



Surveillance of antibiotic resistance in the northern zone of Senegal: Molecular characterization of beta-lactam resistance genes in Enterobacteriaceae (extended spectrum beta-lactamase, plasmid cephalosporinases, carbapenemases).

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ABSTRACT

Introduction: With the increase in bacterial resistance to antibiotics, policies are being implemented on a national scale. These resistances are multiple, especially in enterobacteria, and represent a real public health problem due to the difficulty of their treatment. The objective of this work was to identify ESBL, cephalosporinase and carbapenemase producing enterobacteria in laboratories in the northern part of Senegal and to compare the mechanisms involved. **Materials and methods:** This is a retrospective study conducted at the Saint-Louis Regional Hospital and the Microbiology Unit of the Pasteur Institute of Dakar. Bacterial strains were collected between January and June 2021 in eight bacteriology laboratories in the regions of Saint-Louis, Louga and Matam. Bacterial identification was done by studying morphological, cultural and biochemical characteristics using an API® 20 E gallery and the MALDI-TOFF technique. The antibiogram was performed by the disc diffusion method according to the CA-SFM version 2021 procedure including the search for specific resistances (ESBL). The ESBL genes (CTX, TEM, OXA, and SHV) were identified by polymerase chain reaction (PCR) with specific primers and then analyzed by gel electrophoresis. **Results:** Forty-five strains of Enterobacteriaceae were ESBL producers and each had at least one beta-lactam resistance gene. The CTX-M gene was found in all strains, followed by the OXA (64.44%), TEM (53.33%) and SHV (20%) genes. A simultaneous presence of these 4 genes was noted in 6 isolates (13.33%). Apart from resistance to ertapenem, all strains were sensitive to imipenem. For quinolones, almost all strains (93%) were resistant. **Conclusion:** These results show the need to reinforce the surveillance of ESBL-producing Enterobacteriaceae in the Senegal area. These important data should help to orientate strategies for the reduction of MRBs and their dissemination in the community.

Keywords: Surveillance, ESBL, Molecular characterization, Northern Senegal.

INTRODUCTION

For several decades, bacterial resistance to antibiotics has been increasing, especially in the hospital setting with the emergence of new resistances [1]. Multiple resistances in enterobacteria represent a public health threat related to the difficulty of treating multidrug-resistant bacteria (MDR) infections [2]. The choice of effective antibiotics is made difficult, if not impossible, with reduced therapeutic possibilities. This is compounded by the fact that there are few new molecules and difficulties in obtaining supplies of old molecules [3,4].

One of the most important and widespread mechanisms is the production of an extended spectrum β -lactamase (ESBL) for beta-lactams. Other enzymes are also involved such as cephalosporinases and carbapenemases encoded by specific genes [5-7]. In view of the lack of a national surveillance system, preliminary work was carried out in the northern zone including the regions of Saint Louis, Louga and Matam. The objective was to identify ESBL-, cephalosporinase- and carbapenemase-producing Enterobacteriaceae isolated from

laboratories in the northern zone of Senegal, to identify the mechanisms involved and to compare them.

MATERIALS AND METHODS

The bacterial strains used in this study were extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae isolated in the 8 bacteriology laboratories of the Saint-louis, Louga and Matam regions. These strains came from pathological products of hospitalized or outpatients. The antibiogram was performed by the disk diffusion method on Mueller-Hinton agar and interpreted after measurement of the inhibition diameters in accordance with the recommendations of the antibiogram committee of the French Society of Microbiology CA-SFM version 2021. The search for extended-spectrum beta-lactamase (ESBL) was performed by the classical method based on the detection of the synergy between a disk of amoxicillin + clavulanic acid (AMC) and two disks of third-generation cephalosporin (cefotaxime and ceftazidime) and a disk of aztreonam (ATM).

