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Molecular Characterization of *bla*$_{CTX-M-15}$ Gene Among *bla*$_{TEM}$ and *bla*$_{SHV}$ Variants Isolated on Gram Negative Bacilli Collected in Emergency Units at University Hospital Center, Yalgado Ouedraogo, Burkina Faso

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A B S T R A C T

Extended-spectrum β-lactamase producing Gram negative bacilli constitute a global public health threat. The ESBLs CTX-M, TEM and SHV remain the most frequently isolated worldwide, with high proportions of CTX-M reported and widely distributed in pathogens. Several authors report that the bacterial strains harboring the CTX-M-15 gene are generally multidrug-resistant strains. This study aim to identify the variants of *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ hosted by Gram negative bacilli at hospital center Yalagdo Ouédraogo. This study take on bacterial strains collected in a previous study (DOI: 10.5897/AJB2014.13908). Bacterial strains had been isolated from biological samples such as stools, urine, blood, cerebrospinal fluid, effusion fluids (pus, ascitic fluid, pleural fluid...) and genital swabs.. During this previous study, 259 strains resistant to at least one third-generation cephalosporin had been collected and identified using API20E. This study had shown that some of these bacterial strains harbor *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$. For the present study, from a young bacterial culture, genomic DNA extraction was performed by the boiling method and conventional PCR was used to confirm the presence of resistance genes. PCR products from a total of 17 bacterial strains were subjected to Sanger sequencing for genes variants determination. In the previous study, among 259 strains collected, molecular characterization had shown that 112 strains (65.49%) were positive for *bla*$_{CTX-M}$ screening; 44 strains (25.73%) for *bla*$_{TEM}$ screening and 32 strains (18.71%) for *bla*$_{SHV}$. For the present study, among 17 bacterial strain consisting to 7 *Escherichia coli*, 4 *Klebsiella pneumoniae*, 3 *Pseudomonas aeruginosa*, 1 *Enterobacter sp*, 1 *Citrobacter sp* and 1 *Salmonella tiphy*, *bla*$_{CTX-M-15}$ and *bla*$_{SHV-28}$ genes were found respectively on 82.35% and 5.88% of all bacterial strains analyzed, while the *bla*$_{TEM-1}$ and *bla*$_{SHV-11}$ were each harbored by 23.53% of the isolates. The current situation of bacterial resistance remains dominated by the spread of broad-spectrum beta-lactamases. A high proportion of bacterial strains studied resist by production of ESBLs type CTX-M-15. Measures must be implemented to slow the emergence and spread of ESBL-producing Gram-negative bacilli through the appropriate use of antibiotics.

Introduction

In the 80s, several resistant mutants emerged in bacterial species. Indeed, strains of *Escherichia coli* like those of *Klebsiella pneumoniae* initially resistant to penicillins have become producers of evolved penicillinases allowing them to resist third generation cephalosporins.These new forms of enzyms named Extended Spectrumβ-Lactamases (ESBL) first described, derived of parental TEM and SHV (Mutuku *et al.,* 2022).

In the 90s, emerged new enzymes called CefoTaXimases-München (CTX-M) whose host bacterial strains preferentially hydrolyze cefotaxime (Nicolas-Chanoine 2012; Sarr *et al.,* 2023).

The massive use of third-generation cephalosporins has favored the growth of CTX-M type ESBLs which have now supplanted TEM and SHV type and have become the dominant ESBLs. (Ahmad *et al.,* 2021). Many studies reported that each main cluster of CTX-M genotypes has a progenitor sharing homology with chromosomal genes on *Kluyvera spp* from which $bla_{\text{CTX-M}}$ genes originated (Bevan *et al.,* 2017). CTX-M genotypes have become globally disseminated, with $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$ being the predominant genotypes. CTX-M ESBLs have increased in prevalence since 2000 (Woerthe *et al.,* 2013). ESBL genes are carried by various mobile genetic elements such as transposons, insert sequences, plasmids which facilitate their dissemination in several bacterial species. Several studies have shown that plasmids of the IncF family are the predominant group that carry $bla_{\text{CTX-M-15}}$ (Dhanji *et al.,* 2011; Del carmen rocha-gracia *et al.,* 2022). CTX-M-15, like other class A enzymes, employs an acylation-deacylation mechanism to hydrolyze β-lactam ring, utilizing four invariant residues: Ser70, Lys73, Ser130, and Glu166 (Tooke *et al.,* 2019). The concern linked to the spread of CTX-M-15 gene is that it is often mobilized associeted with other antimicrobial resistance genes like bla_{OXA} or those encoding carbapenemases (Potron *et al.,* 2013).

