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FIRST DETECTION OF SHV-TYPE EXTENDED SPECTRUM B-LACTAMASES IN THE UNIVERSITY HOSPITAL COMPLEX PAEDIATRIC CHARLES DE GAULLE (CHUP-CDG) OF OUAGADOUGOU IN BURKINA FASO

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ABSTRACT

Strains were isolated from urines clinical samples from patients from various service units of University Hospital complex Paediatric Charles De Gaulle (CHUP-CDG) in Burkina Faso. These strains were resistant to at least one oxyimino-cephalosporin. They were identified as producer of extended spectrum β-lactamases (ESBL) by double-disk synergy test between amoxicillin–clavulanate and cefotaxime, ceftriaxone or ceftazidime and by kinetic methods. Crude extract from these isolates showed a broad-substrate profile by hydrolyzing cefuroxime, cefotaxime, ceftriaxone and with a lower degree to cetazidime and cefepime but not imipinem. Sequencing of PCR products amplified with primers designed for bla_{SHV} genes showed that these genes coded for SHV-11, SHV-12, SHV-28, SHV-32, SHV-38, SHV-76 and SHV-99. This is the first description of these enzymes in Burkina Faso.

Keywords: SHV-type extended spectrum β-lactamases, University hospital complex paediatric charles de gaulle.

1. INTRODUCTION

Resistance to antibiotics may be caused by several mechanisms, but the most important of which is the production of β-lactamase that hydrolyzes the β-lactam ring [1, 2]. β-Lactamases (E.C. 3.5.2.6) are the primary agents of bacterial resistance to penicillins and cephalosporins. The enzymes are divided into four major molecular classes, classes A, B, C, and D on the basis of their amino acid sequences [3-5]. All β-lactamases except the zinc-containing class B enzymes are serine-reactive hydrolases that act via an acyl intermediate [3]. To date, a large variety of β-lactamases have been documented in various Gram negative bacilli such as *Pseudomonas* spp. and members of the family *Enterobacteriaceae* [4]. Resistance to β-lactam antibiotics was demonstrated in *Escherichia coli* even before penicillin was released for clinical use. β-lactamases can be mediated by plasmid or chromosome [3]. In the 1960s, the first plasmid-transferable β-lactamase was discovered and named TEM-1 [6]. In this study, we characterize SHV-type extended spectrum β-lactamases produced by cephalosporins resistant strains isolated from samples of urines from CHUP-CDG in Burkina Faso.

2. MATERIALS AND METHODS

2.1. Bacterial Strains

Seven K. pneumoniae, 1 K. oxytoca ,2 Klebsiella sp and 2 Enterobacter sp. strains were collected between July 2010 and Mars 2012, from samples of urines of various service units of CHUP-CDG in Burkina Faso. Isolates were identified using an API 20 E system (bio-Mérieux, Marcy-l'Étoile, France).

2.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the disk diffusion method on Müeller-Hinton agar (Bio Rad, France) as recommended by the Clinical and Laboratory Standards Institute (CLSI) [7]. The double-disk synergy test for confirmation of ESBL activity was carried out as described previously [8, 9], by using amoxicillin–clavulanate against cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ) or aztreonam (ATM). Minimal inhibitory concentrations (MIC) of CRO, CTX, CAZ, cefuroxim (CXM), cefepim (FEP) and imipenem (IPM) were determined by Method of dilution in liquid medium for strains according to CLSI guidelines [7].

2.3. Bacterial DNA Extraction

Genomic DNA was extracted from bacteria using DNAzol®Reagent (Invitrogen/DNA by life technologies) following instructions of the manufacturer.

2.4. Polymerase Chain Reaction (PCR) Amplification of Bla_{shv} Genes and Sequencing

Detection of SHVencoding genes was performed by PCR. The pair of primers SHV F: 5'-

ATGCGTTATATTCGCCTGTG -3' and SHV R: 5'-TTAGCGTTGCCAGTGCTC-3' [10] was used to amplify bla_{SHV} sequences. The DNA amplification program consisted of an initial denaturation step 5 min at 96°C, followed by 30 cycles of denaturation for 1 min at 96°C, annealing for 1 min at 60°C and 1 min at 72°C for polymerization. Final products were extended by incubation for 10 min at 72 °C. PCR products were visualized by agarose gel electrophoresis (Fig.1). Amplicons of 875bp were sequenced by the Company GATC Biotech in Europe, and the resulting sequences were then compared with the sequences from GenBank database.

