

Full Length Research Paper

Molecular characterization and *in silico* analysis of mutations associated with extended-spectrum beta-lactamase resistance in uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* in two hospitals, Côte d'Ivoire

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***Escherichia coli* and *Klebsiella pneumoniae* are pathogens frequently involved in urinary tract infections with high epidemic potential. The increase and spread resistance of these microbes to broad spectrum beta-lactam antibiotics are usually reported and is a real public health concern in Côte d'Ivoire but information on genetic variants and intragenic mutations encoding these resistances are scarce. The aim of this study is to characterize genetic variants and describe the intragenic mutations underlying resistance to broad-spectrum beta-lactam antibiotics in uropathogen *E. coli* and *K. pneumoniae* in HKB and CHR hospitals with different epidemiological facies in Côte d'Ivoire. 39 strains comprising 30 of *E. coli* and 9 strains of *K. pneumoniae* were isolated from which DNA was extracted, amplified and sequenced. ESBLs genes were detected by polymerase chain reaction in 58.8 % of strain analysis. No significant difference was observed between ESBL from HKB and CHR hospitals although HKB and CHR sites present 50 and 56.8% of ESBL respectively. Nucleotide sequences subjected to BLASTn for sequences similarity and homology revealed diversity of resistance genes with dominance of the gene encoding the extended-spectrum β -lactamase CTX-M-15 and the emergence of a new *bla*TEM-9 gene in Côte d'Ivoire. The significant co-expression of ESBLs might impact 3rd generation cephalosporin multi-resistance among pathogenic bacteria infecting patient population. Routine antibiogram practice could guide the choice of optimal antibiotic therapy for successful treatment and delay the occurrence of multidrug resistance in enterobacterial infections.**

Key words: Urinary tract infection, extended-spectrum β -lactamase, gene variants, mutations, antibiotic resistance, Côte d'Ivoire.

INTRODUCTION

Escherichia coli and *Klebsiella pneumoniae* are commensal enterobacteria of the intestinal microflora of humans and warm-blooded animals (Ardakani and Ranjbar, 2016). These microbes are able to colonize the extra-intestinal tracts of the host by acquisition of virulence factors and caused a pathological state in this one (Massot et al., 2016). Several studies showed that *E. coli* and *K. pneumoniae* are the pathogens frequently involved in hospital and community-acquired urinary tract infections (Hyun et al., 2019; Yadav et al., 2019). Because of their high frequency in infections, they are constantly subjected to antibiotics used in irrational, empirical and probabilistic ways (Jena et al., 2017) and broad-spectrum antibiotics, mainly β -lactam molecules, are the most recommended by clinicians (Lima et al., 2020). Given its low cost and availability which result in over use (Ventola, 2015), β -lactam antibiotic experienced resistance leading to therapeutic failures (Friedman et al., 2016). Indeed, bacteria, in a hostile environment generated by drug pressure, develop resistance mechanisms or acquire mobile genetic elements (Munita and Arias, 2016). The most common mechanism of resistance in Enterobacteriaceae is related to expression of extended-spectrum beta lactamases (ESBLs) (Shaikh et al., 2015; Teklu et al., 2019). This resistance now extends to the latest generation of cephalosporins and carbapenems (Kim et al., 2017). *bla*TEM, *bla*SHV and *bla*CTX-M are the main variants of genes coding for resistance in Enterobacteriaceae with the latest one (Ahmad and Khadija, 2019). In Côte d'Ivoire, recent studies have reported the spread of ESBL-producing strains of *E. coli* and *K. pneumoniae* in various infections (Tahou et al., 2017; Gadou et al., 2018) although the setup since 2002 of an observatory of antimicrobial resistance is dedicated to actions contributing to fight against the spread of resistant or multi-resistant bacteria (Ouédraogo et al., 2017). In addition, information on genetic variants and intragenic mutations encoding these resistances are scarce. This study aims to determine gene variants as well as nucleotide and peptide mutations involved in resistance to broad-spectrum beta-lactam antibiotics in uropathogenic *E. coli* and *K. pneumoniae* in Côte d'Ivoire.

MATERIALS AND METHODS

Study sites description

This study was carried out in two hospitals belonging to different

epidemiological facies: the Henriette Konan Bedié Hospital in Abobo (HKB-Abobo) located in Abidjan 03°86'501 N; 06°01'275 and the Regional Hospital Center of Haut-Sassandra (CHR-Daloa) located in Daloa 07°82'734 N; 07°61'563 W (Figure 1). At HKB hospital, medical management of bacterial infections is carried out according to an empirical and probabilistic model due to the lack of antibiograms implementation which could lead to an accurate diagnosis and the prescription of the correct antibiotic. In addition, this hospital is located in one of the most populated municipalities of Abidjan, with an estimated population of about 1.03 million inhabitants (RGPH, 2014) with common practice of self-medication. These factors actively contribute to the spread of a wide variety of bacterial pathogens that are resistant to a large number of antibiotics. CHR hospital in Daloa remains the only hospital in the Haut-Sassandra region which has a medical microbiology laboratory which carries out routinely microbial tests. Consequently, it receives patients from all four geographical points of the region for bacterial infection cases, showing a good distribution of bacterial infection cases in the region.

