

# Molecular typing of extended spectrum $\beta$ -lactamase producing *Klebsiella pneumoniae* strains isolated in the university hospital center of Dakar

## Abstract

*Enterobacteria*, the bacteria most frequently isolated in pathology laboratories, are responsible for the majority of community and nosocomial infections. Beta-lactam antibiotics are used as the first-line treatment for these infections. However, the emergence of strains resistant to this family of antibiotics, due to the production of extended-spectrum betalactamases (ESBLs) in particular, considerably decreases their efficacy. In this study, we aimed to detect the ESBLs secreted by *Klebsiella pneumoniae* (*K. pneumoniae*) at Fan Hospital in Dakar, and to characterize them molecularly. We identified 32 isolates producing ESBLs. The molecular characterization of these strains identified genes encoding CTX-M-15 (96.87%) and TEM (78.13%) enzymes. In 75% of isolates, both CTX-M-15 and TEM genes were identified. None of the 32 isolates carried the OXA-1, CTX-M-9 or CTX-M-25 genes.

The CTX-M-15 gene was thus found in 96.87% of the isolates studied was the most frequently detected ESBL gene in this study.

**Keywords:** *Klebsiella pneumoniae*, ESBL, molecular characterization, Senegal

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## Introduction

*Enterobacteriaceae* bacteria are an important bacterial family in human medicine. They are responsible for nosocomial and community infections.  $\beta$ -lactams antibiotics are the basic treatment for enteric infections.<sup>1</sup> These are increasingly resistant to beta-lactams by the production of extended spectrum  $\beta$ -lactamase (ESBL). The first narrow-spectrum penicillinases (TEM-1/2: Temoneira; SHV-1: sulfhydryl-variable) were detected in *Escherichia coli* and *K. pneumoniae* (*K. pneumoniae*) in the 1960s.<sup>2</sup> They were followed, in the 1980s, by SHV-2, an enzyme hydrolyzing broad-spectrum cephalosporins produced by *K. pneumoniae*.<sup>3</sup> The activity spectra of these enzymes are continually expanding to include other beta-lactams. Also, new enzymes, not derived from either TEMs or SHVs, have appeared and spread rapidly: CTX-M (cefotaximase-Munich) enzymes.<sup>4</sup>

The overall aim of this study was to detect the production of ESBLs by isolates of *K. pneumoniae* in the Bacteriology Laboratory of Fan University Hospital (CHUF) in Dakar. The specific objective was to use molecular biology tests to characterize the genes encoding these enzymes. This part of the work was carried out at the Bacteriology Laboratory of Pierre et Marie Curie University (Paris VI) in France.

## Materials and methods

### Materials Bacterial isolates

The isolates studied were obtained from the Bacteriology Laboratory of the CHUF in Dakar. We studied 32 ESBL-producing isolates of *K. pneumoniae*. Following their isolation and identification between January 2009 and December 2010, these isolates were stored at -80°C until their molecular characterization.

### Bacteriological media and reagents

We used the following media: eosin methylene blue (EMB) agar and Mueller-Hinton (MH) agar for strain isolation and determination

of the antibiogram. The API 20E-Bio Merieux panel was used for the identification of isolates.

Discs bearing the following antibiotics (from BioRad) were tested: amoxicillin, amoxicillin+clavulanic acid, ticarcillin, piperacillin, cephalothin, ceftriaxone, cefotaxime, ceftazidime, aztreonam and imipenem.

### Materials molecular biology reagents

We used Qiagen minikits, an Applied Biosystems 3730XL capillary sequencer (Applied Biosystems), and the BigDye Terminator v3.1 Cycle sequencing kit. The reaction mixture used for PCR consisted of 5 $\mu$ l of 10x Taq buffer, 5 $\mu$ l of 2MM dNTPs, 0.5 $\mu$ l of forward primer, 0.5 $\mu$ l of reverse primer, 0.2 $\mu$ l of Taq polymerase (5U/ $\mu$ l) and 36.8 $\mu$ l H<sub>2</sub>O. We used forward and reverse primers for the TEM, CTX-M-9, CTX-M-15, CTX-M-25 and OXA-1 genes Table 1.<sup>5,6</sup>

## Methods

### Isolation and identification of strains

At the Bacteriology Laboratory of the CHUF in Dakar, isolates obtained from urine, blood, pus and vaginal secretions were identified on the basis of their morphological, culture and biochemical characteristics (API 20E, Biomerieux). Antibiogram analyses were carried out by the disc diffusion method on MH agar. ESBLs were detected in tests of synergy between discs carrying third-generation cephalosporins (ceftriaxone, ceftazidime and cefotaxime) and discs carrying amoxicillin-clavulanic acid. The results were interpreted according to the recommendations of the Comie de l'Antibiogramme de la Societe Française de Microbiologie (CA-SFM).<sup>7</sup>

### Detection and characterization of ESBL genes

This part of the study was carried out at the Bacteriology Laboratory of Pierre et Marie Curie University (Paris VI), France. Total DNA was extracted from the isolates with Qiagen minikits. The DNA was used for PCR to amplify the following genes: TEM, CTX-M-9,

CTX-M-15, CTX-M-25 and OXA-1. PCR products were subjected to electrophoresis in a 3% agarose gel at 100V for 40minutes.

