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Bacteriological Profile of Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae Isolated at the Abass NDAO Hospital Center (CHAN) in Dakar from June 2021 to June 2022

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A B S T R A C T

Infections caused by extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae represent a public health issue in both hospital and community settings. The aim of this study was to assess the emergence of ESBL-producing Enterobacteriaceae at CHAN. We conducted a cross-sectional study involving 309 Enterobacteriaceae isolates collected between June 1, 2021, and June 31, 2022, from both outpatients and inpatients. Conventional bacteriological isolation and identification methods were employed. Antimicrobial susceptibility testing was performed using the disk diffusion method on Mueller-Hinton agar. ESBL production was identified using the synergy test between clavulanic acid and third- and fourth-generation cephalosporins. Urine was the most frequently received specimen type (52.43%), followed by pus samples (36.90%). The ESBL phenotype predominated, accounting for 51% of isolates. Distribution by gender was unequal: 54% in men and 46% in women. *Escherichia coli* was the most frequently isolated species (51.30%), followed by *Klebsiella pneumoniae* (23.95%) and *Proteus* spp. (10.70%). ESBL-producing Enterobacteriaceae infections were observed across all age groups, but individuals over the age of 60 were the most affected (43.31%). Our study reveals very high resistance rates—reaching up to 100%—among ESBL-producing Enterobacteriaceae against beta-lactams, with the exception of imipenem (0.7%). Furthermore, high resistance to quinolones (66%) was observed. However, effective antibiotics include fosfomycin, colistin, chloramphenicol, and aminoglycosides (especially amikacin). Our study reveals a statistically significant relationship between acquiring an ESBL-producing *E. coli* infection and either age or hospitalization (p=0.0002 and p=0.000, respectively). Conversely, this infection is not linked to gender but appears to be due to chance (p=0.2017).

Introduction

Enterobacteriaceae constitute a vast family of Gram-negative bacilli comprising numerous genera and species. These bacteria cause diseases of widely varying severity due to distinct pathogenic mechanisms. In hospital settings, they account for over 30% of morbidity and mortality associated with bacterial infections (Eddair, 2021). To date, numerous ESBLs (>230) have been described worldwide (Cattoir & Bicêtre, 2008). Although these ESBLs have been described in Enterobacteriaceae, they are primarily found in bacterial species such as *Klebsiella pneumoniae* and *Escherichia coli* (Sarr and al., 2021). Consequently, the major challenge today is to limit the spread of ESBL-producing Enterobacteriaceae in both community and, especially, hospital settings. This requires bacterial identification and antimicrobial susceptibility testing. Generally, these Enterobacteriaceae are isolated from various clinical samples, such as urine, pus, vaginal swabs, stool, and blood cultures. It is against this backdrop that we conducted a cross-sectional study on the bacteriological profile of ESBL-producing Enterobacteriaceae isolated at the bacteriology laboratory of the Abass Ndao Hospital Center (CHAN) between June 2021 and June 2022, with the overall objective of assessing the emergence of ESBL-producing Enterobacteriaceae at CHAN.

The present study was undertaken to determine the prevalence of extended-spectrum β-lactamase (ESBL)-producing organisms among patients attending CHAN, to assess the distribution of Enterobacteriaceae isolates according to key epidemiological parameters including age, sex, type of clinical specimen, and patient origin, and to characterize the antibiotic susceptibility patterns of ESBL-producing Enterobacteriaceae in order to provide valuable epidemiological data for effective antimicrobial therapy and infection control strategies.

Materials and Methods

Study setting

This study was conducted at the laboratory of the Abass Ndao Hospital Center (CHAN) in Dakar, Senegal. The medical analysis laboratory is divided into four departments (microbiology, hematology, biochemistry, and immunoserology) and includes a biochemistry unit associated with the hospital's Marc Sankale Diabetes Treatment Center.

Study type and period

This was a cross-sectional study conducted at CHAN over the period from June 1, 2021, to June 31, 2022.

Study population

Our study population consisted of patients who were hospitalized or attended consultations at CHAN during the study period and were suspected of having a bacterial infection. A sample of these patients was selected for the study.

Inclusion and Exclusion Criteria

Inclusion Criteria

This study includes any patient in our population from whom an Enterobacteriaceae has been isolated and for whom information such as the isolated organism, the pathological specimen, age, sex, and patient status is complete, and whose antibiogram reports are available and interpreted, is included.

Exclusion Criteria

Any patient with a negative test result or a positive result with isolation of an organism other than an Enterobacteriaceae is excluded, as is any patient who came outside our study period. Each duplicate is considered only once.

Materials Used

Biological Materials

Our biological materials included pathological specimens such as urine, pus, vaginal secretions, stool, blood cultures, and puncture fluids.

Non-biological materials

These consist of sampling and analysis equipment.

Sampling materials

The sampling materials used consist of: gloves, sterile vials, syringes, speculums, slides, PV trays, cotton swabs, and swabs.

Analytical Equipment

For the analysis, several materials were used: optical microscopes; calibrated loops, Pasteur pipettes, transfer pipettes, hemolysis tubes, Bunsen burner, incubator, centrifuge, vortex mixer, slides and coverslips, test tubes, culture media (CLED, EMB, MH, CS, MMN, UI, KH, BT, SS…), antibiotic discs, forceps, and rulers. For the solutions, we used gentian violet, Lugol's iodine, alcohol, fuchsin, physiological saline, and distilled water.

Methods

The methods used depended on the types of samples to be examined.

Sampling

The type of sample depends on the site of infection: stool, urine, vaginal swab, cerebrospinal fluid (CSF), pus, etc. This is a crucial step in the diagnostic process: the quality of the sample determines the success rate. Biological samples must be collected under aseptic conditions and sent to the laboratory as quickly as possible.

Diagnostic Methodology for Enterobacteriaceae

Macroscopic Examination of the Sample

This serves as a guide and, in some cases, allows verification of the sample's suitability:

- ✓ Turbidity: CSF, urine, etc.;
- ✓ Blood: CSF, urine, etc.;
- ✓ Consistency: stool, vaginal swab, etc.;
- ✓ Appearance: stool, vaginal swab, etc.

Microscopic Examination

Fresh Sample: Direct examination of a fresh sample involves observing a bacterial suspension between a slide and coverslip under a 40x objective. This allows visualization of the presence and motility of the bacteria, as well as the qualitative and quantitative cytology of the pathological products: presence and number of leukocytes, erythrocytes, parasites, crystals, etc.

Microscopic Examination after Gram Staining: This is the reference stain in bacteriology. It allows differentiation of bacteria by their ability to retain gentian violet (Gram-positive) or fuchsin (Gram-negative). Note that Enterobacteriaceae are all Gram-negative bacilli. (Figure 1)

Culture and Isolation

The choice of culture medium depends on the type of sample and the target organism. Several media are used for culturing Enterobacteriaceae. In our study, we used the following media:

Mueller Hinton (MH), which is a standard agar that promotes the growth of all types of bacteria;

EMB (Eosin Methylene Blue): a medium selective for Gram-negative bacteria;

CLED (Cystine Lactose Electrolyte Deficient): a non-selective isolation medium, recommended for urine culture; the method used was the calibrated loop method (10 µL);

Hektoen and SS agar: selective isolation media for Gram-negative bacilli, used for isolating Salmonella and Shigella from stool;

BT (Thioglycolate Broth): an enrichment medium, also used to study the respiratory type of bacteria. After culturing, the plates are incubated in an incubator at 37°C for 18 to 24 hours.