Ethics statement

The study protocol was reviewed and approved by the national ethics committee of Life Sciences and Health in Côte d'Ivoire with the number: N/Ref:106-18/MSHP/ CNESVS-KM, US DPT OF HHS REGISTRATION #: IORG00075 on 30th July 2018. This study is part of the ESTHER project which aims to do a better diagnosis of urinary tract infections in outpatients in addition to microbial surveillance of uropathogens and antimicrobial resistance. Consent was obtained from patients and/or guardians after explaining the objective of the study. The laboratory results were communicated to patients via physicians for better antibiotic prescription.

Study design and sample collection

The sampling of this prospective and descriptive study was performed from October 2018 to April 2019 at HKB and from May to October 2019 at CHR Daloa. During this period, a total of 39 strains including 30 strains of *E. coli* and 9 strains of *K. pneumoniae* were isolated from fresh urine samples from patients with urinary tract infections.

Isolation and identification of bacterial strains

The uropathogenic bacterial strains were isolated on CHROMagar Orientation (Becton Dickinson, Cockeysville, MD), chromogenic medium (Manickam et al., 2013). The identification of *E. coli* and *K. pneumoniae* species was performed using Gram staining tests and classical biochemical tests such as indole, oxidase, catalase, urease, tryptophan deaminase, glucose and lactose fermentation, production of gases from glucose fermentation, degradation of hydrogen peroxide by the production of hydrogen sulfide, use of citrate as the unique source of carbon, motility, lysine deaminase and lysine decarboxylase production (Tandon and Bhargava, 2019). Samples collection details are presented by the algorithm in Figure 2.

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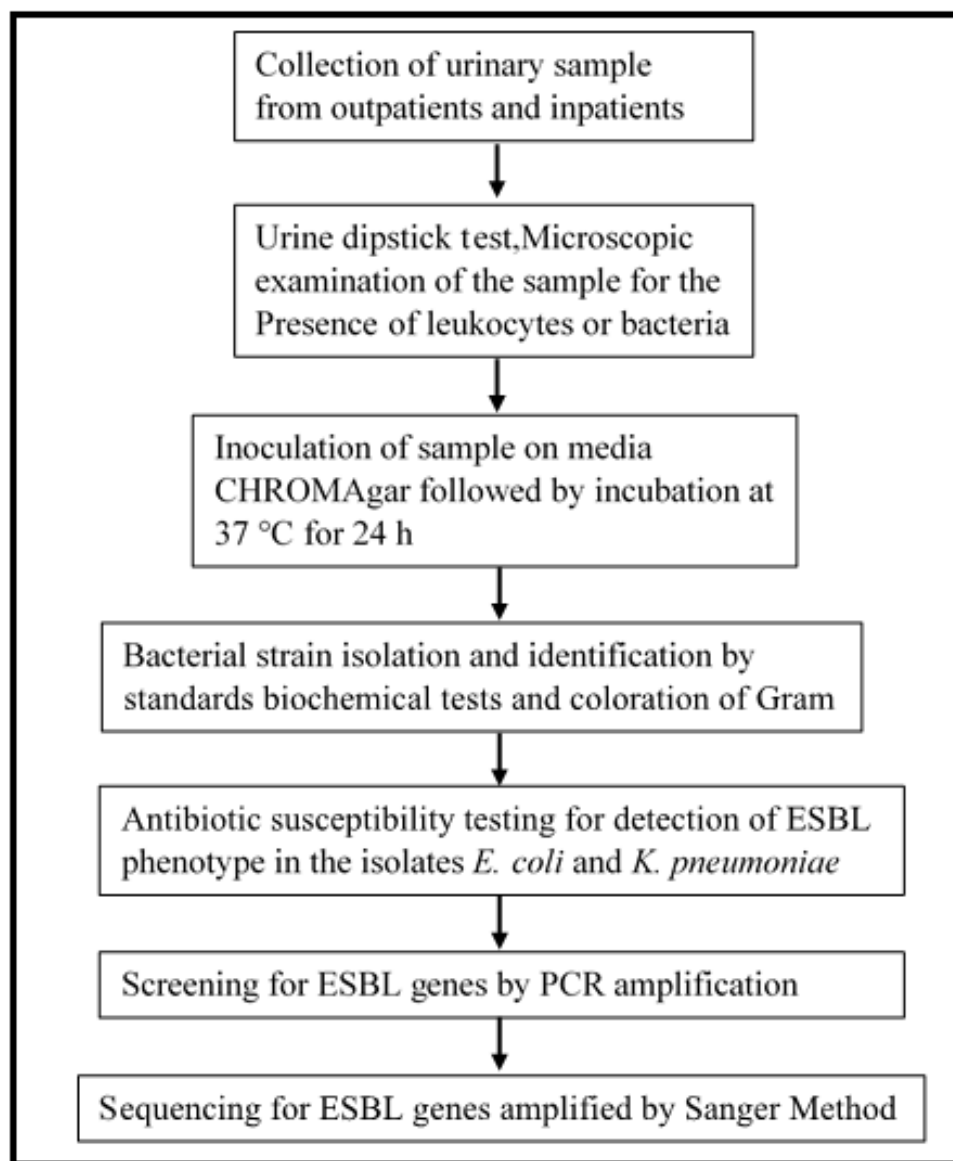


Figure 2. Algorithm for sampling and analysis of collected samples.

Table 1. Primers used for the screening of genes coding for the production of broad-spectrum beta-lactamases.