### Amplicon sequencing

The PCR products were purified with the ExoSAP-IT enzyme. Their nucleotide sequences were determined by direct Sanger sequencing on an Applied Biosystems 3730XL sequencer, with the

Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).<sup>8</sup>

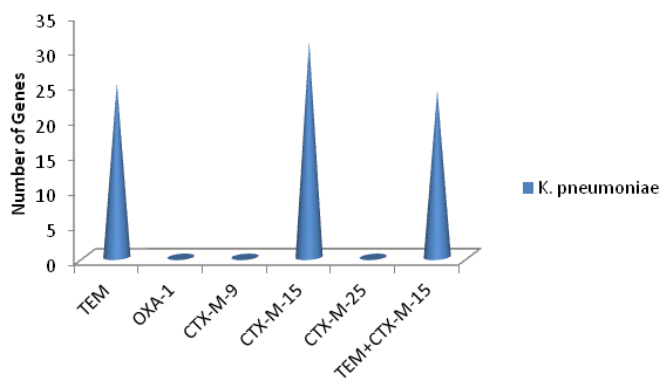
The nucleotide sequences, which were obtained in Fasta format, were then analyzed and compared with sequences deposited in the GenBank database, via the website of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>).

**Table 1** Primers used for amplification

Genes	Upper Primer (5'–3')	Lower Primers (5'–3')
TEM	ATGAGTATTCAACATTTCCG	CCAATGCTTAATCAGTGAGG
OXA-1	TATCAACTTCGCTATTTTTTTA	TTTAGTGTGTTTAGAATGGTGA
CTX-M-9	ATGGTGACAAAGAGAGTGCA	CCCTTCGGCGATGATTCTC
CTX-M-15	GGTTAAAAAATCACTGCGTC	TTACAAACCGTCGGTGACGA
CTX-M-25	ATGATGACTCAGAGCATTCG	TGGGTTACGATTTTCGCCCG

## Results

*K. pneumoniae* isolates were resistant to first and third generation cephalosporins and to aztreonam. However, they remained susceptible to imipenem (100%) and ceftioxin (87.5%) in the standard antibiogram test (disk diffusion in MH agar). A total of 31strains (96.87%) had a champagne cork synergy. This synergy was absent for a single strain that was also resistant to third-generation cephalosporins and aztreonam. PCR amplification identified the following two genes: TEM and CTX-M-15 Figure 1. The CTX-M-15 gene was detected in 31 isolates (96.87%), the TEM gene was detected in 25 isolates (78.13%). We also found that 24 isolates (75%) carried both CTX-M-15 and TEM genes.



**Figure 1** Distribution of genes found in *K. pneumoniae*

## Discussion

Our study is one of the first to report the isolation of *K. pneumoniae* strains carrying the CTX-M-15 gene in Senegal. The CTX-M-15 gene was the most predominant among our strains. It is carried by 96.87% of the isolates. CTX-M-15-type ESBLs have also been found in *K. pneumoniae*,<sup>9</sup> *Salmonella enterica*,<sup>10</sup> *Morganella morganii* and *K. pneumoniae*,<sup>11</sup> in Senegal. It has also been isolated from *K. pneumoniae* strains in Nigeria.<sup>12</sup> In 2004; it was detected in two *K. pneumoniae* strains isolated in Taiwan.<sup>13</sup>

In 2004, it was detected in two isolates of *K. pneumoniae* in Taiwan.<sup>13</sup> CTX-M-type ESBLs now make up the majority of ESBLs in all regions of the world, such that their spread can be described as pandemic.<sup>1</sup>

The secretion of this enzyme by a strain confers high levels of resistance to cefotaxime, ceftriaxone, ceftazidime and aztreonam.<sup>14</sup> Genetic analyses have shown that the progenitor genes of the CTX-M group originated in the genus *Kluyvera*, an enterobacterium only very rarely isolated in medical bacteriology laboratories.<sup>1</sup>

CYX-M-2 enzymes are derived from the natural  $\beta$ -lactamase of *Kluyvera ascorbata*, whereas CTX-M-8 enzymes are derived from *Kluyvera georgiana*.<sup>15</sup>

The TEM enzymes were the next most frequent, found in 78.13% of our isolates. A study carried out in Nigeria in 2013 found this gene in *K. pneumoniae* strains.<sup>16</sup> Some TEM and SHV genes encode enzymes with strong or weak penicillinase activity. For this reason, they must be sequenced to ensure that they really do encode ESBLs.<sup>2</sup> Nevertheless, the criteria for the selection of isolates for this study strongly suggests that the TEM and SHV genes identified in this study do encode ESBLs. The SHV enzyme is naturally present in *K. pneumoniae*.<sup>2</sup>

The synergy test, which can be used to detect isolates producing ESBLs, was negative for two isolates, which were nevertheless found to carry genes encoding ESBLs. This finding confirms the existence of false-negative results for the detection of ESBLs by classic phenotypic tests, as reported elsewhere,<sup>17</sup> highlighting the importance of genotypic tests for the detection of ESBLs in some cases.

## Conclusion

Two major ESBL families were found in *K. pneumoniae* at the CHUF in Dakar: CTX-M-15 and TEM. Of these ESBL, CTX-M-15 was the most frequently detected. The emergence of cross-resistance to several families of antibiotics requires careful surveillance of resistance to prevent therapeutic deadlock situations in the future. Multicenter studies will allow for better characterization of the different types of ESBL produced by *K. pneumoniae* strains circulating in Senegal.

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## Conflicts of interest

We (the authors) declare that there are no conflicts of interest in relation to this article.

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