

# Isolation and Identification of *Escherichia coli* and *Klebsiella pneumoniae* Strains Resistant to the Oxyimino-Cephalosporins and the Monobactam by Production of GES Type Extended Spectrum Bêta-Lactamase (ESBL) at Saint Camille Hospital Center in Ouagadougou, Burkina Faso

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**Background:** Bacterial resistance to beta lactamins is a real public health problem as it complicates treatment strategies. Several types of beta lactamase confer this resistance. Numerous studies report a high prevalence of ESBL producers among Gram-negative bacilli. The objective of this work was to identify the presence of the resistance gene GES in strains of *E. coli* and *K. pneumoniae* in Burkina Faso.

**Methods:** During this study 39 strains of *E. coli* and *K. pneumoniae* resistant to oxyimino-cephalosporin and monobactam were collected in several samples and analyzed to determine the presence of the beta lactamase resistance gene Bla<sub>GES</sub> by classic PCR.

**Results:** In the present study, resistant strains were observed in 21 *E. coli* and 18 *K. pneumoniae*. Among producers of ESBL isolates, the presence of the GES gene was detected up to 63% in *E. coli* and 37% in *K. pneumoniae*.

**Conclusion:** This study highlighted the presence of the GES gene in strains of *E. coli* and *K. pneumoniae* resistant to oxyimino-cephalosporin and monobactam in Burkina Faso. This highlights the presence of new ESBL in Burkina, which is of great interest for the proper care of patients and the control of resistance to antibiotics.

**Keywords:** PCR classic, *GES* gene, broad-spectrum beta-lactamases

## Background

Broad spectrum  $\beta$ -lactamases (ESBLs) are enzymes that confer resistance to oxyimino cephalosporins and aztreonam.<sup>1</sup> In fact, they result from mutations in the genes of the common plasmid b-lactamases (in particular TEM and SHV) which modify the configuration of the enzyme at the level of its active site to increase the affinity and the hydrolytic capacity of b-lactamase for oxyimino compounds.<sup>2</sup> The organisms producing these ESBLs are clinically relevant and remain an important cause of failure of treatment with Oxyimino cephalosporins. ESBLs are mainly produced by Enterobacteriaceae, in particular *Klebsiella pneumoniae*<sup>3</sup> and *Escherichia coli*.<sup>4</sup> Bacteria harboring ESBLs can also acquire and most often present additional resistance to other classes of antibiotics such as monobactam (aztreonam), quinolones, tetracyclines, aminoglycosides, etc., which further limits the therapeutic options and thus pose a therapeutic dilemma.<sup>5</sup>

Among Enterobacteriaceae, *E. coli* and *K. pneumoniae* are the two organisms most isolated from biological samples during bacterial infections.<sup>6</sup> In fact, they are pathogenic enterobacteria in humans that lead to a broad spectrum of nosocomial and community infections such as urinary<sup>7</sup> and genital tract infections, sepsis, pneumonia, peritonitis.<sup>8</sup> The most important problem for patients with *E. coli* and *K. pneumoniae* infections is the emergence of antibacterial resistance.

ESBLs associated with infections were largely of the historically common CTX-M, TEM and SHV types.<sup>9</sup> TEM, SHV and CTX-M ESBLs have been the most found and most documented in bacterial resistance. But the rarer types such as SFO, TLA, PER, BES, GES,<sup>10</sup> found mainly in *Acinetobacter baumannii*, *Serratia fonticola*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* have also been described.

ESBLs of the GES type are increasingly reported in Enterobacteriaceae, in particular *P. aeruginosa*, *E. coli* and *K. pneumoniae*. The GES-1 type was initially described in a strain of *K. pneumoniae* isolated in 1998 in France then in Argentina, Brazil, Portugal and the Netherlands.<sup>11</sup> To date, 9 different variants have been described including GES-2 in South Africa, GES-5 to GES-8 in Greece, GES-3 and GES-4 in Japan, GES-5 in South Korea, China and in Brazil, and GES-9 in France.<sup>11</sup> By a single mutation, GES-2 is the first example of an ESBL with a spectrum of activity extended to carbapenems,<sup>11</sup> which could be at the origin of the emergence of “pan-resistant” microorganisms.

The potential spread of these ESBL-producing Enterobacteriaceae in populations poses a real public health problem as well as a challenge for the management of bacterial infections, which were generally treated empirically with antibiotics such as oxyiminocephalosporins or fluoroquinolones without antibiotic susceptibility testing. From the above, efforts to describe new types of ESBLs such as the GES type would help assess the extent of bacterial resistance by enzyme production and help to contain this formidable scourge. Thus, the main objective of this study was to isolate and identify the strains of *E. coli* and *K. pneumoniae* resistant to oxyiminocephalosporins and monobactam by production of ESBLs of the GES type in patients suffering from infections bacteria at St Camille Hospital in Ouagadougou.

## Methods

### Sampling of *Escherichia coli* and *Klebsiella pneumoniae* Strains

Our sampling took place from September 3, 2018 to October 3, 2018. These were 39 strains of Enterobacteriaceae (21 *E. coli* and 18 *K. pneumoniae*) from patients who brought their samples for bacteriological examination to the laboratory of Saint Camille Hospital in Ouagadougou. The samples for this study were from urine, stool, pus, and vulvar swabs. The samples were from patients at Saint Camille Hospital and from patients from other hospitals. The following data were collected: age, sex, patient's origin and origin of the specimens were recorded for each patient. All samples with enterobacterial strains identified as *Escherichia coli* and *Klebsiella pneumoniae* were included in the study. Both strains were identified using the Api 20E gallery. The bacterial strains we selected were *Escherichia coli* and *Klebsiella pneumoniae* resistant to at least one third generation cephalosporin or monobactam. The sample was taken at the Hôpital Saint Camille (HOSCO) in Ouagadougou, more precisely in the laboratory, in the bacteriology department.