Investigating on bacterial resistance through β-lactamase production, particularly through CTX-M-15 production, is a topic of interest. Several studies conducted in Africa have revealed bacterial resistances through the production of extended-spectrum β-lactamases. In Cameroon, a study conducted by Lonchel and collaborators reported high proportions of strains carrying the gene CTX-M-15 among *enterobacteriaceae* isolates (Lonchel *et al.,* 2013). Genes of ESBL, particularly CTX-M-15 and SHV-12, have also been reported in Tanzania; these genes were isolated from hospital strains (Blomberg *et al.,* 2005). In West Africa, extensive studies on ESBLs including the TEM, SHV, and CTX-M types, have already been conducted in several countries such as Benin, Nigeria and Senegal (Ahoyo *et al.,* 2007; Yushsha'u *et al.,* 2007; Harrois *et al.,* 2013). In Burkina Faso, the description of ESBLs genes, although fairly recent, is very abundant. Bla_{TEM}, bla_{SHV} and bla_{CTX-M} have been described on Gram negative bacilli isolated from hospital Center (Mètuor Dabiré *et al.,* 2014; Zongo *et al.,* 2015; Dembélé *et al.,*2020) and from environment (Muhigwa *et al.,* 2023). $Bla_{CTX-M-15}$ was first described in Burkina Faso on *Enterobacteriacea* among clinical isolates (Ouédraogo *et al.,* 2016)

In view of the above, our study aimed to contribute to providing information on β-lactamase gene variants, particularly $bla_{\text{CTX-M}}$ gene variants disseminated in our hospitals.

Materials and Methods

Bacterial cultures

LB (Luria-Bertani) broth was used for the culture of strains conserved in LB-glycerol broth. For that, a volume of 10 µL of suspension of each bacterial strain initially conserved in LB-glycerol was used to inoculate 4 mL of LB contained in a sterile test tube, then incubated at 37°C for 24 hours. From this culture 2µl were used to inoculate MH (Mueller-Hinton) agar in Petri dishes which were incubated for 24 hours. The pure bacterial colonies obtained were used for subsequent DNA extraction.

DNA extraction and amplification of bla gene

For molecular characterization total genomic DNA was obtained using a boiling method (Ribeiro Junior *et al.,* 2016). Single colony was suspended in a total volume of 100 µL of distilled water. Bacterial cell suspension was heated at 100°C, during 10 minutes. After boiling, bacterial suspension was placed in ice bath and centrifugated at 13000 g for 5 minutes. The resulting supernatant was used as a DNA solution. The DNA concentration was then determined using the Nanodrop spectrophotometer. A portion of the supernatant was used for amplification and the remainder was stored at -80 °C.

Amplification of DNA was performed with primers supplied by Promega under following sequences indicated in Table 1.

PCR Conditions

Mixed PCR for one reaction has the following components: 5 µl of Green buffer 5X; 1 µl of each Primer 10µM; 0.65µl of dNTPs 10 mM; 0.12 µl of Gotaq 0.5 U/µl and 15.25 µl of purified water. Reaction volume is completed to 25 µl with 2 µl of DNA template Primers supplied by Promega according to each β-lactamase gene type are in Table 1. PCR was carried out under the following conditions on SensoQest Labcycler, GmbH, Germany: initial denaturation step at 96°C for 5 min, followed by 35 cycles consisting of denaturation at 96°C for 1 min, annealing at 58°C, 60°C and 50°C for TEM, SHV and CTX-M at 1 min, primer extension at 72°C for 1 min and final extension for 10 min. Migration of PCR products watched by Green buffer (Eugentec) was performed on agar gel of prepared with BET at 0. 25% as final concentration.

Agarose Electrophoresis Gel and Visualization

To ascertain the presence of these genes, PCR products were analyzed by electrophoresis using 1.5% agarose gel. For that, 1.5g of agarose was dissolved in 100mL of a 1.5X Tris-Acetate-EDTA solution, then heated in the microwave for 5 minutes. After heating and slight cooling, a volume of 5µL of ethidium bromide (BET) was added. The gel obtained was then poured before solidification, into an electrophoresis tank each time with appropriate combs used to dig wells for depositing the samples. To assess the expected sizes of the bands, migration of PCR products was carried out for at 90 V for 45 minutes along with a 100 bp molecular ladder (Inqaba Biotec, Ibadan, Nigeria) and intern quality control samples. Electrophoresis gel was visualised under UV light using a gel documentation UVP PhotoDoc-lt Imaging System (Inqaba Biotec, Pretoria, South Africa).