Genes	Primers	Sequences (5'- 3')	References	Amplicon size (bp)
<i>bla</i> TEM	R	CTCAAGGATCTTACCGCTGTTG	Zhu et al. (2013)	112
	F	TTCCTGTTTTTGCTCACCCAG		
<i>bla</i> CTX-M	R	TTTATCCCCACAACCCAG	Zhu et al. (2013)	701
	F	AATCACTGCGTCAGTTCAC		
<i>bla</i> SHV	R	CGCAGATAAATCACCACAATG	Zhu et al. (2013)	768
	F	TCGCCTGTGTATTATCTCCC		

Table 2. Distribution of broad-spectrum beta-lactamase-producing isolates according to the collection site and the species studied.

		ESBL-Phenotypes			χ^2	P
		N	ESBL + (%)	ESBL - (%)		
Sites						
	Abobo HKB	22	11(50)	11(50)	0.05	0.82 ^a
	Daloa CHR	17	10 (58.8)	7 (41.2)		
	Total	39	21 (53.8)	18 (46.2)		
Species						
	<i>E. coli</i>	30	17 (56.7)	13 (43.3)	----	0.70 ^b
	<i>K. pneumoniae</i>	9	4(44.4)	5 (55.6)		
	Total	39	21(53.8)	18 (46.2)		

N: total number of isolates; ESBL+: extended-spectrum beta-lactamase production; ESBL-: no extended-spectrum beta-lactamase production; χ^2 : Chi-square constant; p: probability associated with the statistical test; a: independence Chi-square approximation; b: Fisher's exact test.

Information (NCBI) using the BLASTN local alignment search tool available online (<http://www.ncbi.nih.gov>). Protein sequences derived from the genes were aligned using DNA Baser Assembler 5.15.0 and analyzed in order to identify the CTX-M and TEM type according to sequences leading to detection of the mutations underlying resistance to ESBLs. The statistical Chi-square test of independence and the Fisher exact test were performed to compare the proportions of ESBLs strains. Significant differences are observed when the probability value (*p*) associated with the statistical tests is strictly less than 0.05.

RESULTS

Phenotypic determination of ESBLs

Figure 3 shows the phenotypic detection of a broad-spectrum beta-lactamase-producing bacterial strain using the double-synergy method on Müller Hinton agar. ESBLs production is detected by the appearance of a particular champagne cork image. The distribution of broad-spectrum beta-lactamase-producing isolates is indicated in Table 2. Results showed that 21 (53.8%) out of 39 study strains produced ESBL. *E. coli* and *K. pneumoniae* species expressed 56.7 and 44.4% at least one ESBL respectively. At the HKB hospital in Abobo, 50 % of strains analyzed were BLSE-producing positive, while this phenotype was expressed in 58.8 % of the strains at the CHR-Daloa. Although the prevalence of ESBL strains is slightly higher at CHR hospital in Daloa, the difference was not statistically significant (*P* = 0.82). This result showed that ESBL production was not significantly associated with bacterial specie in this study (Table 2).

Molecular detection of ESBLs resistance

The genes coding for resistance to ESBL, namely *bla*TEM, *bla*SHV and *bla*CTX-M were tested for the 21 strains with the ESBL phenotype. The electrophoretic profiles of the detected BLSE genes are shown in Figure 4. Genotyping revealed the presence of two types of ESBL genes: The *bla*TEM gene encoding the TEM enzyme and the *bla*CTX-M gene encoding the CTX-M enzyme. The *bla*TEM and *bla*CTX-M genes are characterized by DNA fragments of 112 and 701 base pairs respectively (Figure 4a and b), but the *bla*SHV gene was not detected in this study.

Distribution of broad-spectrum beta-lactamases resistance genes in *E. coli* and *K. pneumoniae*

The distribution of the *bla*CTX-M and *bla*TEM genes encoding resistance to the broad-spectrum beta-lactamases detected in *E. coli* and *K. pneumoniae* in this study are shown in Figure 5. In the population of *E. coli* producing extended-spectrum beta-lactamase, the *bla*TEM gene was detected in nine strains (52.94 %), including six strains from HKB Abobo Hospital (66.7%) and three strains (33.3%) from CHR Daloa. Concerning the *bla*CTX-M gene, an overall frequency of occurrence of 70.6% is obtained in these bacterial species. In the collection from Abobo HKB hospital, the proportion of *E. coli* strains expressing the *bla*CTX-M gene was 58.3% (7/12), while at Daloa CHR, the proportion was 41.7%(5/12). Among the four ESBL-producing strains of *K. pneumoniae*, an overall occurrence frequency of 75 %