### Detection of Resistant Strains and Strains Producing ESBL

Disk diffusion antibiogram tests were performed on all strains confirmed to be *Escherichia coli* and *Klebsiella pneumoniae*. The antibiotics were tested on Petri dishes containing Muller Hinton agar by depositing the discs of antibiotics so as to reveal the image of synergy of action representing a champagne stopper between the oxyiminocephalosporins (cefotaxime, ceftazidime or ceftriaxone) and amoxicillin plus clavulanic acid<sup>12</sup> characteristics of the ESBL profile. The following Liofilchem antibiotic discs were used: aztreonam (30 µg) for monobactams, cefotaxime (30 µg), ceftriaxone (30 µg) and ceftazidime (30 µg) for oxyimino cephalosporins.

## Molecular Detection of ESBL Producing Strains

### Extraction of DNA from Resistant *E. coli* and *K. pneumoniae* Strains

The boiling method is a simple method of extracting bacterial DNA for molecular techniques without using an industrial extraction kit.<sup>13</sup> It is a simple method that consists of heating the bacteria to a temperature of 100 degrees, in order to

burst the bacteria and release its genetic material, then centrifuging to separate the DNA from the debris and recover the genetic material.

## Protocol

Strains stored in Luria bertani storage medium were awakened by culturing on MH medium for 18–24h in an oven at 37°C. An isolated colony was removed from the MH Petri dishes and suspended in 200ul sterile distilled water previously aliquoted into labeled Eppendorf tubes. The resulting suspension was immersed in a water bath (100°C for 15 min) to release the genetic material. After immersion in a water bath (MEMMERT, Rost fret), the suspension was centrifuged (NF 048 centrifuge) at 12,000 rpm for 10 min to separate the genetic material from the debris and the supernatant containing the liberated DNA was transferred to a new Eppendorf tube, assayed and stored at –80° C until use.

## Quantification and Verification of the Purity of the Extracted DNA

PCR, qPCR, reverse transcription (RT) or next generation sequencing (NGS) are sometimes tricky to implement and require that the quality of the nucleic acids be checked beforehand to ensure the reliability and accuracy of the results. For this purpose, after the extraction of the bacterial DNA, we checked the amount of DNA obtained and the purity of this DNA using the Nanodrop. Samples whose purity and quantity were not good were repeated until a satisfactory quantity and purity were obtained.

## Detection of the GES Gene by Classic PCR

PCR (Polymerase Chain Reaction) is an in vitro test for DNA replication that allows a “target” DNA sequence to be selectively amplified several million times in a few hours.

All isolates with resistance to the tested antibiotics or with a synergy image were screened for the gene encoding  $\beta$ -lactamase of the bla PER, bla GES and bla SFO family. The crude DNA extracts obtained after extraction were used for gene detection and Firepol Master Mix PCR was used. The reaction volume was prepared as follows: 4  $\mu$ L Firepol master mix + 0.5  $\mu$ L F primer + 0.5  $\mu$ L R primer + 14  $\mu$ L PCR water + 1  $\mu$ L DNA extract for a final reaction volume of 20ul. Positive and negative controls were used when performing conventional PCR. Gene detection was performed by conventional PCR with the GeneAmp System PCR 9700 Thermal Cycler (Applied Biosystems, California, USA) and the following amplification programs were used to search for the genes:

Initial denaturation at 95 ° C for 7 min,

$$\left\{ \begin{array}{l} \text{Denaturation at } 94^{\circ}\text{C for 60s,} \\ \text{Hybridization at } 58^{\circ}\text{C for 60s,} \\ \text{Extension at } 72^{\circ}\text{C for 1min,} \end{array} \right\} \text{ During 35 cycles}$$

Final extension at 72 ° C for 10 minutes.

The following primers were used (Table 1).

## Identification of the Gene by Agarose Gel Electrophoresis

Agarose gel electrophoresis is a method used in molecular biology to separate DNA, RNA or proteins according to their molecular weight. The agarose gel electrophoresis technique is based on the separation of negatively charged nucleic acids under the effect of an electric field. This separation takes place through the agarose gel matrix: smaller molecules move faster and will migrate further than larger molecules. The PCR Product obtained after amplification were

**Table 1** Table Showing the Primers Used

Gene Detected	Primer	Primer Sequences (5'- 3')	Fragments (Pb)	Reference
bla <sub>GES</sub>	GES- F GES- R	ATGCGCTTCATTCACGCAC CTATTTGTCCGTGCTCAGG	863	[14]

subjected to electrophoretic migration at 100 volts for 30 min on a 1.5% agarose gel in the presence of ethidium bromide (BET).

## Preparation of TBE 1X from TBE 10X

To do this, 50mL of TBE 10X is mixed with 450mL of distilled water, the resulting solution (TBE, 1X) is homogenized.

## Preparation of the 1.5% Agarose Gel

- Dissolve 1.5g of agarose in 100mL of TBE 1X;
- Heating of the obtained solution with a microwave (SEVERIN 700) at 495w for 3 minutes;
- Allow to cool for about 10 minutes;
- Add 15µL of Ethidium Bromide (BET) (10 mg/mL) to the gel and mix;
- Carefully pour the gel into the transparent holder containing the combs, then dry at laboratory temperature.

## Electrophoretic Migration

The transparent support containing the gel was placed in the electrophoresis tank containing migration buffer which is TBE 1X. The GES gene is 863 bp in size. The 1000 Pb molecular weight marker was used as a reference and the bands obtained were observed under UV light from the GENE FLASH device.

## Results

### Identification of *Escherichia coli* and *Klebsiella pneumoniae* Strains Resistant to Oxyimino-Cephalosporins and Monobactam

During this study, 39 strains of enterobacteria (21 *E. coli* and 18 *K. pneumoniae*) resistant to at least one oxyimino-cephalosporin or to aztreonam and/or showing an image of synergistic action were collected (Figure 1).

The bacterial species we collected came mainly from inpatients (51%) of the Saint Camille Hospital (HOSCO) in Ouagadougou. The distribution of bacterial species according to the gender of the patients is represented in Table 2.

Each subscripted letter indicates a subset of gender categories whose column proportions do not differ significantly from each other at the 0.05 level.

Women represented the majority of our bacterial species (46%), but in terms of distribution within bacterial species, they represented 30% of *K. pneumoniae* and 15% of *E. coli*. Some patients did not specify their gender when sending in their specimens (12%). The sex M/F ratio was 0.71.

The distribution of bacterial species according to the age of the patients is represented in Table 3.