Purification of PCR products

Evry sample of DNA was subjected to several PCR process in order to get sufficient quantity for the sequencing. Purification of PCR products was realized using kit of NucleoSpin® Gel and PCR Clean-up (Taraka Bio USA, Inc.) that allows to remove any contaminations. For this purpose, 40µL of each PCR product was homogenized in 80µL of binding buffer NTI. This mixture was centrifuged 11000 g during 30 seconds into NucleoSpin® Gel and PCR Clean-up column allowing DNA to bind silicate membrane of the column contained in a collecting tube. Contaminations were removed by two centrifugations 11000 g, 30 s with 700µL of ethanolic wash buffer NT3. After drying step of silicate membrane, pure DNA incubated 1min using 30µL of alkaline buffer NE was eluted during last centrifugation 11000 g, 1min. Just as at the end of the extraction, the pure DNA of each PCR product was quantified with Nanodrop spectrophotometer.

DNA Sequencing

Twenty-seven (27) amplicons from 17 bacterial strains hosting either $bla_{\text{CTX-M}}$, bla_{TEM} or bla_{SHV} gene were subjected to Sanger *sequencing*). PCR products comprising the coding regions of the different genes were sequenced using the following primers: TEM-F and TEM-R for blaTEM, SHV-F and SHV-R for blaSHV, CTX-M-F and CTX-M-R for blaCTX-M. The "R" primers anneal at the 5' end, within the coding region and the "F" primers anneal at the 3' end within the coding region of each gene. Thus, sequencing in both directions made it possible to obtain the complete sequence of each gene and to correct sequencing errors. The sequencing of the different genes was carried out by *GATC Biotech* (http://www.gatc-biotech.com/fr/home. Bioinformatic analysis of the sequences was carried out using ClustalW algorithm in *Alignment Explorer of* main MEGA.6 window (Aligning Sequences) and blastn software available on https://blast.ncbi.nlm.nih.gov/.

Statistical Analysis

The collected data were processed with Excel 2016. Antibiotic resistance rates were calculated using the formula P = 100n/N (n being the number of antibiotic-resistant strains and N the total number of strains studied). The same formula was used to calculate the prevalence of each gene as well as the prevalence of gene coexistence (n being the number of strains carrying the gene and N the total number of strains carrying resistance genes).

Results and Discussion

During the previous study, at the end of isolation, identification, and antibiotic sensitivity testing, resistant

bacterial strains collected are distributed among the bacterial species as follows: *Escherichia coli* 50.97% (n=132), *Klebsiella pneumoniae* 16.60% (n=43), *Pseudomonas aeruginosa* 13.13% (n=34), Enterobacter sp 9.65% (n=25), *Citrobacter sp* 4.23% (n=11), *Acinetobacter baumannii* 2.70% (n=7), *Proteus mirabilis* 2.31% (n=6), and *Salmonella Typhi* 0.4% (n=1). Among these strains, 171 resistant isolates were confirmed as ESBLs producers by synergy test. High proportions of these isolates were resistant to cefotaxime (71.36%), ceftriaxone (40.96%) and ceftazidime. (20.26%). Most proportions of bacterial strains resistant to cefotaxime were observed in *Escherichia coli* (91.30%) and *Klebsiella pneumoniae* species (66.66%).

Amplification of β-lactamase-encoding genes

Electrophoretic migration, using molecular weight markers, allowed to isolate the three types of genes sought localized at 1000 bp for bla_{TEM}, bla_{CTX-M} and at arroun 875 bp for bla_{SHV}.(Pagani *et al.,* 2003) (Figure1, Figure2)

Molecular characterization of bla_{CTX-15}

In order to identify variants of the bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes harbored by our isolates, twenty seven (27) PCR products were subjected to sequencing. Sequence mapping (identification of start and stop codons) allowed to determine the coding portion of the isolated genes. For the search of variant forms of the bla_{CTX-M} gene, the alignment (using the MEGA.6 software) of the sequences corresponding revealed almost identical nucleotide sequences on the coding sections consisting of 875 nucleotides for fourteen PCR products (figure 3). Note that the nucleotide sequences of two CTX-M amplicons appeared truncated and were removed from the analysis.

