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Original Article

Emerging trends and resistance patterns of extended-spectrum beta-lactamases producing *Enterobacteriaceae*: an epidemiological insight from Ibn Tofail hospital in Marrakesh, Morocco

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Abstract

Introduction: The epidemiology of ESBL infection varies widely from one region to another and rapidly evolves across hospital and community boundaries. Therefore, obtaining an updated local picture of the epidemiology of ESBL-producing microorganisms and analyzing trends in their dissemination is essential. The main aim of this study was to assess the prevalence and the antibiotic resistance profiles of ESBL-producing *Enterobacteriaceae* (ESBL-E) at Ibn Tofail Hospital in Marrakesh.

Methodology: To our knowledge, the present study is the first to conduct a descriptive analysis from January 1, 2010, to December 31, 2022, including all ESBL-E strains isolated in the microbiology laboratory. Antimicrobial susceptibility testing was determined using the standardized Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The double-disc synergy test confirmed the presence of ESBL.

Results: Among 3672 *Enterobacteriaceae* strains isolated, 20% were ESBL producers. *Klebsiella pneumoniae* accounted for 45.9% of ESBL-E, followed by *Escherichia coli* (25.3%) and *Enterobacter cloacae* (12.8%). The surgical and intensive care units were most affected. Urine samples were the most common source (42.4%). ESBL-E strains exhibited high resistance to tobramycin (80.3%), gentamicin (72.9%), and ciprofloxacin (73.7%), but maintained sensitivity to imipenem (15.6% resistance) and amikacin (21.9%). Significant differences were detected between non-ESBL and ESBL regarding all tested antibiotics. Male patients were significantly more affected by ESBL-E than females.

Conclusions: The increasing incidence of ESBL-E has become a significant concern. Monitoring their epidemiological and resistance profiles is crucial for guiding antibiotic therapy and preventing the development of further resistant strains.

Key words: *Enterobacteriaceae*; expanded-spectrum; beta-lactamases; epidemiology; antibiotics; resistance.

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Introduction

Enterobacteriaceae are a large family of Gram-negative bacteria [1] responsible for various community-acquired and nosocomial human infections such as sepsis and urinary tract infections [2]. Treatment of these infections often involves the use of antibiotics [3]. However, the excessive and abusive use of these therapeutic agents, in recent decades, has led to the development of resistance strains, thus making the treatment more complex and sometimes ineffective [4,5].

Over 35,000 deaths and over 2.8 million cases—or almost one death every 15 minutes—are attributed to antibiotic resistance in the United States each year. In contrast, in the European Union, it results in 25,000 deaths and 2.5 million additional hospital days [6]. Additionally, a Saudi Arabian tertiary care center identified a concerning pattern of rising antibiotic resistance among the bacterial pathogens that caused pediatric urinary tract infections (UTIs) during the COVID-19 pandemic (2019–2020) [7].

Beta-lactamase production is the most critical mechanism in developing beta-lactam resistance in Gram-negative bacteria. β-lactamases are enzymes that destroy the antibacterial activity of β-lactam antibiotics by binding covalently to their carbonyl group and hydrolyzing the β-lactam ring [8,9]. Due to their broad activity spectrum, these enzymes have been termed Extended-Spectrum Beta-Lactamases (ESBL). ESBLs include a group of beta-lactamases that hydrolyze extended-spectrum cephalosporins (mainly third generation), penicillins, and monobactams (aztreonam),

but not cephamycin (cefoxitin and cefotetan) and carbapenems. They are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam [10,11].

ESBLs are encoded by plasmids. The location of ESBL genes facilitates their spread by horizontal gene transfer to similar and different bacterial species [9]. The most critical β-lactamase genes include CTX-M, SHV, and TEM variants, which have broad substrate specificity against ceftazidime, cefotaxime, and ceftriaxone [5].

The World Health Organization (WHO) has recognized the ESBL-producing *Enterobacteriaceae* as a significant threat to public health [12].

Indeed, these microorganisms play a significant role in hospital and community-acquired infections by prolonging hospital stay and increasing morbidity and mortality [13]. Their enzymes have become a critical resistance mechanism as they can spread easily through plasmids, causing epidemics of resistant infections and leading to severe clinical illnesses [8]. The gravity of the ESBL issue lies in the fact that β-lactams are among the most prescribed and widely used antibiotics to treat bacterial infections due to their broad spectrum of activity and minimal side effects [14]. This class of antibiotics has been used empirically by most physicians not only for pre-operative prophylaxis but also for treating common and more severe infections, such as bloodstream infections [15]. It is also worth mentioning that ESBL-producing microorganisms can develop resistance to antibiotic classes other than β-lactams, including trimethoprim-sulfamethoxazole, aminoglycosides, chloramphenicol, and fluoroquinolones [9]. Consequently, the emergence and spread of ESBLs limit treatment options and complicate the selection of appropriate antibiotics for various types of infections [13,15].

The epidemiology of ESBL infection varies widely from one geographical region to another and rapidly evolves across hospital and community boundaries [16,17].

Consequently, acquiring a current local picture of the epidemiology of bacteria that produce ESBL and examining patterns in their spread are crucial [15]. The only way to direct antibiotic treatment and reduce the chance of developing antibiotic-resistant illnesses is to monitor these microbes routinely [18,19]. In this context, the current study conducted a descriptive analysis at Ibn Tofail Hospital, one of the major tertiary-referring hospitals of Marrakesh, to determine the prevalence of ESBL-E and identify their antibiotic resistance profiles. To the best of our knowledge, this is the first study on ESBL-producing *Enterobacteriaceae* to be carried out over a relatively long period (2010-2022) in the region.

This study aimed to assess the prevalence and antibiotic resistance profiles of ESBL-producing *Enterobacteriaceae*. This will help to improve the empirical treatment of patients and thus contribute to better management of multidrug-resistant infections in hospitals.

Methodology

Study setting and data collection

This was a retrospective analysis of data collected over thirteen years (from January 1, 2010, to December 31, 2022) in the microbiology laboratory of the Ibn Tofail tertiary-referring hospital in Marrakesh, Morocco. It included 3672 samples from adult patients (aged 18 years or older).