The majority of bacterial species in our study were from patients in the age range of 0 to 14 years and 25 to 64 years. The distribution of bacterial species according to the patient's pathological products is represented in Table 4.

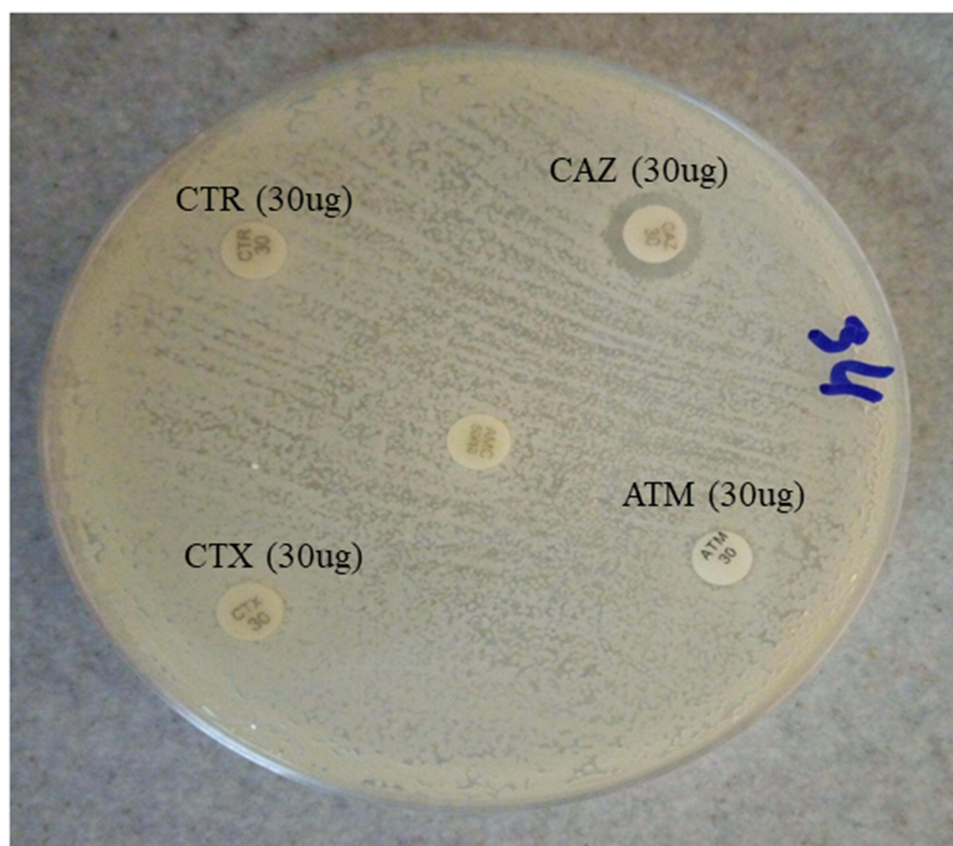
A total of 56% of the bacterial species came from urine. The distribution of bacterial species according to susceptibility to Oxyimino-cephalosporin is shown in Table 5.

The bacterial species *E. coli* and *K. pneumoniae* showed a high level of resistance to the antibiotics tested, ie 50% resistance to CAZ, 68% to CTX and CTR, and 63% to ATM. The distribution of bacterial species according to patient gender and resistance to oxyimino cephalosporins is presented in Table 6.

The strains of *K. pneumoniae* that were harbored by male patients showed the maximum resistance to oxyimino-cephalosporin. While for the *E. coli* strains, the female patients presented the maximum resistance to oxyimino-cephalosporin. The distribution of bacterial species according to patient age and oxyimino cephalosporin resistance is presented in Table 7.

The two bacterial species with the highest number of resistant strains came from patients in the 0–14-year age group, the pediatric age group, and the 25–64-year age group, the adult age group. The distribution of oxyimino cephalosporin resistance of the two bacterial species according to sample type is shown in Table 8.

The strains of *E. coli* and *K. pneumoniae* that showed maximum resistance were from urine.



**Figure 1** Petri dish with a strain of *E. coli* resistant to CRO, CAZ, CTX, ATM.

## Amplification of the GES Gene and Electrophoresis

Research of the GES gene by PCR with specific primers has shown that 30 strains (76%) harbor this gene. Bands of approximately 863 bp were observed after migration and visualization of PCR products (Figure 2).

In our study, we determined that 76% of *Escherichia coli* and *K. pneumoniae* strains harbored the GES gene. The distribution of the two bacterial species according to the presence of the GES gene is shown in Table 9.

The *E. coli* strain harbors the majority of the GES gene (48%). The distribution of the GES gene according to the sex and age of the patients is represented in Tables 10 and 11.

*K. pneumoniae* strains harboring the GES gene were predominantly from patients in the 0–14 age group; whereas *E. coli* strains were predominantly from patients in the 25–64 age group. The distribution of the GES resistance gene according to the type of sample is represented in Table 12.

**Table 2** Distribution of Bacterial Species According to the Gender of the Patients

Bacterial Species		Sex			Total
		None	Women	Men	
<i>Klebsiella pneumoniae</i>	Headcount	1 <sub>a</sub>	12 <sub>a</sub>	5 <sub>a</sub>	18
	% du total	2.0%	30.0%	12.0%	46.0%
<i>E. coli</i>	Headcount	4 <sub>a</sub>	6 <sub>a</sub>	11 <sub>a</sub>	21
	% du total	10.0%	15.0%	28.0%	53.0%
<b>Total</b>	Headcount	5	18	16	39
	% du total	12.0%	46.0%	41.0%	100.0%



**Table 3** Distribution of Bacterial Species According to the Age of the Patients

Age		Bacterial Species		Total
		<i>Klebsiella pneumoniae</i>	<i>E. coli</i>	
0–14 ans	Effectif	7 <sub>a</sub>	5 <sub>a</sub>	12
	% du total	19.0%	13.0%	33.0%
15–24 ans	Effectif	0 <sub>a</sub>	3 <sub>a</sub>	3
	% du total	0.0%	8.0%	8.0%
25–64 ans	Effectif	8 <sub>a</sub>	7 <sub>a</sub>	15
	% du total	22.0%	19.0%	41.0%
65 years and older	Effectif	3 <sub>a</sub>	3 <sub>a</sub>	6
	% du total	8.0%	8.0%	16.0%
Total	Effectif	18	18	36
	% du total	50.0%	50.0%	100.0%

**Notes:** Each subscripted letter indicates a subset of Strain categories whose column proportions do not differ significantly from each other at the 0.05 level.

