CTX-M-15-producing *Morganella morganii* from Hôpital Principal de Dakar, Senegal

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Abstract

We report the detection and molecular characterization of extended spectrum β-lactamases in a series of 112 clinical isolates of *Enterobacteriaceae* from the Hôpital Principal de Dakar, Senegal, including five CTX-M-15-producing *Morganella morganii* isolates, which are reported for the first time in this country.

Keywords: *Enterobacteriaceae*, extended-spectrum β-lactamases, nosocomial infection

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Introduction

Over the last 30 years, hospital-acquired infections caused by Gram-negative bacilli, especially *Enterobacteriaceae*, have become a worldwide problem as a result of the misuse of antibiotics and poor hospital hygiene [1]. Indeed, in health facilities, these opportunistic pathogens are responsible for nosocomial infections including hospital-acquired pneumonia, urinary tract infections, bloodstream infections and wound infections [2,3]. The increase in these infections has been mainly associated with the widespread dissemination of multidrug-resistant bacteria that are highly resistant to the common antibiotics used for the treatment of these infections [4]. However, while the dissemination and high prevalence of antibiotic resistance in Gram-negative bacilli has been described worldwide and could be associated with international travel and tourism [5], few studies have described the prevalence and mechanism of antibiotic resistance in *Enterobacteriaceae* from northern Africa, especially Senegal [3]. Indeed, the few studies conducted in this region were on *Enterobacteriaceae* species including *Shigella flexneri* and *Salmonella enterica*, which are not classified as dominant nosocomial pathogens [6,7]. In this region, we have recently described the emergence of clinical multidrug-resistant Gram-negative bacteria, especially *Acinetobacter baumannii*, resistant to all β-lactams including the carbapenems [8], which has never been reported before in Senegal. Therefore, because of the emergence of multidrug-resistant bacilli such as *A. baumannii* and the paucity of studies focused on antibiotic resistance in clinical nosocomial pathogens, we conducted an epidemiological study to describe the prevalence and dissemination of antibiotic resistance genes in a large collection of clinical *Enterobacteriaceae* strains isolated from the Hôpital Principal de Dakar, Senegal.

Methods

All clinical Enterobacteriaceae isolated and studied here were collected from hospitalized patients between October and December 2011 in the Hôpital Principal de Dakar, Senegal. All of them were isolated from blood cultures, urine samples, superficial pus, protected bronchial samples, or catheters. Bacteria were grown on trypticase soy or Müller–Hinton media for 24 h at 37°C. Bacterial identification was performed using both standard phenotypic methods including API 20E gallery (BioMérieux, Marcy l'Etoile, France) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Autoflex, Bruker Daltonic, Bremen, Germany) with the FLEX CONTROL software (Bruker Daltonic). MALDI-TOF MS identification to species level was validated with score values ≥1.9 as previously reported [9]. Antibiotic susceptibility testing was performed by the disk diffusion method as well as by the Etest (AB Biodisk, Solna, Sweden) method. Thirteen antibiotic disks—amoxicillin, amoxicillin/clavulanic acid, ticarcillin, ceftriaxone, cefpirome, cefepime, imipenem, gentamicin, netilmicin, tobramycin, colistin, pefloxacin and nalidixic acid—were used (Bio-Rad, Marnes la

Coquette, France) on Müller–Hinton agar according to the recommendations of the Comité de l'antibiogramme de la Société Française de Microbiologie (CASFM) (http://www.sfm-microbiologie.org/). The antibiotic susceptibility results were interpreted according to the guidelines of the CASFM. The major and predominant extended-spectrum β-lactamase (ESBL) -encoding genes, including bla_{TEM}, bla_{SHV} and bla_{CTX-M}, were investigated by standard PCR and sequencing using previously described primers [10–12].

Results

Phenotypic properties of the strains

One hundred and twelve clinical isolates were studied including *Morganella morganii* (n = 5), *Escherichia coli* (n = 30), *Enterobacter cloacae* (n = 13) and *Klebsiella pneumoniae* (n = 64). Forty-three (38%) strains were isolated from blood cultures, 41 (37%) were from urine samples, 17 (15%) were from pus and 11 (10%) were from an assortment of samples including bronchoalveolar lavage fluid, catheters and protected bronchial samples (Fig. 1). The identification score values from MALDI-TOF was at least 1.90 for all isolates and therefore all strains were identified to species level, as shown in the MALDI-TOF dendrogram (Fig. 1). The antibiotic susceptibility testing reveals a high incidence of resistance to β-lactam drugs, and up to 90% of isolates were resistant to amoxicillin, amoxicillin/clavulanic acid, ticarcillin and ceftriaxone; 58% of the isolates were resistant to cefepime, but all of them were susceptible to imipenem (Fig. 2a). A more variable incidence of resistance to the other antibiotic families was observed (Fig. 2a). As shown in Fig. 2(a), colistin resistance was observed only in *M. morganii* isolates, which are naturally resistant to this antibiotic drug. Interestingly, as observed from the antibiotic susceptibility testing results, the resistance phenotype of some isolates to the third (ceftriaxone) and fourth (cefpirome) generations of cephalosporins suggests the presence and expression of ESBL-encoding genes in these isolates.

