

One-Day Prevalence of Extended-Spectrum β -Lactamase (ESBL) and Carbapenemase-Producing Bacteria in Fecal Samples from Surgical Patients: A Concerning Trend of Antibiotic Resistance

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Purpose: Extended-spectrum β -lactamase (ESBL) and carbapenemase producing bacteria are of increasing concern due to their multidrug resistance and infection potential. This study determines the one-day prevalence of faecal carriage of ESBL and carbapenemase producing Gram-negative bacilli.

Methods: Fecal samples were collected from 30 post-surgery patients (hospitalized for at least 48 hours) in each of the four hospitals involved in the study and were analyzed for antibiotic-resistant bacteria. Identification was done using Maldi Tof mass spectrometry, and antibiotic susceptibility was tested using disk diffusion and specialized tests for ESBL (double disk synergy technique) and carbapenem (NG-TEST CARBA 5) resistance detection. PCR was conducted on isolates to detect betalactam resistance genes, carbapenemase genes and quinolone resistance genes.

Findings: Out of the 120 patients enrolled, 38.33% (n = 46) and 49.16.33% (n = 59) were found to carry ESBL- and carbapenemase-producing bacteria, respectively, in their fecal samples. Among the isolates, 51.08% (n = 47) exhibited ESBL production, with *Escherichia coli* (44.56%) being the most common species. The identification of bacteria with resistance to carbapenems showed a predominance of the species *Escherichia coli* (44.45%) followed by the species *Klebsiella pneumoniae* (16.06%) and *Acinetobacter baumannii* (13.58%). The study of the association of variables shows a high degree of association ($p < 0.05$) for the factors independent walking and use of a wheelchair with ESBL production. The most frequently detected genes among ESBL producing bacteria were *bla_{CTXM-1}* (91.49%), *qnrB* (70.21%) and *qnrS* (63.82%). *bla_{NDM}* (54.68%) was the most detected carbapenemase genes among carbapenemase producing isolates.

Conclusion: This study demonstrates, for the first time, a significant prevalence of ESBL and carbapenemase producing gram-negative bacteria among surgical patients in Benin, with multiple resistance genes detected. Findings should be interpreted in light of the cross-sectional design and >48-hour hospitalization criterion.

Keywords: ESBL, carbapenemase, fecal carriage, post-surgery patients, one-day prevalence

Introduction

Antimicrobial resistance has become a global concern, posing significant challenges in the treatment of infectious diseases.¹ Beta-lactam antibiotics, including penicillins, cephalosporins, carbapenems, and aztreonam, are commonly prescribed for bacterial infections.² However, the widespread use of third generation cephalosporins to combat Gram-negative bacterial infections has contributed to the development of resistance against beta-lactam antibiotics.³ Consequently, this has led to the emergence of organisms producing Extended-Spectrum Beta-Lactamases (ESBLs) and carbapenemase, further exacerbating the issue.⁴ In 2024, the World Health Organization (WHO) classified

carbapenem-resistant *Pseudomonas aeruginosa*, carbapenem-resistant *Acinetobacter baumannii*, and Carbapenem-resistant *Enterobacterales* (CRE) as “critical” and “high” priority pathogens.⁵

The spread of antibiotic-resistant organisms in health care facilities and in the community is a worrying epidemiological problem that could be influenced by the rate of faecal carriage of antibiotic-resistant organisms.⁶ The *Enterobacterales*, regardless of whether they are community or hospital-acquired, primarily originate from the digestive tract.⁷ This area is a hub for the exchange of resistance genes and antibiotic treatments, which can lead to the overgrowth of resistant bacteria.⁸ When antibiotic resistant isolates colonize the intestinal compartment, the risk of infection due to these bacteria significantly increases.⁹ Additionally, the intestinal compartment may act as a source for ESBL- and carbapenemase-producing resistance genes, which can spread to other *Enterobacterales* through horizontal transmission.¹⁰ Hence, being aware of the frequency of patients carrying resistant bacteria in their digestive tracts can serve as a means of forecasting the extent to which these bacteria may cause infections and spread among people.^{11–13} In the past decade, there has been a global rise in reported cases of hospital-acquired infections caused by Gram-negative bacilli (GNB) that are resistant to carbapenem antibiotics.^{14–17} Hospitalized patients face a double burden as they not only have to deal with their health problems but also with a severe infection of this scale. Hence, this critical situation is a matter of concern for all healthcare systems.^{18,19}

In Africa, ESBL-PE (ESBL Producing *Enterobacterales*) carriage in the gastrointestinal tract ranges from 10% to 50%, highlighting the severity of the situation.²⁰ In Benin, a West African country, government has formally validated its National Action Plan (NAP); however, there are ongoing efforts to implement these measures, and as of now, the NAP has not yet been included on the WHO NAP website.²¹ More resources are required to develop laboratory testing capacity for the accurate detection of antibiotic-resistant bacteria throughout the country.²² While several studies have reported systematic data about AMR prevalence in general or focused on specific sample types, there is a lack of data regarding fecal carriage of antibiotic-resistant bacteria.^{23–28} Recent studies in Benin have reported high rates of multi-drug-resistant pathogens and revealed the first detection of a plasmid-encoded New-Delhi metallo-beta-lactamase-1 (NDM-1) producing *Acinetobacter baumannii* from surgical site infections within hospitals.^{29,30} Prior to this, a study reported the first occurrence of hospital-originated *Pseudomonas aeruginosa* producing carbapenemase VIM-2.³¹ Additionally, there is an increased risk of infection associated with care,^{24,32} and wound contamination by hospital bacteria.^{24,33} Therefore, this study aims to determine the one-day prevalence of faecal carriage of ESBL and carbapenemase producing Gram-negative bacilli, along with associated risk factors and to characterize the resistant isolates.

