International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Study of the biochemical characteristics of Enterobacteriaceae isolated from patients at the Sidi Kacem provincial hospital, Morocco

Elouakfaoui Aziz[*1], Rouani Abdeljabbar[2], Yassine Chaib[3], Mahjoub Aouane[4]*

[1]PhD Student, [3]PhD Student, [4]university professor, Department of Biology Faculty of Science Ibn Tofail University Laboratory of Natural Resources and Sustainable Development, Kenitra, Morocco,

[2]PhD Student, Department of Physics Faculty of Science Ibn Tofail University Laboratory of Materials and Subatomic Physics, Kenitra, Morocco.

Abstract

The dissemination of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in the hospital environment presents a high risk of mortality for patients with chronic immunodeficiency. The aim of our study is to determine the epidemiological profile and level of antibiotic resistance of these bacteria. A prospective study over a 12-month period was carried out in the hospital microbiology laboratory by analyzing biological fluids from hospitalized patients, SPSS version 20 software was used to analyze the results, statistical comparisons were carried out using the chi-square test, any p-value less than 0.05 is considered to be statistically significant. 645 Enterobacteriaceae samples detected in the laboratory, 270 of which were ESBL producers, the epidemiological profile is dominated by the Escherichia coli species (58.13%), urinary tract infections were predominant (53.95%), with an F/M sex ratio of 1.8, the intensive care unit was the most contaminated with 28.84%. 41.86% of strains are resistant to betalactam antibiotics, of which 16% are resistant to carbapenems, 58.4% of strains are resistant to cephalosporins and 51% are resistant to fluoroquinolones, Imipenem and Amikacin remain the most effective antibiotics for treating these infections. These multiresistant bacteria are responsible for the deaths of 2% of patients. **Conclusion:** these results show a high prevalence of infection by ESBL-producing Enterobacteriaceae, so a global approach to combating these infections must be implemented.

Keywords: Enterobacteriaceae, biochemical characteristics, extended-spectrum beta-lactamase (ESBL)

Full length article ***Corresponding Author*, e-mail: *elouakfaouiaziz@gmail.com***

1. Introduction

Nosocomial infections are a global health problem, given their high frequency and socio-economic costs, with significant mortality and morbidity. Among the micro-organisms most responsible for these infections are Enterobacteriaceae, which are responsible for several nosocomial epidemics in hospitals [1].

These bacteria represent one of the most dangerous groups and are frequently isolated, especially in hospitals, because they produce extended-spectrum betalactamases (ESBLs) and carbapenemases (EPCs) and have other mechanisms of resistance to a large number of antibiotics, making it very difficult to treat these infections. These multi-resistant bacteria (MRB) are mainly localized in patients' gastrointestinal tracts, and are transmitted by the contaminated hands of healthcare staff, soiled equipment or through the hospital environment. Components [2].

Enterobacteriaceae are responsible for most nosocomial bacterial infections, especially lung infections, surgical site infections and urinary tract infections. These bacteria are resistant to beta-lactam antibiotics through the production of enzymes called extended-spectrum beta-lactamases (ESBL), which hydrolyze penicillins, cephalosporins and aztreonam. More recently, other resistances have been discovered, such as resistance to carbapenems and fluoroquinolones, due to their past use as reference treatments, leading to a risk of therapeutic impasse [3].

Developing an infection with these multi-resistant bacteria (MRB) is a risk factor that threatens patients' health, since these infections have a higher mortality rate than infections with sensitive strains. They are often associated with delayed effective treatment in patients with co-morbidities. An additional cost caused by this type of infection as a result of over-consumption of resources due to prolonged

hospitalization, with possible physical and social consequences and even the death of the patient [4].
The proliferation of MRB in hospitals depends on a number of factors: an uncontrolled environment, the absence of control protocols, non-compliance with good hygiene practices, the absence of regular monitoring for these bacteria, The extensive and frequently abusive use of antibiotics, coupled with an imbalance in hospital hygiene, allows these infections to develop rapidly, resulting in several epidemics [5]. The nosocomial infection control committee (CLIN) and the operational hygiene team (EOH) are the two inseparable pillars of the strategy to be implemented to control the spread of MRBs. The nosocomial infection control committee (NICC) and the operational hygiene team (OET) are the two inseparable pillars of the strategy to be implemented to control the spread of MRBs [6].
As part of the surveillance and fight against the emergence of extended spectrum betalactamase and carbapenemase-producing Enterobacteriaceae, this prospective study was carried out on the various biological samples taken from hospitalized patients over a one-year period in the microbiology laboratory of the provincial hospital center of Sidi Kacem, with the aim of establishing an epidemiological profile and the degree of antibiotic resistance of isolated enterobacterial strains.