**Table 4** Distribution of Bacterial Species According to the Patient's Pathological Products

Bacterial Species		Pathological Products			Total
		Urine	Stool	Vaginal Sample	
<i>Klebsiella pneumoniae</i>	Effectif	8 <sub>a</sub>	9 <sub>a</sub>	1 <sub>a</sub>	18
	% du total	20.0%	23.1%	2.0%	46.0%
<i>E. coli</i>	Effectif	14 <sub>a</sub>	7 <sub>a</sub>	0 <sub>a</sub>	21
	% du total	35.0%	17.0%	0.0%	53.0%
Total	Effectif	22	16	1	39
	% du total	56.0%	41.0%	2.0%	100.0%

**Notes:** Each subscripted letter indicates a subset of Pathological products categories whose column proportions do not differ significantly from each other at the 0.05 level.

**Table 5** Distribution of Bacterial Species According to Susceptibility to Oxyimino-Cephalosporin

Bacterial Species		CAZ		CTR		CTX		ATM		Total
		None	CAZ	None	CTR	None	CTX	None	ATM	
<i>K. pneumoniae</i>	Effectif	10 <sub>a</sub>	7 <sub>a</sub>	6 <sub>a</sub>	11 <sub>a</sub>	7 <sub>a</sub>	10 <sub>a</sub>	6 <sub>a</sub>	11 <sub>a</sub>	17
	% of total	26.0%	18.0%	15.0%	28.0%	18.0%	26.0%	15.0%	28.0%	44.0%
<i>E. coli</i>	Effectif	9 <sub>a</sub>	12 <sub>a</sub>	6 <sub>a</sub>	15 <sub>a</sub>	5 <sub>a</sub>	16 <sub>a</sub>	8 <sub>a</sub>	13 <sub>a</sub>	21
	% of total	23.0%	31.0%	15.0%	39.0%	13.0%	42.0%	21.1%	34.0%	55.0%
Total	Effectif	19	19	12	26	12	26	14	24	38
	% of total	50.0%	50.0%	31.0%	68.0%	31.0%	68.0%	36.0%	63.0%	100.0%

**Notes:** Each subscripted letter indicates a subset of ATM, CAZ, CTR, CTX categories whose column proportions do not differ significantly from each other at the 0.05 level.

*K. pneumoniae* strains harboring the GES gene were primarily from stool samples (33%), while *E. coli* strains were primarily from urine samples (57%). The only vaginal swab included in our study harbored a strain of *K. pneumoniae* and they harbored the GES gene.

**Table 6** Distribution of Bacterial Species According to Patient Gender and Resistance to Oxyimino-Cephalosporin

Bacterial Species			CAZ		CTX		CTR		ATM	
			None	CAZ	None	CTX	None	CTR	None	ATM
<i>K. pneumoniae</i>	None	Effectif	1 <sub>a</sub>	0 <sub>a</sub>	0 <sub>a</sub>	1 <sub>a</sub>	0 <sub>a</sub>	1 <sub>a</sub>	1 <sub>a</sub>	0 <sub>a</sub>
		% of total	5.0%	0.0%	0.0%	5.0%	0.0%	5.0%	5.0%	0.0%
	Women	Effectif	6 <sub>a</sub>	5 <sub>a</sub>	6 <sub>a</sub>	5 <sub>a</sub>	5 <sub>a</sub>	6 <sub>a</sub>	3 <sub>a</sub>	8 <sub>a</sub>
		% of total	35.0%	29.0%	35.0%	29.0%	29.0%	35.0%	17.0%	47.1%
	Men	Effectif	3 <sub>a</sub>	2 <sub>a</sub>	1 <sub>a</sub>	4 <sub>a</sub>	1 <sub>a</sub>	4 <sub>a</sub>	2 <sub>a</sub>	3 <sub>a</sub>
		% of total	17.0%	11.0%	5.0%	23.0%	5.0%	23.0%	11.0%	17.0%
<i>E. coli</i>	None	Effectif	10	7	7	10	6	11	6	11
		% of total	58.0%	41.0%	41.0%	58.0%	35.0%	64.0%	35.0%	64.0%
		Effectif	2 <sub>a</sub>	2 <sub>a</sub>	1 <sub>a</sub>	3 <sub>a</sub>	1 <sub>a</sub>	3 <sub>a</sub>	2 <sub>a</sub>	2 <sub>a</sub>
		% of total	9.0%	9.0%	4.0%	14.0%	4.0%	14.0%	9.0%	9.0%
	Women	Effectif	3 <sub>a</sub>	3 <sub>a</sub>	2 <sub>a</sub>	4 <sub>a</sub>	2 <sub>a</sub>	4 <sub>a</sub>	2 <sub>a</sub>	4 <sub>a</sub>
		% of total	14.0%	14.0%	9.0%	19.0%	9.0%	19.0%	9.0%	19.0%
	Men	Effectif	4 <sub>a</sub>	7 <sub>a</sub>	2 <sub>a</sub>	9 <sub>a</sub>	3 <sub>a</sub>	8 <sub>a</sub>	4 <sub>a</sub>	7 <sub>a</sub>
		% of total	19.0%	33.0%	9.0%	42.0%	14.0%	38.1%	19.0%	33.0%
		Effectif	9	12	5	16	6	15	8	13
		% of total	42.0%	57.0%	23.0%	76.0%	28.0%	71.0%	38.1%	61.0%
	Total	Effectif	19	19	12	26	12	26	14	24
		% of total	50.0%	50.0%	31.0%	68.0%	31.0%	68.0%	36.0%	63.0%

**Notes:** Each subscripted letter indicates a subset of CAZ, CTX, CTR, ATM categories whose column proportions do not differ significantly from each other at the 0.05 level.