Reading and Identification

After incubation, if the strain is pure, it is identified. In the case of a polymicrobial culture, re-isolation is performed if necessary. In the case of a urine culture, it is essential to count the colonies because there is a bacteremia threshold of $\geq 10^5$ bacteria/ml (one colony corresponds to 10^4 bacteria/ml), and the culture must be monomicrobial.

Oxidase Test

This is a diagnostic test used for the differentiation and identification of microbial species, particularly Gram-negative bacteria, based on the presence of the cytochrome oxidase enzyme. One of the characteristics of Enterobacteriaceae is that they do not possess cytochrome oxidase (oxidase test is always negative).

Biochemical Test (Classic Mini Gallery)

The biochemical tests addressed in our study correspond to the classic mini gallery for Gram-negative bacteria.

After incubation of the media for 24 hours at 37°C, a color change is observed, indicating whether or not the media have degraded sugars (glucose, lactose) by the bacteria, whether or not Simmons citrate has been used as the sole carbon source, the presence or absence of certain enzymes such as urease and tryptophanase deaminase, and the production or absence of H2S (hydrogen sulfide).

Figure 2 shows some media before (A) and after (B) incubation.

Antibiotic Susceptibility Testing: Antibiotic Susceptibility Testing

An antibiotic susceptibility test is a technique for studying the susceptibility of bacteria to antibiotics. It measures the ability of an antibiotic to inhibit bacterial growth in vitro. In a standard antibiogram, after inoculation, amoxicillin + clavulanic acid (AMC) is placed in a Petri dish surrounded by the four generations of cephalosporins.

After 18 to 24 hours of incubation, the appearance of synergy between AMC and one of the third-generation (ceftazidime or cefotaxime) or fourth-generation (cefepime) cephalosporins is sufficient to conclude that the strain produces ESBLs.

In the absence of synergy, the strain's resistance to all beta-lactams except carbapenems and cephamycins allows us to conclude that it secretes ESBLs.

Data Analysis

Data processing was performed using Epi Info version 7.2.5 and Excel (Microsoft Office 2013). The document was written in Microsoft Word. Zotero software was used for references and the bibliography.

Differences between groups were tested using the Chi-square (χ^2) test, and the significance level was set at $p < 0.05$. Logistic regression was performed to investigate a possible association between risk factors and carriage of ESBL-producing Enterobacteriaceae.

Results and Discussion

During our study, from June 2021 to June 2022, a total of 1030 samples related to the study were received at the CHAN laboratory.

Epidemiological Aspects

Population Distribution According to Reason for Consultation

Most of our patients (33.88%) came for a consultation (pregnancy, infertility, check-up, etc.), 27.09% for signs of urinary tract infection (UTI, UTI, etc.), 16.50% already had an infection (suppuration, abscess, etc.), 3.10% had threatened miscarriages, and 7.87% had comorbidities (diabetes, hypertension, etc.) (Table I).

Population Distribution According to Patient Status

Our population consisted primarily of hospitalized patients (62.62%), while those from other hospital settings (outpatients) represented 37.38%.

Population Distribution According to Age Groups

Patients ranged in age from 0 to 96 years, with a median age of 40 years. Figure 3 shows that the most represented age group was 21 to 40 years, with 439 patients (42.62% of the population), followed by those over 60 years of age (n=297, 28.83%).

Population Distribution by Sex

Samples were collected more frequently from men (n=582), representing 56.5%, while samples from women totaled 448, or 43.5%.

Population Distribution by Pathological Product

As in most medical analysis laboratories, the most frequently received pathological product during our study was urine (n=675), representing 66% of all samples. Vaginal swabs (21%) and pus (12%) were the second most common. Other types of samples (stool, blood culture, puncture fluid, etc.) represented only 1% of the total (Figure 4).

Distribution of Isolated Strains

Distribution of Strains According to Patient Status

During our study period, 309 Enterobacteriaceae were isolated. Table II shows a clear predominance of Escherichia coli, representing 50.80% of the strains (n=157), followed by Klebsiella pneumoniae at 23.95% (n=74), then Proteus ssp. at 10.70% (n=33), and Klebsiella oxytoca at 7.44% (n=23).

The other Enterobacteriaceae constitute 7.11%, or 22 strains. Among these isolated strains, 238 were of internal origin, representing 77%, while 71 strains were of external origin, representing 23%. Whether of hospital or community origin, Escherichia coli and Klebsiella pneumoniae remain in all cases the predominant species.

Distribution of strains according to pathological samples

Enterobacteriaceae were most frequently found in urine samples, with an isolation rate of 52.43% (n=162), followed by pus samples at 36.90% (n=114). In contrast, only 19 enterobacteriaceae were isolated from urine samples (6.15% of the total), and 4.53% (n=14) in the other types of samples (Figure 5).

Distribution of strains by sex

In our study, we observed a predominance of infections in men, who harbored 54% of the isolated strains, compared to 46% for women, for a sex ratio of 1.17.

Distribution of strains by age group

Figure 6 reveals that patients over 60 years of age were the most infected, with a strain isolation rate of 39.80%. Next were those aged between 41 and 60 years (27.18%), and those aged 21 to 40 years (24.92%). In contrast, only 8.09% of strains were isolated from those under 20 years of age.

Antibiotic Profile

Distribution of strains according to resistance phenotype

This study shows that the ESBL phenotype is predominant with a prevalence of 51% (n=157), followed by the high-level penicillinase (HLP) phenotype with 34% (n=105) and the low-level penicillinase (LLP) phenotype with 10%.

The wild-type phenotype represents 5% (Figure 7).

Distribution of ESBLs according to Enterobacteriaceae species

We identified 157 Enterobacteriaceae species that produced an ESBL, with E. coli being the most represented species at 47.77% (n=75),

Followed by Klebsiella pneumoniae at 24.20% (n=38) and Proteus spp (12.74%) (Table III).

Distribution of ESBL-producing Enterobacteriaceae according to patient status

According to our results, 80.9% of ESBL-producing Enterobacteriaceae infections are hospital-acquired, while 19.1% are community-acquired.

Distribution of ESBL-producing Enterobacteriaceae according to pathological samples

ESBL-producing Enterobacteriaceae were predominantly isolated from urine samples and surgical sites, with respective frequencies of 45.86% (n=72) and 45.22% (n=71) (Figure 8).

Distribution of ESBL-producing Enterobacteriaceae by Age Group

Figure 9 shows that ESBL-producing Enterobacteriaceae infections are much more frequent in people over 60 years of age, with an isolation rate of 43.31% (n=68).

In patients aged 41 to 60 years, this rate is 30.57% (n=48), it is 19.10% in those aged 21 to 41 years, and 7% in those under 20 years of age.

Distribution of ESBL-producing Enterobacteriaceae by Sex

ESBL-producing Enterobacteriaceae were found in both men and women, with respective rates of 54.2% and 46.8%.