2. Materials and methods

2.1 Study design

A prospective study was carried out at the microbiology laboratory of the provincial hospital of Sidi Kacem over a period of one year, from the first of May 2022 to the end of April 2023, in order to establish an epidemiological and antibiotic resistance profile of the different Enterobacteriaceae strains isolated from hospitalized patients.

2.2 Study setting

The province of Sidi Kacem is the capital of the Rabat-Salé-Kénitra region of Morocco, covering an area of around 7042 km². The population of the province is estimated at 740,000 inhabitants according to the 2017 population census. limited by the provinces of Ouezzane, Sidi Slimane, Meknès and the province of Khemiset. The microbiology laboratory at the provincial hospital in Sidi Kacem plays an active role in diagnosing cases of infection by multi-antibiotic-resistant bacteria, either nosocomial or community-acquired. Our study was carried out as part of the prevention of nosocomial infections caused by multi-resistant bacteria by detecting the different strains of multi-resistant enterobacteria present in patients and determining their degree of antibiotic resistance.

2.3 Study population

The population of our study is composed of all hospitalized patients suspected of being infected by enterobacteria and who have undergone sampling of a biological fluid such as pus, urine, blood, saliva, pleural puncture fluid, ascites fluid, etc., for eventual microbiological analysis in search of enterobacteria strains multiresistant to antibiotics.

2.4 Definition

Nosocomial strains: hospitalized patients who contract enterobacterial infection during hospitalization.
community strains: patients who have contracted an enterobacterial infection outside the hospital, which is the reason for their hospitalization.

2.5 Sampling method

For patients with several positive samples for the same strain of Enterobacteriaceae during the same hospitalization, a single sample was taken into account to avoid duplication of cases. Excluded from our sample were all analyses of patients colonized by other types of bacteria other than Enterobacteriaceae, and samples from patients who were not hospitalized and who came from outside the hospital.

2.6 Data collection method

Every patient diagnosed as a carrier of an enterobacteria strain by analysis of the biological fluid is investigated using a pre-established guide to collect the following information: personal characteristics (gender, age, marital status, level of education, etc.), factors favouring infection (chronic illnesses, diabetes, asthma, cancer, immunosuppressants such as corticosteroids, drugs, self-medication, invasive procedures, etc.). Hospital admission date, hospitalization department, sampling types, length of hospitalization. Antibiotic susceptibility test results are collected for each positive enterobacterial sample. The data is collected by a team of 2 nurses and a doctor.

2.7 Microbiological analysis

In the laboratory, the samples received were processed rapidly and the Enterobacteriaceae strains were identified using the classic biochemical gallery and API 20E (Biomerieux, France). The Mueller Hinton agar diffusion method was used for antibiogram tests, based on the guidelines and advice of the antibiogram committee of the French Microbiology Society (CASFM, 2018) [7]. Extended-spectrum beta-lactamase (ESBL)-producing strains were detected by the synergy test between a central disc of amoxicillin + clavulanic acid 30 mm away from discs of cefotaxime, ceftazidime, cefepime and aztreonam. The presence of ESBL was noted in the presence of a "champagne cork" appearance.

2.8 Statistical analysis

The data collected were analyzed using SPSS version 20 software, and descriptive statistics were produced in percentage form for the qualitative variables. The chi-square test was used to compare and correlate categorical variables. For correlation results, any p-value less than 0.05 is considered statistically significant.

2.9 Ethical considerations

Our study is validated by a provincial health ethics commission and authorized by the hospital director. The confidentiality of collected information and the anonymity of the participants were guaranteed. The survey team was trained to respect ethical procedures such as professional secrecy, the security of the information collected and anonymity, etc. The consent of all patients surveyed was sought and respected.

