



First Detection of PER-Type Extended-Spectrum β -lactamases at Saint Camille Hospital Center of Ouagadougou, Burkina Faso

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Abstract: Resistance to a wide variety of common antimicrobials is observed among clinical strains designated as extended-spectrum β -lactamase (ESBL) producers. They produce enzymatic proteins that effectively inactivate cephalosporins and aztreonam and are a serious global health problem that complicates treatment strategies. Many studies report a high prevalence of ESBL producers among Gram-negative bacilli. The purpose of this work was to identify of PER resistance gene in enterobacterial strains. Gram-negative bacilli resistant to at least one third-generation cephalosporin, Aztreonam or showing a synergistic image between amoxicillin + clavulanic acid and a third generation cephalosporin were isolated during an antibiogram. Antibiotic resistance was detected for the following antibiotics: Ceftriaxone, Cefotaxime, Ceftazidime and Aztreonam. A classical polymerase chain reaction (PCR) analysis of the β -lactamase PER (*Pseudomonas* Extended Resistance) gene was performed using specific primers in 60 ESBL-producing isolates. Among 250 strains of Gram negative bacilli collected, 60 strains (24%) showed resistance to antibiotics used. Stool samples are a major source of ESBL producers. The highest prevalence of resistant strains was observed in *Escherichia coli* with a rate of 35%. Among the producers of ESBL isolates, the presence of the PER gene was detected in the present study by up to 15% in 6 bacterial species. This study represents the first detection of the PER gene in Burkina Faso.

Keywords: Broad-spectrum Beta-lactamases (ESBL), PER Gene, PCR

1. Introduction

Enterobacteria, which are mainly responsible for community and nosocomial bacterial infections, have developed mechanisms of resistance face to the action of the antibiotics used against them. This resistance can be explained by the excessive and unregulated use of antibiotics, where they are prescribed excessively [1]. This problem is not solved even by the discovery of new, more effective antibiotics and the multi-resistance is increasing becoming common [2].

The majority of ESBLs are the result of genetic mutations

of natural β -lactamases [3]. They are very active against penicillins and moderately active against first generation cephalosporins. The genetic mutations at the origin of ESBLs broaden the spectrum of these enzymes and also affect third-generation cephalosporins (ceftazidime, cefotaxime and ceftriaxone, etc.) and monobactams (aztreonam). Virtually all Gram-negative germs have a chromosomal gene that codes for a cephalosporinase [4]. ESBLs are mainly found in the *Enterobacteriaceae*, mainly *Escherichia coli* and genus *Klebsiella*, more rarely *Serratia*, *Citrobacter*, *Enterobacter*, *Morganella*, *Proteus*, *Salmonella*, *Shigella* or non-fermentative Gram-negative bacteria such as *Pseudomonas*

spp., *Acinetobacter* and others. The majority of ESBLs are derived from TEM and SHV enzymes, but new ESBLs have been described such as CTX-M (cefotaximase), OXA (oxacillinase), PER (*Pseudomonas* Extended Resistance), VEB (Vietnam extended-spectrum β -lactamase), GES (extended-spectrum β -lactamase Guyana), TLA (TEM Like Activity), BES (Brazilian Extended Spectrum β -lactamases), SFO (*Serratia fonticola*) and FEC (Fecal *E. coli*) [5]. The rarer types such as SFO, TLA, PER, BES, GES, are found in *Acinetobacter baumannii*, *Serratia fonticola*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* [6]. ESBLs, because of their mode of distribution, constitute a major threat for West African countries where weak socio-economic conditions result in poor hygiene conditions, favoring the spread of resistance. It is therefore necessary to determine the presence of genes conferring these resistances. Studies in Burkina have already identified the presence of TEM, SHV and CTX-M genes, which are responsible for bacterial resistance in enterobacteria [7]. These genes, although widespread, are not the only ones responsible for bacterial resistance as determined in other countries [5]. In a context of escalating antibiotic resistance in our countries, we must not neglect the search for new ESBL. With this in mind, this study was conducted with the aim of detecting the presence PER gene in ESBL-producing gram-negative enterobacterial strains at Saint Camille Hospital in Ouagadougou, Burkina Faso.

2. Material and Methods

2.1. Isolation and Identification of Strains

The bacterial strains are gram-negative bacilli resistant to a third-generation cephalosporin, or to a monobactam. These strains were collected between September and October 2018 at the bacteriology department of the Saint Camille hospital laboratory. Strains were isolated from urine, pus, stool and vulvar specimens, from internal or external patients at HOSCO. The identification was carried out using the API 20E tests (BioMérieux S. A., Marcy l'Etoile, France).