The Ibn Tofail tertiary-referring hospital is a health structure attached to the Mohammed VI University Hospital Center in Marrakesh. It has a capacity of 409 beds.

The study samples, clinical and laboratory data (including ESBL-producing bacterial species and antibiotic resistance) were identified through the database of the clinical microbiological laboratory of patients admitted to the hospital from January 2010 to December 2022. Duplicates corresponding to the same strain collected from the same patient were removed.

The ESBL-E strains were isolated from various samples (urine, pus, indwelling catheters, central venous lines, blood, genital excreta, sputum, tracheobronchial lavage, and cerebrospinal fluids) received at the microbiology laboratory from hospitalized patients for diagnostic purposes.

The bacteriological samples were collected from the various hospital departments, including the medical departments (cardiology, endocrinology, dermatology, nephrology, internal medicine, neurology, rheumatology, pneumology), surgical departments (cardiovascular surgery, maxillofacial surgery, general surgery, traumatology, plastic surgery, neurosurgery, urology, gynecology-obstetrics), and intensive care units.

Culture and identification of isolates

In the microbiology laboratory, samples were aseptically inoculated onto appropriate culture media (MacConkey agar, blood agar, and chocolate agar) based on the type and source of the specimen. They were then incubated in aerobic conditions at 37 ºC for 18 to 24 hours. *Enterobacteriaceae* were identified

based on colony morphology, Gram stain results, the oxidase test, and biochemical tests using standard Api 20E galleries (BioMérieux), which were used to determine enterobacterial genera and species [20].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was determined using the standardized Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MH; BioMérieux). This method consists of placing several antibiotic-impregnated discs on one or more plates previously inoculated with the bacterial suspension, and the antibiotic rapidly diffuses concentrically around each disk. The plates were incubated at 35 ± 2°C for 20 ± 4 hours in aerobic conditions. After incubation, the diameter of the inhibition zones was determined, and the results were interpreted as sensitive (S) or resistant (R) according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [21].

ESBL Confirmation Test

The presence of ESBL was confirmed by the double-disc synergy test (DDST) as recommended by the EUCAST. The synergy test was performed on the standard antibiogram between two disks, including a disc of cefotaxime (5 µg), ceftazidime (10 µg), cefepime (30 µg), and a disc containing clavulanic acid (amoxicillin-clavulanic acid (20 µg / 10 µg)), placed 30 mm away from the cephalosporin disks on a MH agar plate already inoculated with the bacterial suspension [22]. Then, the plates were incubated at 35 ± 2 °C for 20 ± 4 hours. The appearance of a champagne cork synergy effect indicated the presence of ESBL.

Statistical analysis

Segmented linear regression and standard linear regression analyses were employed in R to characterize the temporal trends in ESBL-producing *Enterobacteriaceae* (ESBL-E) prevalence over the study period (2010-2022). Additionally, the Wilcoxon-Mann-Whitney non-parametric test was utilized to statistically compare the distributions of the two bacterial species, *Escherichia coli* and *Klebsiella pneumoniae*, during the same timeframe. SPSS (version 25 for Windows; SPSS Inc., Chicago, IL) was also used to perform the Chi-square test (χ^2) to examine the relationship between ESBL-E and non-ESBL-E regarding different categorical variables. The probability of error (p) was expressed as follows: p value > 0.05: non-significant, p value ≤ 0.05: significant, and p value < 0.01: highly significant.

Ethical approval

The study was approved by the Ethics Committee of the Marrakesh University Hospital Center (UHC), approval number 69/2024, granted on January 22, 2024

Results

A retrospective analysis of all positive cultures for *Enterobacteriaceae* collected at Ibn Tofail Hospital in Marrakesh from January 1, 2010, to December 31, 2022, was conducted. A total of 3,672 strains of *Enterobacteriaceae* were isolated, including 732 ESBL

Table 1. Comparison between non-ESBL and ESBL regarding gender distribution and type of bacteria.

		Non-ESBL	ESBL	Test value	*p*	Sig.
Gender	Female	1372 (48.3%)	289 (39.0%)	18.100*	0.000	HS
	Male	1470 (51.7%)	443 (61.0%)			
Enterobacteriaceae						
Escherichia coli		1351 (46.4%)	185 (25.3%)	108.487*	0.000	HS
Klebsiella pneumoniae		588 (20.2%)	336 (45.9%)	202.665*	0.000	HS
Enterobacter cloacae		259 (8.9%)	94 (12.8%)	10.225*	0.001	HS
Enterobacter spp.		170 (5.8%)	78 (10.7%)	25.256*	0.000	HS
Proteus mirabilis		203 (6.9%)	2 (0.3%)	48.497*	0.000	HS
Proteus spp.		79 (2.7%)	4 (0.5%)	12.400*	0.000	HS
Proteus vulgaris		32 (1.1%)	6 (0.8%)	0.452*	0.501	NS
Citrobacter freundii		28 (1.0%)	5 (0.7%)	0.516*	0.473	NS
Citrobacter koseri		26 (0.9%)	6 (0.8%)	0.005*	0.944	NS
Morganella morganii		28 (1.0%)	1 (0.1%)	5.065*	0.024	S
Providencia stuartii		19 (0.7%)	4 (0.5%)	0.109*	0.741	NS
Serratia marcescens		25 (0.9%)	0 (0.0%)	5.583*	0.018	S
Klebsiella oxytoca		26 (0.9%)	7(1.0%)	0.092*	0.762	NS
Klebsiella aerogenes		14 (0.5%)	3 (0.4%)	0.066*	0.797	NS
Providencia rettgeri		18 (0.6%)	0 (0.0%)	3.291*	0.070	NS
Cronobacter sakazakii		10 (0.3%)	1 (0.1%)	0.838*	0.360	NS
Salmonella spp.		4 (0.1%)	0 (0.0%)	1.010*	0.315	NS
Serratia odorifera		3 (0.1%)	0 (0.0%)	0.757*	0.384	NS
Proteus penneri		3 (0.1%)	0 (0.0%)	0.757*	0.384	NS

Sig.: significance, $p > 0.05$: Non-significant; $p \leq 0.05$: Significant; $p \leq 0.01$: Highly significant, *: Chi-square test.

Figure 1. Percentage of ESBL-E among isolated *Enterobacteriaceae*.