Molecular investigation of β-lactamase-encoding genes

The standard PCR and sequencing analysis using universal primers targeting all bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes revealed the surprising presence of the $bla_{CTX-M-15}$ ESBL-encoding gene in all 112 isolates (Fig. 2b; see Supporting information, Table S1). Fifty-four and 57 out of the 64 strains of *K. pneumoniae* harboured the bla_{TEM-1} and bla_{SHV} genes, respectively, including bla_{SHV-11}, bla_{SHV-12} and bla_{SHV-28} (Fig. 2b; Table S1). Four and 18 out of the 30 *E. coli* isolates, respectively, harboured the bla_{TEM-1} and bla_{SHV} genes. Among the 13 *Enterobacter cloacae* isolates, two harboured bla_{SHV} genes and 11 harboured the bla_{TEM-1} gene; finally, three of the five *M. morganii* harboured the bla_{TEM-1} gene and no bla_{SHV} genes were found in these isolates (Fig. 2b; Table S1).

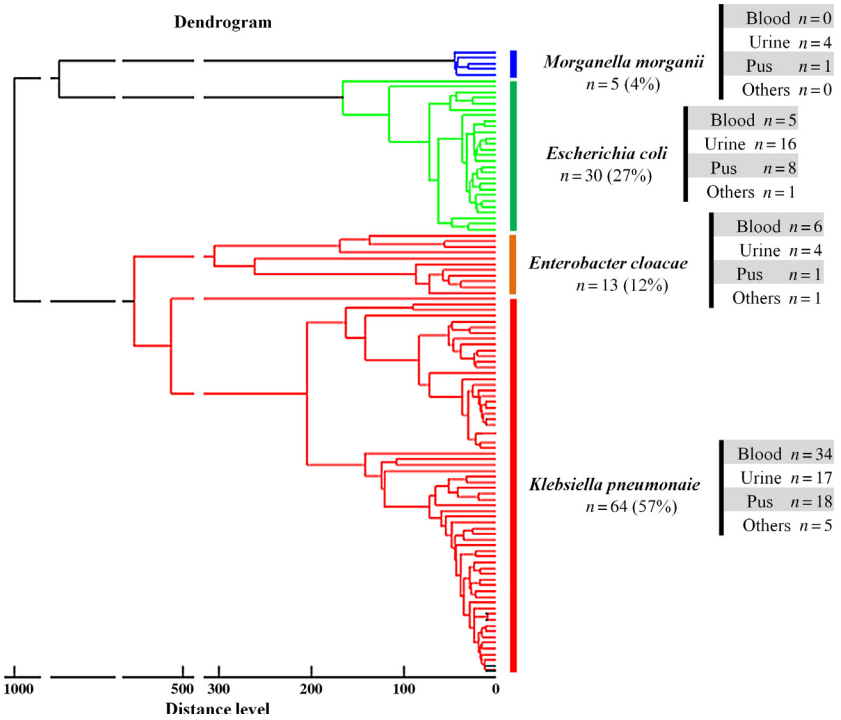

FIG. 1. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry dendrogram and isolation sources of the strains.