To obtain the genetic material we performed a re-isolation on TCS agar (Tryptocasein-Soybean) for 18-24H. Then, an extraction of the genomic DNA was performed with QIAGEN reagents (Qiamap DNA Mini Kit Cat. 51304 Qiagen) according to the manufacturer's protocol and the DNA was stored at - 20° C.

The resistance genes that code for ESBL were tested by PCR with specific primers for each gene. The primers tested (bla CTX-M, bla TEM, bla SHV and bla OXA) are listed in Table 1.

Table 1: Characteristics of the Primers

Genes	Primer sequence (5'-3')	Size	Temperature
bla CTX-M	ATGTGCAGTACCAGTAARGTCATGGC TGGGTRAARTARGTSACCAGAAYSACC	700 pb	57° C
bla TEM	CCAATGCTTATTCAGTGAGG ATGAGTATTCAACATTTCCG	850 pb	57° C
bla SHV	TTATCTCCCTGTTAGCCACC GATTTGCTGATTCGCTCGG	800 pb	57° C
bla OXA	ATGAAAAACACAATACATATC AATTTAGTGTGTTTAGAATGG	890 pb	57° C

Data were entered in Excel 2013 and analyzed by whonet version 5.6 software.

RESULTS

The study included 45 strains of ESBL-producing Enterobacteriaceae isolated from 8 bacteriology laboratories. The majority of the study population was female (see Table III). The sex ratio was 0.87 (21 men versus 24 women).

The mean age of the patients was 47 years with extremes ranging from 1 month to 77 years. The [41-60] age groups were the most represented followed by the over 60 years (Figure 1).

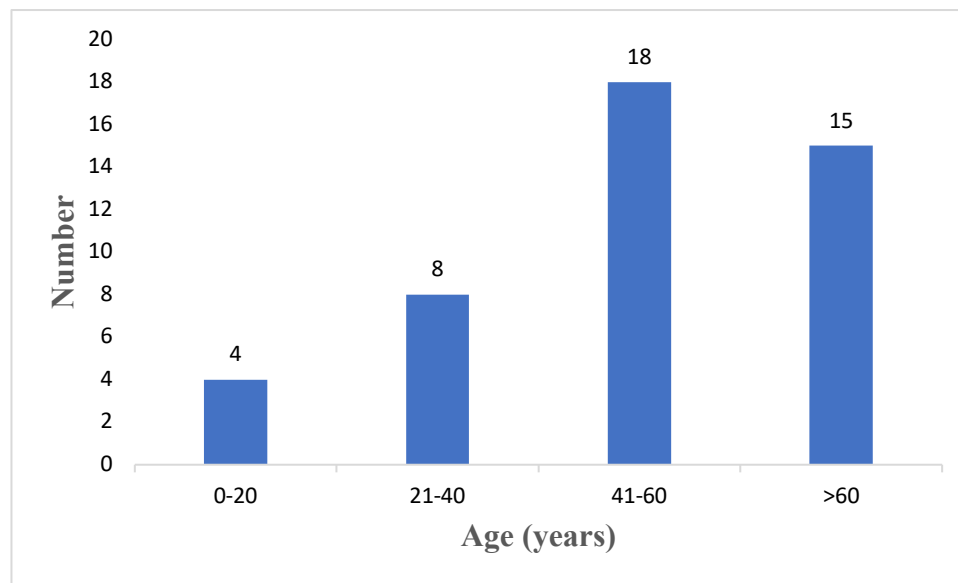


Figure 1: Distribution of the population according to age groups.

The study population was predominantly inpatients, with 26 patients (57.78%) versus 19 patients (42.22%) outpatients (see Figure 2). However, the Biondar laboratory and the Saint-Louis regional laboratory do not have an inpatient department.