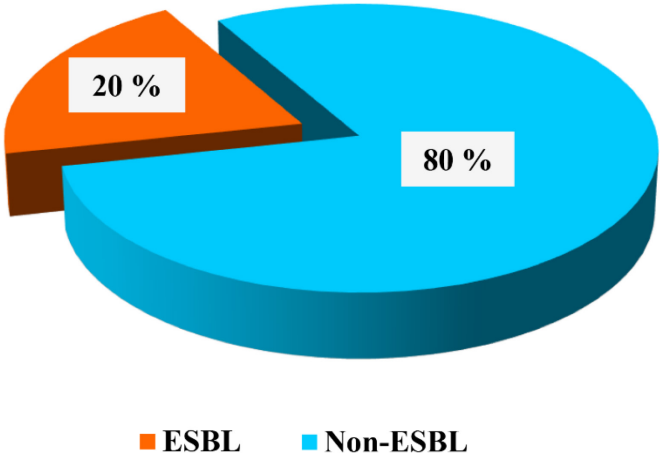
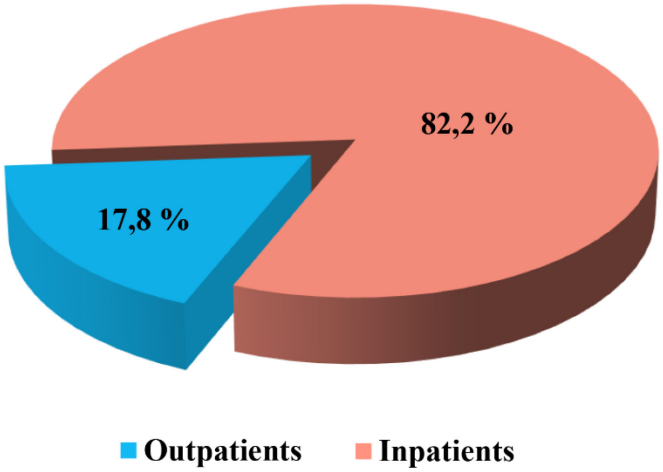


Figure 2. Distribution of ESBL-E according to various hospital departments.



producers, resulting in an overall isolation frequency of 20% (Figure 1).

Distribution of ESBL-E according to gender

The gender distribution of adult ESBL-E infections revealed a significant disparity, with 61% of cases occurring in men and 39% in women, corresponding to a male-to-female ratio of 1.53. A chi-squared test revealed this gender difference to be highly significant. Additionally, the comparison between ESBL-E and non-ESBL-E infections revealed a highly significant difference in gender distribution, with a p of less than 0.010. This extremely low p indicates the skewed gender distribution is unlikely to have arisen by chance. This suggests that significant underlying factors contribute to the disproportionately higher prevalence of ESBL-E infections among the male patient population (Table 1).

Distribution of ESBL-E according to various hospital departments

The current study evaluated the prevalence of ESBL-E and non-ESBL-E isolates across various hospital departments (Figure 2). A predominance of ESBL-E was observed in surgical departments, accounting for a total of 34.2% (251/732), with 13.4% attributed to traumatology, 5.7% to urology, and 5.6% to the plastic surgery department. The intensive care units contributed with a share of 27.7% (203/732). The medical department was also involved, generating 20.2% (148/732) of cases, with notable contributions from the nephrology and dermatology departments at 7.9% and 2.9%, respectively (Table 2). ESBL-producing *Enterobacteriaceae* prevalence varied

Table 2. Comparison between non-ESBL and ESBL groups regarding hospital departments.

Ward	Non-ESBL	ESBL	Test value	*p*	Sig.
Outpatients	1069 (36.6%)	130 (17.8%)	94.093*	0.000	HS
Intensive care units	626 (21.4%)	203 (27.7%)	13.281*	0.000	HS
Medical departments	426 (14.6%)	148 (20.2%)			
Dermatology	85 (2.9%)	21 (2.9%)	0.003*	0.956	NS
Nephrology	127 (4.3%)	58 (7.9%)	15.583*	0.000	HS
Neurology	36 (1.2%)	19 (2.6%)	7.342*	0.007	HS
Endocrinology	42 (1.4%)	6 (0.8%)	1.723*	0.189	NS
Rheumatology	33 (1.1%)	10 (1.4%)	0.282*	0.595	NS
Cardiology	44 (1.5%)	6 (0.8%)	2.042*	0.153	NS
Pneumology	12 (0.4%)	12 (1.6%)	13.543*	0.000	HS
Internal medicine	47 (1.6%)	16 (2.2%)	1.151*	0.283	NS
Surgical departments	801 (27.4%)	251 (34.2%)			
Neurosurgery	49 (1.7%)	23 (3.1%)	6.505*	0.010	S
Visceral surgery	85 (2.9%)	13 (1.8%)	2.879*	0.089	NS
Cardiovascular surgery	49 (1.7%)	17 (2.3%)	1.375*	0.241	NS
Gynecology-obstetrics	34 (1.2%)	14 (1.9%)	2.533*	0.111	NS
Maxillofacial surgery	19 (0.7%)	3 (0.4%)	13.215*	0.000	HS
Plastic surgery	97 (3.3%)	41 (5.6%)	8.384*	0.003	HS
Traumatology	337 (11.5%)	98 (13.4%)	93.000*	0.000	HS
Urology	131 (4.5%)	42 (5.7%)	2.042*	0.153	NS

Sig.: significance, $p > 0.05$: Non-significant; $p \leq 0.05$: Significant; $p \leq 0.01$: Highly significant, *: Chi-square test.

significantly across hospital departments (p = 0.002). Furthermore, the present study evaluated the differences in the prevalence of ESBL-E and non-ESBL-E isolates across various hospital departments. Significant differences were found in the following departments: Intensive Care Units, Nephrology, Neurology, Neurosurgery, Maxillofacial Surgery, Plastic Surgery, and Traumatology ($p < 0.01$) (Table 2).