Resistance of ESBL-producing Enterobacteriaceae to Antibiotics

Regarding the antimicrobial resistance profile of the ESBL-producing isolates, we observed very high resistance to all beta-lactams. Indeed, the ESBL-producing Enterobacteriaceae were resistant up to 100% against amoxicillin, amoxicillin + clavulanic acid, ticarcillin, and first-, third-, and fourth-generation cephalosporins. As for cefoxitin and aztreonam, the strains showed resistance rates of 76% and 68%, respectively, against these antibiotics. ESBL-producing Enterobacteriaceae also showed strong resistance to cotrimoxazole and quinolones, with respective percentages of 79% and 66%. In contrast, significant susceptibility rates were observed to fosfomycin (76%), colistin (82%), and aminoglycosides (74%), with amikacin proving to be the most effective at 94%. Regarding imipenem, all our strains retained excellent susceptibility except for two Klebsiella pneumoniae strains. (Figure 10)

Association between ESBLs and socio-demographic variables

Statistical tests allowed us to obtain, with a 95% confidence interval, p-values less than 0.05 for the association between ESBLs and variables such as age and origin of the patient (hospitalized), with p=0.0002 and p=0.0000 respectively. As for the association between ESBLs and sex, we found a p-value greater than 0.05; namely p=0.2017 (Table IV).

This was a retrospective, cross-sectional study conducted in the bacteriology unit of the Abass NDAO Hospital Center (CHAN) laboratory in Dakar between June 1, 2021, and June 30, 2022. The study focused on the bacteriological profile of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae isolated at the CHAN laboratory. The primary objective of this work was to investigate the emergence of ESBL-producing Enterobacteriaceae isolated at CHAN. Our study results show that general health, infertility, pregnancy, and follow-up check-ups were the most frequently recorded clinical indications on patient analysis request forms (35.31%). The median patient age was 40 years (range: 0–96 years), with the 21–40 age group being the most represented. Enterobacteriaceae isolates were predominant in males (54%), with a male-to-female ratio of 1.17. Our findings are similar to those reported by Sarr (2019) in a study on the prevalence of ESBL-producing and/or imipenem-resistant Enterobacteriaceae strains at the FANN laboratory (Dakar), where the age range was 1–98 years and the male-to-female ratio was 1.13. Conversely, our results differ from those of a study conducted in Morocco, where women were more frequently infected (57%) and the male-to-female ratio was 0.75 (Eddair, 2021). The distribution of strains by age shows that the elderly is most frequently associated with bacterial infections, with a rate of 39.80%, followed by the 41–60 age group at 27.18%. These results align with findings from Sabor (2017) at Fann (Dakar), where the most affected age group was those over 60 (610 cases, or 25.37%), followed by the 46–60 age group (402 cases, or 16.72%); Sy *et al.,* (2021) at the Thiès Regional Hospital, who reported a prevalence of 40.60% among those over 60; and Rania & Rania (2017) in Algeria, where the infection rate for the over-60 population was 48%. This high infection rate among the elderly may be attributed to their increased vulnerability to infections resulting from the frailty and aging of their immune systems. In our study, the majority of isolated strains originated from urine samples (52.43%), followed by pus samples (36.90%). These results align with those reported by Sabor (2017) at another hospital in Dakar (Fann), where 52% of isolates were of urinary origin. Our findings also concur with those from a study in Cameroon by Ebongue *et al.,* (2015), in which the majority of strains came from urine (68.7%), followed by purulent samples (13.5%). This distribution is typical for a bacteriology laboratory, as urine culture (ECBU) is the most frequently requested test. Among the isolated Enterobacteriaceae strains, *Escherichia coli* ranked first with an isolation frequency of 51.30%, followed by *Klebsiella pneumoniae* (23.95%) and *Proteus* spp. (10.70%). Our results align with those reported by Sabor (2017) in Dakar (*E. coli*: 40.2%, *K. pneumoniae*: 12.76%). Results obtained by Dia and al. (2015) at the Dakar University Hospital (Senegal)—covering the period from January 2012 to September 2014—showed that Enterobacteriaceae were the most common group, with a predominance of *E. coli* (39.92%) followed by *K. pneumoniae* (17.96%); similarly, Gaudré *et al.,* (2019) reported in Mali that *E. coli* was the most frequently isolated organism (42.9%), followed by *K. pneumoniae* (16.3%). In Algeria, Souna (2011) reported figures of 37.1% for *E. coli* and 21.4% for *K. pneumoniae*, again placing *E. coli* in the leading position. This typical ranking for the species may be explained by its ease of adhesion to cells (Sy *et al.,* 2021), its prevalence in commensal flora (Ladjal & Baghdadi, 2021), and the fact that urine samples are the most

common type submitted to medical analysis laboratories, where this species is predominantly isolated (Benamira *et al.,* 2007). In contrast, a retrospective, descriptive study conducted at the Fann University Hospital (CHNU de FANN) on the profile of healthcare-associated infections and antimicrobial resistance in an infectious disease department found *Klebsiella pneumoniae* to be the predominant species (28%), followed by *Escherichia coli* (25%) and *Enterobacter* spp. (24%) (Massaly and al., 2022). The predominance of this species in this study could be due to the presence of patients with pulmonary infections within the study population. The results of our study show a relatively high prevalence rate of ESBL-producing Enterobacteriaceae—approximately 51%, or one in every two strains. In Dakar, a prevalence of 38.7% was found at the Fann University Hospital (CHNU) in a 2019 study by Sarr focusing on Enterobacteriaceae producing ESBLs and/or exhibiting imipenem resistance. Similarly, an ESBL-producing Enterobacteriaceae rate of 56.3% was recorded at the Point G University Hospital (Bamako) in a study by Kansaye (2020) on beta-lactam resistance phenotypes in Enterobacteriaceae strains isolated from pus, blood cultures, and stool samples. Our data align with the very high ESBL prevalence rates observed in these two studies. However, other studies have reported prevalence rates lower than ours. This is the case for a study conducted at the Aristide Le Dantec University Hospital by Tine (2013) on the prevalence of ESBL-producing Enterobacteriaceae between 2011 and 2013, which found a rate of 24.3%. Frequencies of 29% and 13.65% were observed in Bamako and Morocco, respectively (Eddair, 2021). The frequency of ESBL production among Enterobacteriaceae strains varies unevenly across the globe. This geographical variation can be attributed to differences in epidemiological factors, antibiotic usage policies, and hospital hygiene measures across institutions (Sabor, 2017). The high rate of ESBL production observed in our study could be due to the widespread introduction of third-generation cephalosporins into the therapeutic arsenal, as well as the overuse and inappropriate use of antibiotics—the primary driver of antibiotic resistance emergence. Indeed, a statistically significant correlation was identified between the consumption of third-generation cephalosporins and the rates of ESBL-producing resistant Enterobacteriaceae between 2004 and 2011 ($p = 0.002$) (Chaouch and al., 2014). Regarding ESBL-producing strains, *Escherichia coli* was the most prevalent species at 47.77% (n=75), followed by *Klebsiella pneumoniae* at 24.20% (n=38). Our results align with those reported in Saint-Louis (Senegal) by Lô (2015), who found an ESBL-producing *E. coli* prevalence of 51.96% and a *K. pneumoniae* prevalence of 31.31%. In Togo, a study by Toudji and al. (2017) found that 51.13% of *E. coli* isolates produced ESBLs, compared to 30.10% of *Klebsiella* spp. However, some studies show *Enterobacter* spp. surpassing *Klebsiella pneumoniae* and *E. coli*. This was the case in a study on the epidemiology and medico-economic impact of healthcare-associated infections caused by ESBL-producing Enterobacteriaceae in Senegal, where *Enterobacter* spp. accounted for the highest proportion of ESBL producers (40%), followed by *Klebsiella pneumoniae* (29%) and *E. coli* (8%) (Ndir, 2015). In our study, the majority of ESBL-producing strains were isolated from urine samples (45.86%), followed by pus samples (45.22%). This distribution of ESBLs across clinical specimens reflects the trend observed in most diagnostic laboratories. At the Aristide Le Dantec University Hospital, an ESBL prevalence of 59% was found in urine, compared to 25% in pus (Camara *et al.,* 2014).