2.2. Antibiotic Sensitivity Test and ESBL Detection

Antibiotic sensitivity was tested by the disk diffusion method. Antibiotics were tested on Petri dishes containing Muller Hinton agar. The antibiotic discs used were: aztreonam (30 μ g) for monobactam, cefotaxime (30 μ g), ceftriaxone (30 μ g) and ceftazidime (30 μ g) for third-generation cephalosporins. Isolates resistant to at least one antibiotic in clinical trials were collected, purified by transplanting and stored at -20 °C for further analysis. In order to also determine the production of ESBL, the antibiotic discs were deposited in such a way as to reveal the synergy image representing a champagne plug characteristic of the ESBL profile through a synergy test between the third generation of cephalosporins (cefotaxime or ceftazidime) and amoxicillin plus clavulanic acid [8] (Figure 1).

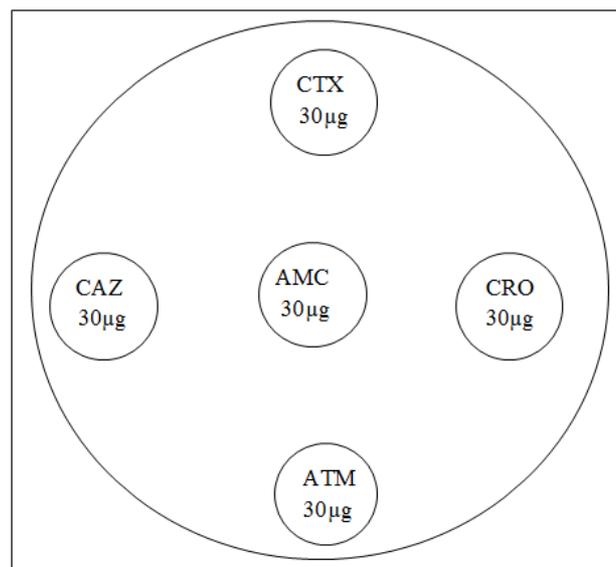


Figure 1. Disposition of antibiotic discs for synergy testing.

2.3. Molecular Characterization of Isolates Producing ESBL

2.3.1. Bacterial DNA Extraction

The extraction was done by the boiling method. The conserved strains are reactivated by culture on the MH medium for 18-24H. An isolated colony is taken from the Petri dishes and suspended in 200 μ l of distilled water previously aliquoted in labeled eppendorf tubes. The suspension thus obtained is immersed in a water bath (100°C. for 15 min) in order to release the genetic material. The suspension is then centrifuged at 12,000 rpm for 10 minutes and the supernatant containing the released DNA is transferred to a new eppendorf tube and stored at -20°C.

2.3.2. PER Gene Detection

All isolates with antibiotic resistance tested, or showing a synergy image were screened for the gene encoding the β -lactamase of the PER family. The crude DNA extracts obtained after extraction were used for the detection of the gene and the Green Master Mix PCR was used. The reaction volume was prepared as follows: 12.5 μ l of Green master mix + 0.5 μ l of primer F + 0.5 μ l of primer R + 10.5 μ l of PCR + 1 μ l of DNA extracted. PCR was performed with the Gene Amp System PCR 9700 thermocycler (Applied Biosystems, California, USA). The following primers sequences were used: PER-F: ATGAATGTCATTATAAAAGC and PER-R: AATTTGGGCTTAGGGCAGAA.

The amplification was carried out according to the following program: Initial denaturation at 95°C for 5 min, followed by denaturation at 95°C for 60s, hybridization at 50°C for 60s, extension at 68°C for 1mn, to finish with a final extension at 68°C for 5mn. This cycle was repeated 30 times.

2.3.3. Agarose Gel Electrophoresis

Electrophoretic migration at 120 volts for 30 minutes was performed on a 1% agarose gel in the presence of ethidium

bromide. A molecular weight marker of 1000 Pb was used as a reference. The resulting bands were observed under UV light from the GENE FLASH apparatus.

3. Results

3.1. Bacterial Strains and Antibiotic Susceptibility Testing

In this study, out of 250 strains isolated, 60 strains of Enterobacteriaceae resistant to at least one cephalosporin third generation or aztreonam and / or showing a synergy image were detected (Figure 2 and Figure 3).



Figure 2. Petri dish with a strain of enterobacteria resistant at CTR, CAZ, CTX, ATM.



Figure 3. Petri dish representing a synergy image characteristic of an ESBL.