Distribution of ESBL-E across different types of collected samples

The current study evaluated the distribution of ESBL-E according to the nature of the collected samples. All clinical samples found infected with ESBL-E were mainly composed of urine (42.4%), followed by pus (26.5%), blood (11.6%), respiratory samples (sputum and broncho-alveolar lavages) (7.7%), indwelling devices (intravenous catheters and urinary catheters) (8.2%), and other samples (cerebrospinal fluid, peritoneal fluid, articular fluid and vaginal samples) (Table 3). Differences between the prevalence of ESBL-E and non-ESBL-E isolates across all collected samples were also evaluated. Urine, Pus, Blood, IV Catheter, and Cerebrospinal fluid samples showed highly significant differences in the prevalence of ESBL-E and non-ESBL-E isolates, with a $p < 0.01$ (Table 3).

Distribution of ESBL-E according to the nature of samples and hospital department

The distribution of ESBL-E according to the nature of the samples and hospital wards showed that, in surgical departments, ESBL-E were mainly identified in pus samples, accounting for 63% of the total samples, followed by urine samples with a rate of 18%. In intensive care units, ESBL-E were mainly detected in blood, with a percentage of 27%, followed by respiratory samples (24%), and indwelling devices (catheters and urinary catheters) (22%). Regarding the medical departments, ESBL-E were primarily detected in urine samples, accounting for 78% of the total samples, followed by blood samples (11%) (Figure 3).

Distribution of ESBL-E according to different types of isolated bacterial species

The distribution of ESBL-producing *Enterobacteriaceae* across various bacterial species revealed a predominance of *Klebsiella pneumoniae*, accounting for 336 of 732 cases (45.9%), followed by *Escherichia coli* (185 of 732, 25.3%) and *Enterobacter cloacae* (94 of 732, 12.8%) (Table 1). The same findings were accurate for non-ESBL isolates regarding the same three bacterial species, with predominance of *Escherichia coli*, accounting for 46.4% of cases, followed by *Klebsiella pneumoniae* (20.2%) and *Enterobacter cloacae* (8.9%). It is worth noting that the differences between ESBL and non-ESBL were significant for the following species: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae, Enterobacter spp., Proteus mirabilis, Proteus spp., Morganella morganii,* and *Serratia marcescens.* (Table 1)

Figure 3. Distribution of ESBL-E according to the nature of the samples and hospital department.

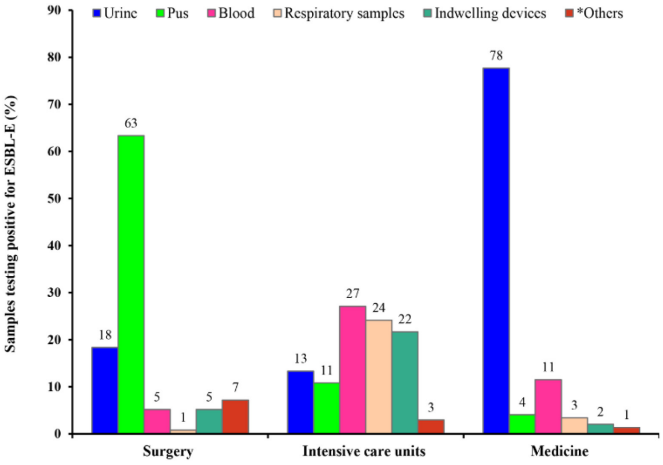


Table 3. Comparison between non-ESBL and ESBL groups regarding sample type.

Samples	Non-ESBL	ESBL	Test value	*p*	Sig.
Urine	1722 (58.7%)	311 (42.4%)	63.050*	0.000	HS
Pus	634 (21.6%)	194 (26.5%)	7.894*	0.004	HS
Blood	174 (5.9%)	85 (11.6%)	28.654*	0.000	HS
Respiratory samples	192 (6.6%)	56 (7.7%)	1.112*	0.291	NS
IV Catheter	126 (4.3%)	54 (7.4%)	11.847*	0.001	HS
Cerebrospinal fluid	27 (0.9%)	22 (3.0%)	19.255*	0.000	HS
Urinary catheter	16 (0.5%)	6 (0.8%)	0.733*	0.392	NS
Vaginal samples	20 (0.7%)	1 (0.1%)	3.064*	0.080	NS
Peritoneal fluid	17 (0.6%)	2 (0.3%)	1.070*	0.301	NS
Articular fluid	1 (0.0%)	1 (0.1%)	1.126*	0.288	NS

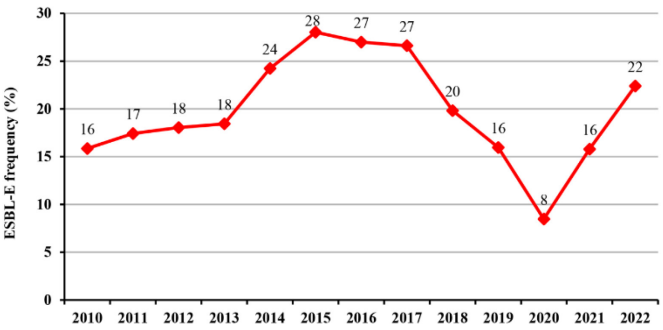
Sig.: significance, $p > 0.05$: Non-significant; $p \leq 0.05$: Significant; $p \leq 0.01$: Highly significant, *: Chi-square test.

Prevalence of ESBL-E according to different periods of the study

The evaluation of the prevalence of ESBL-producing *Enterobacteriaceae* over the study period revealed two distinct periods, from 2010 to 2012 and from 2013 to 2022. Initially, from 2010 to 2016, a gradual increase in the prevalence of these bacteria was observed, increasing from 16% in 2010 to a peak of 28% in 2015, before slightly decreasing to 27% the following year. This result was confirmed by a segmented linear regression analysis, which revealed a significant change in the evolution of ESBL-producing *Enterobacteriaceae* over the study period, with an estimated breakpoint occurring in 2015.

Thereafter, the trend was reversed in 2017, marking a gradual decline. This decline began with reorganization and structural changes at ITH, which led to the closure of certain medical services within the hospital, as well as a halt in the activity and a reduction in microbiological sampling from those services. The situation worsened in 2020 amid the COVID-19 pandemic. The suspension of outpatient consultations during this period resulted in a significant reduction in microbiological samples from outpatients, contributing to a decline in the ESBL-E rate to 8%. However, since 2021, the ESBL-E rate has gradually increased, reaching 22% in 2022, coinciding with the return to normal hospital activities (Figure 4).

