Molecular typing of extended spectrum β-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 beta-lactamasases (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

**Introduction**

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

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*. They were followed through 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. Disks bearing the following antibiotics (from BioRad) were tested: amoxicillin, amoxicillin+clavulanic acid, ticarcillin, piperacillin, cephalothin, ceftriaxone, cefotaxime, cefazidime, 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 5µl of 10x Taq buffer, 5µl of 2MM dNTPs, 0.5µl of forward primer, 0.5µl of reverse primer, 0.2µl of Taq polymerase (5U/µl) and 36.8µl H₂O. We used forward and reverse primers for the TEM, CTX-M-9, CTX-M-15, CTX-M-25 and OXA-1 genes Table 1.

**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, cefotaxime and cefotaxime) and discs carrying amoxicillin-clavulanic acid. The results were interpreted according to the recommendations of the Comité de l’Antibiogramme de la Société Française de Microbiologie (CA-SFM).

**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,
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Results

*K. pneumoniae* isolates were resistant to first and third generation cephalosporins and to aztreonam. However, they remained susceptible to imipenem (100%) and cefoxitin (87.5%) in the standard antibiogram test (disc diffusion in MH agar). A total of 31 strains (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.

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*, *Salmonella enterica*, *Morganella morgannii* and *K. pneumoniae*, in Senegal. It has also been isolated from *K. pneumoniae* strains in Nigeria. In 2004; it was detected in two *K. pneumoniae* strains isolated in Taiwan. In 2004, it was detected in two isolates of *K. pneumoniae* in Taiwan, 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.

Amplon 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). 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).

![Figure 1](image_url) Distribution of genes found in *K. pneumoniae*

| Genes    | Upper Primer (5'–3') | Lower Primers (5'–3') |
|----------|----------------------|-----------------------|
| TEM      | ATGAGTATTCACATTTCCG  | CCAATGCTTAATCAGTGAGG  |
| OXA-1    | TATTCACCTGCAATTCTTA  | TTATGCTTTGATGACCA     |
| CTX-M-9  | ATGGTAAATACAGATTGAG  | CCCCCCTGGGGTATGCTTC    |
| CTX-M-15 | GGTAAAATCTAATGTCGTC  | TTACAAACCGTCGGTGACGA   |
| CTX-M-25 | ATGATGACCTCAGACCTCG  | TGGGTACGATTTTCGCCGC    |

Table 1 Primers used for amplification

The secretion of this enzyme by a strain confers high levels of resistance to cefotaxime, ceftriaxone, ceftazidime and aztreonam. Genetic analyses have shown that the progenitor genes of the CTX-M group originated in the genus *Klebsiella*, an entero bacterium only very rarely isolated in medical bacteriology laboratories. CYX-M-2 enzymes are derived from the natural β-lactamase of *Klebsiella ascorbata*, whereas CTX-M-8 enzymes are derived from *Klebsiella georgiana*.

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. 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. 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*.

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, 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.

Acknowledgments

None.

Conflicts of interest

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

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References

1. Bonnet R. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother. 2004;48:1–14.

2. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001;14:933–951.

3. Chin FL, Shih KH, Chao HC, et al. Genotypic detection and molecular epidemiology of extended-spectrum-lactamase-producing Escherichia coli and Klebsiella pneumoniae in a regional hospital in central Taiwan. J Med Microbiol. 2010;59:665–671.

4. Canton R, Gonzalez AJM, Galan JC. CTX-M enzymes: origin and diffusion. Front Microbiol. 2012;3(110):1–19.

5. Roschanski N, Fischer J, Guerra B, et al. Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and CIT-type AmpCs in Enterobacteriaceae. PLoS One. 2014;9(7).

6. Iroha IR, Esimone CO, Neumann S, et al. First description of Escherichia coli producing CTX-M-15-extended spectrum beta-lactamase (ESBL) in out-patients from south eastern Nigeria. An. Clin Microbiol Antimicrob. 2012;11:19.

7. Antibiotic committee of the French Society of Microbiology (CA-SFM). Recommendations. 2014.

8. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci. 1977;74:5463–5467.

9. Moquet O, Bouchiat C, Kinana A, et al. Class D OXA-48 carbapenemase in multidrug-resistant enterobacteria, Senegal. Emerg Infect Dis. 2011;17:143–144.

10. Weill FX, Claude JDPP, Demartin M, et al. Characterization of extended-spectrum β-lactamase (CTX-M-15)-producing strains of Salmonella enterica isolated in France and Senegal. FEMS Microbiol Lett. 2004;238:353–358.

11. Diene SM, Fenollar F, Fall B, et al. CTX-M-15-producing Morganella morganii from Hopital Principal de Dakar, Senegal. New Microbes New Infect. 2014;2:46–49.

12. Soge OQ, Queenan AM, Ojo KK, et al. CTX-M-15 extended-spectrum β-lactamase from Nigerian Klebsiella pneumoniae. J Antimicrob Chemother. 2006;57:24–30.

13. Yu WL, Cheng KC, Wu LT, et al. Emergence of two Klebsiella pneumoniae isolates harboring plasmid-mediated CTX-M-15 β-lactamase in Taiwan. Antimicrob Agents Chemother. 2004;48:362–363.

14. Poirel L, Gniadkowski M, Nordmann P. Biochemical analysis of the ceftazidime-hydrolyzing extended-spectrum β-lactamase CTX-M-15 and of its structurally related β-lactamase CTX-M-3. J Antimicrob Chemother. 2002;50:1031–1034.

15. Elhani D. The widening challenge of extended-spectrum-lactamases. An Biol Clin. 2012;70:117–140.

16. Ogbolu DO, Terry Alli OA, Olanipekun LB, et al. Faecal carriage of extended-spectrum beta-lactamase (ESBL)-producing commensal Klebsiella pneumoniae and Escherichia coli from hospital out-patients in Southern Nigeria. Int J Med Sci. 2013;5(3):97–105.

17. Sasirekha B. Prevalence of ESBL, AmpC β-lactamases and MRSA among uropathogens and its antibiogram. EXCLI J. 2013;12:81–88.