Methods

Study Design and Sampling

This cross-sectional study, conducted in December 2022, focused on fecal samples from post-surgery patients (accidents and injury-related cases) in tertiary hospitals in Benin. A consecutive sampling approach was employed, where stool/rectal samples were collected from every consenting patient who had been hospitalized for more than 48 hours, until the target sample size of 30 was reached in each hospital. This 48-hour cutoff was used to distinguish hospital-acquired from community-acquired colonization. Four hospitals (Central Hospital and University of Mother and Child Lagoon of Cotonou, Departmental Hospital Center of Zou and the Hills, Departmental Hospital Center of Borgou-Alibori, and Hospital Saint Jean de Dieu of Tanguéta) participated in the study, with patients with digestive pathologies excluded. The sample size was determined based on the bed capacity of the surgery wards in each hospital, ranging from 39 to 47 beds. All samples were collected on the same day, following strict aseptic protocols. A questionnaire was administered to gather sociodemographic information, clinical data, and medical history. The study received prospective ethical approval from the Ethics and Research Committee of the Institute of Applied Biomedical Sciences (CER-ISBA) under approval number 154. Written informed consent was obtained from each participant or their parent/guardian before participation, with a clear explanation of the study's objectives. All research activities were conducted in accordance with the Declaration of Helsinki.

Species Identification, Antibiotic Susceptibility Testing, ESBL and Carbapenemase Detection

Fecal samples were screened for betalactam and carbapenem resistance using CHROMID[®] ESBL and CHROMID[®] CARBA SMART agar media (Bio-Rad, USA). After 24 hours of incubation at 37°C, distinct colonies were selected based on their characteristic color on the selective media. Pure cultures were obtained using again CHROMID[®] ESBL and CHROMID[®] CARBA SMART agar. Gram-negative bacilli, excluding *Stenotrophomonas maltophilia*, were identified using Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Bremen, Germany). Antibiotic susceptibility testing was performed with inoculum from a subculture on Mueller–Hinton agar using the disc diffusion method on Mueller–Hinton agar, following recommendations from the European Committee on Antimicrobial Susceptibility Testing.³⁴ Various antibiotic discs manufactured by Bio-Rad were used for the testing. ESBL production was confirmed using the double disk synergy technique. For *Enterobacterales*, discs (Bio-Rad, United States) containing cephalosporins including cefotaxime 30 µg, ceftazidime 30µg, cefepime 30µg were applied next to clavulanic acid disc following recommendations from EUCAST.³⁵ For *Pseudomonas* spp, imipenem double-disc synergy test (DDS-IPM) with discs containing ceftazidime 30µg, cefepime 30µg and a disc with imipenem 10µg as the ESBL inhibitor was used.³⁶ *Acinetobacter* spp were tested for ESBL production by placing discs containing piperacillin (30 µg) and a combination of piperacilline + tazobactam (30–6µg) on agar plates.³⁷ Resistance to one of carbapenems tested was used as an indicator for the continuation of tests for detecting carbapenemase production. On these strains, the carbapenemase production test was carried out using the rapid diagnostic kit NG-TEST CARBA 5 (Hardy Diagnostics, California, United States) capable of detecting KPC, OXA-48-like, VIM, IMP, and NDM carbapenemases. Reference strain of *Escherichia coli* ATCC 25922 was used as quality control for the antibiotic susceptibility test.

Detection of Antibiotic Resistance Genes

Bacterial DNA was extracted from strains isolated from ESBL agar using boiling method. Multiplex PCR was performed to identify isolates carrying betalactamase encoding genes,³⁸ carbapenemase encoding genes³⁹ and PMQR genes.⁴⁰ Each reaction was prepared in a total volume of 25 µL using OneTaq[®] Quick-Load[®] 2X Master Mix with Standard Buffer (Biolabs, South Africa) and following the manufacturer instructions. The DNA fragments underwent electrophoresis in a 2% agarose gel, and the results were interpreted by comparing the migration of the fragments to marker sizes. The reference strain *Escherichia coli* ATCC 25922 was used as a negative control, while clinical strains producing ESBL and carbapenemase served as positive controls for the PCR assays.

Statistical Analysis

Data entry was performed using Excel 2019, and statistical analysis was conducted using Stata SE 11 software. Univariate analysis was initially conducted, selecting variables with a p-value less than 0.05 as potentially associated with ESBL and carbapenemase producing bacteria carriage. Results were reported as odds ratio (OR) with corresponding 95% confidence intervals, using an interpretation threshold of $\alpha = 0.05$. The chi-square test was also performed to determine the degree of association between factors and independent variables.