Figure 4. Prevalence of ESBL-E according to different periods of the study.

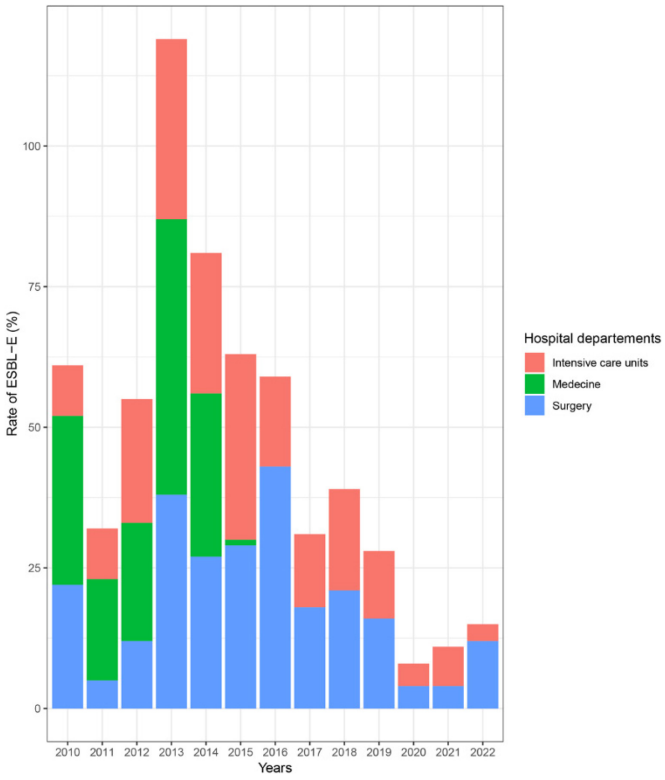


Analysis of ESBL-producing *Enterobacteriaceae* prevalence was divided into two periods: before and after 2015. Linear regression analysis revealed a significant annual increase of 2.33% in these isolates before 2015 ($p = 0.008$). After 2015, a non-significant yearly decline of 0.89% was observed ($p > 0.01$).

Prevalence of ESBL-E according to available hospital facilities

The distribution of ESBL-E strains across various hospital departments from 2010 to 2022 was also evaluated, revealing two distinct periods. Between 2010 and 2015, all hospital services were available, namely medical, surgical, and intensive care services. During this period, the prevalence of isolated ESBL-E was mainly high in medical departments. In contrast, the period from 2015 to 2022 was characterized by structural hospital modifications. The most significant change was the absence of medical services at ITH, leaving only surgery and intensive care units operational. This transformation has led to an increase in activity within these remaining departments, and consequently, an increase in the number of samples received and ESBL-E isolated from surgical and intensive care units (Figure 5).

Figure 5. Prevalence of ESBL-E according to available hospital facilities.



Evolution of the prevalence of ESBL-producing Klebsiella pneumoniae and Escherichia coli isolated between 2010 and 2022

The prevalence of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* strains isolated between 2010 and 2022 is illustrated in Figure 6, which shows the relative frequencies (in percentages) of ESBL-producing strains of *Klebsiella pneumoniae* and *Escherichia coli* over this period. In 2010, *Escherichia coli* accounted for 38.4% of the total isolates, while *Klebsiella pneumoniae* made up 61.5%. Over the

following years, the relative frequency of *Escherichia coli* fluctuated, reaching 50% in 2020 before declining to 16.6% in 2021 and then increasing to 25% in 2022.

Conversely, the frequency of *Klebsiella pneumoniae* increased, peaking at 83.3% in 2021 and 75% in 2022, after dipping to 50% in 2020. Overall, the data show an inverse trend between the two bacterial species, with *Klebsiella pneumoniae* becoming more dominant over the 13 years shown in Figure 6. The Wilcoxon-Mann-Whitney test revealed a significant difference in the distribution of *E. coli* and *K. pneumoniae* ($p < 0.01$). The frequencies of these two species evolved with distinct and opposite trends between 2010 and 2022.

Antibiotic co-resistance profile of all studied isolates

One of the most critical aspects of the current study is the analysis of the antibiotic co-resistance profile of different isolates (Table 4). This analysis showed a considerable prevalence of resistance to various antibiotics: all ESBL isolates were resistant to amoxicillin, amoxicillin-clavulanic acid, ticarcillin, mecillinam, cephalothin, and ceftazidime. Overall, 85.7% of isolates were resistant to trimethoprim-sulfamethoxazole, 80.3% to tobramycin, 73.7% to ciprofloxacin, 72.1% to fosfomycin, and 72.9% to gentamicin. On the other hand, ESBL-E strains retained good sensitivity to imipenem (15.6% resistance) and amikacin (21.9%), and no strain was found to be resistant to colistin. All types of antibiotics exhibited significant differences between ESBL and non-ESBL strains in terms of sensitivity and resistance patterns (Table 4).

Figure 6. Prevalence of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* isolated between 2010 and 2022.

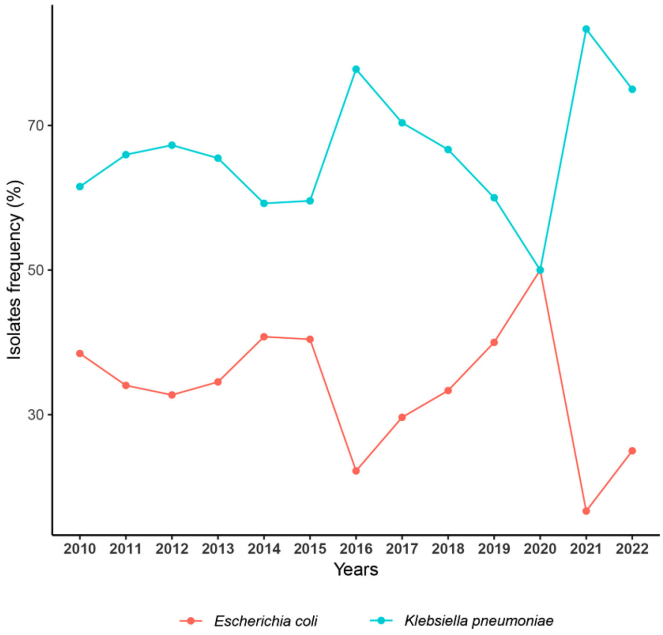


Table 4. Comparison between non-ESBL and ESBL groups regarding sensitivity to antibiotics.