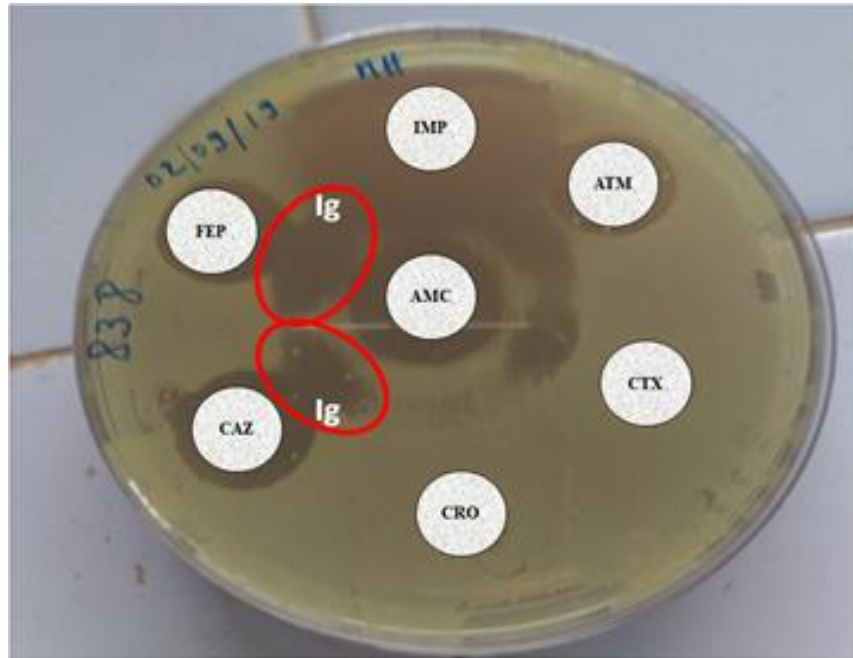


Figure 3. Synergistic image between third generation cephalosporins showing the phenotypic production of extended spectrum beta-lactamase in the bacterial species studied. CAZ: ceftazidime; CRO: ceftriaxone; ATM: aztreonam; CTX: cefotaxime; IMP: imipenem; FEP: cefepime; AMC: amoxicillin + clavulanic acid; Ig: image of synergy.

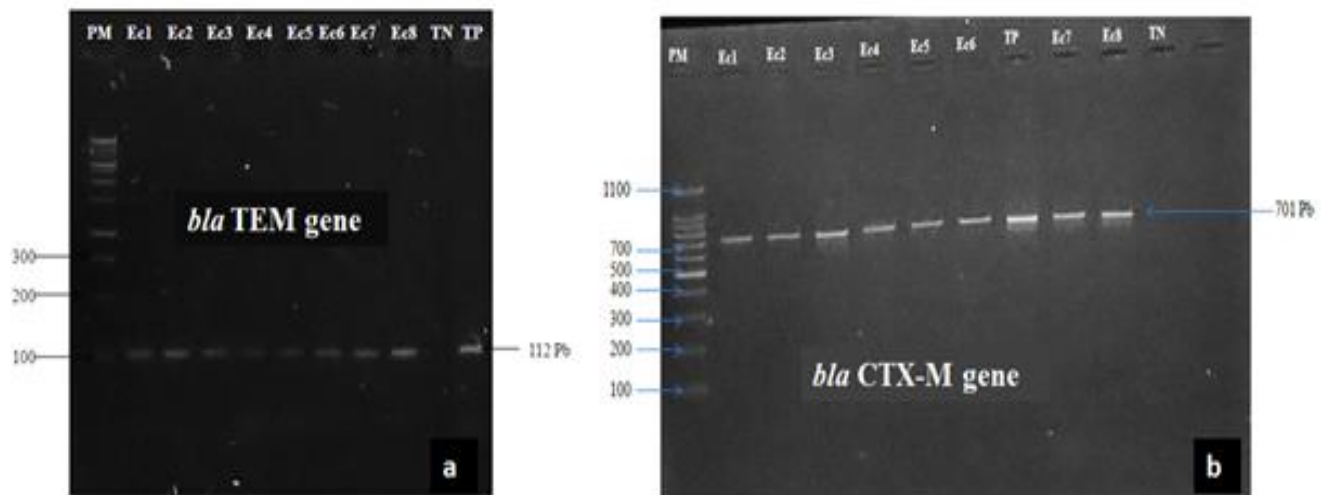


Figure 4. Electrophoretic profile on 1.5 % agarose gel of the *bla*TEM (a) and *bla*CTX-M (b) amplified genes. PM: Molecular weight marker (100bp, Invitrogen); Ec1 to Ec8: Samples tested positive to *bla*CTX-M; TP: Positive control (KCCY 1816); TN: Negative control.

(3 strains) is obtained for the *bla*TEM gene. This gene was detected in all ESBL-producing strains from HKB hospital in Abobo (100%). However, *K. pneumoniae* strain

isolated at CHR Daloa did not carry the *bla*TEM gene. The *bla*CTX-M gene was detected in 3 strains of which two were from HKB hospital in Abobo. The unique strain