3. RESULTS

3.1. B-Lactam Susceptibility Profile and Minimum Inhibitory Concentration Determination

The different strains showed a significant degree of multiresistance to various antibiotics (Table 1). All the 12 strains were resistant to CXM, CTX and CRO and with a lower degree to CAZ and FEP but were susceptible to IPM. The disk diffusion method showed synergy between ceftazidime, cefotaxime, ceftriaxone, and amoxicillin–clavulanic acid against the strains, suggesting the presence of a class A ESBL [8, 9, 11]

3.2. Amplification of B-Lactamase-Encoding $Bla_{ctx\text{-}M}$ Genes and Sequence Analysis

PCR analysis confirmed the presence of an 875 bp bla_{SHV} genes in all strains. Sequences analyses of the nucleotide sequence showed the occurrence of $bla_{SHV\text{-}11}$ (Sequence ID: gb|GU064384.1|) in 6 samples, $bla_{SHV\text{-}12}$ (Sequence ID: gb|GU732834.1|),$bla_{SHV\text{-}28}$ (Sequence ID: gb|HQ877609.1|) , $bla_{SHV\text{-}32}$ (Sequence ID: gb|AY037778.1|) , $bla_{SHV\text{-}38}$ (Sequence ID: gb|GQ407125.1|) , $bla_{SHV\text{-}76}$ (Sequence ID: emb|AM176551.2|) and $bla_{SHV\text{-}99}$ (Sequence ID: emb|AM922305.1|) in 1 sample each one (Table 2).

4. DISCUSSION

In this report, we mentioned the occurrence of several strains carrying $bla_{SHV\text{-}11,\text{-}12,\text{-}28,\text{-}32,\text{-}38,\text{-}76\ and\ \text{-}99}$ genes for the first time in Burkina Faso. Strains were found to be resistant to oxyimino-cephalosporins, FEP and exhibited a positive double-disc synergy test, indicating the presence of an ESBL [11]. Multiresistance has often been described for ESBL producing clinical isolates [12-17]. When a PCR assay for SHV-type genes was used, 875 bp long amplicons were detected in each strain. Sequences analysis showed that PCR products correspond to $bla_{SHV\text{-}11,\text{-}12,\text{-}28,\text{-}32,\text{-}38,\text{-}76\ or\ \text{-}99}$.

Although SHV-11-type β-lactamase is a penicillinase, the enzyme activity measured by a spectrophotometer as previously described [18] showed a hydrolytic activities against cloxacilline, cephalotin and oxymino cephalosporins such as cefotaxime and ceftriaxon.

The hydrolysis of the extended spectrum cephalosporins by the crude extract of SHV-11 producing strains is due to the hyper production of SHV-11 coded simultaneously by plasmidic and chromosomal genes [19]. In fact, modifications of outer membrane proteins [20], and hyperprodution of SHV-1 enzyme due to high gene copy number [21], or a single base pair change in the promoter sequence can compensate for the low specific activity of the β-lactamase to an extended spectrum β-lactams and monobactams [8, 20].

SHV-type β-lactamases contributed poorly to the resistance profile of the clinical isolates, which was mostly reflected by the expression of a CTX-M-type enzyme. SHV-types ESBL were reported in several countries but never in Burkina Faso. For instance β-lactamases.

SHV-98, SHV-99 and SHV-100 were produced by clinical *Klebsiella pneumoniae* strains in an Algerian hospital [22]. SHV-28 was reported at the Southwest Hospital of the Third Military

Medical College in China in 2002 (GenBank AF538324), and it was the first report of SHV-28. [23]. reported the presence of SHV-28 in two strains in Korea [23]. SHV-28 was also demonstrated by Tofteland, et al. [24]. in Norway in 2006 [24].

Ndugulile, et al. [25]. reported the presence of SHV-28 in Africa for the first time in 2005 [25]. SHV-28 was also reported in SOUTH INDIA [26]. SHV-12 was produced by *Enterobacteriaceae* in Tanzanian [27]. The β-lactamase SHV-38 is the first example of a chromosome-encoded SHV-type enzyme with an extended hydrolysis profile that also includes, like the other ESBLs of the SHV-type, some expanded-spectrum cephalosporins [28, 29].

In this study, the genes of SHV-types β-lactamases are carried by *klebsiella* and *Enterobacter*. But in other countries, the genes of SHV-types β-lactamases are found in other bacterial such as *Shigella dysenteriae* in India [30], *Escherichia coli* in Korea [31], *Pantoea spp* in Tanzanian [27].

The easy horizontal and vertical spread of β-lactamases worldwide will therefore dictate future research. Thus, considering the diversity of the enzymic characteristics of β-lactamases, the biochemical characterization of new enzymes is essential to understand their individual contribution to resistance phenotypes of pathogens which have an impact on public health [32].

Careful attention to infection control and rational use of antimicrobials may help prevent further spread of these multidrug-resistant bacteria.