According to our study, the majority of ESBL-producing Enterobacteriaceae (ESBL-E) are of nosocomial origin (80.9%), compared to 19.1% of community origin. Nevertheless, the prevalence among outpatients is significant. Our figures are similar to those from a study conducted in Morocco, where 81% of ESBL strains were of hospital origin. Similarly, Gadou (2019) reported a 70% prevalence of hospital-acquired ESBL-E. This predominance in the hospital setting may be attributed to the fact that these patients are at higher risk of contracting the infection due to factors such as prolonged hospitalization, disease severity, the use of various invasive devices (urinary catheters, central venous catheters, mechanical ventilation, intubation, etc.), and multiple antibiotic treatments, particularly with broad-spectrum cephalosporins (Foulal, 2013). Until the late 1990s, ESBL-producing Enterobacteriaceae were primarily "hospital-associated" species that spread clonally among hospitalized patients. However, since the early 2000s, the emergence and explosive dissemination of new ESBLs have been observed worldwide, accompanied by a radical shift in epidemiology. Indeed, the spread of these enzymes within the species *Escherichia coli* has disrupted the traditional epidemiology of ESBLs, leading to the dissemination of such strains in the community (Grall *et al.,* 2011).

Consequently, the rates observed in the community appear to result from selective pressure caused by the excessive prescribing and misuse of broad-spectrum

antibiotics (such as over-the-counter dispensing and self-medication), alongside the impact of poorly regulated food systems and the increasing use of antibiotics in agriculture and livestock farming. In our study, the acquisition of an ESBL-producing infection was independent of patient gender, as men and women were represented almost equally (54.2% women vs. 46.8% men) (p=0.2). However, patients over the age of 60 accounted for the highest proportion of infections (43.31%). A study conducted by Diarra in 2019 found higher infection rates among women and patients aged 15 to 30; these findings differ from ours.

The ESBL-producing Enterobacteriaceae (ESBL-E) isolated in our study exhibited very high resistance to beta-lactams (amoxicillin, amoxicillin-clavulanic acid, ticarcillin, piperacillin, and first-, third-, and fourth-generation cephalosporins), with resistance rates of 100%, compared to 76% for cefoxitin and 68% for aztreonam. However, two strains of ESBL-producing *Klebsiella pneumoniae* were found to be resistant to carbapenems (imipenem), representing a resistance rate of 0.7%. Our results align with those reported by Foulal (2013) in Rabat, where ESBL-producing strains showed 100% resistance to ticarcillin, piperacillin, and first-, third-, and fourth-generation cephalosporins, as well as a resistance rate to imipenem slightly higher than that observed in our study (3.34%). Similarly high resistance rates were found in a study conducted in Bamako by Kansaye (2020), although the values for third- and fourth-generation cephalosporins (88%) were lower than ours. This is consistent with the literature stating that ESBL-producing Enterobacteriaceae are resistant to all beta-lactams except cephamycins and carbapenems. This resistance of Enterobacteriaceae to beta-lactams may be attributed to the fact that beta-lactams are the most frequently prescribed class of antibiotics. Indeed, a study on antibiotic prescribing patterns in the Internal Medicine Department of Abass Ndao Hospital in Dakar showed that this class of antibiotics accounted for 45.3% of prescriptions (Sow *et al.,* 2020). Some studies suggest reducing the empirical use of third-generation cephalosporins (3GCs), which are available at low cost in hospital pharmacies. However, this restriction may be viewed as a "false solution." Indeed, it would likely lead to increased use of carbapenems—last-resort drugs effective against ESBL-producing Enterobacteriaceae—which could, in turn, foster the emergence of carbapenem-resistant bacteria (also known as highly antibiotic-resistant bacteria). This is precisely the case in our study, where two *Klebsiella pneumoniae* strains are resistant to all beta-lactams, including imipenem. Such a situation could lead to a therapeutic dead end and prove fatal for the patient. Consequently, epidemiological surveillance of these strains is essential.

The ESBL-producing isolates also demonstrated high resistance to co-trimoxazole (79%) and quinolones (66%), specifically 79% for nalidixic acid and 66% for ciprofloxacin. These prevalence rates remain lower than those observed in a study conducted in Saint-Louis, where resistance was 66% for nalidixic acid and 60% for ciprofloxacin (Lo, 2015). This multidrug resistance in the strains can be attributed to the fact that ESBL genes—typically plasmid-borne—are frequently associated with genes conferring resistance to other antibiotics, particularly aminoglycosides and fluoroquinolones (Shi *et al.,* 2015).

Susceptibility to certain aminoglycosides, particularly amikacin, appears to be preserved (94%), unlike that for gentamicin (58%) and kanamycin (59%); this supports the use of amikacin for empirical treatment. These figures align with those reported by Kansaye (2020), who found 94% susceptibility to amikacin and 48% to gentamicin. The apparently preserved efficacy of aminoglycosides may be explained by their route of administration—often parenteral—which limits their overuse. Colistin and fosfomycin demonstrate good antimicrobial activity against ESBL-producing organisms, with susceptibility rates of 89% and 76%, respectively. This efficacy of fosfomycin was confirmed in a study conducted in Algeria, where all ESBL-producing strains were susceptible to the drug. Statistical analysis reveals that patient age and origin are strongly associated with the presence of ESBL-producing Enterobacteriaceae ($p < 0.05$; $p = 0.0002$ and $p = 0.0000$, respectively).