Results

Factors Associated with the Carriage of ESBL and Carbapenemase Producing Gram Negative Bacteria

Table 1 described the factors associated with the carriage of ESBL Producing Gram negative bacteria. Out of the 120 patients enrolled in the study, 46 were identified as carriers of ESBL-producing bacteria, resulting in a prevalence of ESBL carriage at 38.33%. The study of the association of variables shows a high degree of association ($p < 0.05$) with ESBL carriage for the factors: independent walking and use of a wheelchair (Table 1).

Table 2 described the factors associated with the carriage of carbapenem-resistant bacteria. Out of the 120 patients enrolled in the study, 59 were identified as carriers of carbapenem-resistant bacteria, resulting in a prevalence of 49.16%. Analysis of the association between variables revealed no statistically significant associations ($p > 0.05$) among the factors studied (Table 2).

Table 1 Factors Associated with the Carriage of ESBL Producing Bacteria

	ESBL (+) (46)	ESBL (-) (74)	Total (120)	Chi ²	Odds Ratio	Z	P> z
Length of hospital stay at the moment of sampling (Days)							
02–10	16	25	41	0.78	1.17	0.37	0.70
11–20	15	18	33				
21–30	9	21	30				
31–40	5	9	14				
>40	1	1	2				
Sex							
Male	27	44	71	1	0.92	−0.36	0.71
Female	19	30	49				
Age							
00–15	5	4	9	0.79	1.14	0.69	0.48
16–30	19	30	49				
31–45	11	23	34				
46–60	7	11	18				
>60	4	6	10				
Presence of immunosuppression (HIV)							
YES	5	11	16	0.72	0.59	−0.80	0.42
Independent walking							
YES	25	29	54	0.15	4.43	2.04	0.04*
Use of a wheelchair							
YES	9	9	18	0.40	4.48	2.09	0.03*
Bedridden patient							
YES	21	44	65	0.19	1.51	0.62	0.53
Diabetic patient							
YES	5	9	14	1	0.66	−0.58	0.55
Presence of sign of infection							
YES	6	10	16	1	0.79	−0.33	0.73
Antibiotic Treatment in the days preceding sampling							
YES	24	42	66	0.76	0.25	−1.66	0.09
Antibiotics Family							
Betalactam	6	18		0.44	0.70	−1.19	0.23
Betalactam and Nitroimidazole	11	10					
Nitroimidazole	4	8					
Quinolone	3	5					

(Continued)

Table 1 (Continued).

	ESBL (+) (46)	ESBL (-) (74)	Total (120)	Chi ²	Odds Ratio	Z	P> z
History of hospitalization in the last 6 months							
YES	9	9	18	0.40	2.26	-1.19	0.23
Patient with faecal incontinence							
YES	4	5	9	0.97	2.13	0.76	0.44
Patients with urinary incontinence							
YES	2	6	8	0.66	0.49	-0.60	0.54

Notes: (*) indicates a statistically significant factor ($p < 0.05$).

Table 2 Factors Associated with the Carriage of Carbapenem-Resistant Bacteria

	Carba R (59)	Carba S (61)	Total (120)	Chi ²	Odds Ratio	Z	P> z
Length of hospital stay at the moment of sampling (Days)							
02–10	22	19	41	0.902	1.01	0.08	0.937
11–20	14	19	33				
21–30	16	15	31				
31–40	6	7	13				
>40	1	1	2				
Sex							
Male	28	43	71	0.01*	0.45	−1.80	0.072
Female	31	18	49				
Age							
00–15	2	7	9	0.270	0.804	−1.11	0.268
16–30	24	25	49				
31–45	18	16	34				
46–60	11	6	17				
>60	4	7	11				
Presence of immunosuppression (HIV)							
YES	10	6	16	0.252	1.531	0.660	0.510
Independent walking							
YES	32	22	54	0.045*	0.84	−0.26	0.797
Use of a wheelchair							
YES	4	14	18	0.013*	0.159	−2.37	0.018

(Continued)

Table 2 (Continued).

	Carba R (59)	Carba S (61)	Total (120)	Chi ²	Odds Ratio	Z	P> z
Bedridden patient							
YES	27	38	65	0.069	0.054	−0.92	0.360
Diabetic patient							
YES	6	8	14	0.614	0.663	−0.59	0.557
Presence of sign of infection							
YES	10	6	16	0.252	2.753	1.50	0.134
Antibiotic Treatment in the days preceding sampling							
YES	32	34	66	0.869	1.388	0.73	0.468
History of hospitalization in the last 6 months							
YES	10	8	18	0.556	0.845	−0.26	0.796
Antibiotic treatment last 6 months							
YES	3	2	5	0.621	1.97	0.59	0.555
Patient with faecal incontinence							
YES	3	6	9	0.323	0.486	−0.72	0.472
Patients with urinary incontinence							
YES	3	5	8	0.494	0.580	−0.52	0.603

Notes: (*) indicates a statistically significant factor ($p < 0.05$).