Among the 60 strains of resistant enterobacteria, the following species were described. (Table 1).

Table 1. Frequency of bacterial strains.

Species	Number	Percentage (%)
Escherichia coli	21	35
Klebsiella pneumoniae	18	30
Enterobacter cloacae	6	10
Enterobacter aerogenes	1	1, 67
Citrobacter freundii	3	5
Citrobacter brakii	1	1, 67
Citrobacter youngae	1	1, 67
Proteus mirabilis	4	6, 66
Salmonella arizona	1	1, 67
Serratia marescens	4	6, 66
TOTAL	60	100

The maximum of resistant strains came from cytobacteriological examinations of the urine (50%). The maximum of resistant strains was from out patients at Saint Camille Hospital with 51.64%. The age of patients harboring strains with resistance ranged from 22 days to 95 years with an average age of 36.66 years. The sex ratio H / F was 0.71.

The distribution of the strains according to their resistance or to the presence of a synergistic image is represented in Table 2.

Table 2. The distribution of strains according to their resistance or the presence of a synergistic image.

Strains	ATM resistant	CAZ resistant	CTX resistant	CTR resistant	Synergy image
Escherichia coli	13	12	15	15	4
Klebsiella pneumoniae	12	8	11	12	0
Enterobacter cloacae	6	4	4	4	0
Enterobacter aerogenes	0	1	0	0	0
Citrobacter freundii	1	2	3	1	0
Citrobacter brakii	0	0	0	1	0
Citrobacter youngae	0	0	0	0	1
Proteus mirabilis	0	0	2	2	2
Salmonella arizona	1	0	1	0	0
Serratia marescens	3	3	3	3	0
TOTAL	36	30	39	38	7

3.2. PER Gene Amplification and Electrophoresis

The search of the PER gene by PCR with specific primers showed that 9 strains (15%) harbor this gene. About 1000 bp bands were observed after migration and visualization of the PCR products. (Figure 4)

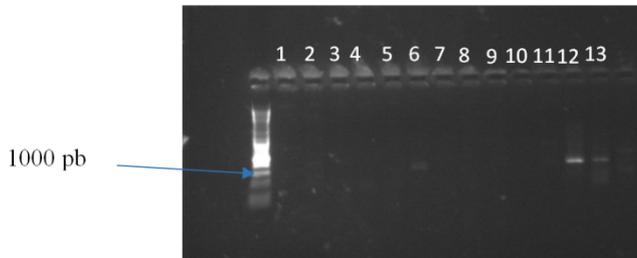


Figure 4. Agarose gel representing the migration of PCR products from gene of PER.

Legend: M = Molecular weight marker, numbers 1-8 represent the samples: 1 = Uro8; 2 = Uro9; 3 = Uro10; 4 = Uro11; 5 = Uro12; 6 = Uro13; 7 = Uro14, 8 = Uro 15; number 9 represents the negative control; numbers 10-13 represent the samples: 10 = Uro16; 11 = Uro17; 12 = Uro18; 13 = Uro19. The direction of migration of electrophoresis is from top to bottom.

Among the results, 44.44% of the PER gene was found in patients over 50 years of age and 11.11% were less than 5 years old. According to sexual division, 77.77% were found in the male group. Isolates that produce PER-type ESBL are much more common in feces with 66.66%.

The distribution of genes according to bacterial species is shown in Table 3:

Table 3. Gene distributions according to bacterial species.

Species	Gene PER
<i>E. coli</i>	2
<i>k. pneumoniae</i>	0
<i>Enterobacter cloacae</i>	3
<i>Salmonella arizona</i>	1
<i>Citrobacter youngae</i>	0
<i>Citrobacter freundii</i>	1
<i>Serratia marescens</i>	1
<i>Citrobacter brakii</i>	0
<i>Enterobacter aerogenes</i>	1
TOTAL	9

