RESISTANCES TO THE OXYIMINO-CEPHALOSPORINS BY CTX-M-15 PRODUCING KLEBSIELLA ISOLATED FROM THE URINES SAMPLES OF PATIENTS IN THE UNIVERSITY HOSPITAL COMPLEX PAEDIATRIC CHARLES DE GAULLE (CHUP-CDG) OF OUAGADOUGOU IN BURKINA FASO

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ABSTRACT
Seventeen oxyimino-cephalosporins-resistant Klebsiella 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. The ESBL was identified as CTX-M-15 for the 17 strains by sequencing of PCR.
products amplified with primers designed for blaCTX-M genes. This is the first description of this enzyme in Burkina Faso.

Keywords: β-lactamases, CTX-M-15; ESBL, Oxyimino-Cephalosporins, Klebsiella.

1. INTRODUCTION

Cefotaximase first isolate in Munich (CTX-M)-type extended-spectrum β-lactamases (ESBL) constitutes a worldwide growing group of enzymes encoded by blaCTX-M genes located on diverse plasmids belonging to the IncFII group (Eckert et al., 2004; Pieboji et al., 2005; Carattoli Alessandra, 2009). This family of plasmid-mediated ESBL belongs to Ambler class A and functional group 2be of the Bush-Jacoby and Medeiros classification (AMBLER, 1980; BUSH et al., 1995). They are capable of hydrolyzing expanded-spectrum cephalosporins and are inhibited by clavulanic acid, sulbactam, and tazobactam. In addition, they confer a high level resistance to cefotaxime but have a low level activity towards ceftazidime (Bonnet, 2004; Eckert et al., 2004). The CTX-M β-lactamases are the most widespread ESBL enzymes (Mena et al., 2006), distributed both over wide geographic areas and among a wide range of clinical bacteria, in particular, members of the family of Enterobacteriaceae. They were initially reported in the second half of the 1980s, and their rate of dissemination among bacteria and in most parts of the world has increased dramatically since 1995 mostly due to positive selection exerted by use of antimicrobials (Radice et al., 2002; Quinteros et al., 2003; Hernandez et al., 2005; Pieboji et al., 2005; Tumbarello et al., 2006). At present, the CTX-M family comprises 142 enzymes (www.lahey.org/studies/other.asp). A phylogenic study revealed five major groups of CTX-M enzymes according to their amino acid sequences (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25 group) (Bonnet, 2004; Munday et al., 2004; Oliver et al., 2005; Mena et al., 2006).

blaCTX-M genes have been found to originate from the chromosomal β-lactamase genes of Kluyvera. For instance, CTX-M-2, CTX-M-8 and CTX-M-9 clusters derive from Kluyvera ascorbata’s KLUA-1, K. georgiana’s KLUG-1, and K. georgiana’s KLUY enzymes, respectively (Humeniuk et al., 2002; Poirel et al., 2002; Olson et al., 2005). Besides, a chromosome encoded CTX-M-3 from a Kluyvera ascorbata strain seems to be the closest enzyme and most probable origin of the CTX-M-1 group (Rodríguez et al., 2004).

The aim of the study is the characterization of ESBL among clinical isolates of 17 different oxyimino-cephalosporin-resistant Klebsiella strains isolated from samples of urines from CHUP-CDG in Burkina Faso.

2. MATERIALS AND METHODS

2.1. Bacterial Strains

Twelve K. pneumoniae, 2 K. oxytoca and 3 Klebsiella 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 Müller-Hinton agar (Bio Rad, France) as recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2006). The double-disk synergy test for confirmation of ESBL activity was carried out as described previously (Jacoby and Han, 1996; Livermore et al., 2001), 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 (CLSI, 2006).

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 blaCTX-M Genes

Detection of CTX-M encoding genes was performed by PCR. The pair of primers CTX-M F: 5’-GTTACAATGTGAGAAGCAG-3’ and CTX-M R: 5’-CGGTTCGTACTATACAGTAC-3’ (Pagani et al., 2003) was used to amplify blaCTX-M 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 50°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. Amplicons of 953bp 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. β-Lactam Susceptibility Profile and Minimum Inhibitory Concentration Determination

The different Klebsiella strains showed a significant degree of multiresistance to various antibiotics (Table 1). All the 17 Klebsiella 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 (Jarlier et al., 1988; Jacoby and Han, 1996; Livermore et al., 2001) (Fig.1)