		Non-ESBL	ESBL	Test value	*p*	Sig.
Amoxicillin	Resistant	2700 (95.0%)	732 (100.0%)	32.819*	0	HS
	Sensitive	142 (4.9%)	0 (0.0%)			
Amoxicillin-clavulanic acid	Resistant	1905(67.0%)	732 (100.0%)	327.286*	0	HS
	Sensitive	937 (32.9%)	0 (0.0%)			
Ticarcillin	Resistant	2616 (92.0%)	732 (100.0%)	64.583*	0	HS
	Sensitive	226 (7.9%)	0 (0.0%)			
Mecillinam	Resistant	550 (40.4%)	732 (100.0%)	719.370*	0	HS
	Sensitive	809 (59.5%)	0 (0.0%)			
Cephalotin	Resistant	437(34.6%)	732 (100.0%)	846.331*	0	HS
	Sensitive	824 (65.3%)	0 (0.0%)			
Ceftazidime	Resistant	739 (26.0%)	732 (100%)	1325.779*	0	HS
	Sensitive	2103(73.9%)	0 (0.0%)			
Imipenem	Resistant	285(10.0%)	114 (15.6%)	17.962*	0	HS
	Sensitive	2557 (89.9%)	618 (84.4%)			
Trimethoprim-sulfamethoxazole	Resistant	872 (37.5%)	627 (85.7%)	516.231*	0	HS
	Sensitive	1450 (62.4%)	105 (14.3%)			
Ciprofloxacin	Resistant	661 (25.1%)	540 (73.7%)	724.575*	0	HS
	Sensitive	1971 (74.9%)	192 (26.2%)			
Amikacin	Resistant	118 (4.1%)	160 (21.9%)	261.792*	0	HS
	Sensitive	2713 (95.8%)	572 (78.1%)			
Tobramycin	Resistant	487 (19.8%)	588(80.3%)	957.041*	0	HS
	Sensitive	1972 (80.2%)	144 (19.7%)			
Fosfomycin	Resistant	141 (11.6%)	528 (72.1%)	836.568*	0	HS
	Sensitive	1073 (88.4%)	204 (27.8%)			
Gentamicin	Resistant	778 (32.1%)	534 (72.9%)	387.878*	0	HS
	Sensitive	1647 (67.9%)	198 (27.0%)			
Colistin	Resistant	236 (12.9%)	0 (0.0%)	387.367*	0	HS
	Sensitive	1593 (87.1%)	732 (100.0%)			

Sig.: significance, $p > 0.05$: Non-significant; $p \leq 0.05$: Significant; $p \leq 0.01$: Highly significant, *: Chi-square test.

Discussion

The spread of ESBL-E constitutes a significant challenge for public health, due to their increasing resistance to antibiotics and their involvement in various types of infections [12,13]. Effective management of this problem requires continuous monitoring, a thorough understanding of local epidemiological profiles, and constant adaptation of treatment protocols as the situation evolves.

In this study, the overall prevalence of ESBL-producing *Enterobacteriaceae* strains was 20%. This result is lower than that reported in the city of Settat, Morocco (24.6%) [23], but higher than that in the city of Meknes, Morocco (12.2%) [24]. However, the result of all these cities of Morocco remains lower than the prevalences reported in other countries, as Tunisia and Lebanon (66.6%) [25], Niger (42.0%) [26], Brazil (30.3%) [27], and India (48.27%) [28]. These differences indicate that the distribution of ESBL-producing organisms differs between geographic regions [19]. This could be attributed, in part, to variations in antibiotic prescribing practices and protocols adopted in different areas [29], socioeconomic standards of various cities and countries, and available healthcare facilities.

This study revealed a significantly higher prevalence of ESBL-E infections in men (61%) compared to women (39%), with a gender ratio of 1.53. This result is controversial, as it is comparable to several previous studies on one side [19,26,27,30,31], but contrasts with other research that has observed a predominance of ESBL-E infection in women on the other side [32–34]. This discrepancy between different studies could be attributed to differences in the regions where the studies were conducted (developing versus developed countries), variations in sample size, type, study design, and duration. Although gender may be a risk factor associated with ESBL-E infections, the precise nature of this relationship remains ill-defined [31]. Further studies are needed to understand these associations and their underlying mechanisms fully. It is worth noting that males in our community are more likely to engage in outdoor activities and are at a higher risk of road traffic accidents than females (particularly motorcycle accidents, which national authorities have recorded as one of the significant causes of road traffic accidents). This makes males more likely to be admitted to the traumatology and other surgical departments and to receive antibiotic therapy.

Most ESBL-E isolates (82.2%) were primarily from hospitalized patients. On the other hand, 17.8% of these strains originated from the community. These results differ from some previous studies [12,16,26] but are consistent with recorded data from Morocco and other countries [18,30,35]. Indeed, these microorganisms are now recognized as the main agents of infections contracted not only in hospitals, but also within communities worldwide [19].

Again, this discrepancy could be attributed to variations in antibiotic prescribing protocols adopted in different countries [25], socioeconomic standards, healthcare practices, and the availability of healthcare facilities.

The most affected hospitalization services by ESBL-E were primarily those related to surgery, with a rate of 34.2%. These included traumatology (13.4%), urology (5.7%), and plastic surgery (5.6%). Intensive care units were also involved, with a notable prevalence of 27.7%. Other medical services accounted for 20.2% of ESBL-E cases, with nephrology (7.9%) and dermatology (2.9%) being the most prevalent.

