

Surveillance of Resistance to Third-Generation Cephalosporins in Four Regions of Senegal (Saint-Louis, Diourbel, Kaolack, and Dakar) between January 2020 and September 2021

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Abstract

Antibiotic resistance remains a major public health problem, and if no action is taken by 2050, mortality related to multidrug resistance is expected to reach 10 million deaths per year. In Senegal, most studies and interventions on multidrug resistance surveillance have been limited to Dakar. Against this background, the present study was undertaken with the primary aim of surveilling resistance patterns to third-generation cephalosporins (3GCs), the most extensively used antibiotic class in the inpatient setting, particularly in acute care and emergency contexts. **Methods:** This was a prospective study conducted in the hospitals of Diourbel, Kaolack, Le Dantec (Dakar), Matlaboul Fawzeini Hospital in Touba, and the regional laboratory of Kaolack. Bacterial isolates were identified based on morphological, cultural, and biochemical characteristics. Antimicrobial susceptibility testing was performed according to the recommendations of the French Society of Microbiology Antibigram Committee (CA-SFM) in force. Data were first recorded in registers, entered into Excel, and analyzed using SPSS. **Results:** Between January 2020 and September 2021, 329 Enterobacterales isolates resistant to third-generation cephalosporins were collected. These isolates were mainly recovered from Saint-Louis Regional Hospital (n = 130; 39.5%), the Regional Laboratory of Kaolack (n = 55;

16.71%), and Le Dantec Hospital (n = 80; 24.31%). The majority of isolates were obtained from urine samples (n = 178; 54.10%), followed by pus specimens (n = 64; 19.45%). Resistance to 3GCs was mainly mediated by extended-spectrum beta-lactamase (ESBL) production (79.63% of isolates), while 3.34% of isolates were resistant to carbapenems. *Escherichia coli* and *Klebsiella pneumoniae* were the main ESBL-producing species, with prevalences of 55.9% and 25.6%, respectively. **Conclusion:** These findings show that ESBL-producing and 3GC-resistant Enterobacterales represent a major resistance issue reported in clinical laboratories. Antimicrobial resistance control efforts should be implemented at the national level to better understand gene distribution and reduce morbidity and mortality associated with multidrug-resistant bacteria.

Keywords

Antibiotic Resistance, Third-Generation Cephalosporins, ESBL, Senegal

1. Introduction

Antimicrobial resistance remains a growing threat to global public health. It compromises treatment efficacy and increases morbidity and mortality associated with bacterial infections [1]. This resistance results from several contributing factors, including the misuse of antibiotics in both human and veterinary medicine, antibiotic overconsumption, and the role of the environment in the dissemination of resistance mechanisms.

In response to this global challenge, the World Health Organization has prioritized the surveillance of critical bacterial pathogens by classifying them according to decreasing levels of priority (critical, high, and medium priority). The majority of bacterial infections, particularly urinary tract infections, gastrointestinal infections, and septicemia, are caused by Enterobacteriaceae [2]. Among these, *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp. are responsible for approximately 50% to 80% of prostatic infections [3].

These Enterobacteriaceae have developed acquired resistance to third-generation cephalosporins, which are classified as critically important antimicrobials. Their high concentration within the gastrointestinal tract facilitates the exchange and dissemination of resistance genes.

With the emergence of β -lactamases, the development of broad-spectrum cephalosporins significantly improved the management of bacterial infections. However, their clinical use was rapidly followed by the emergence of enzymes capable of hydrolyzing these antibiotics, known as extended-spectrum β -lactamases (ESBLs). The widespread dissemination of these enzymes represents a major step in the global progression of multidrug-resistant bacterial strains.

Additionally, the emergence of plasmid-mediated cephalosporinases and chromosomal AmpC β -lactamases, naturally present in certain Enterobacteriaceae and

capable of horizontal transfer to other species, further contributes to this phenomenon [4].

Consequently, these resistant Enterobacteriaceae play a significant role in healthcare-associated infections, particularly in intensive care settings.