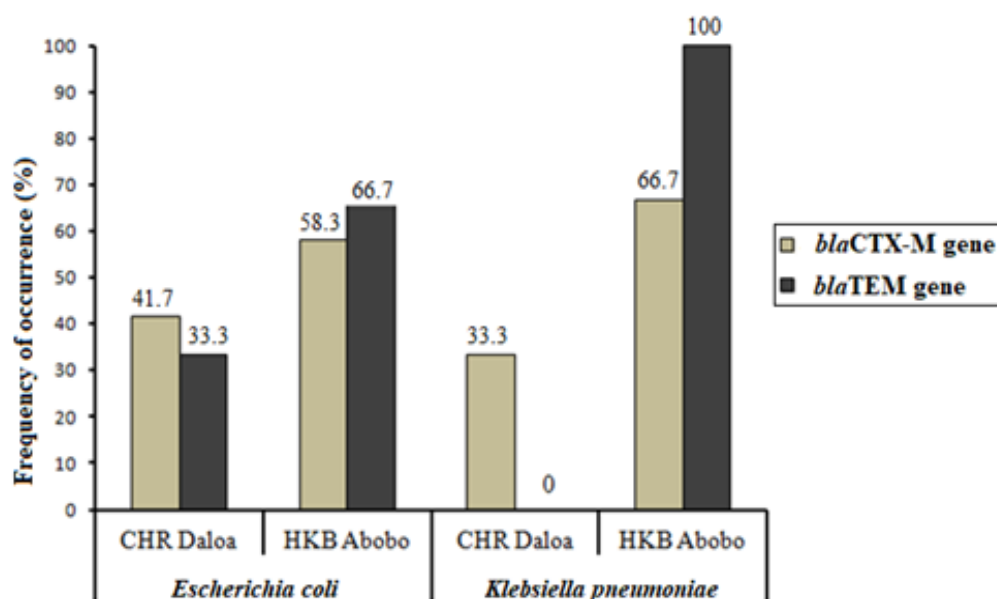


Figure 5. Frequency of occurrence of *blaTEM* and *blaCTX-M* genes detected in *E. coli* and *K. pneumoniae*.

of *K. pneumoniae* collected at CHR Daloa was a carrier of the *blaCTX-M* gene, with 33.3% frequency of occurrence. In addition, co-occurrences for these two genes (*blaTEM* and *blaCTX-M*) were observed in this study in four strains of *E. coli* (66.7%) and two strains of *K. pneumoniae* (33.3%) (Figure 5).

Bioinformatics Analysis of *blaTEM* and *blaCTX-M* genes

Out of twenty-one strains expressing ESBL genes detected and sent for sequencing, fifteen were successfully sequenced. These strains consisted of three strains of *K. pneumoniae* and twelve strains of *E. coli*. Analysis of TEM and CTX-M genes sequences were done to determine their relationship to other TEM and CTX-M gene sequences available in Gen-Bank database using BLAST nucleotide algorithm (<http://www.ncbi.nlm.nih.gov/>). Variants of the *blaTEM* sequences analysis revealed that among the seven strains (*E. coli*₁, *E. coli*₅, *E. coli*₁₄, *E. coli*₆, *E. coli*₁₁, *E. coli*₉ and *Kleb*₂), only one strain (14.3 %) isolated at HKB hospital produced the TEM-1 enzyme (Table 3). The corresponding nucleotide sequence was identified to be 97 % homologous to the coding sequence of the 66 base pair length TEM-1 allele, available in the Genbank under accession number KT415643. Alignment of this sequence revealed two base point mutations with changes in amino

acid at position 38; the adenine of the CCA codon in the reference sequence was substituted by cytosine (A38C) and at position 48 the guanine of the TGT codon in the reference sequence was substituted by thymine (G48T). These nucleotide mutations induced an amino acid change in the translated peptide sequence. Accordingly, at position 13 of this sequence, glutamine mutated to proline (Gln13Pro) and at position 16 tryptophan mutated to cysteine (Trp16Cys) in the newly formed sequence (Figure 6).

Apart from TEM-1 enzyme, the others strain 85.7% revealed the production of the TEM-9 enzyme with 95.2 to 100 % similarity to the reference TEM-9 allele (accession number KY271103) available in Genbank. As illustration, the nucleotide sequence of the *E. coli*_6 isolate shows 98.6 % homology with the reference TEM-9 allele of the *blaTEM* gene (Figure 7, Table 3). A point mutation of bases was detected in position 3 regarding nucleotide alignment where A base in reference sequence was replaced by a C in the query sequence. However, after translation into amino acid sequences, no mutation was observed (Figure 7).

With the *E. coli*_1 strain, alignment results showed a 95.2% of similarity between the nucleotide sequence of the *blaTEM* gene and the reference sequence coding for the TEM-9 allele (accession number KY271103). The alignment of these two sequences revealed a substitution at position 4 of the thymine TGT codon by adenine (T4A) in the query sequence. A gap extension at positions 10

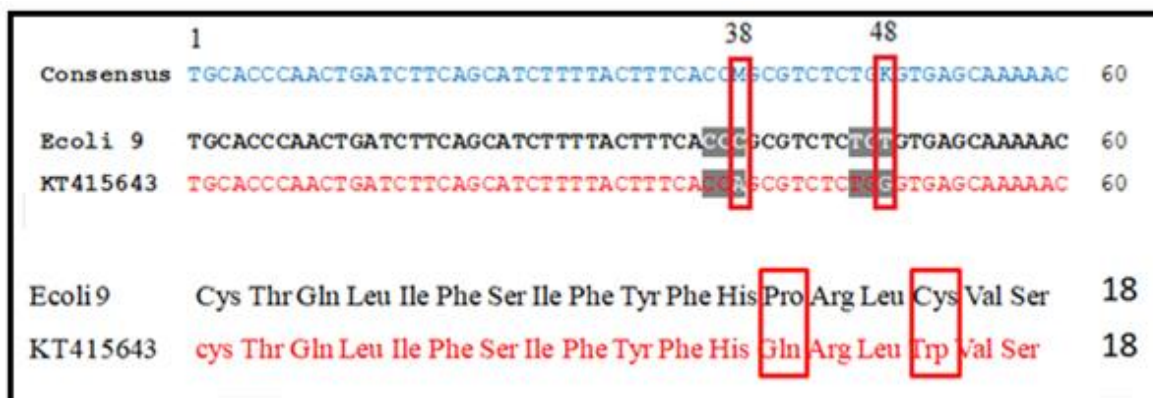


Figure 6. Alignment of the nucleotide and peptide sequences of the *bla*TEM gene of the *E. coli* 9 isolate in black with that of the synthetic reference clone of accession number KT415643 in red. In blue: consensus sequence of the alignment; red box: mutation zone; shaded area: codons concerned.