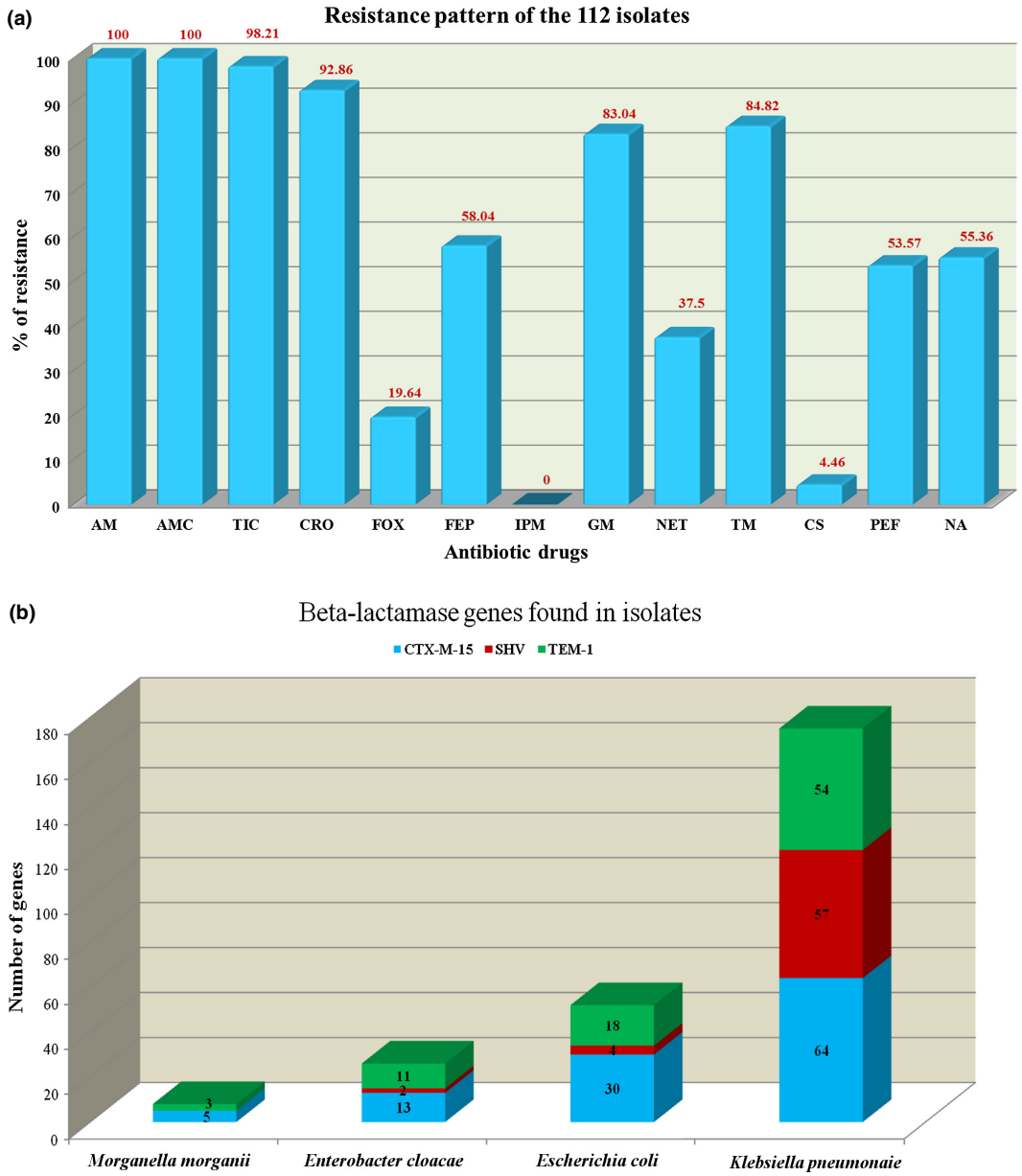

FIG. 2. Antibiotic resistance pattern (a) and β-lactamase encoding genes detected in isolates (b). AM, amoxicillin; AMC, amoxicillin/clavulanic acid; TIC, ticarcillin; CRO, ceftriaxone; FOX, cefpirome; FEP, cefepime; IPM, imipenem; GN, gentamicin; NET, netilmicin, TM, tobramycin; CS, colistin; PEF, pefloxacin; NA, nalidixic acid; S, susceptible; R, resistant; I, intermediate; ND, not determined.

Discussion

To the best of our knowledge, CTX-M-15-producing *M. morganii* isolates are reported herein for the first time in Senegal. There are only two reports of CTX-M-15-producing *Enterobacteriaceae* in clinical isolates in Senegal, including 45 *K. pneumoniae*, one *E. coli* and one *Enterobacter cloacae* [13,14] isolates. Hence, in the present study, we report the largest series of CTX-M-15-producing Enterobacteriaceae from Senegal, showing a high prevalence and dissemination of the CTX-M-15 ESBL-encoding gene in this hospital. Furthermore, this study highlights a probable outbreak concerning *K. pneumoniae* isolates as >60% of the isolates harboured simultaneously the three genes bla_{TEM-1}, $bla_{CTX-M-15}$ and bla_{SHV-28}. This finding is very disturbing because no previous studies have focused on monitoring the prevalence and dissemination of antibiotic resistance genes in this important Dakar hospital. The presence of ESBLs in *M. morganii* is also worrying because these bacteria are intrinsically resistant to colistin. As we have

also recently described the emergence of the OXA-23 carbapenemase gene in multidrug-resistant *A. baumannii* isolates from this hospital [8] as well as the recent emergence of class D OXA-48 carbapenemase in *Enterobacteriaceae* in Senegal [13], it is urgent that local authorities are alerted to this problem to avoid the widespread dissemination of antibiotic-resistance genes and multidrug-resistant bacteria in all hospitals in Senegal.

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Conflict of Interest

None declared.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Phenotypic and genotypic features of the 112 *Enterobacteriaceae* clinical isolates investigated in this study.

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