Figure.1 Morphology of Gram-negative bacilli after Gram staining

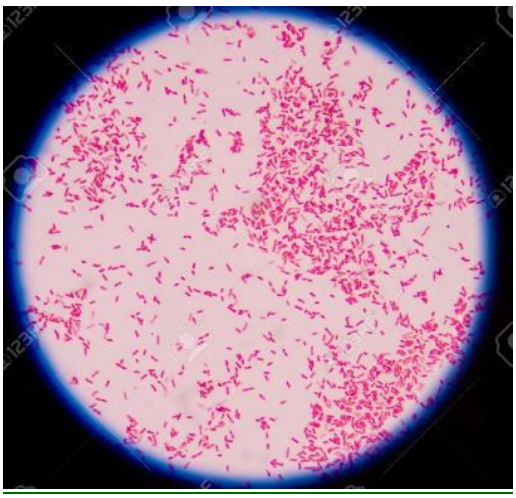
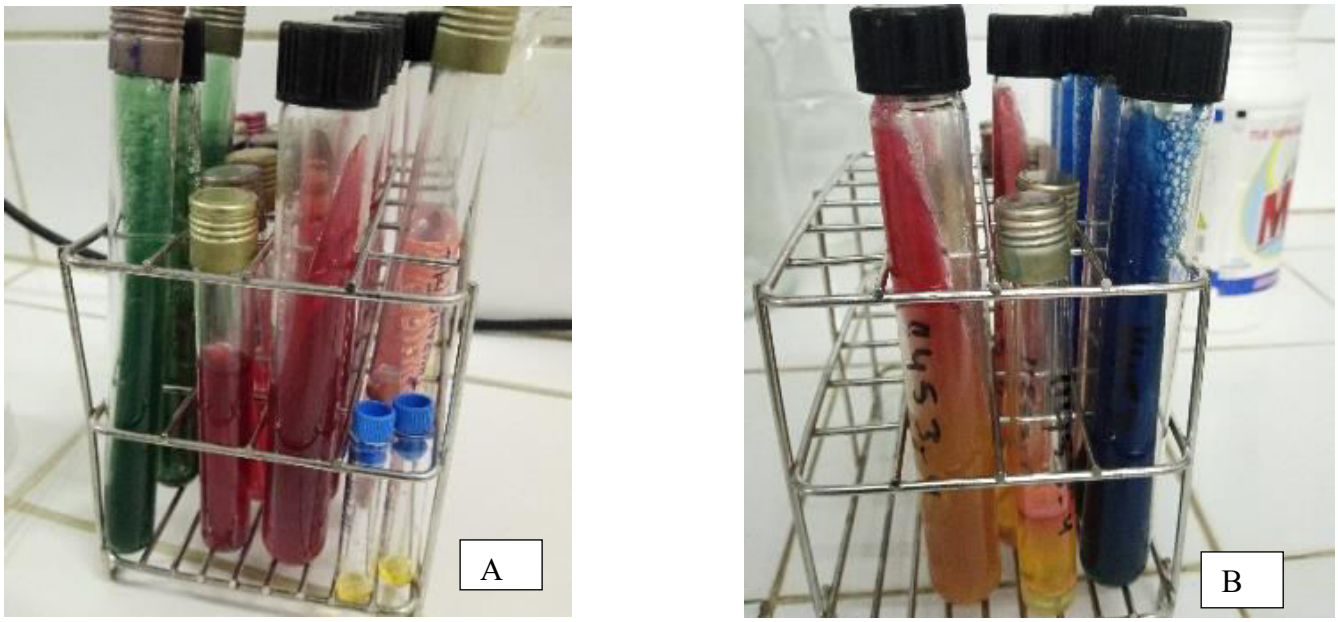
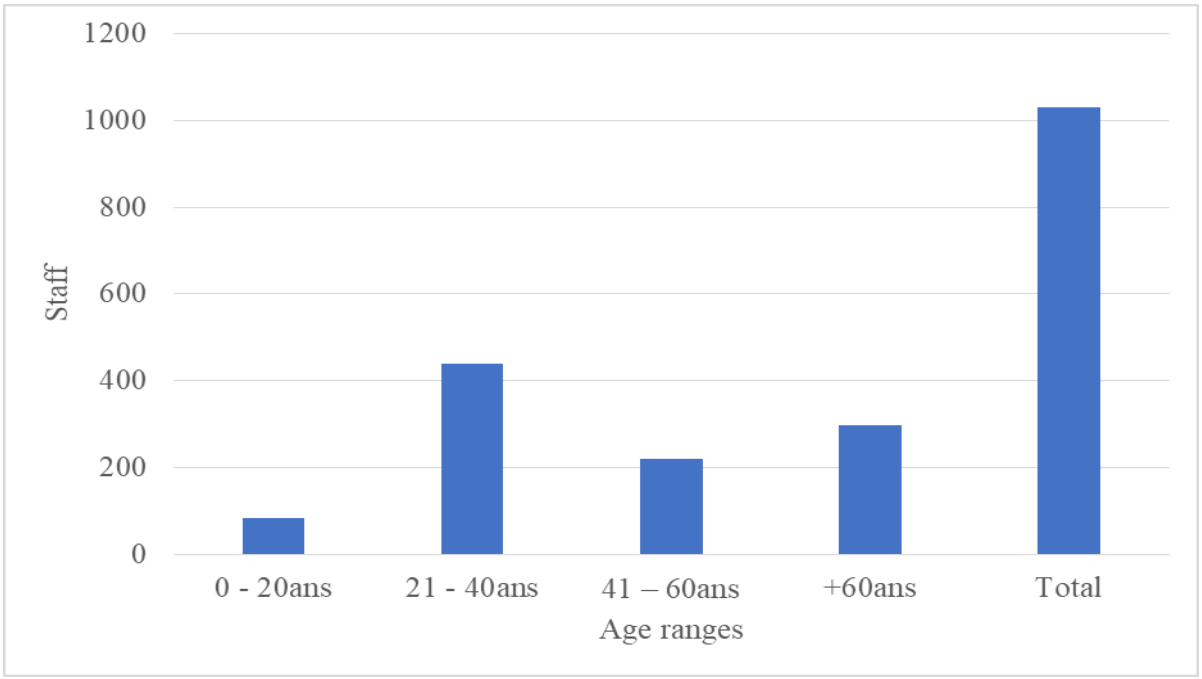


Figure.2 CS, MMN, and KH media before (A) and after (B) incubation (photos from this work)



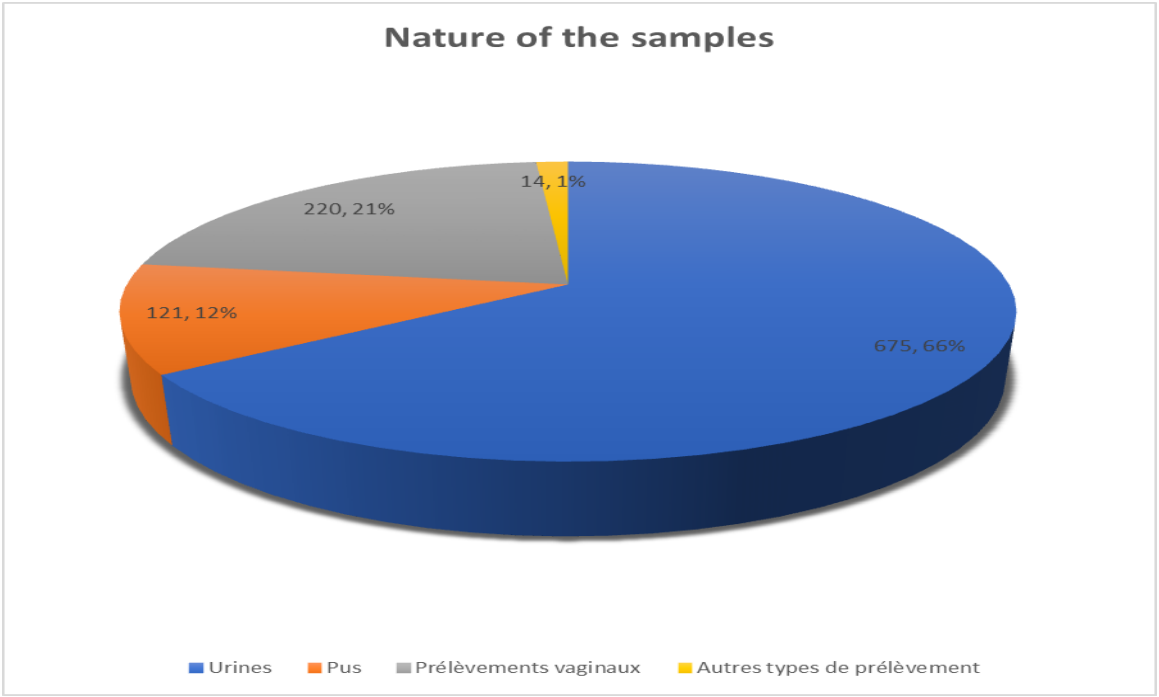
Before incubation (A): from left to right: CS (Simmons citrate), MMN (Mannitol Mobility Nitrate), KH (Hajna Kliger), and IU (indole urea) media
After incubation (B): from left to right: KH, MMN, and CS media