Species Identification and Percentage of Antibiotic Resistance

Table 3 shows the results of the identification of the isolated bacterial species. A total of 92 strains were isolated using ESBL agar. Among the bacterial species isolated from ESBL agar, *Escherichia coli* (44.56%) was the most frequently isolated, followed by *Pseudomonas aeruginosa* (26.08%) and *Acinetobacter baumannii* (10.87%). Notably, all *Enterobacter cloacae* strains (100%), most *Escherichia coli* strains (82.93%), and *Klebsiella pneumoniae* strains (80%) were found to be ESBL producers. A total of 83 strains were isolated using carba agar. The identification of isolates from carba agar showed a predominance of the species *Escherichia coli* (44.45%) followed by the species *Klebsiella pneumoniae* (16.06%) and *Acinetobacter baumannii* (13.58%). A total of 64 (77.10%) isolates were found to be resistant at least to one of the tested carbapenem antibiotics. *Klebsiella pneumoniae* (92.30%) and *Escherichia coli* (75%) were the most represented species.

The resistance percentage of the isolates from ESBL agar is presented in Table 4. Notably, high rates of resistance were observed for ofloxacin (ranging from 97.11% to 100%), amoxicillin + clavulanic acid (ranging from 79.41% to 100%), and cefotaxime (ranging from 50% to 100%) among the tested antibiotics. Most *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae* strains demonstrated multidrug resistance. On the other hand, *Pseudomonas aeruginosa* strains exhibited susceptibility to most of the tested antibiotics, except for ceftulodin. Furthermore, the lowest levels of resistance were observed with antibiotics such as imipenem, ertapenem, and temocillin (Table 4).

The study of the resistance profile of *Enterobacteriales* isolates from carba agar was presented in Table 5. The results showed that all isolates (100%) were resistant to amoxicillin and piperacillin. Similarly, a higher rate of resistance was observed to carbapenem, ranging from 87.50% to 100%. Various resistance rates were observed for other antibiotics.

The study of the resistance profile of *Pseudomonas spp* was presented in Table 6. The results showed that isolate of *Pseudomonas putida* was resistant to all tested antibiotics excepted amikacin. Similarly, 80% (4/5) of *Pseudomonas aeruginosa* isolates exhibited resistance toward all tested antibiotics but some isolates (3/5) were susceptible to amikacin and aztreonam.

Table 3 Distribution of Isolates According to the Culture Media

Organisms	Isolates from ESBL Agar	ESBL Producers	Isolates from CARBA Agar	Carbapenem Resistant Isolates
<i>Acinetobacter baumannii</i>	10 (10.87%)	1 (10%)	12 (13.58%)	8 (66.66%)
<i>Acinetobacter nosocomialis</i>	1 (1.03%)	0 (0%)	1 (1.23%)	1 (100%)
<i>Citrobacter freundii</i>	0%	0%	4 (4.94%)	2 (50%)
<i>Citrobacter amalonaticus</i>	1 (1.03%)	1 (100%)	1 (1.23%)	0%
<i>Enterobacter cloacae</i>	5 (5.43%)	5 (100%)	9 (9.88%)	9 (100%)
<i>Escherichia coli</i>	41 (44.56%)	34 (82.93%)	36 (44.45%)	27 (75%)
<i>Klebsiella pneumoniae</i>	5 (5.43%)	4 (80%)	13 (16.06%)	12 (92.30%)
<i>Klebsiella oxytoca</i>	0%	0%	1 (1.23%)	1 (100%)
<i>Pseudomonas putida</i>	2 (2.17%)	0 (0%)	1 (1.23%)	0%
<i>Pseudomonas aeruginosa</i>	24 (26.08%)	0 (0%)	5 (6.17%)	4 (57.14%)
<i>Morganella morganii</i>	2 (2.17%)	2 (100%)	0%	0%
<i>Providencia stuartii</i>	1 (1.03%)	0 (0%)	0%	0%
Total	92 (100%)	47 (51.08%)	83 (100%)	64 (77.10%)

The study of the antibiotic resistance profile of *Acinetobacter spp* was presented in Table 7. The results showed that isolate of *Acinetobacter nosocomialis* was resistant to all tested antibiotics. Higher resistance rate (>80%) was also observed for *Acinetobacter baumannii* but 41.66% were susceptible to Amikacin.

Distribution of Carbapenemase Types

A total of 64 isolates resistant at least to one of the tested carbapenem antibiotics were selected for NG-TEST CARBA 5 (KPC, OXA-48-like, VIM, IMP, and NDM). All isolates selected were found to be carbapenemase producers. NDM (43.08%) was the most detected type of carbapenemase followed by strains for which carbapenemase type was not detected (30.77%) (Table 8).

Antibiotic Resistance Genes Detection

Tables 9 and 10 present the distribution of resistance genes in carbapenemase and ESBL producing isolates. *bla_{NDM}* (54.68%) was the most detected carbapenemase genes. Among the ESBL-producing isolates, the presence of five ESBL genes was identified and presented in Table 10. The most detected genes were *bla_{CTXM-1}* and *bla_{CTXM-15}*, accounting for 91.49% of the isolates. Additionally, the *bla_{TEM}* (36.17%) and *bla_{OXA-1}* (29.78%) were found in the isolates. Quinolone resistance genes were prevalent among the isolates, with *qnrB* present in 70.21% of the isolates, followed by *qnrS* in 63.82% of the isolates, and *aac(6')-ib-cr* in 53.19% of the isolates. Among the different species, *Escherichia coli* exhibited a high prevalence (97.05%) of the *bla_{CTXM-1}* gene, while *Enterobacter cloacae* had a prevalence of 80% for the same gene. Electrophoresis gel images are shown in supplementary data (Figures S1–S6).