Resistance profile and frequency of the investigated bacterial species

Among the microbial agents responsible of infections,Gram negative bacteria hold a significant place. Infections by these pathogens significantly increases hospitalization time, morbidity, mortality and significant economic costs due to treatment failures (Wang *et al.,* 2022). In our study, enterobacteria particularly *Escherichia coli* and *Klebsiella pneumoniae* represent the mains multiresistant bacteria. A similar results was obtained in India: *E. coli* 73.53% and *K. pneumoniae* 75% (Tsering *et al.,* 2009). These species are also enterobacteria currently isolated in urines samples. The anatomical proximity of the urogenital and anal tracts explains the recurrent transit of enterobacteria from one tract to the other. In urinary tract, *Echerichia coli* strains appear to be the best adapted to the hostility of the bladder which concentrates wide diversity of antibiotics. Most part of these bacterial strains resist to oxyimino cephalosporin like cefotaxime and ceftriaxone which are the antibiotics most suited to the purchasing power of the majority. Unfortunately, their inappropriate use is the cause of the observed resistance (Doma *et al.,* 2020). Besides enterobacteria, strains of *Pseudomonas aeruginosa*, which is a non-fermenting Gram-negative bacteria, were also investigated during our study. Among them, 83.33% and 20.58% were respectively resistant to ceftriaxone and ceftazidime. Long regarded as an opportunistic bacteria, *Pseudomonas aeruginosa* is now described as a nosocomial bacteria affecting immunocompromised subjects in intensive car units.

It exhibits a natural resistance to penicillins, first and second generation cephalosporins, and an acquired resistance to third generation cephalosporins (Kouame *et al.,* 2016). In Côte d'Ivoire, a study conducted by Guessennd and his colleagues reported that 21% of the strains of *Pseudomonas aeruginosa* responsible for postoperative infections were resistant to ceftazidime (Guessennd *et al.,* 2005). For all bacterial strains invsetigated, phenotypic resistances observed on the Petri dishes have been irrefutably confirmed by the molecular characterization of the resistance genes.

Variants of β-lactamase genes isolated

During our previous study, *bla* genes of the TEM type were found in 44 strains (25.73%), whereas those of the blaSHV type were isolated from 32 strains (18.71%). The prevalence of the CTX-M type appears to be higher. It was isolated from 112 samples, representing 65.46% of the strains. These prevalences of *bla* genes are lower than to those of Meeta and collaborators who reported bla_{CTX-M} (82.5%), bla_{TEM} (67.5%) and bla_{SHV} (57.5%) (Meeta *et al.,* 2013) among Gram-negative bacilli isolated at NIMS hospital in India. The sequencing of the previously isolated *bla* genes revealed that our bacterial strains harbor simple β-lactamase genes (TEM-1, TEM-198, SHV1, SHV11) and extended-spectrum β-lactamase genes (SHV28, CTX-M-15).

Table.1 Primers used for amplification of blaTEM, blaSHV and blaCTX-M

bla genes	Primers	Sequences (5'-3')	Amplicon size	References
blaTEM	TEM-F TEM-R	ATAAAATTCTTGAAGACGAAA GACAGTTACCAATGCTTAATCA	1080bp	(Olivier *et al.*, 2002)
blaSHV	SHV-F SHV-R	ATGCGTTATATTCGCCTGTG TTAGCGTTGCCAGTGCTC	875bp	(Olivier *et al.*, 2002)
blaCTX-M	CTX-F CTX-R	GTTACAATGTGTGAGAAGCAG CCGTTTCCGCTATTACAAAC	1041bp	(Pagani *et al.*, 2003)