3. Results and Discussions

3.1 Results

During the 12 months of our study, we received 700 microbiological samples composed of biological fluids from hospitalized patients (pus, urine, blood, etc.) suspected of being infected by bacteria, at the microbiology laboratory of the provincial hospital center. The results show that 645 of the samples analyzed contained enterobacteria, while 30 were a negative culture and 25 contained non-enterobacterial species. A total of five Enterobacteriaceae strains were isolated from the patients' biological fluids. The distribution of isolated strains by hospital department is shown in Table 4. The following bacterial species were isolated: Escherichia coli (58.13%), Klebsiella pneumoniae (21.86%) were predominant, followed by Enterobacter cloacae (9.14%), Proteus vulgaris (6.97%) and Serratia rubidaea (3.87%). we noted that nosocomial infections were more predominant than community infections for all infections detected (Table 1).

The distribution of enterobacteria strains isolated according to the type of biological fluid analyzed showed a predominance of enterobacteria in urinary samples (53.95%) by means of cytobacteriological examinations of urine. These were followed by pus samples (14.10%), blood samples for blood cultures (5.89%), pleural fluid (5.27%), stools (4.65%) and vaginal swabs (4.34%). The lowest results were found for ascites fluid samples (3.87%), cerebrospinal fluid (2.63%) and other effusion fluids (2.17%) (Table 2). The results of antibiogram tests carried out on Enterobacteria strains isolated from hospital patients showed a high level of resistance to all types of antibiotics tested, and show that imipenem, meropenem, chloramphenicol, cefotaxime, amikacin, gentamicin and ciprofloxacin are still the antibiotics most frequently active in vitro, which can be used as a treatment for these infections. Enterobacteriaceae strains of nosocomial origin showed higher percentages of resistance than strains of community origin, with significant p-values ($p<0.05$) for the majority of antibiotics tested such as amoxicillin, first, second and third generation cephalosporins, nalidixic acid, gentamicin and colistin. For the following antibiotics Meropenem, Nitrofurans, Amikacin, Ciprofloxacin, Norfloxacin Amoxicillin + Clavulanic acid, no statistically significant difference in resistance was observed for the two strains of Enterobacteriaceae with p-values greater than 0.05. we noted that 270 isolated strains were producing extended-spectrum beta-lactamase (ESBL), with an overall rate of 41.86% of which 104 enterobacteria strains are carbapenemase (EPC) producers, with an overall prevalence of 16% (Table 3). The distribution of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBLE) strains, including carbapenemase-producing strains, by hospital department showed that all departments are infected by these multi-resistant bacteria, with a predominance in the intensive care unit with a percentage of 28.84% for EPCs and 18.67% for other strains that are sensitive to carbapenems but resistant to other beta-lactam antibiotics, followed by the emergency department with 17.30% for EPC and 13.25% for other ESBLE strains, and the urology department with 12.50% for EPC and 12.04% for ESBLE. In our study, we found that the departments least contaminated by multi-resistant enterobacteria were the following: the pneumology department, with only one strain (0.96%) producing carbapenemase (EPC) and 7 strains (4.21%) producing ESBL, while 70 isolated strains did not produce ESBL, followed by the gastrology department with 4 strains (3.84%) of EPCs, 8 ESLB-producing strains represent (4.81%) and 62 strains not producing ESBL. These results show that there is a large spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBLE) and enterobacteria producing carbapenemase (EPC) in hospital departments, which makes it difficult to treat these cases and threatens the vital prognosis of hospitalized patients (Table 4). The distribution of extended-spectrum beta-lactamase-producing Enterobacteriaceae strains according to the nature of the bacterial species showed that Escherichia coli species are the most resistant to betalactam antibiotics, with 121 ESBL-producing strains (44.81%), 62 of which are carbapenemase-producing strains isolated mainly from patients' urine, followed by Klebsiella pneumoniae with 70 (25.92%) ESBL-producing strains, 31 of which are resistant to carbapenems, and Enterobacter cloacae with 40 ESBL-producing strains, 08 of which are carbapenemase-producing. While 70.90% of Serratia rubidaea strains isolated are sensitive to beta-lactams, including carbapenems. However, half of the Proteus vulgaris strains (23) are ESBL producers, including 3 strains resistant to carbapenems, and the other half (22 strains) are sensitive to betalactam antibiotics (Table 5).