## Discussion

The main objective of this study was to isolate and identify strains of *E. coli* and *K. pneumoniae* that produce ESBLs in patients with bacterial infections at the HOSCO. *Escherichia coli* and *Klebsiella pneumoniae* strains represent the most important enterobacteria in bacterial infections, more specifically urinary tract infections.<sup>15</sup> These infections are often treated with beta-lactams and other antibiotics effective against Enterobacteriaceae. Cephalosporins belong to the  $\beta$ -lactam class of antibiotics and are currently the most commonly used antibiotics to treat infections caused by these two enterobacteria. Both species possess a wide range of resistance mechanisms that make them even more virulent and dangerous to patients.<sup>14,15</sup> Strains of *E. coli* and *K. pneumoniae* can become resistant to beta-lactam antibiotics by producing an extended-spectrum beta-lactamase (ESBL), which is a plasmid-mediated  $\beta$ -lactamase capable of hydrolyzing and inactivating  $\beta$ -lactams such as cephalosporins and monobactams.<sup>17</sup> Highly pathogenic and antibiotic-resistant species of *K. pneumoniae* and *E. coli* are spreading rapidly worldwide.<sup>18,19</sup> Bacterial resistance is largely dependent on population and geographic factors. Strains of *Escherichia coli* and *Klebsiella pneumoniae* have shown high resistance to all antibiotics.

Antibiotic susceptibility results showed in general that these two bacterial species studied have a high rate of resistance to commonly used antibiotics: CRO (68%), CTX (68%), CAZ (50%) and ATM (63%). This corroborates the results of Tiemtoré et al in 2019 who demonstrated that these antibiotics are no longer as effective against enterobacteria. This multiresistance could be explained by the fact that the genes responsible for resistance can be carried by the same plasmid or by the coexistence of several resistance mechanisms.<sup>15</sup> The ESBL phenotype was found in 92.30% of these two bacterial species. These results testify to the emergence and spread of antibiotic-resistant germs generally due to the poor living conditions and hygiene observed in West African countries.<sup>4</sup> This high prevalence of antibiotics can be explained by deficiencies in the medical system (personnel, infrastructure), inappropriate use of antibiotics, socio-economic conditions, and antibiotic therapy in the animal sector.<sup>4</sup> Many patients, because of inaccessibility to health services or financial problems, obtain antibiotics directly from parallel markets. They start the treatment and when they feel better, they stop taking the tablets for another time or pass them on to someone else.<sup>17</sup> Self-medication and

**Table 7** Distribution of Bacterial Species According to Patient Age and Oxyimino Cephalosporin Resistance

Bacterial Species			CTR		CAZ		CTX		ATM	
			None	CTR	None	CAZ	None	CTX	None	ATM
<i>K. pneumoniae</i>	0–14 ans	Effectif	3 <sub>a</sub>	0	4 <sub>a</sub>	3 <sub>a</sub>	3 <sub>a</sub>	4 <sub>a</sub>	4 <sub>a</sub>	3 <sub>a</sub>
		% du total	16.00%	22.00%	22.00%	16.00%	16.00%	22.00%	22.00%	16.00%
	25–64 ans	Effectif	3 <sub>a</sub>	5 <sub>a</sub>	5 <sub>a</sub>	3 <sub>a</sub>	4 <sub>a</sub>	4 <sub>a</sub>	1 <sub>a</sub>	7 <sub>a</sub>
		% du total	16.00%	27.00%	27.00%	16.00%	22.00%	22.00%	5.00%	38.00%
	65 years and older	Effectif	0 <sub>a</sub>	3 <sub>a</sub>	1 <sub>a</sub>	2 <sub>a</sub>	0 <sub>a</sub>	3 <sub>a</sub>	1 <sub>a</sub>	2 <sub>a</sub>
		% du total	0.00%	16.00%	5.00%	11.00%	0.00%	16.00%	5.00%	11.00%
<i>E. coli</i>	0–14 ans	Effectif	1 <sub>a</sub>	4 <sub>a</sub>	2 <sub>a</sub>	3 <sub>a</sub>	1 <sub>a</sub>	4 <sub>a</sub>	3 <sub>a</sub>	2 <sub>a</sub>
		% du total	5.00%	22.00%	11.00%	16.00%	5.00%	22.00%	16.00%	11.00%
	15–24 ans	Effectif	1 <sub>a</sub>	2 <sub>a</sub>	1 <sub>a</sub>	2 <sub>a</sub>	0 <sub>a</sub>	3 <sub>a</sub>	0 <sub>a</sub>	3 <sub>a</sub>
		% du total	5.00%	11.00%	5.00%	11.00%	0.00%	16.00%	0.00%	16.00%
	25–64 ans	Effectif	3 <sub>a</sub>	4 <sub>a</sub>	4 <sub>a</sub>	3 <sub>a</sub>	3 <sub>a</sub>	4 <sub>a</sub>	3 <sub>a</sub>	4 <sub>a</sub>
		% du total	16.00%	22.00%	22.00%	16.00%	16.00%	22.00%	16.00%	22.00%
	65 years and older	Effectif	0 <sub>a</sub>	3 <sub>a</sub>	1 <sub>a</sub>	2 <sub>a</sub>	0 <sub>a</sub>	3 <sub>a</sub>	1 <sub>a</sub>	2 <sub>a</sub>
		% du total	0.00%	16.00%	5.00%	11.00%	0.00%	16.00%	5.00%	11.00%
Total	0–14 ans	Effectif	4 <sub>a</sub>	8 <sub>a</sub>	6 <sub>a</sub>	6 <sub>a</sub>	4 <sub>a</sub>	8 <sub>a</sub>	7 <sub>a</sub>	5 <sub>b</sub>
		% du total	11.00%	22.00%	16.00%	16.00%	11.00%	22.00%	19.00%	13.00%
	15–24 ans	Effectif	1 <sub>a</sub>	2 <sub>a</sub>	1 <sub>a</sub>	2 <sub>a</sub>	0 <sub>a</sub>	3 <sub>a</sub>	0 <sub>a</sub>	3 <sub>a</sub>
		% du total	2.00%	5.00%	2.00%	5.00%	0.00%	8.00%	0.00%	8.00%
	25–64 ans	Effectif	6 <sub>a</sub>	9 <sub>a</sub>	9 <sub>a</sub>	6 <sub>a</sub>	7 <sub>a</sub>	8 <sub>a</sub>	4 <sub>a</sub>	11 <sub>a</sub>
		% dans CTR	54.00%	36.00%	50.00%	33.00%	63.00%	32.00%	30.00%	47.00%
		% du total	16.00%	25.00%	25.00%	16.00%	19.00%	22.00%	11.00%	30.00%
	65 years and older	Effectif	0 <sub>a</sub>	6 <sub>a</sub>	2 <sub>a</sub>	4 <sub>a</sub>	0 <sub>a</sub>	6 <sub>a</sub>	2 <sub>a</sub>	4 <sub>a</sub>
		% du total	0.00%	16.00%	5.00%	11.00%	0.00%	16.00%	5.00%	11.00%
		Effectif	11	25	18	18	11	25	13	23
		% du total	30.00%	69.00%	50.00%	50.00%	30.00%	69.00%	36.00%	63.00%