Table 4 Percentage of Antibiotic Resistance of Isolates Recovered from ESBL Screening Agar

Organisms	AMP 10µg	AMC 20–10µg	TEM 30µg	PRL 30µg	TZP 30–6µg	CF 30µg	FOX 30µg	CTX 5µg	CAZ 10µg	FEP 30µg	ATM 30µg	IMP 10µg	ERT 10µg	TOB 10µg	AK 30µg	OFX 5µg	LEV 5µg
<i>A. baumannii</i> (n=10)	–	–	–	33.33%	0%	–	–	–	0%	33.33%	–	33.33%	–	33.33%	0%	–	33.33%
<i>C. amalonaticus</i> (n=1)	100%	100%	0%	100%	100%	–	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	0%
<i>E. cloacae</i> (n=5)	100%	100%	20%	100%	80%	–	100%	100%	100%	80%	80%	20%	0%	80%	40%	100%	100%
<i>E. coli</i> (n=41)	100%	79.41%	23.52%	100%	26.47%	–	26.47%	85.29%	100%	97.05%	97.05%	14.70%	14.70%	47.05%	11.76%	97.11%	62%
<i>K. pneumoniae</i> (n=5)	100%	100%	25%	100%	50%	–	75%	75%	75%	75%	75%	25%	25%	50%	25%	100%	75%
<i>Pseudomonas</i> spp (n=26)	–	–	–	0%	0%	100%	–	–	0%	0%	0%	0%	–	0%	0%	–	0%
<i>Morganella morganii</i> (n=2)	100%	100%	50%	100%	50%	–	50%	50%	100%	100%	50%	50%	0%	50%	50%	100%	100%

Abbreviations: Amp, Ampicillin; AMC, Amoxicillin-Clavulanic acid; TEM, Temocillin; PRL, Piperacillin; TZP, Piperacillin+Tazobactam; CF, Cefsulodine; FOX, Cefoxitin; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; ATM, Aztreonam; IMP, Imipenem; ERT, Ertapenem; TOB, Tobramycin; AK, Amikacin; OFX, Ofloxacin; LEV, Levofloxacin.

Table 5 Percentage of Antibiotic Resistance of Enterobacterales Isolates Recovered from Carba Screening Agar

Organisms	AM 10µg	AMC 20–10µg	PRL 30µg	TPZ 30–6µg	TEM 30µg	CTX 5µg	FOX 30µg	CAZ 10µg	FEP 30µg	CPD 10µg	ATM 30µg	IMP 10µg	ERT 10µg	CN 30µg	TOB 10µg	AK 30µg	LEV 5µg
<i>Citrobacter amalonaticus</i> (1)	100%	100%	100%	100%	0%	100%	100%	100%	100%	100%	100%	100%	100%	0%	100%	100%	0%
<i>Citrobacter freundii</i> (4)	100%	100%	100%	100%	75%	100%	100%	100%	100%	100%	100%	100%	100%	50%	75%	50%	75%
<i>Enterobacter cloacae</i> (8)	100%	87.50%	100%	87.50%	62.50%	87.50%	100%	87.50%	87.50%	87.50%	75%	100%	87.50%	87.50%	87.50%	37.50%	75%
<i>Escherichia coli</i> (36)	100%	100%	100%	100%	97.22%	97.22%	94.44%	97.22%	94.44%	97.22%	88.88%	94.44%	97.22%	72.22%	72.22%	36.11%	91.66%
<i>Klebsiella oxytoca</i> (1)	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	0%	100%	100%	100%	100%	100%	0%
<i>Klebsiella pneumoniae</i> (13)	100%	100%	100%	100%	100%	100%	92.31%	100%	100%	100%	92.31%	100%	100%	46.15%	92%	61.54%	92%

Abbreviations: AM, Amoxicillin; AMC, Amoxicillin-Clavulanate; PIP, Piperacillin; TPZ, Piperacillin-Tazobactam; TEM, Temocillin; CTX, Cefotaxime; FOX, Cefoxitin; CAZ, Ceftazidime; FEP, Cefepime; CPD, Cefpodoxime; ATM, Aztreonam; IMP, Imipenem; ERT, Ertapenem; CN, Gentamicin; TOB, Tobramycin; AK, Amikacin; LEV, Levofloxacin.

Table 6 Percentage of Antibiotic Resistance of *Pseudomonas* Spp Isolates Recovered from Carba Screening Agar

Organisms	PRL 30µg	TPZ 30–6µg	TIC 75µg	TIM 75–10µg	CAZ 10µg	FEP 30µg	ATM 30µg	TOB 10µg	AK 30µg	IMP 10µg	MEM 10µg	LEV 5µg	CIP 5µg
<i>Pseudomonas putida</i> (1)	100%	100%	100%	100%	100%	100%	100%	100%	0%	100%	100%	100%	100%
<i>Pseudomonas aeruginosa</i> (5)	100%	100%	100%	100%	100%	100%	40%	80%	40%	80%	80%	80%	80%

Abbreviations: PRL, Piperacillin; TPZ, Piperacillin-Tazobactam; TIC, Ticarcillin; TIM, Ticarcillin-Clavulanate; CAZ, Ceftazidime; FEP, Cefepime; ATM, Aztreonam; TOB, Tobramycin; AK, Amikacin; IMP, Imipenem; MEM, Meropenem; LEV, Levofloxacin; CIP, Ciprofloxacin.