Table.2 Variants of *bla* genes harbored by studed isolates

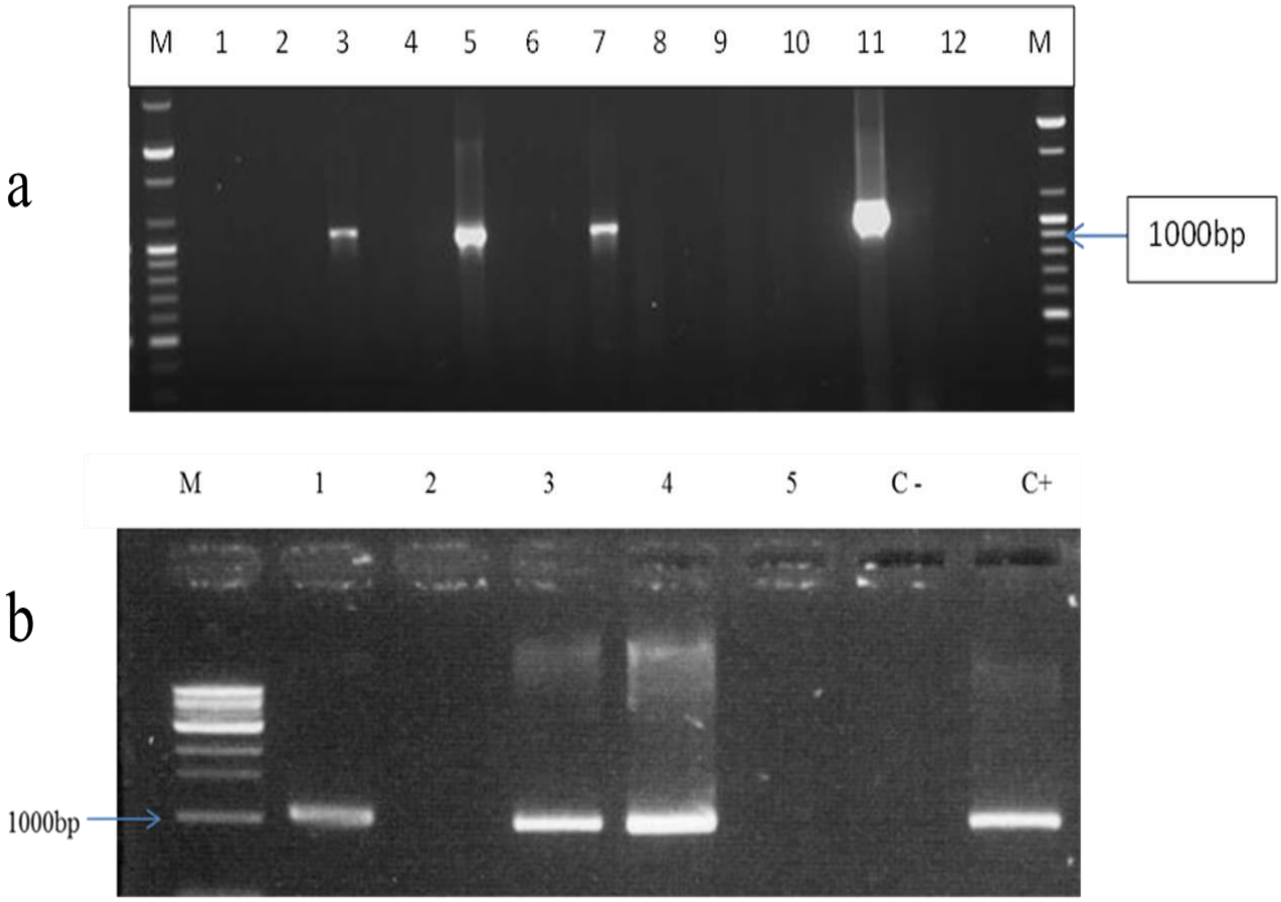
Number of bacterial strains	Identity of strain	Gene *bla* harboring	Results of gene *bla* sequencing
273H	*K. pneumoniae*	*CTX-M*	*CTX-M-15*
450H	*K. pneumoniae*	*TEM, SHV, CTX-M*	*TEM-198,*
			SHV-28
			CTX-M-15
714 Uro	*E. coli*	*TEM, CTX-M*	*TEM-1,*
			CTX-M-15
1226 Uro	*E. coli*	*TEM, CTX-M*	*TEM-1*
			CTX-M-15
681Uro	*K. pneumoniae*	*TEM, CTX-M*	*TEM-1,*
982 Uro	*P. aeruginosa*	*SHV*	*SHV-1,*
93P	*P. aeruginosa*	*SHV, CTX-M*	*SHV-11,*
			CTX-M-15
224P	*Enterobacter sp*	*SHV, CTX-M*	*CTX-M-15*
			SHV-11,
320Uro	*E. coli*	*CTX-M*	*CTX-M-15*
1028Uro	*Citrobacter sp*	*CTX-M*	*CTX-M-15*
565P	*E. coli*	*CTX-M*	*CTX-M-15*
751Uro	*E. coli*	*SHV, CTX-M*	*SHV-11*
632Uro	*K. pneumoniae*	*SHV, CTX-M*	*CTX-M-15*
			SHV-11
176 Uro	*Salmonella* Typhi	*CTX-M*	*CTX-M-15*
763Uro	*P. aeruginosa*	*CTX-M*	*CTX-M-15*
241Uro	*E. coli*	*CTX-M*	*CTX-M-15*
114P	*E. coli*	*TEM, CTX-M*	*CTX-M-15*
			TEM-1