**Notes:** Each subscripted letter indicates a subset of ATM, CAZ, CTR, CTX categories whose column proportions do not differ significantly from each other at the 0.05 level.

ignorance promote this sharing of antibiotics between individuals based on similar clinical signs. Furthermore, the quality of antibiotics, like many other drugs in West African countries such as Burkina Faso, is very often below the required standards. In addition to the risk of therapeutic failure, this poor quality of antibiotics increases the selection of resistant mutants. The causes of this poor quality of medicines are mainly the profit-seeking activities of pharmaceutical laboratories, which put on the market in these countries medicines that are sometimes under-dosed in active ingredients or even counterfeit.<sup>20</sup> Unfortunately, very few countries have regulatory bodies to control the quality of medicines.



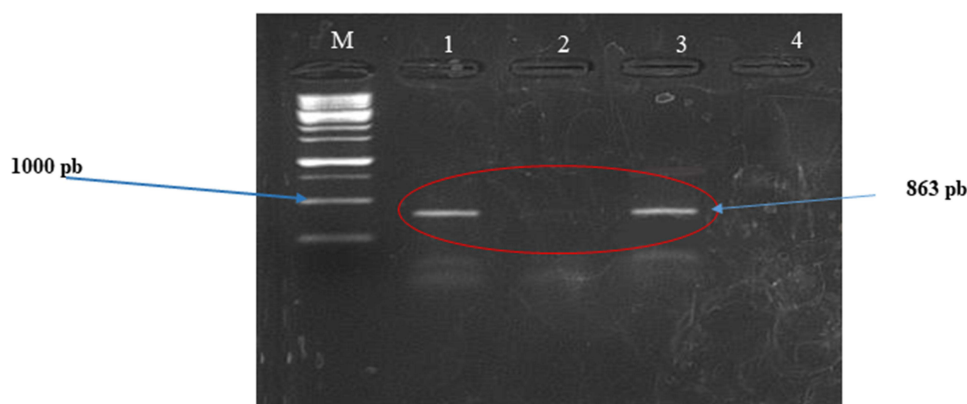
**Table 8** Distribution of Oxyimino Cephalosporin Resistance of the Two Bacterial Species According to Sample Type

Bacterial Species			CAZ		CTX		CTR		ATM	
			None	CAZ	None	CTX	None	CTR	None	ATM
<i>K. pneumoniae</i>	Urine	Effectif	4 <sub>a</sub>	4 <sub>a</sub>	3 <sub>a</sub>	5 <sub>a</sub>	2 <sub>a</sub>	6 <sub>a</sub>	1 <sub>a</sub>	7 <sub>a</sub>
		% of total	22.0%	22.0%	16.0%	27.0%	11.0%	33.0%	5.0%	38.0%
	Stool	Effectif	6 <sub>a</sub>	3 <sub>a</sub>	4 <sub>a</sub>	5 <sub>a</sub>	4 <sub>a</sub>	5 <sub>a</sub>	5 <sub>a</sub>	4 <sub>b</sub>
		% of total	33.0%	16.0%	22.0%	27.0%	22.0%	27.0%	27.0%	22.0%
	Vaginal swab	Effectif	0 <sub>a</sub>	1 <sub>a</sub>	0 <sub>a</sub>	1 <sub>a</sub>	0 <sub>a</sub>	1 <sub>a</sub>	0 <sub>a</sub>	1 <sub>a</sub>
		% of total	0.0%	5.0%	0.0%	5.0%	0.0%	5.0%	0.0%	5.0%
<i>E. coli</i>	Urine	Effectif	10	8	7	11	6	12	6	12
		% of total	55.0%	44.0%	38.0%	61.0%	33.0%	66.0%	33.0%	66.0%
	Stool	Effectif	5 <sub>a</sub>	9 <sub>a</sub>	3 <sub>a</sub>	11 <sub>a</sub>	3 <sub>a</sub>	11 <sub>a</sub>	5 <sub>a</sub>	9 <sub>a</sub>
		% of total	23.0%	42.0%	14.0%	52.0%	14.0%	52.0%	23.0%	42.0%
	TOTAL	Effectif	4 <sub>a</sub>	3 <sub>a</sub>	2 <sub>a</sub>	5 <sub>a</sub>	3 <sub>a</sub>	4 <sub>a</sub>	3 <sub>a</sub>	4 <sub>a</sub>
		% of total	19.0%	14.0%	9.0%	23.0%	14.0%	19.0%	14.0%	19.0%
	TOTAL	Effectif	9	12	5	16	6	15	8	13
		% of total	48.0%	51.0%	30.0%	69.0%	30.0%	69.0%	35.0%	64.0%

**Notes:** Each subscripted letter indicates a subset of CAZ, CTX, CTR, ATM categories whose column proportions do not differ significantly from each other at the 0.05 level.

