

Full Length Research Paper

Coexistence between (TOHO-type and BES-type) extended-spectrum β -lactamase genes of identified enterobacteria at Saint Camille Hospital, Ouagadougou, West Africa

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Received 17 September, 2019; Accepted 10 October, 2019.

Coexistence between the TOHO-type and Brazilian extended-spectrum (BES)-type of extended-spectrum β -lactamase (ESBL)-produced by bacteria caused public health issue. Several studies have been reported on the coexistence between blaTEM, blaCTX-M and blaSHV genes in ESBL in broad spectrum enterobacteria. The present study involved the prevalence of coexistence of blaTOHO and blaBES genes in enterobacteria identified in hospitalized and out-patients at Saint Camille Hospital in Ouagadougou (Burkina Faso). Firstly, the study was led by microbiological identification of enterobacteria, secondly antibiogram was performed by diffusion method and finally the molecular characterization was done by conventional polymerase chain reaction (PCR) to search for antibiotic resistance genes blaTOHO and blaBES. The ultraviolet (UV) lamp (Gene Flash) for the photography of gels allowed the visualization of specific bands of TOHO and BES genes. Among 250 strains of Gram negative bacilli collected, 60 strains (24.1%) showed resistance profile to antibiotics used. Molecular characterization showed the coexistence between blaTOHO and blaBES genes in 53.3% in bacteria strains carried by the patients. The highest prevalence was observed in *Escherichia coli* (34.4%) and *Klebsiella pneumoniae* (21.9%) strains. For the first time in Ouagadougou, Burkina Faso, this study therefore established the coexistence between blaTOHO and blaBES genes in ESBL produced enterobacteria at Saint Camille Hospital.

Key words: Antibiotic, resistance, extended-spectrum β -lactamase (ESBL), genes, TOHO, Brazilian extended-spectrum (BES).

INTRODUCTION

Antimicrobial resistance constitutes a growing danger to human health in the whole world, but hospitals are more

at risk in holding it (Bradford, 2001).

World Health Organization (WHO, 2018)'s first

published antimicrobial resistance surveillance data revealed that there are high rates of resistance to many severe bacterial infections in rich and poor nations. Antimicrobial resistance causes around 700 thousand deaths yearly in the globe and affects health cost (Jasovsky et al., 2016). The main mechanism of the antimicrobial resistance is producing extended-spectrum β -lactamases (ESBLs) enzymes by enterobacteria. A lot of research have been done on the main genes producing ESBLs. The commonest ESBLs include temoneira (TEM), sulfhydryl variable (SHV) and cefotaximase-munich (CTX-M) types (Jacoby and, Munoz-Price, 2005).

The TEM-types ESBLs were derived of TEM-1 and TEM-2. The first resistance gene was reported for the first time in 1965 in *Escherichia coli* taken from a patient in Athens (Greece) called temoneira (hence the designation TEM) (Zubair et al., 2015). With punctual mutations, the SHV-types ESBLs were obtained from the original SHV-1 enzyme. These enzymes originated from *Klebsiella pneumoniae* chromosomal penicillinase blaSHV gene variant (Brisse and Verhoef 2001; Haeggman et al., 2004). Actually, over different 180 blaSHV-types ESBLs have been defined globally (Liakopoulos et al., 2016).

CTX-M ESBLs were described for the first time in 1986 in Japan, Germany and France in 1989 (CTX-M-1) and have since expanded widely in the world (Thomson and Moland, 2000). CTX-M was the dominant type of ESBL in the world (Anna et al., 2014). These different types of ESBLs could coexist in certain enterobacteria. The presence of more than two different types of β -lactamases in the same isolates has been reported from India, Germany, Malaysia and Iran (Bora et al., 2014; Schmiedel et al., 2014; Seyedjavadi et al., 2016; Yahya Mohsen et al., 2016).

Apart from the main ESBLs, there were minor types ESBLs such as TOHO-type, BES-type, *Pseudomonas* extended resistance (PER) type, Vietnam extended-spectrum β -lactamase (VEB) type, Guiana extended-spectrum β -lactamase (GES) type, TEM like activity (TLA) type, and *Serratia fonticola* (SFO) type which were less studied (Cattoir, 2008).

