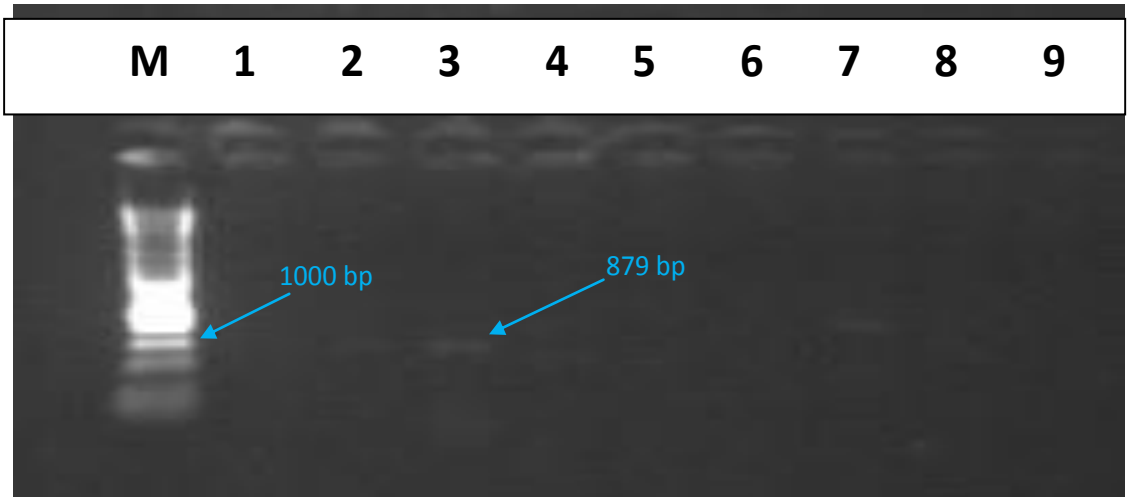

Figure 2. Agarose gel image showing PCR products of *bla*$_{BES}$ gene in identified isolates. Lane (**M**) = Molecular Weight Marker (DNA Ladder (1kb)); Lane (**1-8**) = Samples; Lane (**9**) = Negative control; Lane (**3**) = Positive to *bla*$_{BES}$ gene.

Table 2. Prevalence of the *bla*$_{BES}$ gene according to bacterial species.

Bacterial species	BES gene
Escherichia coli	13
Klebsiella pneumoniae	10
Enterobacter cloacae	5
Proteus mirabilis	3
Serratia marcescens	2
Enterobacter aerogenes	1
Citrobacter freundii	2
Citrobacter brakii	1
Citrobacter youngae	1
Salmonella arizonae	0
Total	38

countries (Ouédraogo, 2017). It is currently admitted that the use of antibiotics, including third-generation cephalosporins for therapeutic purposes is the most important risk factor in the development of bacterial resistance (Metuor-Dabire, 2014).

Others types of resistance mechanisms could explain these levels of antibiotic resistance like the modification of the membrane permeability, the modification of the antibiotic target, the metabolic pathway change or the efflux phenomena.

The molecular characterization of the 60 bacterial strains by PCR revealed the BES gene in 38 (63.3%). Few studies have evaluated the prevalence of the bla_{BES} gene in ESBL-producing Enterobacteria. The bla_{BES} gene was first described in *Serratia marcescens* in Brazil in 1996 (Bonnet et al., 2000). This strain had a very high level of resistance to Aztreonam; distinctly high to Cefotaxime than to Ceftazidime. In this study, 21 strains, more than 55% of the strains carried the BES gene were resistant to Aztreonam. Philippon found that BES-1 ESBLs, characterized by a high level of resistance to Ceftazidime and sometimes to Aztreonam rather than to Cefotaxime, have a less wide distribution than the CTX-M group (Philippon and Arlet, 2006). The presence of the BES gene in our study could be explained by the variation of ESBLs frequencies between different geographical areas or by the genetic support of BES gene. If it was plasmids or integrons, genes could be easily transmitted between several bacteria. It could also be explained by the spread of this gene in West Africa and at Saint Camille Hospital in Ouagadougou).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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