Table 7 Percentage of Antibiotic Resistance of *Acinetobacter* Spp Isolates Recovered from Carba Screening Agar

Organisms	PRL 30µg	TPZ 30–6µg	TIC 75µg	TIM 75–10µg	CAZ 10µg	FEP 30µg	IMP 10µg	CIP 5µg	LEV 5µg	CN 30µg	TOB 10µg	AK 30µg	SXT 1.25– 23.75µg
<i>Acinetobacter baumannii</i> (12)	100%	100%	100%	100%	50%	91.66%	91.66%	91.66%	91.66%	91.66%	83.33%	41.66%	91.66%
<i>Acinetobacter nosocomialis</i> (1)	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

Abbreviations: PRL, Piperacillin; TPZ, Piperacillin-Tazobactam; TIC, Ticarcillin; TIM, Ticarcillin-Clavulanate; CAZ, Ceftazidime; FEP, Cefepime; IMP, Imipenem; CIP, Ciprofloxacin; LEV, Levofloxacin; CN, Gentamicin; TOB, Tobramycin; AK, Amikacin; SXT, Trimethoprim-Sulfamethoxazole.

Table 8 Distribution of Carbapenemase Types Among Species Isolated by Phenotypic Test

Organisms	NDM	OXA48-Like	Negative for Carbapenemase (KPC, OXA-48-Like, VIM, IMP, and NDM)
<i>Citrobacter freundii</i> (2)	50%	50%	0%
<i>Enterobacter cloacae</i> (9)	66.66%	11.11%	22.22%
<i>Escherichia coli</i> (27)	25.92%	59.09%	25.92%
<i>Klebsiella oxytoca</i> (1)	100%	0%	0%
<i>Klebsiella pneumoniae</i> (12)	75%	16.66%	16.66%
<i>Pseudomonas aeruginosa</i> (4)	50%	0%	50%
<i>Acinetobacter baumannii</i> (8)	25%	0%	75%
<i>Acinetobacter nosocomialis</i> (1)	0%	0%	100%
Total (64)	43.08%	26.15%	30.77%

Table 9 Distribution of Detected Carbapenemase Gene Among Species Isolated

Organisms	bla _{NDM} (%)	bla _{OXA-48} (%)
<i>Citrobacter freundii</i> (2)	50	50
<i>Enterobacter cloacae</i> (9)	77.77	11.11
<i>Escherichia coli</i> (27)	33.33	11.11
<i>Klebsiella oxytoca</i> (1)	100	0
<i>Klebsiella pneumoniae</i> (12)	91.66	8.33
<i>Pseudomonas aeruginosa</i> (4)	75	0
<i>Acinetobacter baumannii</i> (8)	25	0
<i>Acinetobacter nosocomialis</i> (1)	100	0
Total (64)	54.68	9.37

Table 10 Distribution of Resistance Genes in ESBL Producing Isolates

Organisms	bla _{TEM} (%)	bla _{SHV} (%)	bla _{OXA-1} (%)	bla _{CTXM-1} (%)	bla _{CTXM-15} (%)	qepA (%)	qnrB (%)	qnrS (%)	aac (%)
<i>Acinetobacter baumannii</i> (1)	0	0	100	100	100	0	100	0	100
<i>Citrobacter amalonaticus</i> (1)	0	0	100	100	100	0	100	0	100
<i>Enterobacter cloacae</i> (5)	40	0	0	80	80	20	80	20	60
<i>Escherichia coli</i> (34)	38.23	2.94	35.29	97.05	97.05	32.35	67.64	82.35	52.94
<i>Klebsiella pneumoniae</i> (4)	50	50	0	50	50	50	75	25	25
<i>Morganella morganii</i> (2)	0	0	0	100	100	0	50	0	50
Total (47)	36.17	6.38	29.78	91.49	91.49	29.78	70.21	63.82	53.19

Discussion

The emergence and global spread of extended-spectrum β -lactamase (ESBL) and carbapenemase producing *Enterobacterales* pose a significant threat to public health.²⁰ The objective of this study was to determine the one-day prevalence of faecal carriage of ESBL and carbapenemase producing Gram-negative bacilli, along with associated risk factors, and to characterize the resistant isolates.