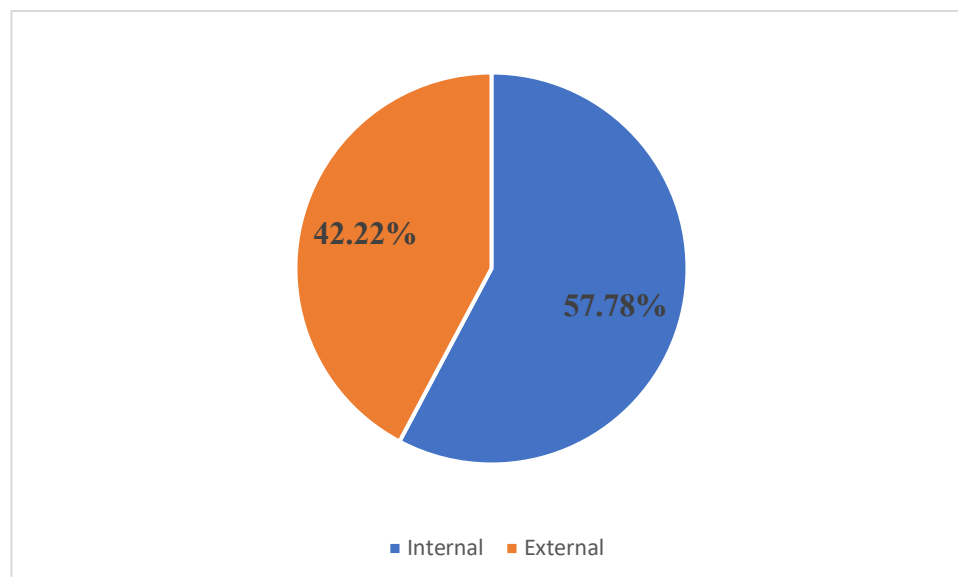


Figure 2: Population distribution by patient origin

The strains isolated were *Escherichia coli* (*E. coli*) followed by *Klebsiella* (*K. spp*) and *Enterobacter spp* (*E. spp*) (Table 2).

Table 2: Distribution of isolated strains according to regions.

Regions	<i>E. coli</i>	<i>K. spp</i>	<i>E. spp</i>	TOTAL
Saint-Louis	19	07	01	27
Louga	07	01	00	08
Matam	05	04	01	10
Total	31 (68.88%)	12 (26.66%)	02 (4.46%)	45 (100%)

The pathological products where bacterial strains were most isolated were urine followed by pus samples (Table 3).

Table 3: Distribution of pathological products according to regions.

Regions	Urine	Pus	Blood culture	PV	Copro	Total
Saint-Louis	19	06	01	00	01	27
Louga	07	00	00	01	00	08
Matam	07	03	00	00	00	10
Total	33	09	01	01	01	45

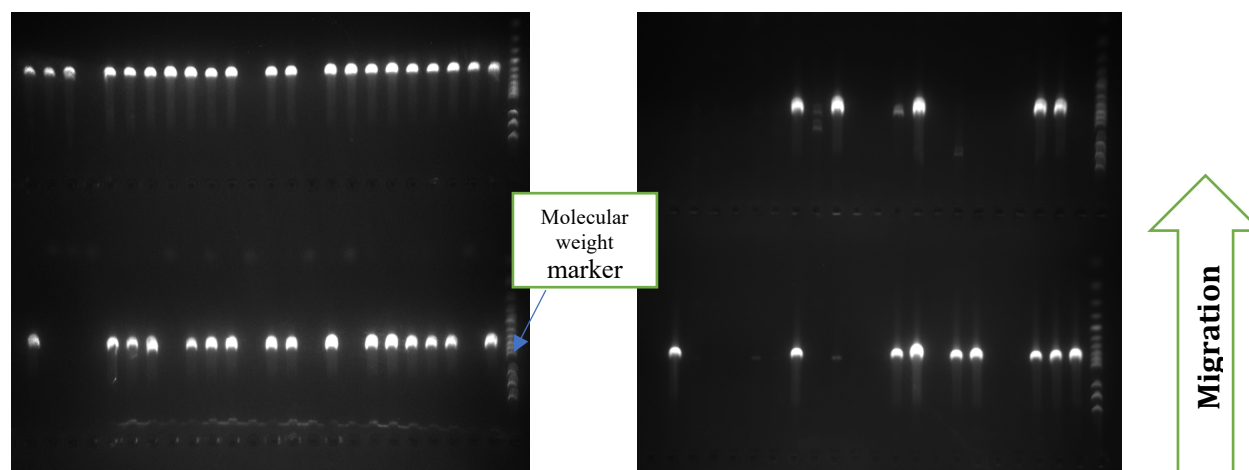
Legend :

PV : Vaginal Swab

Copro : Fecal Culture

With respect to PCR results, each strain had at least one beta-lactam resistance gene.