The frequencies of the different variants of the investigated *bla* genes is recorded in table 3

Table.3 Distribution of *bla* genes variants identified

***Bla* gene isolated**	**Variants of betalactamase genes**	**Incidence (%) on *bla* gene investigated**	**Prévalence (%) in bacterial strains investigated**
***bla*TEM**	*bla*TEM-1	80	23.53
	*bla*TEM-198	20	5.88
***bla*SHV**	*bla*SHV-1	16.67	5.88
	*bla*SHV-11	66.67	23.53
	*bla*SHV-28	16.67	5.88
***bla*CTX-M**	*bla*CTX-M-15	87.50	82.35

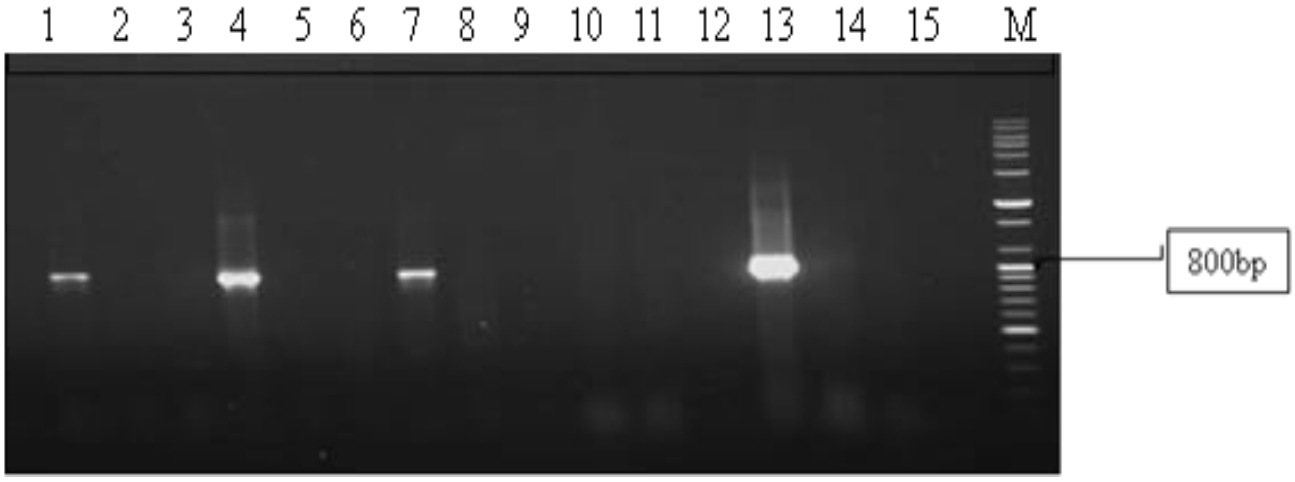
Figure.1 Bands corresponding to *bla*$_{TEM}$ *and bla*$_{CTX-M}$ *genes*



bla$_{TEM}$ gene, numbers: 1 to 10 correspond to the samples, 11 = positive control and 12 = negative control, M= GeneRuler 100bp DNA Ladder

bla$_{CTX-M}$ gene, numbers: 1 to 5 correspond to the samples: 1 = 45 H, 2 = 219P, 3 = 310 P, 4 = 315 Uro, 5 = 225 H; C + = positive control and C- = negative control, M= GeneRuler 100bp DNA Ladder

Figure.2 Bands corresponding to *bla*$_{SHV}$



bla$_{SHV}$ gene: 1 = positive control and 2 = negative control, numbers : 3 to 15 correspond to the samples, M= Gene Ruler 100bp DNA Ladder

blaTEM, blaSHV, and *blaCTX-M* were found in 25.73%, 18.71%, and 65.46% of the bacterial strains, respectively.