3.2. Amplification of β-lactamase-encoding blaCTX-M Genes and Sequence Analysis

PCR analysis confirmed the presence of a 953 bp blaCTX-M gene in all Klebsiella strains. Sequences analyses of the nucleotide sequence showed the occurrence of blaCTX-M.15 (Sequence ID: gb|JQ686199.1) in all samples.
4. DISCUSSION

In this report, we mentioned the occurrence of several Klebsiella strains carrying the \( \text{bla}_{\text{CTX-M-15}} \) gene for the first time in Burkina Faso. Klebsiella were found to be resistant to oxyimino-cephalosporins, FEP and exhibited a positive double-disc synergy test, indicating the presence of an ESBL (Jarlier et al., 1988). Multiresistance has often been described for ESBL (and particularly CTX-M) producing clinical isolates (Rice et al., 1996; Shannon et al., 1998; Asensio et al., 2000; Nathisuwan et al., 2001; Bradford, 2001; Kang et al., 2004). When a PCR assay for CTX-M-type genes was used, 953 bp long amplicons were detected in each strain of Klebsiella. Sequence analysis showed that all PCR products correspond to \( \text{bla}_{\text{CTX-M-15}} \). Like the majority of the CTX-M enzymes, CTX-M-15 has been shown to hydrolize the CTX preferentially to CAZ (Sturenburg et al., 2004). CTX-M-15 was reported in several countries but never in Burkina Faso. For instance at the Charles-Nicolle Hospital in Tunis (Tunisia), 62 enterobacterial strains producing CTX-M \( \beta \)-lactamase were collected between March 2000 and June 2003. All of isolates produce CTX-M-15 or CTX-M-16 (Mamlouk et al., 2006). CTX-M-15 is also found in other bacterial species in other countries. As such E. coli strains CTX-M-15 have been described in Canada, in India, in Kuwait, in France, in Switzerland, in Portugal and in Spain (Coque et al., 2008). In some cases \( \text{bla}_{\text{CTX-M-15}} \) dissemination is related to dispersion of a same plasmid and to the propagation of some clones of Klebsiella. The exchange of plasmids between E. coli and K. pneumonia with high epidemic potential is most probable. Results in favor of these genetic exchanges between bacterial species were obtained in Poland with CTX-M-3 (Baraniak et al., 2002). In this report, the plasmids present in the Klebsiella strains isolated in Burkina Faso were not characterized but many studies showed that dissemination of organisms that produce CTX-M-9, CTX-M-14, CTX-M-15 and CTX-M-32 have been linked with epidemic plasmids associated to those of the incompatibility group IncFII (Lavollay et al., 2006; Novais et al., 2006). Studies realized in Spain and Israel indeed showed that the rate of CTX-M-15 producing E. coli found in saddles of in-patients is 11.8 % and 10.8 % respectively (Valverde et al., 2004; Ben-Ami et al., 2006), which would constitute a tank very significant of \( \text{bla}_{\text{CTX-M-15}} \) plasmids carrying with strong potential of inter-species transfer. In China, in contrast to the diversity of clonal relationships, many local isolates harbored a 90-kb IncFII plasmid carrying \( \text{bla}_{\text{CTX-M-15}} \), suggesting that this plasmid appeared to be a major vehicle mediating the local dissemination of \( \text{bla}_{\text{CTX-M-15}} \) in K. pneumonia (Zhuo et al., 2013). Indeed, previous reports (Canton and Coque, 2006) suggested that plasmid is one major factor responsible for the worldwide spread of \( \text{bla}_{\text{CTX-M-15}} \). For example, in E. coli, many plasmids carrying \( \text{bla}_{\text{CTX-M-15}} \) found in France, Tunis, Bangui and India (Coque et al., 2008) (Karim et al., 2001), shared common features with pC15-1a from Canada (Nicolas-Chanoine et al., 2008). Furthermore, emergence of K. pneumoniae isolates producing CTX-M-15 were also found in European countries and \( \text{bla}_{\text{CTX-M-15}} \) transfer were mediated by IncFII-related plasmids with different sizes among part of them (Machado et al., 2006).