TOHO-type ESBL is a variant of CTX-M2c (Andres et al., 2005). The blaTOHO gene has been described for the first time at Toho University School of Medicine (Japan) in the urine of a one-year-old girl in *E. coli* TUH12191 (Ishii et al., 1995). This gene has been notified for the first time in Argentina in *Shigella flexneri* in the stool of a 33-year-old woman (Andres et al., 2005). TOHO-2 ESBL has also been described as produced by *E. coli* TUH1083. It is classified as a β -lactamase like enzyme of TOHO-1 group rather than mutants of TEM or SHV enzymes (Ling et al., 1998). BES-type ESBL is less

distributed and highly resist Ceftazidime, Aztreonam and not Cefotaxime (Bradford, 2001; Arlet and Philippon, 2003). Bacterial strains that carried these genes caused a variety of nosocomial infections and have become a serious problem in clinical practice (Ma et al., 2002).

The prevalence of coexistence between blaTOHO and blaBES genes in ESBL-producing enterobacteria has not been reported in the literature yet. Researches on β -lactamases at Burkina Faso scale were relatively recent and have already identified the presence of TEM, SHV and CTX-M genes, which are responsible for bacterial resistance in enterobacteria (Zongo et al., 2015). The blaTOHO and blaBES genes were not concerned yet. The objective of this work was to detect cases of coexistence between blaTOHO and blaBES genes in enterobacteria that produce ESBLs at Saint Camille Hospital, Ouagadougou, Burkina Faso.

MATERIALS AND METHODS

Type of study

This work is a cross-sectional one done at Saint Camille Hospital, Ouagadougou, Burkina Faso, from September to November 2018. Stool, urine and vaginal swab samples consisted the samples taken from out-patients or hospitalized patients. 250 samples were obtained from 250 patients. Uri Select medium, Hektoen medium and *Salmonella-Shigella* (SS) media were used to inoculate the samples for the growth of Enterobacteria and then incubated at 37°C for 24 h. Later, for antimicrobial analysis, enterobacteria grown on past media were subcultured on a Mueller-Hinton (MH) medium and incubated at 37°C for 24 h (Gaillot et al., 1999).

Antimicrobial assays

Analytical Profile Index (API 20 E) Identification method was used to identify the bacterial strains. Based on the recommendations of the Committee of antibiogram of the French Society of Microbiology (CASFM/EUCAST 2018), antibiotic susceptibility and resistance test were done on Mueller-Hinton (MH) medium with pure colonies of enterobacteria. Amoxicillin + Clavulanic acid (Augmentin), Cefotaxime, Ceftazidime, Ceftriaxone and Aztreonam were the antibiotic discs utilized. All Augmentin resistant enterobacteria and one third generation cephalosporin were seen in this study as the producers of ESBL (Pereckaite et al., 2018).

Molecular characterization of ESBLs

Extraction of bacterial DNAs

To extract DNAs from bacteria boiling method was utilized (Ribeiro Junior et al., 2016). The strains were reactivated by culturing on the MH medium for 18 h at 24 h. A colony isolated was removed from the Petri dish and suspended in 200 μ l distilled water aliquoted previously in Eppendorf tubes. It was immersed in a water bath to

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Frequency of bacterial species

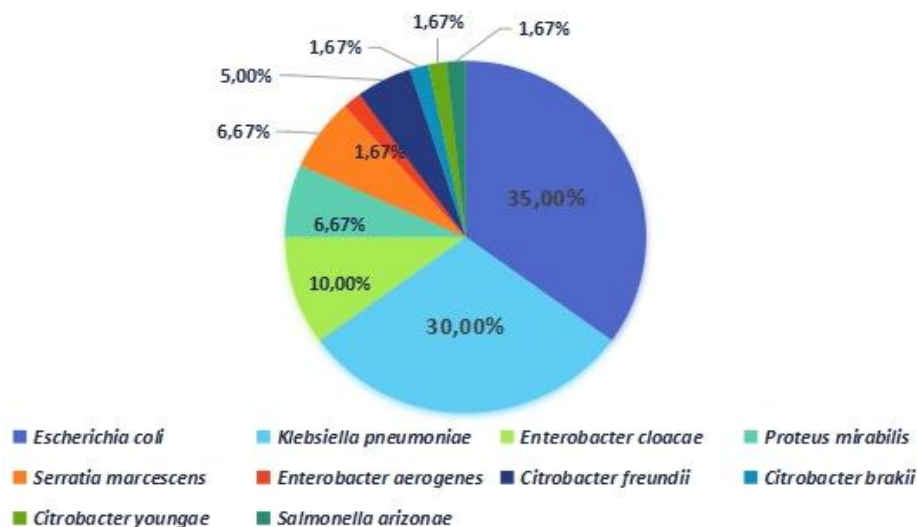


Figure 1. Distribution of bacteria species.

release the genetic material at 100°C for 15 min. Later, the suspension was centrifuged at 12,000 rpm for 10 min and the supernatant having the released DNA was taken to another Eppendorf tube. Nanodrop-type spectrophotometer (BioDrop UV/Vis DUO, Holliston, USA) was used to determine the DNA concentration.