Figure.3 Sequence of *bla*$_{CTX\text{-}M}$ gene amplicons for investigated isolate 320Uro

ATGGTTAAAAAATCACTGCGCCAGTTCACGCTGATGGCGACGGCAACCGTCA
CGCTGTTGTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCA
AAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATT
GATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTG
CGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAA
GTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGA
CCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCAC
TGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAA
TAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGA
CAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACA
CCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCA
AACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGC
GCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAG
GCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCT
ATGGCACCACCAACGATATCGCGGTGATCTGGCCAAAAGATCGTGCGCCGCT
GATTCTGGTCACTTACTTCACCCAGCCTCAACCTAAGGCAGAAAAGCCGTCG
CGATGTATTAGCGTCGGCGGCTCGTCACCGACGGTTTGTAATAG

ATG= initiator codon, TAG= stop codon

For all the amplicons studied, bioinformatics analysis of the sequences (on the site http://wwww.ncbi.nih.gov), revealed the different types of *bla* genes to which they correspond and whose accession numbers are opposite: *bla*$_{CTX\text{-}M\text{-}15}$: Sequence ID: gb|KF891471.1| (figure 4), *bla*$_{TEM\text{-}1}$: Sequence ID: gb|JQ941741.1|, *bla*$_{SHV\text{-}1}$: Sequence ID: gb|GU064389.1|, *bla*$_{SHV\text{-}11}$: Sequence ID: gb|GU064384.1|, *bla*$_{SHV\text{-}28}$: Sequence ID: gb|HQ877609.1| and*bla*$_{TEM\text{-}198}$: Sequence ID: dbj|AB700703.1|. The variants of β-lactamase genes harbored by our isolates are recorded in table 2. At term of the sequencing, 82.35% of the strains concerned harbor the *bla*$_{CTX\text{-}M\text{-}15}$ gene, while the *bla*$_{TEM\text{-}1}$ and *bla*$_{SHV\text{-}11}$ are each harbored by 23.53% of the isolates (table 2).

Figure.4 Alignment of the *bla*$_{\text{CTX-M}}$ amplicon sequence (Query=320Uro) with that of the *blaCTX-M-15* gene ID: gb|KF891471.1| of *Escherichia coli* P25104 www.ncbi.nlm.nih.gov/nuccore/672443044

Bioinformatic analysis showed that the identity score between the query and subject sequences ranges from 99 to 100% for all analyzed sequences.

bla$_{TEM-1}$, parental β-lactamase gene was identified in 80% and TEM-198 in 20% of our strains harboring *bla*$_{TEM}$. These β-lactamases were found in 23.53% and 5.88% of all the strains. This last result is lower than the observation of Shah and colleagues in Pakistan who found TEM-1 responsible for 80% of resistance to β-lactams among the isolates studied (Shah *et al.,* 2004). *bla*$_{TEM-1}$ encodes penicillinases whose some of which are resistant to β-lactamase inhibitors (TRI) (Canton *et al.,* 2008, Kumar and Kumar 2021). The isolation of this gene suggests that the phenotypic resistance of isolates observed on Petri dishes aigainst amoxicillin-clavulanic acid may be linked to the production of TEM Resistant to Inhibitors (TRI).

bla$_{SHV-1}$ and *bla*$_{SHV-11}$ were respectively isolated on 5.88% and 23.53% of all ours trains subjected to sequencing. *bla*$_{SHV-1}$ was previously reported in Burkina Faso by Kpoda and colleagues during a study on *Enterobacteriaceae* (Kpoda *et al.,* 20218). The *bla*$_{\text{SHV-11}}$ gene was previously detected in Burkina Faso on strains of *Klebsiella pneumoniae* isolated from urinary tract infection (Zeba *et al.,* 2004). This β-lactamase variant appears to be the most widespread of the SHV type in our country. , it was also isolated from 66.66% of our strains screened harboring *bla*$_{SHV}$. A concomitant study conducted at the Charles-De-Gaulles pediatric university hospital revealed a prevalence of 50% for the *bla*$_{\text{SHV-11}}$ gene among the strains studied. (Mètuor-Dabire *et al.,* 2014). *bla*$_{\text{SHV-28}}$ gene encoding extended spectrum β-lactamase, was found on 16.66% of our isolates (table 3). This result is below that of Alfaresi and Elkoush who reported that 28.88% of strains harbor the *bla*$_{\text{SHV-28}}$ gene (Alfaresi and Elkoush 2010). SHV-28 is an extended-spectrum β-lactamases that was first reported at the Southwest Hospital of the Third Military Medical College in China in 2002 (GenBank AF538324). Han *et al.,* reported the presence of SHV-28 and KPC-2, CTX-M-15 coproducing in *Klebsilla pneumonia* an Intensive Care Unit of a Tertiary Hospital in China (Han *et al.,* 2021). Ndugulile *et al.,* reported the presence of SHV-28 in Africa for the first time in 2005 (Ndugulile *et al.,* 2005)