The CTX-M gene was found in all 45 (100%) ESBL-producing strains (Table 4).

**Figure 3: Electrophoresis results of the CTX-M gene and the OXA gene.**

The TEM gene was found in 24 strains (53.33%) of enterobacteria (Table 4).

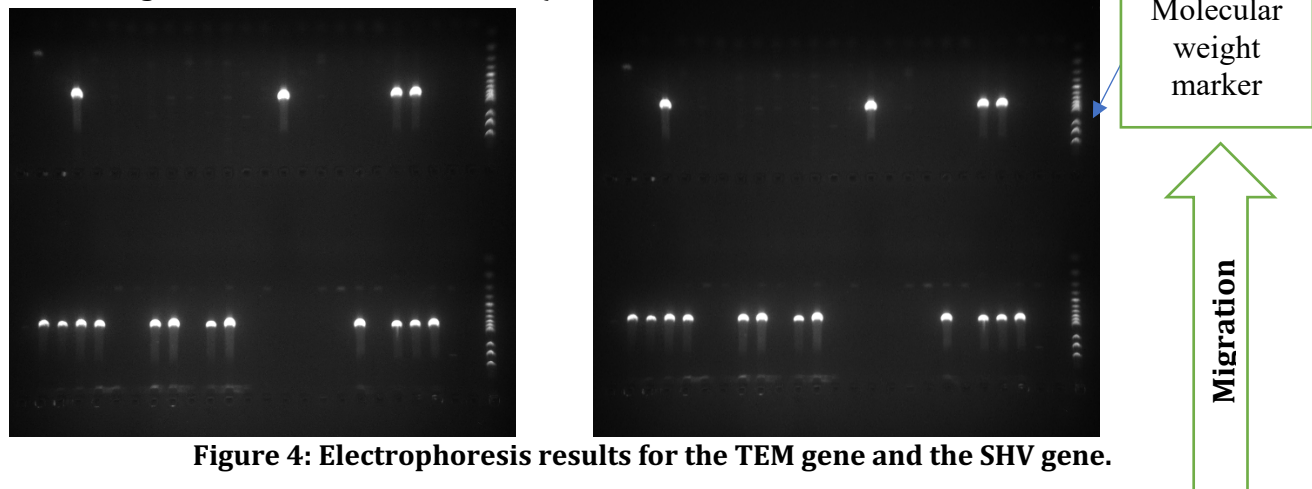


Figure 4: Electrophoresis results for the TEM gene and the SHV gene.

The SHV gene was found in 9 strains of *Enterobacteria* (20%). All SHV gene positive strains were *Klebsiella pneumoniae* (Table 4).

The OXA gene was found in 29 strains of *Enterobacteria* (64.44%). This gene was more isolated in *Escherichia coli* (Table 4). In addition, six strains simultaneously possessed all four genes CTX-M, TEM, SHV and OXA (13.33%).

Table 4: Repair of identified resistance genes

<i>Species</i>	CTX-M	TEM	OXA	SHV
<i>E. coli</i>	32	13	19	-
<i>K. pneumoniae</i>	12	10	09	09
<i>E. cloacae</i>	01	01	01	-
Total	45	24	29	09

All 45 strains of *Enterobacteria* were tested for susceptibility to imipenem and ertapenem. All these strains of enterobacteria studied were sensitive to imipenem. For ertapenem, all strains were sensitive except one which was resistant (2.22%). The resistant strain was an *E. coli* carrying the CTX-M, OXA and TEM genes. For fluoroquinolones, almost all strains were resistant to ciprofloxacin (93%). The sensitive strains had only the TEM and CTX-M resistance genes.