In this study, a culture-dependent approach was used to detect ESBL-producing- and carbapenem-resistant Gram-negative bacteria. The results of this study revealed a significant prevalence of carriage of ESBL-producing bacteria (38.33%), indicating a potential risk for nosocomial infections and the dissemination of antimicrobial resistance genes in the community. Lower rates of ESBL-PE carriage prevalence were reported in other African countries such as Gabon (11.8% to 16.7%), Cameroon (15% to 18%), Central African Republic (19.3%), and Nigeria (20.9%).^{41–44} In Madagascar, the prevalence of ESBL-PE among individuals was found to be 10.1%.⁴⁵ On the other hand, a higher prevalence of ESBL-PE was reported in Burkina Faso.⁴⁶ Disparities in prevalence rates among countries and the increasing prevalence of ESBL-producing bacteria in Africa have been highlighted.⁴⁷ However, our findings do not align with their estimation that the overall rate of ESBL-PE in clinical samples was below 15%. Concerning carbapenem resistance detection, out of the 120 samples that were collected, a total of 81 strains were isolated. As 59 were identified as carriers of carbapenem-resistant bacteria, the prevalence of carbapenem resistance carriage is therefore 49.16%. A study conducted in Egypt revealed that 62.7% of *Enterobacterales* isolates were resistant to carbapenems, which is consistent with our findings.⁴⁸ Similarly, high rates of carbapenem resistance were observed in South Africa (68%),⁴⁹ and Sudan (83%).⁵⁰ Respectively, 28.6% and 35% rates of resistance were observed in Uganda and Tanzania.^{51,52} The prevalence of ESBL-PE values among individuals in different African countries varies significantly not only between countries, as underlined by the authors, but also between cities, sites (such as rural versus urban areas, and hospitals versus communities), and over different years. This variability should be highlighted even if the provided data remain indicative. The unrestricted use of antibiotics, which is prevalent among most of the population in low- and middle-income countries (LMICs) in Africa especially in Benin, is likely to lead to an increase in carbapenem resistance in the region.⁵³ In this study, the samples were collected within one day (24 hours), and it is not possible to estimate whether these results show an intermittent or regular situation in the study area. Sampling at different times of the year could give a more reliable profile of the situation.

In this study, no significant association was found between these risk factors and carriage of ESBL-producing bacteria. Similar investigations focusing on carbapenemase-producing isolates did not yield comparable results.^{54,55} The study of the association of variables shows a high degree of association ($p < 0.05$) for the factors sex, independent walking, and use of a wheelchair. Patients who use wheelchairs or have limited mobility spend generally more time in healthcare facilities, potentially increasing their exposure to resistant isolates. Also, young patient age has been regarded as a risk factor for Carbapenem-resistant *Enterobacterales* (CRE) infection.⁵⁰ Length of hospital stay, sex, age, presence of immunosuppression, independent walking, bedridden patient, diabetic patient, presence of sign of infection, antibiotic treatment, history of hospitalization in the last 6 months, antibiotic treatment last 6 months, patient with faecal/urinary incontinence were the other risk factors examined in this survey. Neither of these risk factors was significantly associated with carbapenemase producing strains carriage. Numerous studies have shown interest in assessing the risk factors associated to carbapenemase producing isolates carriage but none of them have found similar results.^{54,55}

In total, 51.08% of the isolated strains were found to be ESBL producers. The identification of ESBL producing bacteria species revealed a predominance of *Escherichia coli* (82.93%), followed by *Enterobacter cloacae* (100%; $n = 5$) and *Klebsiella pneumoniae* (80%; $n = 4$). Notably, *Enterobacter cloacae*, *Escherichia coli*, and *Klebsiella pneumoniae* strains exhibited high proportions (100%, 82.93%, and 80% respectively) of ESBL production. Similar species predominance was observed in recent studies.^{44,56} Other studies conducted in Cameroon and China have reported a higher diversity of ESBL-PE species, including *Enterobacter spp.* and *Citrobacter spp.*^{57,58} Nevertheless, *E. coli* consistently emerges as the most identified species during colonization by ESBL-PE. It is worth noting that these bacteria, which pass through the human intestine, have the potential to acquire resistance through horizontal gene transfer.⁴⁶ The identification of bacteria with resistance to carbapenems showed a predominance of the species *Escherichia coli* (44.45%) followed by the species *Klebsiella pneumoniae* (16.06%) and *Acinetobacter baumannii* (13.58%). Same isolates were reported in

various studies.^{48–52} Patients with carbapenem-resistant *Enterobacterales* often face treatment failure, prolonged hospital stay, high expenses and a high possibility of death.⁵⁹

The antibiotic resistance phenotype results of isolates from ESBL agar showed high rate of resistance for ofloxacin (ranging from 97.11% to 100%), amoxicillin + clavulanic acid (ranging from 79.41% to 100%), and cefotaxime (ranging from 50% to 100%) among the tested antibiotics. Multidrug resistance was common among *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella pneumoniae* strains. Similar observations were made,⁴⁴ indicating significant resistance not only to β -lactam antibiotics due to ESBL production but also to other antibiotic families, including quinolones. In this study, the carbapenems (imipenem and ertapenem) showed the highest effectiveness against ESBL-producing Gram-negative bacteria (GNBs). These carbapenems are considered expensive last-resort drugs in the region but have demonstrated favorable activity against GNBs that produce this enzyme.^{60–62} This finding is consistent with a report which indicates the widespread distribution of ESBL producers among *Enterobacterales*.⁵⁰ The study of antibiotic susceptibility patterns of carbapenem-resistant isolates revealed that the majority of isolates were resistant to all tested antibiotics, including carbapenems. Distribution of carba types according to species isolated and ESBL production showed that 52.31% of isolates were ESBL producers, but none non-*Enterobacterales* was found to be ESBL producing. All isolates selected were found to be carbapenemase producers. NDM (43.08%) was the most detected type of carbapenemase followed by other types (30.77%) using the aforementioned phenotypic method. The NDM enzyme was frequently encountered among *Enterobacterales* as one of the most common carbapenemases, and it was also observed in *A. baumannii* and *P. aeruginosa* isolates.^{63,64} Most of non-*Enterobacterales* were found to produce other types of carbapenemase. This is consistent with a study reporting that carbapenemase producers are becoming highly distributed among *Enterobacterales*.⁵⁰ Non-*Enterobacterales* including *Pseudomonas* spp employ mainly porin expression reduction and increased chromosomal cephalosporinase activity against carbapenems.⁶⁵