Figure.3 Distribution of the population by age group



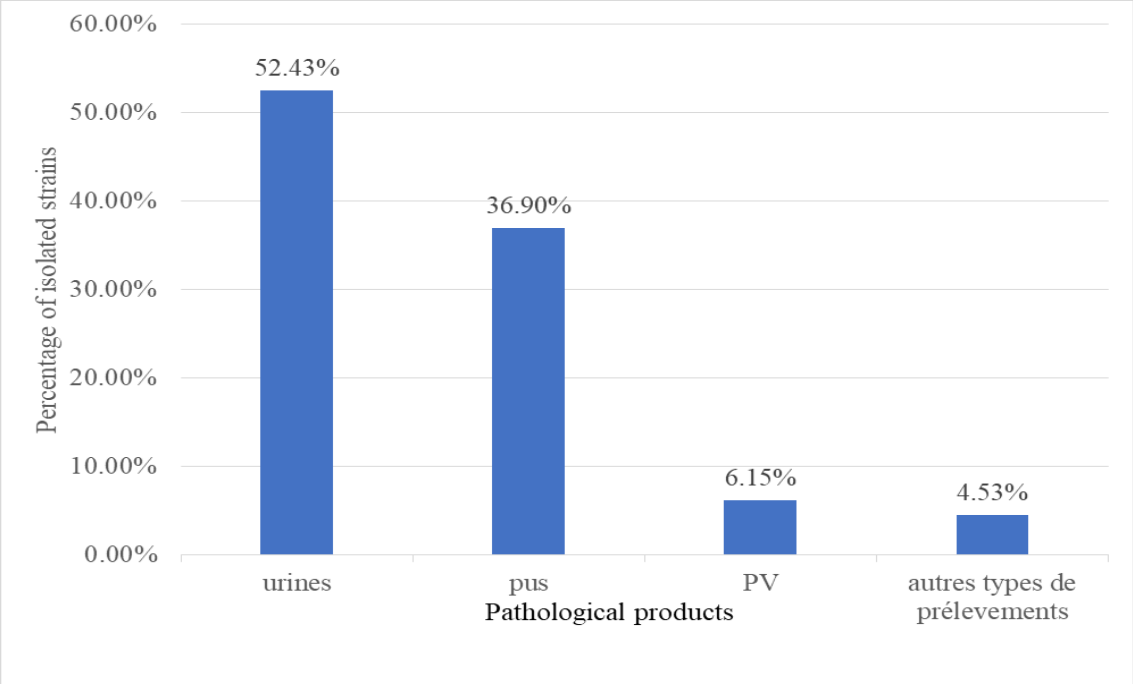
Ans = years

Figure.4 Distribution of the population according to pathological products



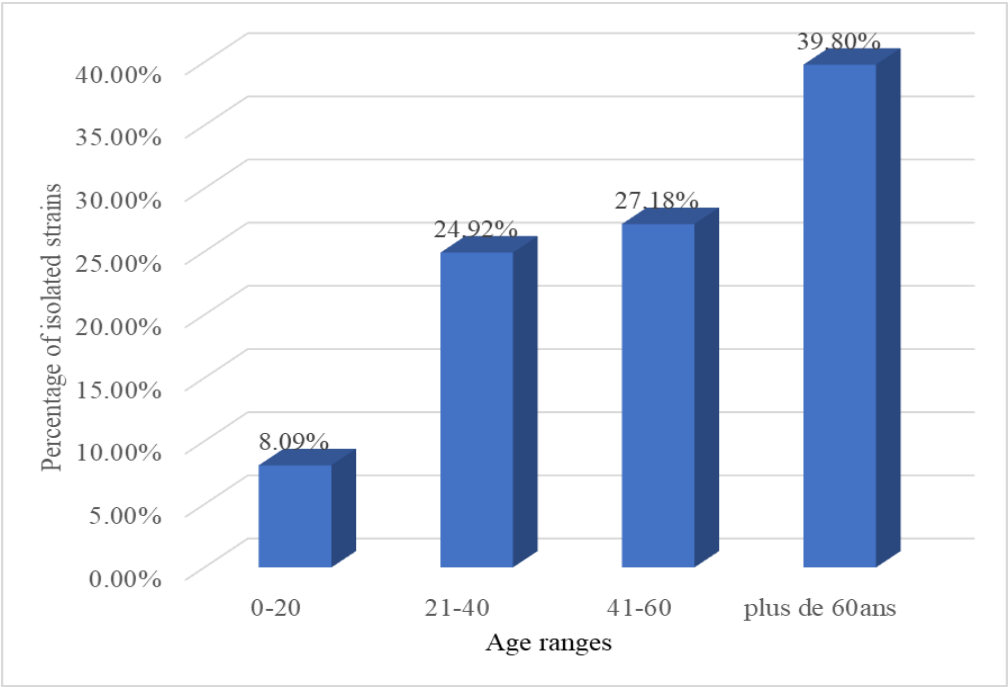
Urines = urine
Pus = Put
Prélevements vaginaux = vaginal swabs
Autres types de prélèvement = Other types of sampling

Figure.5 Distribution of strains according to pathological products



Urines = urine
Pus = put
PV = vaginal swabs
Autres types de prélèvement = Other types of sampling