Molecular analysis

25 µl of the Master Mix, DNA and primers (TOHO and BES) constituted the reaction medium for PCR. 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. We used Gene Amp Thermocycler PCR System 9700.

The sequences of the primers used in this study provided by Applied Biosystems were TOHO-1A 5'-ATGTGCAGT ACCAGTAA-3' and TOHO-1B 5'-TAGGTCACCAGAACCAG-3' for blaTOHO with 876 bp as molecular weight (Laurent et al., 2000). For blaBES, we used BES-F 5' TAATAACCCTGACCAAGCCTA 3' and BES-R 5' CCCTTTCAAAGTCATAAATC 3' with 879 bp as molecular weight (Bonnet et al., 2000).

Electrophoresis in agarose gel

Agarose gel (1%) used for electrophoresis was made using 1X TBE buffer by adding 8 µl Ethidium bromide (BET) 0.5 µg/ml. This made the bands to be visualized in the UV light. A molecular weight marker (1 kb) was used to perform an electrophoretic migration (110 mv) for 30 min on the PCR products. UV light (Gene Flash) was to visualize the fragments and the images were recorded (Lee et al., 2012).

Data processing

The hospital data were entered in Excel 2013 and Standard Statistical Package for Social Sciences (SPSS) version 17.0

for Windows and the EPI Info version 6.0 software were used to analyze the data. All tests were statistically significant at P-value < 0.05 (WHO, 2019).

RESULTS

A total of 250 samples constituted by 169 stools, 79 urines and 2 vaginal swab samples were collected. Out of 250 samples collected, 60 (24.09%) had shown an antibiotic resistance profile by ESBL production. Among these 60 patients 26 were male and 34 were female, with a sex ratio of 0.75. The ages ranged from 16 months to 95 years with an average age of 36 years. There were 28 hospitalized patients and 32 out-patients.

Figure 1 shows the distribution of strains taking part in bacterial resistance by ESBL production.

Results for the sensitivity/resistance of the 60 enterobacteria isolates to the antibiotics tested revealed that 46 strains (76.6%) resist Cefotaxime, 45(75%) resist Ceftriaxone, 42(70%) resist Aztreonam and 35 (58.3%) resist Ceftazidime. Table 1 shows the resistance profiles by bacterial strains. All the strains resist Amoxicillin + Clavulanic acid (Augmentin®).

Molecular characterization of the ESBLs by PCR showed that 32 (53.3%) strains were carried blaTOHO and blaBES genes in the same time as displayed by the electrophoresis bands (Figure 2). The distribution to coexistence of blaTOHO and blaBES genes according to the bacterial species is shown in Table 2.

DISCUSSION

The findings are similar regarding the prevalence of

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

Bacterial strain	% ATM	% CAZ	% CTX	% CTR	% Synergy image
<i>Escherichia coli</i>	35.72	34.29	34.78	37.78	57.14
<i>Klebsiella pneumoniae</i>	33.33	28.58	30.44	33.34	0
<i>Enterobacter cloacae</i>	14.29	14.29	8.70	8.89	0
<i>Proteus mirabilis</i>	0	0	4.35	4.44	28.57
<i>Serratia marcescens</i>	9.52	11.43	8.70	8.89	0
<i>Enterobacter aerogenes</i>	2.38	2.85	2.17	2.22	0
<i>Citrobacter freundii</i>	2.38	5.71	6.52	2.22	0
<i>Citrobacter brakii</i>	0	0	2.17	2.22	0
<i>Citrobacter youngae</i>	0	0	0	0	14.29
<i>Salmonella arizonae</i>	2.38	2.85	2.17	0	0