Of the 18 strains of *K. pneumoniae* collected, 17 or 94.44% showed resistance to at least one of the antibiotics tested, but showed higher resistance to Ceftriaxone (68%) and Cefotaxime (68%). This corroborates the results of Ahmadi et al in 2022 who also showed higher resistance to Ceftriaxone. *K. pneumoniae* strains with resistance to at least one Oxyimino-cephalosporin and or aztreonam were mainly found in female patients, this can be explained by the fact that female patients represented the majority of our samples harboring *K. pneumoniae*. Similarly, concerning the age of the patients, the antibiotic resistances were mainly found in the age groups where they were collected. Regarding pathological products, *K. pneumoniae* strains with resistance to at least one Oxyimino-cephalosporin and or aztreonam were mainly found in urine, which corroborates the results of several studies that have shown that *Klebsiella pneumoniae* is one of the main causes of resistant urinary tract infections.<sup>3,16</sup>

*E. coli* is the most common Gram-negative bacterial pathogen among resistant bacteria and causes a wide range of diseases affecting all age groups. Indeed in our study we determined *E. coli* strains showing resistance to all antibiotics tested, from patients of all age groups. Multidrug resistant, extremely drug resistant and pan drug resistant *E. coli* strains have indeed been reported worldwide, and this is becoming a critical global problem.<sup>21</sup> Resistant *E. coli* strains were



**Figure 2** Agarose gel with the GES gene. M = Molecular weight marker, numbers 1: 1 = Positive control; 2 = Negative control; 3 = GES gene; 4 = Vaginal swab, the red circle indicates the position of the GES gene bands. The meaning of Electrophoresis migration is from top to bottom.

**Table 9** Distribution of the Two Bacterial Species According to the Presence of the GES Gene

Bacterial Species		GES		Total
		None	GES	
<i>Klebsiella pneumoniae</i>	Effectif	7 <sub>a</sub>	11 <sub>b</sub>	18
	% dans GES	77.0%	36.0%	46.0%
	% du total	17.0%	28.0%	46.0%
<i>E. coli</i>	Effectif	2 <sub>a</sub>	19 <sub>b</sub>	21
	% dans GES	22.0%	63.0%	53.0%
	% of total	5.0%	48.0%	53.0%
Total	Effectif	9	30	39
	% dans GES	100.0%	100.0%	100.0%
	% of total	23.1%	76.0%	100.0%

**Notes:** Each subscripted letter indicates a subset of GES categories whose column proportions do not differ significantly from each other at the 0.05 level.

**Table 10** Distribution of the GES Gene According to the Sex of the Patients

Bacterial Species				Sex			Total
				None	Women	Men	
<i>Klebsiella pneumoniae</i>	GES	None	Effectif	0 <sub>a</sub>	4 <sub>a</sub>	3 <sub>a</sub>	7
			% du total	0.0%	22.0%	16.0%	38.0%
			Effectif	1 <sub>a</sub>	8 <sub>a</sub>	2 <sub>a</sub>	11
	GES	GES	% du total	5.0%	44.0%	11.0%	61.0%
			Effectif	1	12	5	18
			% du total	5.0%	66.0%	27.0%	100.0%
<i>E. coli</i>	GES	None	Effectif	2 <sub>a</sub>	0 <sub>a, b</sub>	0 <sub>b</sub>	2
			% du total	9.0%	0.0%	0.0%	9.0%
			Effectif	2 <sub>a</sub>	6 <sub>a, b</sub>	11 <sub>b</sub>	19
	GES	GES	% du total	9.0%	28.0%	52.0%	90.0%
			Effectif	4	6	11	21
			% du total	19.0%	28.0%	52.0%	100.0%
Total	Total	Total	Effectif	5	18	16	39
			% du total	12.0%	46.0%	41.0%	100.0%

**Notes:** Each subscripted letter indicates a subset of gender categories whose column proportions do not differ significantly from each other at the 0.05 level.

found primarily in urine, which may be explained by the fact that *E. coli* is responsible for more than 85% of all urinary tract infections, according to a study published in March 2012 in the journal *Emerging Infectious Diseases*. Resistant *E. coli* strains were found mainly in male patients, which can be explained by the fact that the majority of *E. coli* isolates in our study were from male patients. This may be explained by the fact that the majority of *E. coli* isolates in our study were from male patients, as women are usually more prone to urinary tract infections due to their short urethras.

*E. coli* isolates often exhibit resistance patterns typical of ESBL producers. In this study, the majority of the isolates studied showed high resistance to all antibiotics tested.

Our study demonstrated the presence of the GES gene in *E. coli* and *K. pneumoniae* strains. A similar study by Ryoo et al in 2005 also reported *K. pneumoniae* and *E. coli* isolates harboring the GES gene in Korea. GES ESBLs have emerged from the shadows to become a recognized resistance threat. GES-1 ESBL was first described in 1998 in a *K. pneumoniae* isolate collected in France from a patient recently hospitalized in French Guyana.<sup>22</sup> *K. pneumoniae* remains the major reservoir of these widespread enzymes worldwide, for unknown reasons. However, extensive international travel and the spread of humans, food products, and animals have been proposed as possible causes of

**Table 11** Distribution of the GES Gene According to the Sex and Age of the Patients

Bacterial Species			GES		Total
			None	GES	
<i>K. pneumoniae</i>	0–14 ans	Effectif	2 <sub>a</sub>	5 <sub>a</sub>	7
		% of total	11.0%	27.0%	38.0%
	25–64 ans	Effectif	4 <sub>a</sub>	4 <sub>a</sub>	8
		% of total	22.0%	22.0%	44.0%
	65 years and older	Effectif	1 <sub>a</sub>	2 <sub>a</sub>	3
		% of total	5.0%	11.0%	16.0%
		Effectif	7	11	18
		% of total	38.0%	61.0%	100.0%
		Effectif	–	5	5
<i>E. coli</i>	0–14 ans	% of total	–	27.0%	27.0%
		Effectif	–	3	3
	15–24 ans	% of total	–	16.0%	16.0%
		Effectif	–	7	7
	25–64 ans	% of total	–	38.0%	38.0%
		Effectif	–	3	3
	65 years and older	% of total	–	16.0%	16.0%
		Effectif	–	18	18
		% of total	–	100.0%	100.0%
		Effectif	–	18	18
Total	0–14 ans	% of total	–	100.0%	100.0%
		Effectif	2 <sub>a</sub>	10 <sub>a</sub>	12
	15–24 ans	% of total	5.0%	27.0%	33.0%
		Effectif	0 <sub>a</sub>	3 <sub>a</sub>	3
	25–64 ans	% of total	0.0%	8.0%	8.0%
		Effectif	4 <sub>a</sub>	11 <sub>a</sub>	15
	65 years and older	% of total	11.0%	30.0%	41.0%
		Effectif	1 <sub>a</sub>	5 <sub>a</sub>	6
		% of total	2.0%	13.0%	16.0%
		Effectif	7	29	36
		% du total	19.0%	80.0%	100.0%