4. Discussion

The objective of this study was to detect ESBL-producing enterobacteria at Saint Camille Hospital in Ouagadougou. We observed the ESBL phenotype in seven (07) bacterial genera. The prevalence of resistant strains was found in 24% of the 250 strains of bacteria isolated in the said center. It was 35% in *E. coli*, 30% in *Klebsiella pneumoniae*, 10% in *Enterobacter cloacae*, 1.67% in *Enterobacter aerogenes*, 6.66% in *Proteus mirabilis*; 5% at *Citrobacter freundii*; 1.67% in *Citrobacter brakii*, 1.67% in *Citrobacter youngae*, 1.66% in *Salmonella arizona* and 6.66% in *Serratia marescens*. All the bacteria studied showed a high level of resistance to the antibiotics used. Several multiresistant strains have been

observed. This multidrug resistance can be explained by the fact that the genes responsible for these resistances can be carried by the same plasmid, by the coexistence of several resistance mechanisms, or by the production of several enzymatic types [9, 10]. The resistance percentages observed in this study are very high for some antibiotics [cefotaxime (65%), ceftriaxone (63.33%), ceftazidime (50%) and aztreonam (60%)] compared to those observed in developed countries but are approaching resistance rates reported in the developing countries [11]. This high level of resistance is a consequence of the acquisition of antibiotic resistance factors that is generally secondary to the misuse and uncontrolled use of these antibiotics [12, 13]. The absence of routine surveillance in most countries and in the West African region does not allow a good estimate of ESBL proportions among strains isolated during infectious processes. However, some studies report the reality of the problem as in Ghana where half of the enterobacteria (49.4%) isolated from the various infections diagnosed at Korle-Bu Hospital were producing ESBL [12]. In Togo 66% of *E. coli* strains in the urinary infections had the ESBL phenotype [14]. These results demonstrate the emergence and dissemination of antibiotic-resistant germs, generally because of the poor living and hygiene conditions observed in developing countries [2]. Our results on antibiotic susceptibility [cefotaxime (65%), ceftriaxone (63.33%), ceftazidime (50%) and aztreonam (60%)] confirm the results of work already reported on the high prevalence of enterobacteria resistance. β -lactams in developing countries, which is higher than those in developed countries. In this study, the majority of ESBL producing strains (51.67%) were isolated from out-patients at Saint Camille Hospital. This can be explained by the lack of strict rules for the acquisition of antibiotics in developing countries which means that everyone can have access to even broad-spectrum antibiotics without any medical prescription [15]. In all West African countries, antibiotics are sold, like many other medicines, in popular markets. These drugs are known as street drugs [16, 17]. Self-medication and ignorance promote this sharing of antibiotics between individuals based on similar clinical signs.

The CTX, SHV and TEM genes involved in bacterial resistance are the most found and documented genes in West Africa, but outside of these genes, rarer genes existing are equally responsible of resistance, and it is one of these genes the PER gene we have do research [18- 20].

Our results made it possible to highlight for the first time in Burkina and in West Africa the presence of the PER gene. Broad-spectrum β -lactamase (ESBL) PER was detected for the first time in 1993 in a *Pseudomonas aeruginosa* isolate from a Turkish patient in France [21]. This enzyme is weakly related to other ESBLs and confers resistance to penicillins, cefotaxime, ceftriaxone, ceftazidime and monobactam-aztreonam, but avoids carbapenem and cephamycin resistance. Its activity is inhibited by clavulanic acid [22]. The *bla*_{PER} gene is widespread in *Acinetobacter spp.*, *P. aeruginosa* and *Salmonella enterica*, serovar, *Typhimurium* in Turkey [23]

and he has also been detected in the strains of *Providencia rettgeri* in that country [24]. In addition, PER-1 has been identified in *Acinetobacter sp.* isolated in Korea [25]. In Italy, PER-1 was detected in isolates of *P. aeruginosa*, *Alcaligenes faecalis* and *Proteus mirabilis* [26, 27]. In our study, the PER gene was found in *E. coli*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Serratia marescens*, *Citrobacter freundii* and *Salmonella arizona* with a predominance in *Enterobacter cloacae*. The majority of our PER-producing strains were not only resistant to third-generation cephalosporins but also to monobactams, but also exhibited a multiresistant phenotype, which corroborates the results obtained by Pagani et al in 2004 in Italy.

5. Conclusion

The aim of this work was to detect enterobacteria (ESBL) producing extended-spectrum beta-lactamases of the PER type at Saint Camille Hospital in Ouagadougou. The results reveal a number of elements. The isolated bacteria have high levels of beta-lactam resistance tested. The prevalence of resistant bacteria at cephalosporin third generation, aztreonam and / or with a synergistic image is 24%. In light of all this information, it appears that the ESBL threat exists at the Saint Camille Hospital Center in Ouagadougou (HOSCO). The PER type was identified for the first time in Burkina Faso mainly in *Enterobacter cloacae* (42.85%). Our work has the merit of sounding the alarm on bacterial resistance by producing rare ESBLs.

In view of these results, it seems that ESBLs play an important role in bacterial resistance, which is accentuated by the appearance of mutants of these often more dangerous enzymes that compromise health care.

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