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

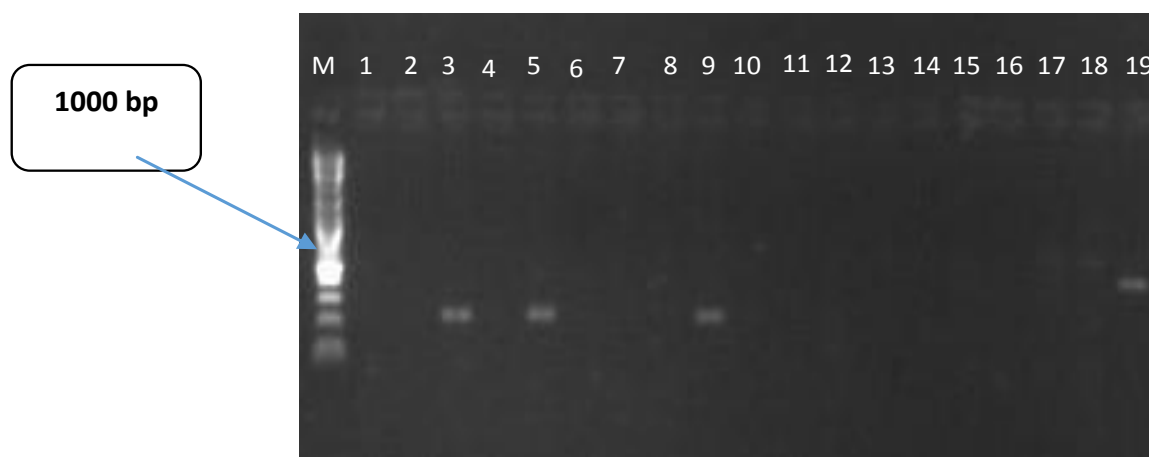


Figure 2. Agarose gel image showing PCR products of *bla*_{TOHO} and *bla*_{BES} genes in identified isolates. Lane (M) = Molecular Weight Marker (DNA Ladder (1kb)); Lane (1-19) = Samples; Lane (10) = negative control; Lanes 3, 5 and 9 = positive to *bla*_{TOHO} gene (mw = 876 bp); Lane 19 = positive to *bla*_{BES} gene (mw = 879 bp).

Table 2. Prevalence to coexistence of *bla*_{TOHO} and *bla*_{BES} genes according to the isolated bacterial species.

Bacterial strain	Coexistence of <i>bla</i> _{TOHO} and <i>bla</i> _{BES} genes	%
<i>Escherichia coli</i>	11	34.37
<i>Klebsiella pneumoniae</i>	7	21.87
<i>Enterobacter cloacae</i>	4	12.5
<i>Proteus mirabilis</i>	3	9.37
<i>Serratia marcescens</i>	2	6.25
<i>Enterobacter aerogenes</i>	1	3.13
<i>Citrobacter freundii</i>	2	6.25
<i>Citrobacter brakii</i>	1	3.13
<i>Citrobacter youngae</i>	1	3.13
Total	32	100

some strains that cause resistance to antibiotic as seen in other works, especially those at Laghouat Hospital, Algeria; 43% *E. coli*, 30% *K. pneumoniae*, 20%

Enterobacter cloacae and 7% *Citrobacter freundii* (Lagha, 2015). Other studies that were done at the Charles De Gaulle Paediatric Teaching Hospital (CHUP/CDG) of

Ouagadougou, Burkina Faso revealed 47.22% of *Escherichia coli*; 15.55% *K. pneumoniae* and 3.33% *Klebsiella oxytoca* (Mètuor-Dabiré, 2014). The types of ESBLs seen in these works were CTX-M, SHV and TEM. The prevalence of ESBLs produced by *Escherichia coli* and *K. pneumoniae* was studied in South America (45.4 to 51.9%) (Villegas et al., 2008) and Saudi Arabia (55%) (Al-Agamy et al., 2009).

These findings show that the total occurrence of ESBLs produced by enterobacteria varies greatly based on geographical zones, nations and various clinics. However, *E. coli* and *K. pneumoniae* with high level of ESBL production were the bacterial strains mainly concerned with antibiotic resistance (Lagha, 2015; Villegas et al., 2008).

The antibiotic susceptibility profile of the 60 strains tested revealed resistance to most β -lactams antibiotics: 46 strains (76.6%) resist Cefotaxime, 45 (75%) resist Ceftriaxone, 42(70%) resist Aztreonam and 37 (58.3%) resist Ceftazidime. These could be attributed to improper use of antibiotics. Currently, it is shown using antibiotics, plus third-generation cephalosporin for treatment is very risky in the advancement of bacterial resistance (Mètuor-Dabiré, 2014). Other types of resistance mechanisms can cause these levels of antibiotic resistance such as modifying the permeability of the membrane, modifying antibiotic target, changing metabolic pathway or the efflux phenomena (Munita and Arias, 2016).