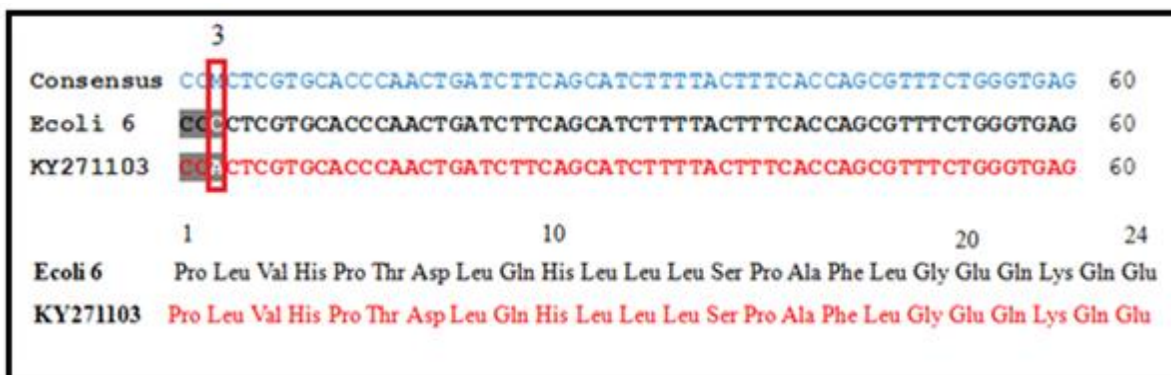


Figure 7. Alignment of the nucleotide and peptide sequence of the *bla*TEM gene of the *E. coli* 6 isolate in black with that of the reference strain *Escherichia coli* ECO584 of accession number KY271103 (in red). In blue: consensus sequence of the alignment; red box: mutation zone; shaded area: codons concerned.

and 11 and an insertion of cytosine at position 55 were also observed in *E. coli*_1 strain sequence (Figure 7). The peptide sequence resulting from this alignment showed numerous amino acid changes from position 2 to 19 and from 21 to 27 (Figure 8). For *E. coli*_5, *E. coli*_11, *E. coli*_14 and *Kleb*_2 isolates a 100% similarity was obtained with the reference TEM-9 allele of the *bla*TEM gene accessible at KY271103 in the Genbank. The TEM-1 and TEM-9 variants of the *bla*TEM gene detected in this study are mainly produced by strains from HKB hospital.

Alignment of the nucleotide sequences of the *bla*CTX-M gene of the studied strains revealed a single variant of this gene, CTX-M-15 with 100% identity to the coding reference sequence (accession number MN816278) in Genbank (Figure 9). CTX-M-15 is the only CTX-M-like

enzyme produced by the studied strains to resist broad-spectrum beta-lactams. This variant was detected in 10 uropathogenic strains (66.7%) versus seven strains (46.7%) for the *bla*TEM variant. These strains comprised one strain of *K. pneumoniae* from each of the two study sites and eight strains of *E. coli* (four from HKB hospital in Abobo and four from the CHR hospital in Daloa).

DISCUSSION

Faced with drug pressure, bacteria develop resistance mechanisms whose expression is encoded by genetic factors subject to mutations. In this study, 53.8% of strains analyzed produced at least one broad-spectrum beta-lactamase. Of the two bacteria species studied, *E.*

Table 3. Characteristics of the coding sequences obtained after alignment of the amplified sequences to Genbank references.