Figure.6 Distribution of strains according to age group



Plus de 60 ans = Over 60 years old

Figure.7 Distribution of isolated strains according to resistance phenotype

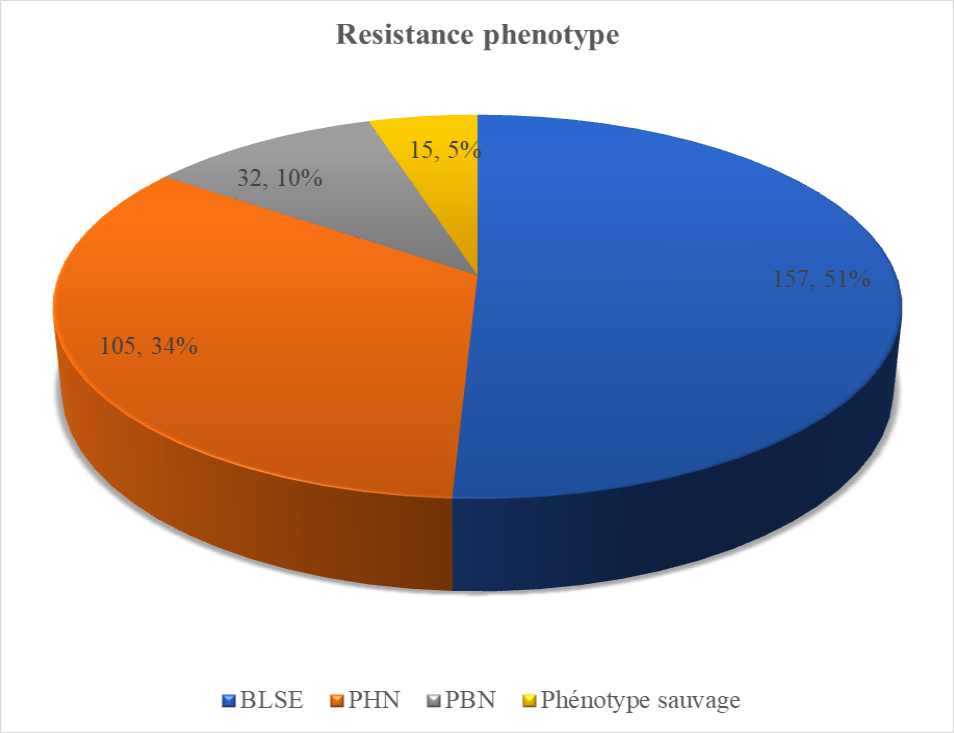
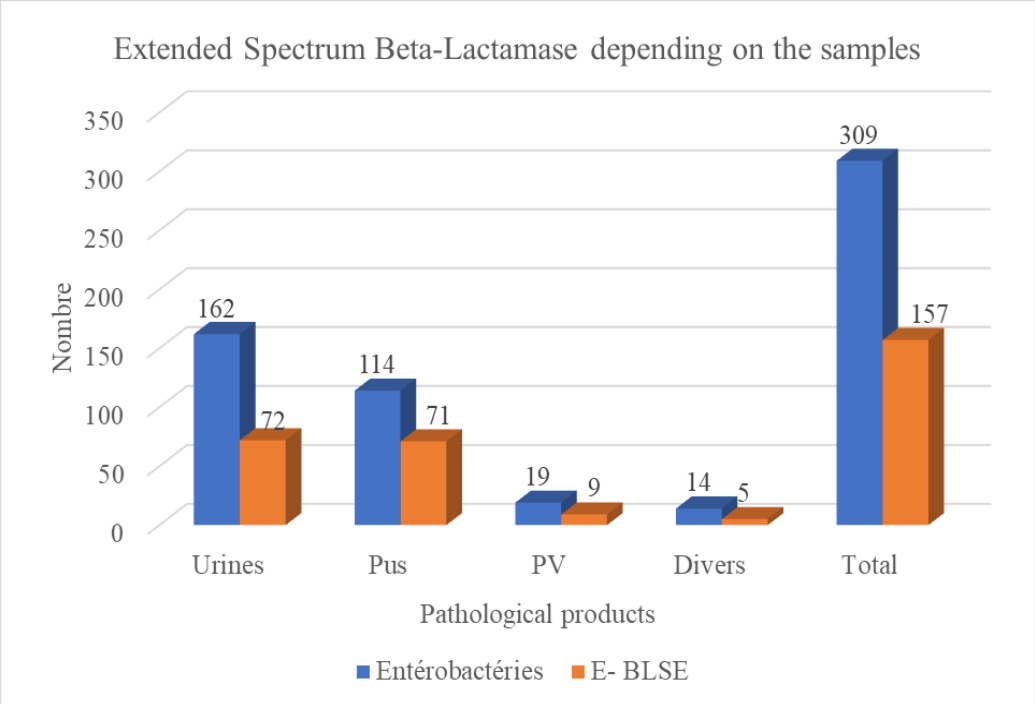
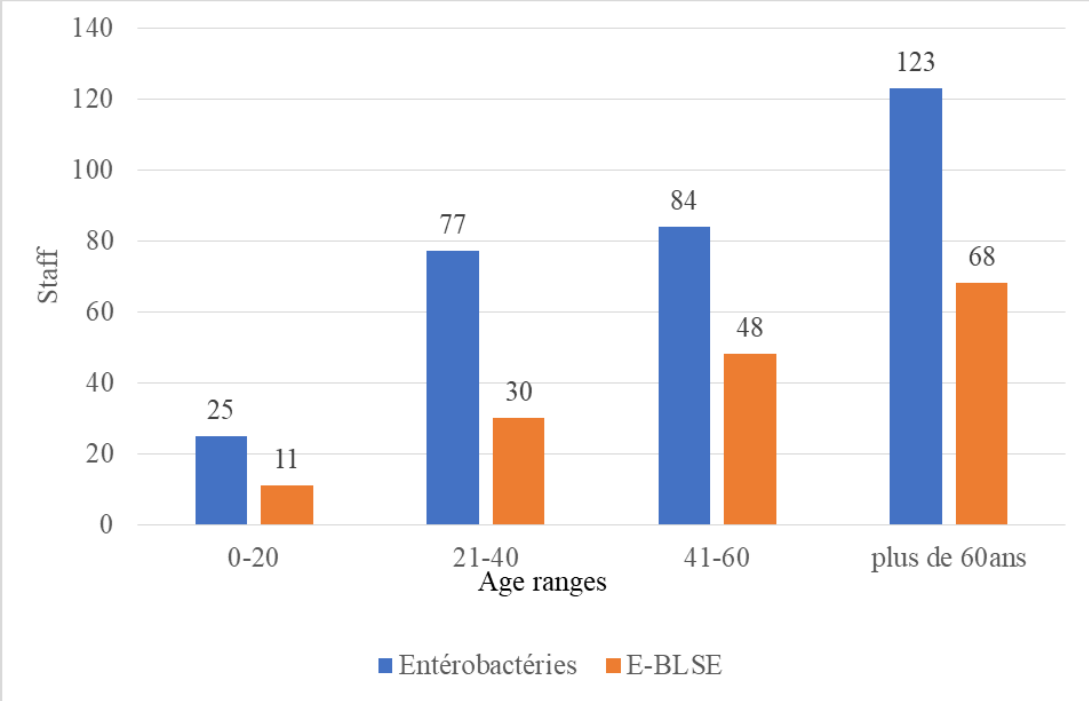


Figure.8 Distribution of ESBLs according to pathological products



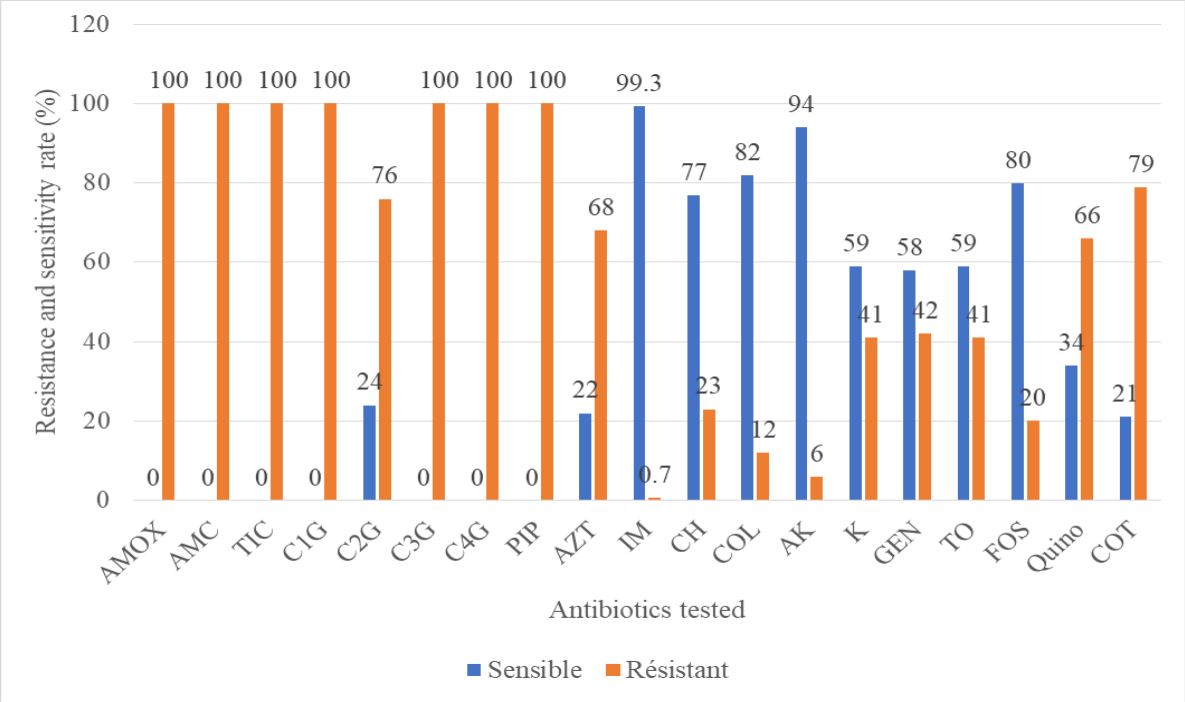
Entérobactéries = Enterobacteriacae
E-BLSE = ESBLs