In Senegal, the extensive and sometimes inappropriate use of third-generation cephalosporins raises concerns regarding the emergence and spread of resistant bacterial strains. However, available epidemiological data remain fragmented and limited in several regional capitals, while being entirely unavailable in certain regions.

Within this context, this preliminary study aimed to analyze bacterial resistance profiles to third-generation cephalosporins across four regions of Senegal, identify factors promoting the emergence of resistant strains, and propose appropriate surveillance strategies.

The primary objective was to evaluate the frequency of Enterobacteriaceae resistance to third-generation cephalosporins over a 20-month period in four regions of Senegal.

2. Methodology

- Study Design and Setting

This was a prospective, cross-sectional, and descriptive study conducted over a 20-month period from January 2020 to September 2021 in one university teaching hospital and four regional referral hospitals in Senegal: Le Dantec Hospital, Saint-Louis Regional Hospital, Diourbel Regional Hospital, Kaolack Regional Laboratory, and Matlaboul Fawzeini Hospital (Touba). All third-generation cephalosporin-resistant Enterobacterales isolates identified consecutively at each participating site during the study period were included; only the first isolate per patient and per bacterial species was retained to exclude duplicates. Both inpatient and outpatient samples were eligible for inclusion.

- Bacterial Identification and Antimicrobial Susceptibility Testing

Bacterial isolate identification was performed using a standardized methodology harmonized across all participating laboratories, based on conventional morphological, cultural, and biochemical characteristics. Following identification, antimicrobial susceptibility testing was carried out using the disk diffusion method in accordance with the recommendations of the Antibioqram Committee of the French Society for Microbiology (CA-SFM, 2021). ESBL production was suspected phenotypically by the presence of a synergy image (“champagne-cork” or keyhole effect) between the amoxicillin-clavulanate disk and third-generation cephalosporin or aztreonam disks, in accordance with CA-SFM recommendations. High-level cephalosporinase (AmpC-type) production was suspected in isolates showing resistance to third-generation cephalosporins without synergy with clavulanate, together with resistance to cefoxitin. Carbapenem resistance was defined according to CA-SFM clinical breakpoints for imipenem, meropenem, and ertapenem; none of these phenotypically inferred mechanisms was confirmed by

molecular or biochemical methods.

- Data Collection and Analysis

Data were initially collected from laboratory bench registers and subsequently entered into and analyzed using Microsoft Excel. Isolates originated from both hospitalized and ambulatory (community) patients attending the participating facilities; the specific clinical department or specimen request source was not systematically recorded for all sites during this preliminary surveillance and could therefore not be analyzed.

3. Results

Over the 20-month study period, a total of 329 Enterobacteriaceae strains resistant to third-generation cephalosporins were isolated from the participating healthcare facilities.

The highest numbers of third-generation cephalosporin-resistant Enterobacteriaceae were reported by the Saint-Louis Regional Hospital, Aristide Le Dantec Hospital, and Kaolack Regional Laboratory (**Figure 1**).

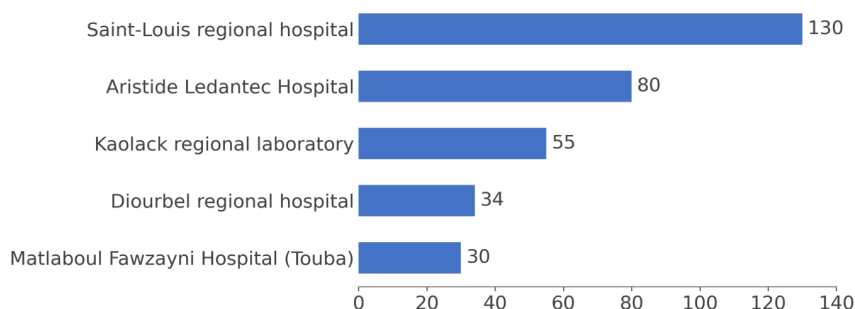


Figure 1. Distribution of isolates according to the healthcare facilities of origin.