Isolates	Collection Sites	Reference Organism	Acession No	% GC	Variants	% Identity	E-value	Sequence length (bp)
<i>E. coli</i> 1	HKB hospital	<i>E. coli</i> . ECO0584	KY271103	46.3	<i>bla</i> _{TEM-9}	95.2	2.11,10 ⁻²⁵	83
<i>E. coli</i> 5	HKB hospital	<i>E. coli</i> .ECO0584	KY271103.1	43.5	<i>bla</i> _{TEM-9}	100	9.45,10 ⁻²²	62
Kleb2	HKB hospital	<i>E. coli</i> .ECO0584	KY271103.1	48.9	<i>bla</i> _{TEM-9}	100	9.97,10 ⁻²⁷	72
<i>E. coli</i> 14	HKB hospital	<i>E. coli</i> .ECO0584	KY271103	42.9	<i>bla</i> _{TEM-9}	100	3.82,10 ⁻²²	63
<i>E. coli</i> 6	HKB hospital	<i>E. coli</i> .ECO0584	KY271103	46.6	<i>bla</i> _{TEM-9}	98.6	5.72,10 ⁻²⁶	73
<i>E. coli</i> 11	HKB hospital	<i>E. coli</i> .ECO0584	KY271103	42.9	<i>bla</i> _{TEM-9}	100	2.71,10 ⁻²²	63
<i>E. coli</i> 9	HKB hospital	Clone synthétique	KT415643	45.5	<i>bla</i> _{TEM-1}	97	2.71,10 ⁻²²	66
<i>E. coli</i> 6	HKB hospital	<i>E. coli</i> .LM8	MN816278	55.1	<i>bla</i> _{CTX-M-15}	100	0	604
<i>E. coli</i> 11	HKB hospital	<i>E. coli</i> .LM8	MN816278	55.1	<i>bla</i> _{CTX-M-15}	100	0	590
Kleb8	HKB hospital	<i>E. coli</i> .LM8	MN816278	55.1	<i>bla</i> _{CTX-M-15}	100	0	604
<i>E. coli</i> 8	HKB hospital	<i>E. coli</i> .LM8	MN816278	55.3	<i>bla</i> _{CTX-M-15}	100	0	506
<i>E. coli</i> 2	HKB hospital	<i>E. coli</i> .LM8	MN816278	54.9	<i>bla</i> _{CTX-M-15}	100	0	576
<i>E. coli</i> 29	Daola CHR	<i>E. coli</i> .LM8	MN816278	54.6	<i>bla</i> _{CTX-M-15}	100	0	577
Kleb9	Daola CHR	<i>K. pneumoniae</i> .NC1	MN786379	54.5	<i>bla</i> _{CTX-M-15}	100	0	560
<i>E. coli</i> 24	Daola CHR	<i>E. coli</i> .LM8	MN816278	55.3	<i>bla</i> _{CTX-M-15}	100	0	602
<i>E. coli</i> 27	Daola CHR	<i>E. coli</i> .LM8	MN816278	54.5	<i>bla</i> _{CTX-M-15}	100	0	571
<i>E. coli</i> 20	Daola CHR	<i>E. coli</i> .LM8	MN816278	55.1	<i>bla</i> _{CTX-M-15}	100	0	604

% GC: Guanine+Cytosine content, bp : bases pairs, HKB : Henriette Konan Bedie, CHR : Regional Hospital Center.

coli is the one which presents the greatest prevalence of resistance (56.7%) even if the 44% presented by *K. pneumoniae* are not negligible. The high prevalence of ESBLs in this study, as well as some reported in Côte d' Ivoire (Guessennd et al., 2011; Gadou et al., 2018) and in other parts of Africa (Togo et al., 2014; Saravanan et al., 2018; Ouchar et al., 2019), show that the spread of ESBL-producing Enterobacteriaceae in an African context remains a very worrisome phenomenon. The high proportion of uropathogenic strains producing ESBL is thought to be related to the widespread use of third-generation cephalosporins and lack of adequate urinary tract infection (UTI) treatment

regarding dosing regimens (Halaji et al., 2020). Indeed, the prevalence of the resistant phenotype is often an accurate reflection of antibiotic prescribing habits (Llor and Bjerrum, 2014). The molecular profile of the 21 strains is characterized by the expression of two main types of ESBL enzymes, namely CTX-M and TEM. The *bla*_{CTX-M} gene was the most involved in ESBL resistance in *E. coli*, while, *bla*_{TEM} gene was detected at 75% in ESBL resistance in *K. pneumoniae*. Expression of these genes may be the cause of failures observed in UTI treatment, particularly using extended-spectrum beta-lactam drugs (Hagel et al., 2013). The concomitant expression of *bla*_{TEM} and *bla*_{CTX-M} genes revealed in this

study could be due to cross-resistance to penicillins and acquired cephalosporins, suggesting the multi-resistant character of these strains (Ahmad and Khadija, 2019).

In silico analysis of the DNA and protein sequences of the *bla*_{TEM} gene revealed two enzyme variants, TEM-1 and TEM-9. The TEM-1 variant observed with proline/glutamine substitutions is an original plasmid-mediated beta-lactamase from which all other TEM variants are derived by mutation of one or more amino acids (Ur Rahman et al., 2018). Commonly found in *E. coli*, TEM-1 is able to hydrolyze penicillins and narrow spectrum cephalosporins such as cephalothin (Ur Rahman et al., 2018; Galindo-

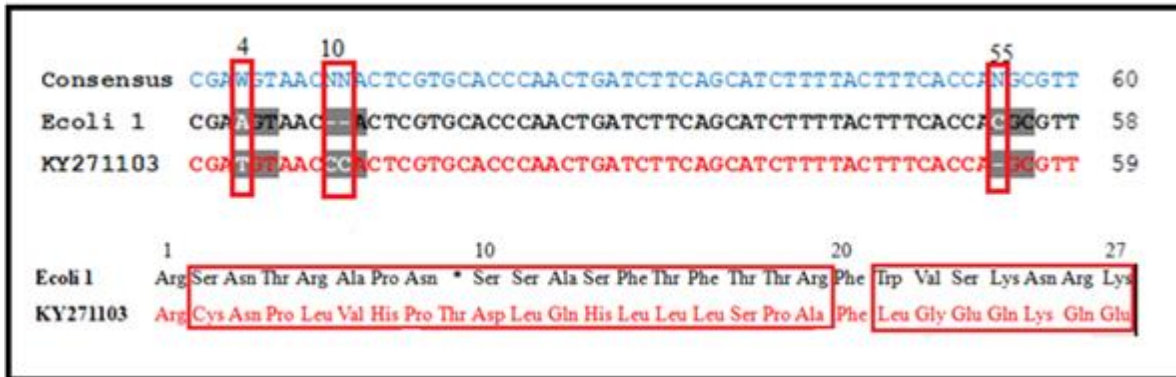


Figure 8. Alignment of the nucleotide and peptide sequence of the *bla*_{TEM} gene of the *E. coli*₁ isolate in black with that of the reference strain *Escherichia coli* ECO0584, accession number KY271103 (in red). In blue: consensus sequence of the alignment; red box: mutation zone; shaded area: codons concerned.