The study extensively examined the presence of ESBL genes among the isolates. The most frequently detected ESBL genes were *bla*_{CTXM-1} and *bla*_{CTXM-15} (91.49%), followed by *bla*_{TEM} (36.17%). These genes are associated with resistance to a broad range of β -lactam antibiotics. Similar findings have been reported in other studies conducted in Cameroon (96% of isolates), Indonesia (94.5%), and Tunisia (91%), indicating the global dominance of CTX-M-type ESBLs.^{57,66,67} The study also investigated the presence of other resistance genes associated with quinolone and carbapenem resistance. The isolates exhibited the presence of quinolone resistance genes, such as *qnrB* (70.21%), *qnrS* (63.82%), and *aac(6')-ib-cr* (53.19%), indicating the potential for reduced efficacy of fluoroquinolone antibiotics in treating infections caused by ESBL-producing bacteria. This result supports the concept co-expression due to the isolates harboring several antibiotic resistance genes. Furthermore, the presence of *qnr* genes and *aac(6')-ib-cr* alongside ESBL-encoding genes further strengthens the possibility of co-selection.⁶⁸ These findings confirm the multidrug-resistant nature of ESBL-PE, which severely limits available therapeutic options and contributes to the widespread use of carbapenems. The detection of carbapenemase-encoding genes in a study is vital for understanding and addressing the growing problem of antibiotic resistance. It provides critical information for infection control, treatment decisions, and the development of strategies to preserve the effectiveness of antibiotics in the face of rising resistance. In this study, *bla*_{NDM} (54.68%) was the most detected carbapenemase genes mainly in *Klebsiella pneumoniae* (91.66%), *Enterobacter cloacae* (77.77%) and *Pseudomonas aeruginosa* (75%). Our results are consistent with others studies in Sudan, Senegal, and South Africa.^{49,50,59} This confirms that *bla*_{NDM} could demonstrate a capacity for rapid dissemination.⁶⁹ The plasmids that carry carbapenemase genes, such as *bla*_{NDM}, exhibit significant diversity and can harbor numerous additional resistance genes, including ESBL-alleles. The presence of multiple resistance genes in certain isolates, as observed in this study, indicates the presence of multidrug-resistant pathogens. These pathogens are responsible for treatment failures, outbreaks of infections, and increased treatment costs.

The primary aim was to determine the prevalence of ESBL and carbapenemase-producing bacteria at a single time point in hospitalized post-surgical patients, providing baseline data for future longitudinal studies on acquisition dynamics. The one-day design and >48-hour hospitalization criterion limit differentiation between community- and hospital-acquired colonization. Without admission screening or follow-up, acquisition timing and transmission pathways cannot be confirmed. Also, Limited clinical and hospital data restrict analysis of risk factors and transmission routes. Therefore, conclusions about nosocomial spread should be interpreted cautiously. The absence of whole genome

sequencing prevents detailed epidemiological and transmission analysis. Future studies with longitudinal sampling, comprehensive data, and genomic tools are needed to clarify acquisition and guide interventions.

Conclusion

This study reports a high one-day prevalence of fecal carriage of extended-spectrum β -lactamase (ESBL) and carbapenemase-producing Gram-negative bacteria among post-surgical patients hospitalized for over 48 hours in Benin. The predominance of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, combined with high resistance to β -lactams and fluoroquinolones, underscores the clinical and public health significance of these pathogens. Genotypic analysis revealed frequent detection of *bla*_{CTX-M} and *bla*_{NDM}, along with co-resistance genes, indicating the circulation of multidrug-resistant organisms in the hospital setting. While this cross-sectional study cannot confirm nosocomial acquisition, it provides important baseline data to inform future surveillance and infection control efforts in Beninese hospitals.

Abbreviations

CTX-M: Cefotaxime-Munich; ESBL: Extended-Spectrum β -lactamase; ESBL-PE: ESBL Producing *Enterobacterales*; GNB: Gram Negative Bacilli; MBL: Metallo-beta-lactamase; NDM: New Delhi Metallo-beta-lactamase.

Data Sharing Statement

All data generated and/or analyzed during the current study are included in this published article. The datasets used and/or analyzed during this study are also available from the corresponding author on reasonable request.

Ethical Approval

The study proposal was reviewed and approved by the Ethics and Research Committee of the Institute of Applied Biomedical Sciences (CER-ISBA) under number 154. Written informed consent was obtained from each patient or their parent/guardian before participation, accompanied by a concise explanation of the study's objective. The research work (sampling from hospitalized patients, sample processing and data analysis) in our study was conducted in accordance with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors declare no conflict of interest.

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