In conclusion, our study highlighted the CTX-M-15 type ESBL responsible for resistance to the oxyimino-cephalosporins of Klebsiella strains isolated from the urines from sick children in CHUP-CDG of Ouagadougou. The increase of consumption of cefotaxime and ceftazidime could
have contributed to the emergence of CTX-M enzymes encoding genes among *Klebsiella* strains in Burkina hospitals. It is anticipated that CTX-M-15 producing *Klebsiella* strains will become an eventual epidemiological problem in CHUP-CDG in Burkina Faso.

The bladder is a site of the human organism where high concentrations are observed in certain antibiotics (quinolones and β-lactamines) (NCCLS, 1999). ESBL producing strains were highlighted in the urines. This result corroborates those of Philippon *et al.* (1993) and of Bermudes *et al.* (1997) which reports that the majority of strains ESBL come from the urine.

The dissemination of *Klebsiella* strains CTX-M-15 type ESBL producing represents a world problem of public health insofar as this species is very easily implied in nosocomial epidemics. Carbapenems often represent the single therapeutic alternative. However, the emergence of resistance to carbapenems was already reported in *K. pneumoniae* strains and other species of Enterobacteria producing different variable of CTX-M-type (Elliott *et al.*, 2006; Woodford *et al.*, 2007; Chen *et al.*, 2008; Oteo *et al.*, 2008); these resistances complicate the assumption of responsibility of the patients.

**5. ACKNOWLEDGEMENTS**

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Isolates</th>
<th>MIC (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>CXM</td>
<td>CTX</td>
</tr>
<tr>
<td>Uries 44</td>
<td><em>K. pneumoniae</em></td>
<td>200&lt; MIC ≤ 300</td>
</tr>
<tr>
<td>Uries 46</td>
<td><em>K. pneumoniae</em></td>
<td>200&lt; MIC ≤ 300</td>
</tr>
<tr>
<td>Uries 120</td>
<td><em>K. pneumoniae</em></td>
<td>&gt;300</td>
</tr>
<tr>
<td>Uries 130</td>
<td><em>K. pneumoniae</em></td>
<td>100&lt; MIC ≤ 200</td>
</tr>
<tr>
<td>Uries 218</td>
<td><em>K. pneumoniae</em></td>
<td>&gt;300</td>
</tr>
<tr>
<td>Uries 292</td>
<td><em>K. pneumoniae</em></td>
<td>200&lt; MIC ≤ 300</td>
</tr>
<tr>
<td>Uries 466</td>
<td><em>K. pneumoniae</em></td>
<td>200&lt; MIC ≤ 300</td>
</tr>
<tr>
<td>Uries 534</td>
<td><em>K. pneumoniae</em></td>
<td>200&lt; MIC ≤ 300</td>
</tr>
<tr>
<td>Uries 538</td>
<td><em>K. pneumoniae</em></td>
<td>100&lt; MIC ≤ 200</td>
</tr>
<tr>
<td>Uries 736</td>
<td><em>K. pneumoniae</em></td>
<td>&gt;300</td>
</tr>
<tr>
<td>Uries 774</td>
<td><em>K. pneumoniae</em></td>
<td>&gt;300</td>
</tr>
<tr>
<td>Uries 778</td>
<td><em>K. pneumoniae</em></td>
<td>100&lt; MIC ≤ 200</td>
</tr>
<tr>
<td>Uries 203</td>
<td>Klebsiella <em>sp.</em></td>
<td>200&lt; MIC ≤ 300</td>
</tr>
<tr>
<td>Uries 336</td>
<td>Klebsiella <em>sp.</em></td>
<td>200&lt; MIC ≤ 300</td>
</tr>
<tr>
<td>Uries 715</td>
<td>Klebsiella <em>sp.</em></td>
<td>200&lt; MIC ≤ 300</td>
</tr>
<tr>
<td>Uries 362</td>
<td><em>K. oxytoca</em></td>
<td>50&lt; MIC ≤ 100</td>
</tr>
<tr>
<td>Uries 613</td>
<td><em>K. oxytoca</em></td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

MIC : Minimal inhibitory concentrations; CXM : Cefuroxim ; CTX : Cefotaxim ; CRO: Ceftriaxon; CAZ: Ceftazidim; FEP: Cefepim; IPM: Imipenem
**Fig-1.** Double-disk synergy test for confirmation of ESBL activity: *K. pneumoniae* isolated from urine 736 (a) and *K. pneumoniae* isolated from urine 120 (b)

**REFERENCES**


CLSI, C.L.s.i., 2006. Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement, clsi document m100-s16. Wayne, PA: CLSI.