The predominance of ESBL-producing isolates observed in surgical departments aligns with several other studies, including those carried out in Morocco (55.6%) [23], Bangladesh (43.58%) [36], and Myanmar (58.62%) [37]. The restructuring of ITH can explain this finding, which was implemented in 2015. As part of this reorganization, only the surgical and intensive care departments remained operational. This change resulted in the absence of medical services and led to the saturation of surgical services. Several factors can contribute to the dissemination of ESBL-producing *Enterobacteriaceae* in surgical departments. First, surgical patients are exposed to the systematic use of antibiotic prophylaxis or postoperative antibiotic therapy, which favors the development of multi-resistant strains [38]. Prolonged hospital stays increase patient exposure to multidrug-resistant (MDR) bacteria present in the hospital environment. Similarly, invasive surgical procedures, such as the use of indwelling catheters, endotracheal tubes, and nasogastric tubes, can create entry routes for these bacteria. Additionally, serious illnesses associated with a suppressed immune system increase the prevalence of bacterial infections, including those caused by strains that are resistant to antibiotics. Finally, ESBL producers may move from one hospital department to another as a result of healthcare personnel switching between departments, potentially spreading these bacteria across the hospital. [39].

The distribution of isolates from various clinical samples showed that urine was the primary source of ESBL-producing isolates (42.4%). This result is comparable to those found in Romania (37.75%) [11],

Brazil (46.8%) [27], and Burkina Faso (50%) [40]. However, it is lower than those found in Morocco (88.9%) [23], Ethiopia (63.85 %) [41], Saudi Arabia (70.3%) [42], and the United States (96%) [43]. These findings are explained by the fact that *Enterobacteriaceae* are the most common cause of urinary tract infections, both in the community and in healthcare settings [44]. Typically, these infections begin with periurethral contamination by enterobacteria present in normal human intestinal flora, followed by colonization of the urethra and subsequent migration of the pathogen to the bladder [45].

The present study revealed a predominance of ESBL-E in pus samples from surgical departments, accounting for 63% of the total. This observation may be attributed to surgical site infections that occur within 30 days of surgery, affecting the surgical incision and/or deep tissues [46]. Multidrug-resistant Gram-negative bacterial strains are increasingly associated with infections involving pus in hospitals [47]. The emergence of these infections is often linked to the proliferation of microbes in the surgical site, favored by poor preoperative preparation, wound contamination, and inadequate use of prophylactic antibiotics [48].

In intensive care units, the primary source of detection of ESBL-E was blood (27%), followed by respiratory samples (sputum and bronchoalveolar lavage) (24%), and indwelling devices (such as catheters and urinary catheters) (22%). The predominance of beta-lactamase-producing isolates in these samples may be attributed to increased rates of bacteremia, pneumonia, and device-related infections caused by multidrug-resistant (MDR) bacteria, particularly extended-spectrum beta-lactamase (ESBL)- producing bacteria.

These results are comparable to those of a study conducted in the same intensive care unit at the ITH in Marrakesh, where the most frequent infections were pneumonia (39%), bacteremia (29%), and catheter-associated infections (17%). This study highlighted risk factors for the acquisition of MDR bacteria, including ESBL-E, such as inadequate patient isolation precautions, prolonged ward stay, and inappropriate antibiotic therapy. However, other factors such as age, nasogastric intubation, and duration of curative antibiotic therapy did not appear to contribute to the acquisition of MDR [49].

The most prevalent ESBL-producing bacterial species identified in the present study were *Klebsiella pneumoniae* (45.9%), followed by *Escherichia coli* (25.3%) and *Enterobacter cloacae* (12.8%). Several studies have reported the predominance of the species *Klebsiella pneumoniae* with comparable prevalences, notably those carried out in Morocco (44.5%) [23], Brazil (37.5%) [27], Senegal (49.1%) [50], India (50.9%) [32], and Ethiopia (54.5%) [19]. This rate, however, was much lower than that found in a study carried out in Algeria (22.45%) [11].

This species is known for its multi-resistance to antibiotics and is strongly associated with nosocomial infections. Furthermore, ESBL-producing *Klebsiella pneumoniae* has been identified as one of the most prevalent pathogens [51]. This bacterium plays a key role in the spread of antibiotic resistance genes. Colonizing the human oropharynx and gastrointestinal tract can lead to various infections, including bacteremia and surgical infections, and promote opportunistic infections in immunocompromised patients. This germ forms biofilms, thus resisting the immune system and antibiotics. Its survival and immune evasion during infection are encouraged by its virulence factors [52].

The current study revealed an alarming increase in the frequency of ESBL-E isolation in the hospital between 2010 and 2016. Indeed, during this period, a notable increase in the prevalence of these bacteria was recorded. The prevalence rate increased from 16% in 2010 to a peak of 28% in 2015, before stabilizing slightly at 27% in 2016. These results were validated by segmented linear regression analysis, which revealed a significant change in trends of ESBL-producing *Enterobacteriaceae,* with an estimated breakpoint in 2015. Several studies confirmed this temporal evolution, although the frequencies may vary. In France, a survey conducted from 1999 to 2019 reported a maximum rate of ESBL-E of 29% in 2016 [53]. However, other research shows lower peak rates, such as Avicenne hospital in Marrakesh, Morocco, between 2008 and 2012, which recorded a peak prevalence of 13% in 2012 [35]. Similarly, the Mohammed V military hospital in Rabat, Morocco, reported a maximum percentage of 11.16% ESBL-E isolation between 2012 and 2018, as noted in 2018 [54]. Furthermore, a study carried out in the Netherlands between 2010 and 2014 revealed a maximum rate of 6.6% in 2014 [55]. The increasing rates of ESBL-producing *Enterobacteriaceae* strains over time can be attributed to the misuse of antibiotic treatment, particularly in countries where self-prescribed antibiotics are permitted and widely used [5].

The study period, from 2016 to 2022, revealed a notable decline in the prevalence of ESBL-E. This drop coincided with the hospital's organizational changes, specifically the absence of medical services. This

change appears to have influenced the prevalence of ESBL-E infection. The rate, which was 27% in 2016, decreased considerably to reach a minimum of 8% in 2020. This decline could be primarily attributed to the interruption of outpatient consultations during the COVID-19 pandemic, which resulted in the cessation of microbiological samples from outpatients. Since 2021, with the gradual resumption of hospital activities, ESBL-E rates have increased, reaching 22% in 2022.