The majority of cephalosporin-resistant Enterobacteriaceae were isolated from urine samples ($n = 178$; 54%) and pus specimens ($n = 64$; 19%). Additional isolates were recovered from blood cultures ($n = 32$; 10%), stool samples ($n = 5$; 1%), vaginal secretions ($n = 9$; 3%), and cerebrospinal fluid (CSF) samples ($n = 3$; 1%) (**Figure 2**).

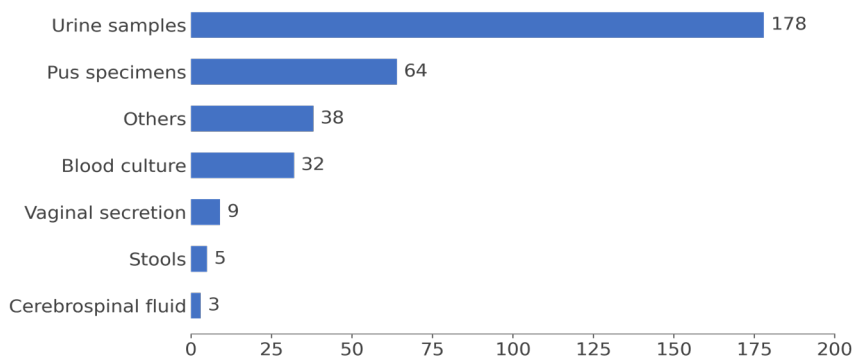


Figure 2. Distribution of isolates according to the pathological specimen.

Among all third-generation cephalosporin-resistant species, *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. largely predominated, with respective prevalences of 54.4% (n = 179), 26.1% (n = 86), and 13.9% (n = 46) (**Figure 3**).

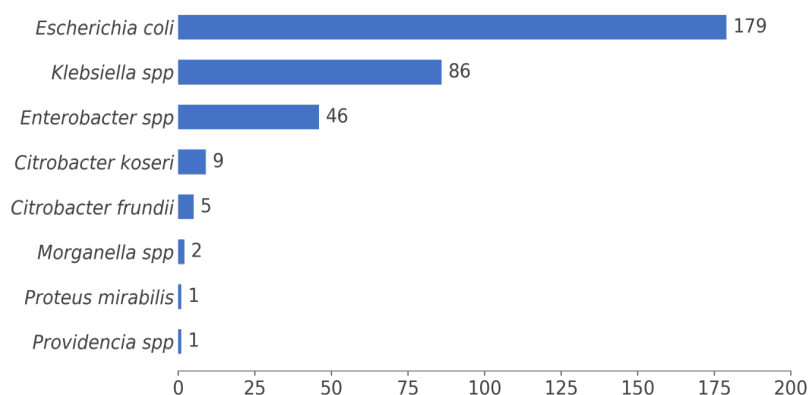


Figure 3. Distribution of third-generation cephalosporin resistance according to species.

The production of extended-spectrum beta-lactamases (ESBL) was the most frequently identified mechanism associated with third-generation cephalosporin (3GC) resistance, with a prevalence of 79.6% (n = 262). Other mechanisms, such as high-level cephalosporinase production, were also detected (**Figure 4**).