The molecular characterization of the 60 bacteria strains by classical PCR revealed the coexistence of TOHO and BES genes in 32 (53.3%) of enterobacteria strains, which makes antibiotic resistance more serious. The antibiotic resistance profile of these different bacteria strains with TOHO and BES genes coexistence was not significantly different from the antibiotic resistance profile of the bacteria strains producing the TOHO and BES enzymes separately in the present study.

TOHO-1 enzymes have been described for the first time in Japan (Tetsuya et al., 1997) and were structurally very close to CTX-M and were therefore classified among this group (Bonnet et al., 2004). This type of ESBL (CTX-M) was frequently encountered in hospitals (Paterson and Bonomo, 2005). This could explain the presence of TOHO enzymes in the present study. The first detection of TOHO-1 outside Japan was reported by Andres (2005) in *S. flexneri* in the stool of a 33-year-old woman in Argentina (Andres et al., 2005). This bacterial strain expressed an enzyme belonging to CTXM2c whose DNA sequencing gave TOHO-1. There were two types of TOHO enzymes (TOHO-1 and TOHO-2) and their precise prevalence has never been reported in an epidemiological study and the truth of the sequence has been questioned because it was so closely related to CTX-M-2 (Hawkey, 2008).

BES-1 was distantly related to either the CTX-M or GES-1 enzyme that has been isolated from South American isolates. The origin of BES-1 thus remains

unknown (Bonnet et al., 2000).

BES-1 was first described in *Serratia marcescens* in Brazil in 1996. This strain (*S. marcescens*) had a very high level of resistance to Aztreonam; distinctly high to Cefotaxime than to Ceftazidime (Bonnet et al., 2000).

In the present study, 21 strains, more than 55% of the strains carrying 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 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 (Lagha, 2015).

In the presence of coexistence between blaTOHO and blaBES genes in the present study, about antibiotic-resistant bacteria strains could be explained by the spread of these genes in Africa and at Saint Camille Hospital in Ouagadougou, HOSCO (Burkina Faso) or through improper use of antibiotic in animal and human health. The genetic support of TOHO and BES genes carried by enterobacteria in the present study if there were plasmids or integrons could be easily transmitted between several bacteria. Most studies found in the literature have described the isolated presence of the TOHO and BES genes in ESBL-producing enterobacteria (Tatsuro et al., 2002; Liakopoulos et al., 2016).

The present study did not show a significant association between TOHO and BES genes with a particular bacterial species. The distribution of these coexistences was homogeneous in all the bacterial strains of our study (Table 2). *E. coli* and *K. pneumoniae* were the bacterial species which showed in the study the most prevalence of coexistence genes (TOHO and BES).

The study of Ibrahimagic et al. (2017) showed the coexistence of more than two types of β -lactamases detected in 77.3% of in-patients and 45.2% in out-patients. Among the isolates of the in-patients, *Klebsiella* species and *E. coli* were the most common to produce more than two types of genes, respectively in 65 and 12% of cases. These results showed that the coexistence of β -lactam resistance genes is frequent but their distribution between bacteria like *K. pneumoniae* and *E. coli* is not homogeneous depending on the geographical area (Canton et al., 2012). Others studies have found 11 multidrug-resistant ESBL-producing strains harboring both CTX-M and SHV as well as TEM and SHV (Mètuor-Dabiré et al., 2018). Our study is the first to have highlighted the coexistence between two types of ESBL, TOHO and BES.

CONCLUSION

This study made it possible to know the distribution of the coexistence of certain antibiotic resistance genes (TOHO

and BES) in Enterobacteriaceae encountered at Saint Camille Hospital in Ouagadougou (Burkina Faso). It also revealed that alongside the CTX-M, TEM and SHV genes, there were other rare types such as TOHO and BES which coexist in Burkina Faso.

For an effective fight against the emergence and diffusion of these bacterial strains multi-resistant to antibiotics, we recommend the population to use antibiotics in a reasonable way and the proper use of hygiene measures at hospitals and doctors should monitor treatment with antibiotics. Our future investigations will focus on the sequencing of the coexistence genes of BES and TOHO.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to thank the CERBA/LABIOGENE, University Joseph Ki-Zerbo. They also express their deep gratitude to the Italian Episcopal Conference (IEC).

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