5. ACKNOWLEDGEMENTS

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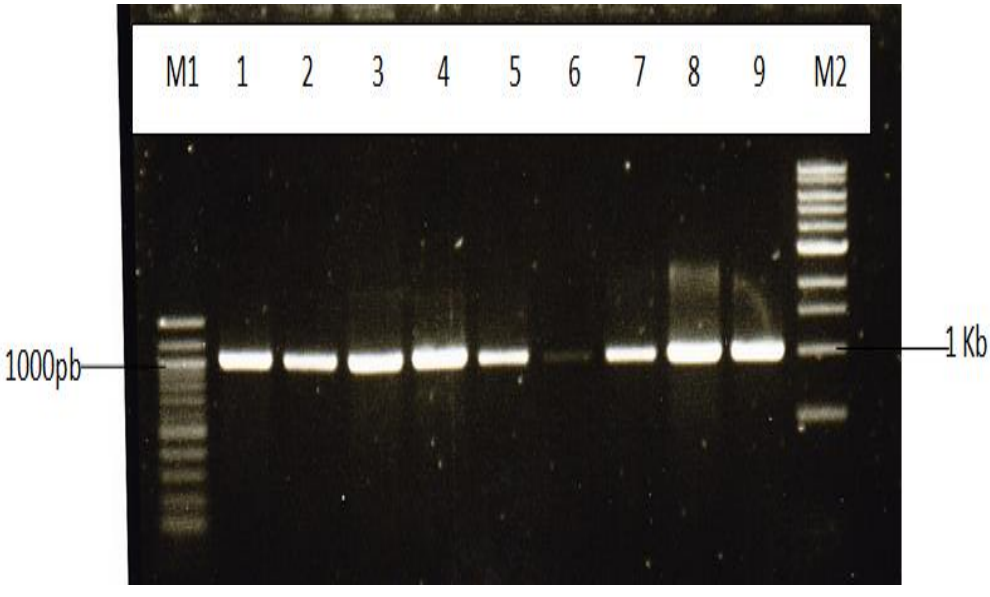
Table-1. Bacterial isolates and minimum inhibitory concentration determination of 6 antibiotics

Samples	Isolates	MIC (µg/ml)					
		CXM	CTX	CRO	CAZ	FEP	IPM
Urines 46	*K.pneumoniae*	200< MIC ≤ 300	200< MIC ≤ 300	100< MIC ≤ 200	≤ 25	≤ 25	≤ 25
Urines 120	*K. pneumoniae*	>300	>300	200< MIC ≤ 300	25< MIC ≤ 50	50< MIC ≤ 100	≤ 25
Urines 213	*K.pneumoniae*	>300	200< MIC ≤ 300	200< MIC≤ 300	50< MIC ≤ 100	50< MIC ≤ 100	≤ 25
Urines 466	*K.pneumoniae*	200< MIC ≤ 300	200< MIC≤ 300	>300	25< MIC≤ 50	≤ 25	≤ 25
Urines 538	*K.pneumoniae*	100< MIC ≤ 200	100< MIC ≤ 200	100< MI C≤ 200	50< MIC ≤ 100	50< MIC ≤ 100	≤ 25
Urines 774	*K.pneumoniae*	>300	200< MIC ≤ 300	100< MIC ≤ 200	50< MIC ≤ 100	50< MIC ≤ 100	≤ 25
Urines 778	*K.pneumoniae*	100< MIC ≤ 200	200< MIC ≤ 300	100< MIC ≤ 200	50< MIC≤ 100	50< MIC≤ 200	≤ 25
Urines 336	*Klesiella sp*	200< MIC ≤ 300	200< MIC ≤ 300	>300	25<MIC ≤ 50	≤ 25	≤ 25
Urines 715	*Klesiella sp*	200< MIC ≤ 300	100< MIC≤ 200	>300	50< MIC ≤ 100	50< MIC ≤ 100	≤ 25
Urines 613	*K.oxytoca*	>300	>300	>300	25< MIC≤ 50	25< MIC ≤ 50	≤ 25
Urines 556	*Enterobacter sp*	200< CMI ≤ 300	200< CMI ≤ 300	100< CMI ≤ 200	50< CMI ≤ 100	≤ 25	≤ 25
Urines 1012	*Enterobacter sp*	>300	200< CMI ≤ 300	100< CMI ≤ 200	25< CMI ≤ 50	50< CMI ≤ 100	≤ 25

MIC : Minimal inhibitory concentrations;CXM : Cefuroxim ; CTX : Cefotaxim ; CRO: Ceftriaxon; CAZ: Ceftazidim; FEP: Cefepim; IPM: Imipenem

Fig-1. Agarose gel of SVH PCR products



M1, Marker of molecular weight (GeneRuler 100bp DNA Ladder), numbers 1-7 represent the samples:1=Uro774; 2=Uro778; 3=Uro538; 4=Uro120; 5=Uro213; 6=Uro466; 7=Uro336; 8=Uro715; 9=Uro46

Table-2. Bacterial isolates and SHV β-lactamases-types

isolates	**β-lactamases-types**	
	SHV	**genes**
K.pneumoniae 774	+	SHV-12
K.pneumoniae778	+	SHV-32
K.pneumoniae 538	+	SHV-76
Enterobacter sp1012	+	SHV-11
Enterobacter sp556	+	SHV-11
Klesiella sp715	+	SHV-11
K.pneumoniae46	+	SHV-99
K.oxytoca613	+	SHV-1 1
K.pneumoniae 120	+	SHV-38
K.pneumoniae 213	+	SHV-11
K.pneumoniae466	+	SHV-28
Klesiella sp336	+	SHV-11