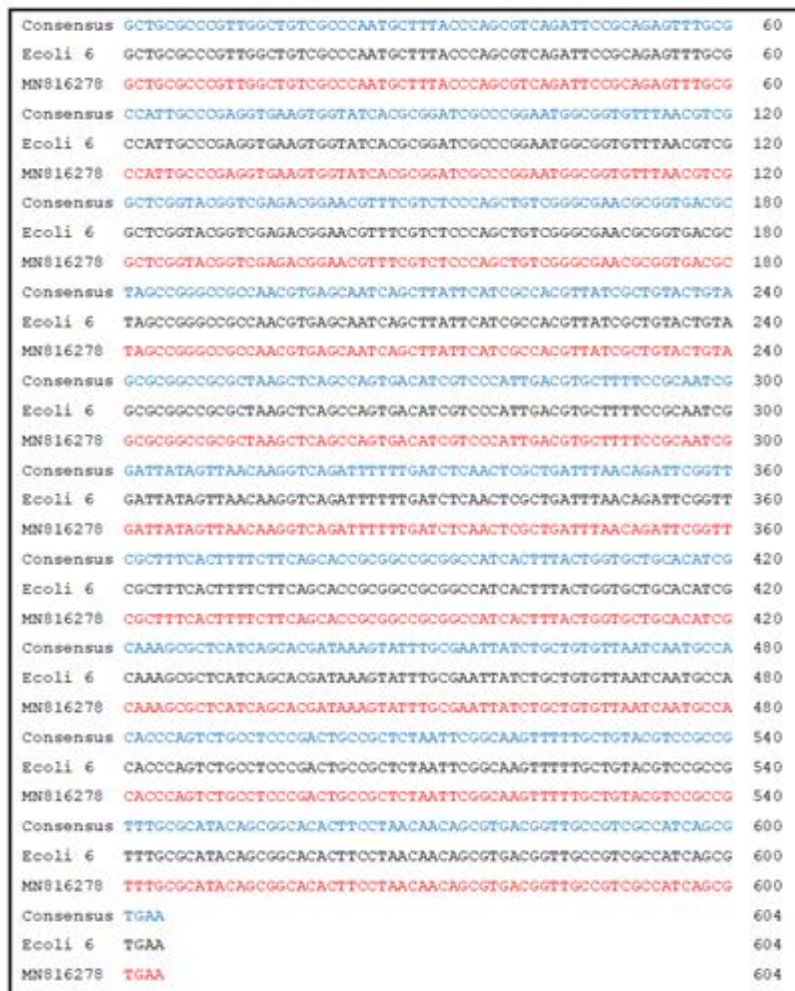


Figure 9. Alignment of *E. coli* 6 nucleotide sequence of *bla*_{CTX-M} with that of the reference strain *E. coli* LM8 (accession number MN816278).

Méndez, 2020). TEM-1 has also been reported in Côte d'Ivoire from previous studies (Guessennd et al. 2008; Tahou et al., 2017) in *Klebsiella pneumoniae*. According to the scientific literature, the TEM-9 variant is detected for the first time in Côte d'Ivoire. This variant was derived from TEM-1 by four amino acid substitutions and was first characterized by Mabilat et al. (1990) in a clinical strain of *K. pneumoniae* at the Royal Hospital in Hallamshire in England. This mutation leads to resistance to third-generation cephalosporins, particularly ceftazidime (Jahani et al., 2017). In addition to *bla*TEM, *bla* CTX-M was also detected with the CTX-M-15 variant being the only one observed in this study. Initially reported in India in 1999, this variant predominates the current molecular epidemiology of ESBLs in *K. pneumoniae* and *E. coli* involved in both community UTI and nosocomial infections (Chong et al., 2018). The co-expression of the TEM-9 and CTX-M-15 variants, observed in the *E. coli*_{1_1} strain in this study is evidence of the multi-drug resistant nature of the strains circulating in the African region, due to the ability of this organism to hydrolyze larger substrates and an extended transmission of these genetic resistance factors (Parajuli et al., 2016).

Conclusion

This study showed a high proportion of ESBL-producing strains in UTI involving *E. coli* and *K. pneumoniae* species. Molecular epidemiology and *in silico* analysis of antibiotic resistance in these two uropathogens is characterized by a diversity of beta-lactam resistance genes with the emergence for the first time of *bla*TEM-9 gene variant in Côte d'Ivoire and the dominance of the gene encoding ESBL CTX-M-15, confirming the high diffusion of this variant worldwide. Furthermore, the study highlighted significant co-expression of ESBLs imparting 3rd generation cephalosporin resistance among pathogenic bacteria infecting patient population. Routine antibiogram practice could guide the choice of optimal antibiotic therapy for successful treatment and delay the occurrence of multidrug resistance in Enterobacterial infections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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