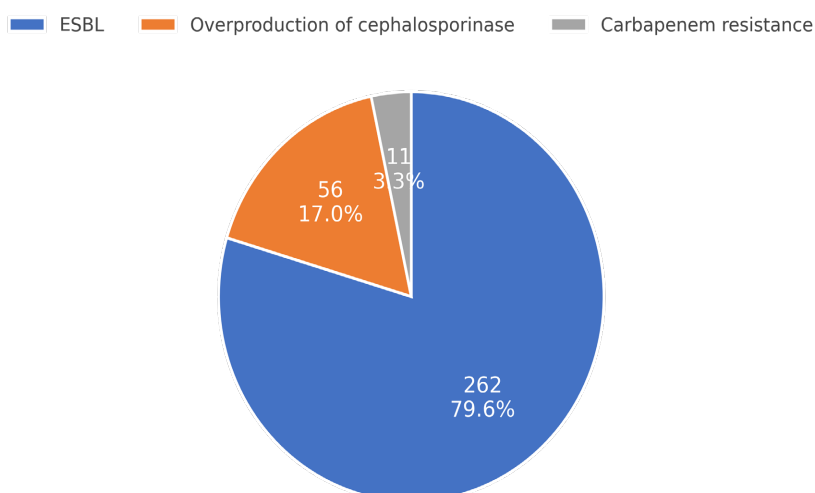


Figure 4. Distribution of isolates according to the determinant of third-generation cephalosporin resistance.

Escherichia coli, *Klebsiella* spp., and *Enterobacter* spp. were the predominant species involved in third-generation cephalosporin (3GC) resistance mediated by ESBL production (**Table 1**).

ESBL-producing strains were more frequently isolated from samples originating from Saint-Louis, Le Dantec, and Kaolack (**Table 2**).

Table 1. Distribution of ESBL-producing species.

ESBL-producing isolates	Number	Prevalence (%)
<i>Escherichia coli</i>	147	55.9
<i>Klebsiella</i> spp.	67	25.6
<i>Enterobacter</i> spp.	38	14.5
<i>Citrobacter koseri</i>	6	2.2
<i>Morganella</i> spp.	2	1.2 0.8
<i>Citrobacter freundii</i>	1	0.3
<i>Proteus mirabilis</i>	1	0.3
Total	262	100

Table 2. Distribution of ESBL-producing strains according to the study site.

Sites	ESBL	Prevalence (%)
Saint-Louis Regional Hospital	123	46.9
Le Dantec National University Hospital (CHNU)	63	24.0
Kaolack Regional laboratory	35	13.4
Matlaboul Fawzeini Hospital (Touba)	22	8.4
Diourbel Regional Hospital	19	7.3
Total	262	100

4. Discussion

This prospective, cross-sectional, descriptive study focused on the surveillance of third-generation cephalosporin (3GC) resistance among Enterobacterales in four regions of the country. It was conducted over a 20-month period between 2020 and 2021, coinciding with the first wave of the COVID-19 pandemic and marked by a reduction in healthcare-seeking behavior in the general population. This may explain the low number of samples and the observed regional disparities.

The healthcare facilities with the highest number of isolates were located in Dakar (Le Dantec National University Hospital) and Saint-Louis (Regional Hospital). This difference compared to other sites may be explained by the fact that these two institutions are tertiary referral hospitals with high patient attendance and a broad range of specialties.

Distribution of species according to specimen type

Regarding the distribution of isolates according to specimen type, urinary tract infections were by far the most frequent, with a prevalence of 54.1%. Several studies in Europe and in the sub-region have reported the same trend [5] [6] [7]. Urinary tract infections represent one of the main reasons for consultation, microbiological investigation, and intensive antibiotic use. Consequently, they have an impact on healthcare costs and contribute to the selection of multidrug-resistant strains in both hospital and community settings [8] [9]. It is estimated that 150

million urinary tract infections occur worldwide each year [9] [10]. Indeed, the urinary tract constitutes the most common entry site for Enterobacterales infections [11].

Distribution of 3GC-resistant species

Resistance to 3GC mainly involved *Escherichia coli* and *Klebsiella* spp., far ahead of other species, with respective prevalences of 54.4% (n = 179) and 26.1% (n = 86).

In Lomé (Togo), Salah *et al.* (2021) reported increasing 3GC resistance rates over an 8-year period, involving 30% of *Escherichia coli* isolates and 42% of *Klebsiella* spp. isolates [6].