The evolution of ESBL-producing *K. pneumoniae* and *Escherichia coli* isolates over 13 years showed an opposite trend. In 2010, *E. coli* accounted for 38.46% of isolates, while *K. pneumoniae* represented 61.54%. The prevalence of *E. coli* fluctuated, peaking at 50% in 2020 and then dropping to 25% in 2022. Conversely, the frequency of *K. pneumoniae* increased, reaching a maximum of 83.33% in 2021 and 75% in 2022, after declining to 50% in 2020. A similar study conducted in Poland (2017-2021) showed this reverse trend, with *K. pneumoniae* frequency increasing from 30.3% in 2017 to 50% in 2021, and *E. coli* fluctuating between 7.9% in 2017 and 9.4% in 2021 [56].

Several factors can explain the opposing trends in these two species. Competition for resources and ecological niches plays an important role. The scarcity of resources or changing conditions can increase the competitiveness of one species, thereby decreasing the prevalence of the other. Additionally, the selective pressure exerted by antibiotics also favors one species over another [57].

In the present work, ESBL-E strains revealed high co-resistance rates for most of the tested antibiotics. Resistance rates of 72.9%, 80.3%, and 21.9% were noted for gentamicin, tobramycin, and amikacin, respectively.

These results were compatible with other studies carried out in the intensive care unit of our hospital (gentamicin 71%, tobramycin 86%) [49]. On the other hand, our results were higher than those recorded in Morocco (gentamicin, 35.41%; tobramycin, 52.77%) [58] and in Niger (gentamicin, 66.7%; tobramycin, 61.9%) [26].

The resistance rate of ESBL-E to amikacin (21.9%) found by the current study is similar to that recorded in Meknes, Morocco (23.61%) [58]. However, it is higher than the values reported in some African studies [23,41,54]. But lower than those noted in others [26,35].

The high sensitivity to amikacin shown by the present study encourages the meticulous use of this antibiotic to combat infections caused by ESBL-producing *Enterobacteriaceae,* particularly in the face of increasing resistance to classic aminoglycosides (gentamicin, tobramycin). Indeed, the high sensitivity to amikacin is linked to its limited use, which limits the selective pressure that favors the emergence of resistance to amikacin [36].

ESBL-producing isolates were also found to be resistant to sulfamethoxazole-trimethoprim, at a rate of 85.7%. This rate is comparable to that reported in some literature [12,19,35,41,49,58]. However, it is higher than the rate recorded in Rabat, Morocco (35%) [54] and lower than that noted in a study carried out in Settat, Morocco (97.8%) [23].

The resistance of ESBL-producing strains to ciprofloxacin was 73.7%. This rate is comparable to those reported in other studies [35,40,49,58]. Nevertheless, some studies have shown higher resistance rates to ciprofloxacin [23,26]. A survey conducted in Ethiopia reported a lower percentage (46.3%) [41]. The increasing resistance of ESBL-E to this class of antibiotic worldwide is attributed mainly to its excessive use in daily medical practice [35,59].

As for fosfomycin, the co-resistance rate among ESBL-E was 72.1%. This result is higher than those reported in several Moroccan studies [35,54,58].

The increasing resistance of ESBL-E to various types of antibiotics is primarily a result of several factors, including the excessive and often inappropriate use of antibiotics, whether in the hospital setting, with the prescription of broad-spectrum antibiotics without an antibiogram, or in the community, with self-medication and easy access to these drugs without prescription. [18,31,41,59,60].

The widespread use of antibiotics in agriculture and livestock farming exacerbates the situation, potentially leading to the transmission of resistant bacteria to humans through the food chain. Furthermore, multi-resistance to antibiotics is facilitated by the transfer of resistance genes located on plasmids, which spread rapidly between bacteria. Added to this are socio-economic factors, such as a lack of education regarding the rational use of antibiotics, poverty, and inadequate healthcare facilities, leading to self-medication, ineffective control measures, and insufficiently restrictive policies regarding antibiotic use, both in healthcare settings and in the community [2,17,19,59,60].

Co-resistance to antibiotics other than beta-lactams could be explained by the presence of genes coding for ESBL production on mobile genetic elements. These elements can also carry genes conferring resistance to antibiotics other than beta-lactams [27,36].

The increased resistance of ESBL-E to several

families of antibiotics limits therapeutic choices and leads to the increasing use of carbapenems to combat these infections, particularly imipenem [35]. According to the current study, 15.6% of ESBL-E isolates were resistant to imipenem. This resistance is mainly due to the production of carbapenemase by these *Enterobacteriaceae.* The resistance rate observed is in agreement with other Moroccan studies [23,35] but is higher than those reported in other studies [22,36,54] and is lower than that recorded in Benin (27.18%) [60]. Carbapenems currently remain the most effective antibiotics for treating infections caused by ESBL-E. However, in the absence of new alternative antibiotics, the rational use of carbapenems is imperative to prevent the emergence of resistant strains [35].

ESBL-producing isolates were 100% susceptible to colistin. This observation is consistent with data obtained from a study conducted in Meknes, Morocco, where a sensitivity of 100% was also reported [59]. This result highlights the crucial role of this antibiotic as a first-choice treatment for serious infections caused by ESBL-producing bacteria, particularly when these organisms are resistant to carbapenems.

We acknowledge a few limitations of this study. First, there is a lack of some data, particularly regarding patient age, comorbidities, antibiotic use, and some information on non-ESBL isolates. Second, a lack of PCR techniques for identifying specific genes concerning ESBL isolates. Third, a detailed patient history (especially about the time of hospital admission and duration of hospital stay) is missing. However, the exceptionally long duration, retrograde nature of the study, and structural reorganization with the closure of some facilities established during the survey can collectively explain the cause of these restrictions.

Conclusions

This study reveals an alarming increase in ESBL-producing *Enterobacteriaceae* in Marrakech, Morocco, highlighting their growing resistance to antibiotics and, consequently, their significant impact on public health. Faced with this situation, urgent and concerted action is required. This involves implementing a global and coordinated strategy. This strategy should include improving hospital hygiene practices, raising awareness about the judicious use of antibiotics, continuous monitoring of bacterial resistance to antibiotics, implementing strict infection prevention and control measures, and supporting research for the development of new antibiotics. Close collaboration between all health stakeholders, authorities, patients, and the agricultural sector is essential to combat the spread of antibiotic resistance and preserve the effectiveness of available treatments.

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Conflict of interest

No conflict of interest is declared.

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