DISCUSSIONS

The main objective of this study was to monitor antibiotic resistance in the northern zone of Senegal by identifying the enterobacteria involved, the genes responsible and by making a comparison between the different regions. The absence of national data and the high frequency of resistance justify the usefulness of this study. In terms of population coverage, a lack of laboratories is noted in Louga and Matam. Also, these laboratories are often confronted with shortages of culture media, identification reagents and antibiotic disks. The consequence is the non-detection of resistance and the underestimation of its frequency, which is a hindrance to resistance surveillance.

In the three regions concerned, ESBL-producing strains isolated during the collection period were preserved in 15% glycerol brain broth and then stored at -20°C before being transported to Saint-Louis. The identification and antibiogram were repeated for confirmation before the gene search by PCR. All strains had the CTX-M gene and the Saint-Louis region had by far the highest number with nearly 60%, followed by Matam (22%) and Louga (18%). CTX-Ms have spread globally since the late 1980s and currently represent the major genes. There is a pandemic of CTX-M ESBLs with more than 150 CTX-M allelic variants and their frequency has increased dramatically [8].

In 2018 in Dakar (CHU Fann), Diagne et al. found the CTX-M gene in 90.6% of *E. coli* strains [9]. In 2016, in Morocco Mennane et al found that the CTX-M gene was largely in the majority in enterobacteria strains [10]. The same was true in another Moroccan study in 2017 with 70% of the CTX-M gene [11].

In addition to CTX-M, the OXA type beta-lactamase was observed in 64% as for Diagne et al who had obtained and 59% of the OXA-1 gene on *E. coli* strains [9]. A similarly high prevalence was noted in the study of DIA ML (63%) [12], on the other hand a low prevalence was noted in the study of Olley M et al in Nigeria (6.7%) [13]. Few epidemiological data have been performed to evaluate the dissemination of the OXA gene but it has often been described in association with group 1 CTX-M [14]. Other genes found were the TEM gene (53%) and the SHV gene (20%). In the 1990s, the main ESBLs were derived from TEM and SHV enzymes and were mainly distributed among hospital clones of *Klebsiella pneumoniae* and *Enterobacter spp* [15]. These genes have also been reported all over the world with variable prevalences from one country to another. In 2015, an Algerian study revealed a prevalence of the TEM-4 gene at 10% [16], while in Benin with a higher prevalence of 72.4% was observed with also the SHV gene [17]. In Nigeria in 2020 the prevalences found were 55% and 35% respectively for the TEM and SHV genes [18]. Outside Africa, in West Azerbaijan in 2021, the prevalences of the TEM and SHV genes were respectively 49.3% and 43.7% for 120 strains [19] and in Iraq, it was 15.4% in 2021 for the TEM gene [20].

Fortunately, the majority of strains were sensitive to imipenem, however, 87% resistance was observed for ciprofloxacin. Similarly, in Morocco, El Bouamri et al, reported 82% resistance to ciprofloxacin and 10% resistance to imipenem in ESBL *Enterobacteriaceae* [21]; the same was true for Sbiti and Léotard with 92.5% and 88% resistance to ciprofloxacin respectively [22,23]. On the other hand, a low level of resistance to ciprofloxacin was noted in Senegal by Sire JM et al in 2007 (15.5%) [24].

CONCLUSION

Antimicrobial resistance is a real public health problem in the world, especially in the African context where the means of diagnosis are limited, associated with the abusive use of antibiotics in human and animal health and in agri-foodstuffs. The nature of the genes found and their simultaneous presence in the same strain expose the occurrence of multidrug resistance. A policy of prevention of the diffusion of these BMR must be implemented and will require the availability of a bank of strains to monitor the evolution of resistance.

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