**Notes:** Each subscripted letter indicates a subset of GES categories whose column proportions do not differ significantly from each other at the 0.05 level.

the dispersed global distribution that is currently observed.<sup>23</sup> However, selective research interests may be another important reason.<sup>24</sup> GES has 23 variants that are described. In this study, we report clinical isolates of *K. pneumoniae*, *E. coli*, producing the Ambler class A enzyme, GES. This enzyme has been reported in Europe in *K. pneumoniae*<sup>25</sup> and *Pseudomonas aeruginosa*.<sup>26</sup> Unlike most ESBLs, GES - 1 does not hydrolyze aztreonam (Naas et al 2008), but our results showed the presence of the GES gene for aztreonam-resistant enterobacteria. These results corroborate those of Amana et al 2019, who found aztreonam resistant strains producing the GES type ESBL. Several GES subtypes have been described with their own properties which could explain why the GES type found hydrolyzes aztreonam. Indeed, the GES-1 subtype has a hydrolysis profile similar to that of other Ambler class A ESBL inhibited by clavulanate,<sup>27</sup> including activity against penicillins and broad spectrum cephalosporins, with higher activity against Ceftazidime than Cefotaxime. In our study enterobacterial strains harboring this gene showed high activity against both antibiotics. Moreover, GES-1 spares cephamycins and carbapenems and is inhibited by clavulanate, tazobactam and Imipenem.<sup>25</sup> However, unlike most ESBLs, GES-1 does not hydrolyze Monobactams. GES-2, which differs from GES-1 by a single Gly170Asn substitution located within the catalytic site loop, additionally hydrolyzes carbapenems, but at a low level.<sup>28</sup> In our study some enterobacteria harboring the GES gene were resistant to Imipenem, the presence of GES-2 could explain this fact. A Gly170Ser change was identified in GES-4, GES-5 and GES-6 and resulted in hydrolysis of carbapenems and cephamycins. GES-9, which differs from GES-1 by a change in Gly243Ser, does not hydrolyze

**Table 12** Distribution of the GES Resistance Gene According to the Type of Sample

Bacterial Species			GES		Total
			None	GES	
K pneumoniae	Urine	Effectif	4 <sub>a</sub>	4 <sub>a</sub>	8
		% of total	22.0%	22.0%	44.0%
	Stool	Effectif	3 <sub>a</sub>	6 <sub>a</sub>	9
		% of total	16.0%	33.0%	50.0%
	Vaginal swab	Effectif	0 <sub>a</sub>	1 <sub>a</sub>	1
		% of total	0.0%	5.0%	5.0%
E. coli	Urine	Effectif	7	11	18
		% of total	38.0%	61.0%	100.0%
	Stool	Effectif	2 <sub>a</sub>	12 <sub>a</sub>	14
		% of total	9.0%	57.0%	66.0%
	Vaginal swab	Effectif	0 <sub>a</sub>	7 <sub>a</sub>	7
		% of total	0.0%	33.0%	33.0%
Total	Urine	Effectif	2	19	21
		% of total	9.0%	90.0%	100.0%
	Stool	Effectif	6 <sub>a</sub>	16 <sub>a</sub>	22
		% of total	15.0%	41.0%	56.0%
	Vaginal swab	Effectif	3 <sub>a</sub>	13 <sub>a</sub>	16
		% of total	7.0%	33.0%	41.0%
	Vaginal swab	Effectif	0 <sub>a</sub>	1 <sub>a</sub>	1
		% of total	0.0%	2.0%	2.0%
		Effectif	9	30	39
		% of total	23.1%	76.0%	100.0%

**Notes:** Each subscripted letter indicates a subset of GES categories whose column proportions do not differ significantly from each other at the 0.05 level.

carbapenems but was found to possess activity against Monobactam.<sup>11</sup> The Enterobacteriaceae in our study were resistant to aztreonam (77%), the presence of this GES-9 type in our strains could explain this resistance. But GES-11 which differs from GES-1 by two amino acid substitutions, including the Gly243Ala change, also has increased activity against aztreonam and has recently been identified in *A. baumannii*.<sup>29</sup> It is possible that several subtypes of GHGs are present in our study. In order to differentiate them a sequencing will have to be done. It was reported that the GES-1 gene was located on a class 1 integron named In 52 which was characterized by a conserved 5' segment containing an *intI1* gene with two putative promoters, P(1) and P(2), for the coordinated expression of downstream antibiotic resistance genes and an *attI1* recombination site; five antibiotic gene cassettes, *bla*<sub>GES-1</sub>.<sup>25</sup>

## Conclusion

The objective of this study was to isolate and identify strains of *Escherichia coli* and *Klebsiella pneumoniae* resistant to oxyimino-cephalosporins and monobactam by production of ESBP type-GES at Saint Camille Hospital Center in Ouagadougou, Burkina Faso effects these two enterobacteria are the most found during infections bacterial. From our results, it appears that these two enterobacteria have a high resistance to oxyimino-cephalosporins which are the antibiotics of first intention in our country during bacterial infections. The GES gene was also found in 92.30% of our strains. In view of these results, we can confirm the presence in our country of strains of *E. coli* and *K. pneumoniae* resistant to antibiotics by production of so-called rare ESBP of the GES types. The presence of rare resistance genes could further compromise the health of populations by extending bacterial resistance to other families of antibiotics such as monobactams. Scientific papers on these enzymes tend to focus heavily on the research aspects, with little data on the clinical, treatment and control of infections. Therefore, it is critically important that the clinical aspects of infections with

GES-producing isolates be studied and documented in order to successfully control them, especially those with carbapenemase properties.

## Ethics

The institutional ethic committee of CERBA/LABIOGENE reviewed and approved the study protocol.

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## Author Contributions

All authors made a significant contribution to the reported work, whether in conception, study design, execution, data acquisition, analysis, and interpretation, or all of these areas; took part in the writing, editing, or critical review of the article; gave final approval to the version to be published; and agreed to the publication of the article. Regarding the version to be published, the authors have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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