In 2024, a FLASH genomic epidemiology survey of 3GC-resistant Enterobacterales in an urban setting (France) identified *E. coli* (n = 133; 42%) as the most frequent community species, followed by community- and hospital-acquired *Klebsiella pneumoniae* (18.9%) [12]. A 10-year review conducted at the Principal Hospital of Dakar on *E. coli* isolates reported 3GC resistance rates of 27%, with an increase from 28% to 41% between 2012 and 2021 [13].

At the Infectious Diseases Department of Fann Hospital (Dakar), a co-dominance of *Klebsiella pneumoniae* and *Escherichia coli* was observed [11].

Indeed, 3GCs are often misused and inappropriately used as first-line agents, particularly in hospital settings, leading to the selection of resistant bacteria, especially in the gastrointestinal tract where *Escherichia coli* and *Klebsiella pneumoniae* predominate. Following breaches in hygiene or mucosal barriers, these selected multidrug-resistant bacteria, in a carrier state, may cause autoinfection or be transmitted to other individuals [14]. They may also be present in the hospital environment, contributing to healthcare-associated infections.

In Europe, a study involving 4376 non-intensive care patients showed a 9.5% prevalence of digestive carriage of 3GC resistance, with *E. coli* predominating at 79%. Colonization with 3GC-resistant Enterobacterales, antibiotic use within the previous 6 months, and prolonged hospitalization were among the factors associated with 3GC resistance [15].

Distribution according to resistance determinants

Extended-spectrum beta-lactamase (ESBL) production was the most frequently identified mechanism associated with 3GC resistance, with a prevalence of 79.6%. Similar findings were reported by Le Hello *et al.* (2024), where ESBL production accounted for 76.4% of 3GC resistance, while AmpC-type cephalosporinase production was observed in 25% of strains [12].

In the carriage study, ESBLs belonging to CTX-M-1 (67.3%) and CTX-M-9 (16.8%) groups were the most frequently encountered β -lactamases associated with 3GC resistance [15].

3GC-resistant bacteria were first described in the early 1980s, shortly after the introduction of these antibiotics into clinical practice. These were *Klebsiella pneumoniae* strains carrying plasmid-mediated resistance capable of hydrolyzing all beta-lactams except cephamycins and carbapenems.

Since then, this resistance mechanism has rapidly spread among community Enterobacterales, particularly *Escherichia coli*. Within 10 years, this commensal intestinal bacterium (10^8 CFU/g of stool) became the most frequently involved species in ESBL-mediated resistance. The enzyme involved is often a CTX-M-type cephalosporinase. Resistance is acquired through plasmid transfer from environmental bacteria not pathogenic to humans but naturally harboring this resistance mechanism, such as *Kluyvera*, as well as through inter-Enterobacterales transmission [4].

Initially considered a hospital-associated problem, the widespread community dissemination of this resistance mechanism now represents a major public health concern [16] [17]. The circulation of plasmid-mediated cephalosporinases of the DHA-1 and CMY-2 types was observed in Dakar and Saint-Louis in 2017 [18]-[20].

5. Conclusions

This study has several limitations. It relied on laboratory-based surveillance without systematic clinical correlation, and ESBL, high-level cephalosporinase, and carbapenem resistance mechanisms were inferred phenotypically without molecular confirmation. The study period coincided with the COVID-19 pandemic, which likely reduced healthcare-seeking behavior and sample volume, potentially affecting the observed regional distribution. Consequently, these findings do not allow inference of individual risk factors or population-level resistance rates and should be interpreted as preliminary.

3GC resistance mainly concerns Enterobacterales and represents a major issue in antimicrobial resistance surveillance. This preliminary work showed that the burden of resistance was similar across the different targeted healthcare facilities. Antimicrobial stewardship programs should, therefore, be implemented at the national level to reduce morbidity and mortality.

However, geographical disparities were observed, and epidemiological determinants should be further investigated to better understand the spread of resistance in these regions. These data should be integrated into national antimicrobial resistance control policies in order to strengthen effective surveillance of bacterial resistance to third-generation cephalosporins in Senegal.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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