Figure.9 Distribution of ESBL E by age group



Entérobactéries = Enterobacteriacae
E-BLSE = ESBLs

Figure.10 Antibiotic resistance of ESBL-secreting strains



Sensible = Sensitivity
Resistant = Resistance

Table.1 Distribution of the population according to the reason for consultation

Reasons for consultation	Number of patients	Percentage
Assessment (pregnancy, infertility…)	**349**	**33, 88%**
Signs of a urinary tract infection	279	27,09%
Threatened premature abortion (TPA)	32	3,10%
Pus infections	170	16,50%
Comorbidities	76	7,38%
Other reasons for consultation	60	5,83%
Not specified	64	6,21%
Total	1030	100%

Table.2 Distribution of strains according to patient origin

Patient status	**Isolated strains**							
Patient status	***E. coli***	***K. pneumoniae***	***K. oxytoca***	***Enterobacter Ssp***	***Proteus Ssp***	***Providencia Ssp***	***Citrobacter Spp***	**Total**
Internal	36	20	4	3	6	1	1	71
External	121	54	19	7	27	10	0	238
Total	157	74	23	10	33	11	1	309
Rate (%)	**50,80**	23,95	7,45	3,23	10,68	3,56	0,32	100

Table.3 Distribution of ESBLs according to Enterobacteriaceae species

Species	Number	Percentage (%)
Escherichia coli	75	47,77
Klebsiella pneumoniae	38	24,20
Enterobacter ssp	6	3,82
Klebsiella oxytoca	10	6,37
Proteus spp	20	12,74
Providencia ssp	8	5,10
Citrobacter ssp	0	0
Total	**157**	**100**

Table.4 Association between BLSE and socio-demographic variables

Variables	**Odds Ratio**	**Confidence interval (95%)**		**Z statistics**	**P-value**
		Lower limit	**Upper limit**		
Age	0,7126	0,5948	0,8538	4,3061	0,0002
Sex	1,2538	0,0860	1,7743	1,2767	0,2017
Hospitalized patients	0,3528	0,2302	0,5407	-4,7825	0,0000

Conversely, regarding the association between ESBL status and gender, statistical tests showed that acquiring an infection with ESBL-producing Enterobacteriaceae is not linked to gender but is instead attributable to chance (p = 0.2017). A study conducted in the Congo found statistically significant associations between intestinal acquisition of ESBL-producing Enterobacteriaceae and factors such as age (p = 0.04) and the participants' place of origin (p = 0.03) (Bisangamo *et al.,* 2018), whereas the association between ESBL acquisition and gender

was attributable to chance (p = 0.46). Our results align with those reported by these authors.

Antibiotic use has risen significantly in recent years, thereby substantially altering bacterial ecology. These compounds, which have saved so many human lives, risk becoming ineffective due to a worrying increase in bacterial resistance and a scarcity of new products reaching the market. This resistance phenomenon is particularly relevant to Enterobacteriaceae, a family of great importance in human pathology (Sabor, 2017). This study, conducted between June 2021 and June 2022, aimed to assess the emergence of ESBL-producing Enterobacteriaceae isolated at the laboratory of the Abbas Ndao Hospital Center in Dakar. It was a cross-sectional study involving 309 Enterobacteriaceae isolates from both inpatients and outpatients. The study analyzed various clinical specimens, including urine, vaginal secretions, pus, stool, and blood. Urine was the most frequently received specimen type (52.43%), followed by pus samples (36.90%). The ESBL phenotype was the most common (51%), followed by the non-ESBL phenotype (37.98%). Men accounted for the majority of infections (54%), and individuals over the age of 60 represented the age group most affected by these ESBL-producing Enterobacteriaceae infections. ESBL-producing Enterobacteriaceae infections were far more frequently observed in the hospital setting (80.9%) than in the community setting (19.1%). Our results show that *E. coli* is the most frequently isolated species (51.30%), followed by *Klebsiella pneumoniae* (23.95%) and *Proteus* spp. (10.70%). Regarding the antibiotic resistance of ESBL-producing strains, 100% resistance was observed against all beta-lactams except IMP (0.7%), ATZ (68%), and FOX (76%). Furthermore, ESBL-producing isolates demonstrated high resistance to quinolones (66%). In contrast, fosfomycin, colistin, chloramphenicol, and aminoglycosides such as amikacin showed very good efficacy against ESBL-producing Enterobacteriaceae, with susceptibility rates ranging from 76% to 94%. Statistical analysis revealed that the risk of acquiring an ESBL-producing Enterobacteriaceae infection increases with age or hospitalization ($p < 0.05$), whereas it is not associated with gender ($p = 0.2017 > 0.05$). This study revealed an extremely high prevalence of ESBL-producing bacteria, as well as imipenem resistance—albeit at a low level—that warrants epidemiological surveillance. The emergence of these multidrug-resistant strains is now an established fact in Senegal and worldwide. Given this alarming situation and the associated risks—such as therapeutic dead-ends, increased morbidity and mortality, higher economic costs, and the establishment of highly resistant bacteria within hospital wards—improving hospital hygiene has become imperative to limit the occurrence of hospital-acquired outbreaks.

Therefore, as part of measures aimed at limiting the occurrence of hospital-acquired outbreaks caused by these ESBL-producing Enterobacteriaceae strains, we recommend:

- ✓ Early screening for ESBL-producing bacterial infections.
- ✓ A policy promoting the appropriate use of antibiotics (see Antibiotic Use Guide).
- ✓ Strengthening Infection Prevention and Control (IPC) policies within facilities by supporting the CLINs (Hospital Committees for the Fight against Nosocomial Infections) responsible for these initiatives.
- ✓ Simple, targeted, and precise measures known to all healthcare personnel, such as hand hygiene, disinfection and sterilization of reusable equipment, the use of single-use equipment, etc.
- ✓ Thorough cooking of meat (addressing the issue of antibiotic use in animals).
- ✓ Collective awareness, cooperation across all departments, and comprehensive training for healthcare teams to minimize the risk of cross-transmission.

Our study on the bacteriological profile of ESBL-producing Enterobacteriaceae has yielded preliminary results. Confirmation of beta-lactamase phenotypes using molecular methods is essential, particularly given that the corresponding genes are often carried by mobile genetic elements such as plasmids, integrons, transposons, or gene cassettes.

Consequently, the next steps should include:

- ➢ Conducting a multicenter study involving facilities from various regions to further assess the emergence of these beta-lactamases.
- ➢ Establishing an epidemiological profile through genotyping to identify the classes and types of beta-lactamases present in Enterobacteriaceae isolates

Author Contributions

Arfang DIAMANKA: Investigation, formal analysis, writing—original draft. Amadou NDIAYE: Validation, methodology, writing—reviewing. Daba DIONE:—Formal analysis, writing—review and editing. Insa Diaw FALL: Investigation, writing—reviewing. Ngor FAYE: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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