Prevalence and molecular characterization of bla$_{\text{CTX-M-15}}$ gene in investigated isolates

The *bla*$_{\text{CTX-M-15}}$ gene was found on 82.35% of the bacterial strains analyzed in our study. Within the bacterial species, it is hosted by 85.71% of the *E. coli* strains and by 75% of those of *Klebsiella pneumoniae*. CTX-M-15 has been characterized as the most epidemic variant of beta-lactamases (Naas *et al.,* 2017).The ESBL CTX-M-15 as well as the ESBL CTX-M-16 and CTX-M-19 confer a strong resistance to oxi-iminocephalosporins in particular to ceftazidime (Bonnet 2004). blaCTX-15 is commonly reported in Burkina Faso in several studies. In 2016, Ouedraogo *et al.,* reported hih prevalence of 94% of bacterial strains harboring blaCTX-M-15 among extended spectrum β-lactamase producing enterobacteriaceae (Ouédraogo *et al.,* 2016).

Investigating on CTX-M-15-producing bacterial strains is subject worthy of interest. Indeed, several authors report that the bacterial strains harboring the CTX-M-15 gene are generally multidrug-resistant strains. Genes qnrA, qnrB, and qnrS responsible for resistance to quinolones and fluoroquinolones have been identified on plasmids of the IncFII group, associated with the gene blaCTX-M-15 and genes conferring resistance to aminoglycosides (Strahilevitz *et al.,* 2009). The isolation of the variant indicates that several antibiotic molecules may be losing their antibacterial properties.

Analysis of the blaCTX-M-15 sequence using ORF finder at: ncbi.nlm.nih.gov provided the sequence of the enzyme protein synthesized by our isolates, which consists of 291 amino acids. This sequence is identical to the sequence of the CTX-M-15 protein: NCBI Reference Sequence WP_000239590.1, produced by *Escherichia coli*.

In conclusion, the current situation of bacterial resistance remains dominated by the spread of broad-spectrum beta-lactamases. These enzymes have rendered several antibiotic molecules ineffective, including third-generation cephalosprins. The resistance phenotype of bacterial strains observed during our study at the Yalgado university hospital center OUEDRAOGO reflects this current situation. A high proportion of bacterial strains studied resist by ESBLs v (CTX-M-15 and SHV-28), or simple beta-lactamases like (TEM-1, SHV-11) production in emergency units. This study, although conducted in a single hospital center, helps to provide information on bacterial resistance in Burkina Faso, also sounding the alarm on the phenomenon in order to found solutions to reverse bacterial resistance trends. To this end, the bacteriological diagnosis of suspected infections and the identification of the clinical categories of bacterial strains through antibiotic sensitivity tests should be carried out as much as possible before any antibiotic therapy. Hospital hygiene measures must be rigorously

implemented in order to limit the spread of resistant bacterial strains in hospital and community environments. Furthermore, an appropriate policy regarding antibiotics should facilitate the control of distribution and dosages to be administered in order to limit their misuse.

Study Limitations

The present study concerned a single hospital center because it followed a previous study that was limited. This previous study, which had collected bacterial strains, initially planned to cover four hospital centers including the university hospitals (Yalgado Ouédraogo and Sanou Sourou of Bobo Dioulasso) and the regional hospitals of Kaya and Ouahigouya. At the end of the collection period, the number of strains collected in the other centers was almost nil or insignificant.

These centers were simply removed from the study. As for the low number of strains involved in the sequencing, it should be pointed out that this number was related to the financial capabilities of the laboratory.

Authors' Contributions

Zongo Koudbi Jacob Conceptualization, Methodology, Investigation; Kaboré Boukaré Writing -original draft; Metuor Dabiré, Sanou Idrissa: Formal analysis, Investigation; Zeba Boukaré: Writing-Reviewing, supervision of the study.

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Conflict of interests

The authors declare no competing interests.

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Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

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