3.2 Discussion

Hospitalization is a major risk factor for nosocomial infections because the hospital environment is colonized by antibiotic-resistant pathogenic bacteria, transmission is often inevitable between patients or via healthcare staff or medical objects and devices. Hygiene conditions and co-morbidities favour infections, with the emergence of multi-resistant bacteria [8]. Enterobacteriaceae are frequently isolated in hospital bacteriology laboratory, Escherichia coli, Klebsiella pneumoniae and Enterobacter cloacae are the species most frequently isolated from hospitalized patients. Betalactam antibiotics have long been the standard treatment for these infections, but the rise in resistance rates to this family of antibiotics is increasingly worrying, especially with the emergence of carbapenem-resistant strains [9]. During our 12-month study, a total of 700 biological samples from 700 hospitalized patients were received in the bacteriology laboratory, enterobacteria are present in 645 biological samples analyzed, 30 samples were a negative culture and 25 contained species other than Enterobacteriaceae, a positivity rate for enterobacteria detected of around 92.14%. The patient characteristics show that the majority (68.52%) are female, the most frequent age group is over 60 years old, with a percentage of 43%, followed by the 30 to 60 age group with 31% and finally patients under 30 years old with 26%, including 9% of hospitalized children.

In terms of co-morbidities, 20% of patients are diabetics, 15% are hypertensives and 6% are immunosuppressed. While more than half of them received invasive procedures such as surgery, central venous catheters, vesical probes, tracheal intubation, etc. 13 patients died as a result of complications from infection with multi-resistant enterobacteria, representing 2.01% of all hospitalized patients. In general, the frequency of infection seems to increase with age, there is therefore a relationship between infectious risk and age. Several of factors involved in the

increase of the incidence of Enterobacteriaceae infections in the elderly: Decreased immunity, bedrest, the medication effect, dehydration (particularly urinary tract infections), Presence of invasive practices, etc. Most studies show that infection rates vary between different age groups, the 35 to 60 age group contains the most patients infected. This is in accord with the results of the study by Dia et al carried out in Dakar in 2015 [10].

In our study, five species of enterobacteria were isolated, while Escherichia coli dominated the epidemiological profile with 375 strains (58.13%) of all isolated bacteria, of which 295 strains (45.73%) were of nosocomial origin and 80 strains (12.40%) were of community origin. 91% of the urinary tract infections (UTIs) detected during the study were due to Escherichia coli species, the ascending pathophysiology of urinary tract infections (UTIs), as well as the high level of colonization of the perineum by this bacteria, associated with specific uropathogenic factors such as bacterial adhesins capable of binding to the urinary epithelium, this explain the high rate of infection. Secondly, the Klebsiella pneumoniae species, with 141 strains detected (21.86%), of which 120 strains (18.60%) were of nosocomial origin and 21 strains (3.25%) were of community origin. The least isolated species is Serratia rubidaea with only 25 (3.87%) strains. It was noted that nosocomial strains represented the majority, with a percentage of 80.15% (517 strains), whereas community strains represented only 19.84% (128 strains), This result can be explained by the high prevalence of nosocomial infections detected in several studies and by contamination of the hospital environment. Several studies have found similar results, such as the study by Maddi Safa Nourhane and Menasra Lamia in 2022 [11], on enterobacteria infections and their antibiotic resistance, the study showed that E. coli was the most frequently isolated bacterial species, accounting for 38% of all studied species. In second place was Klebsiella sp. with (22%). In third and fourth place were Proteus sp. (14%), Morganella sp. and Enterobacter cloacae with a percentage of (10%). For the rest of the strains, the percentage obtained during the study period was not significant.

The study by Lahlou Amine and al in 2009 [12], at the Moulay-Ismail military hospital in Meknes showed the isolation of the following Enterobacteriaceae: Escherichia coli is the predominant species (80%), followed by Klebsiella spp. (10%) and various other species (10%): Proteus mirabilis, Citrobacter freundii, Enterobacter cloacae. The study by Hajar ben abdallah and al in 2005 [13], at the Fattouma Bourguiba University Hospital in Monastir showed that 32.3% and 67.7% of the strains came from hospitalized patients and consultant patients respectively. E. coli ranked first with (76%), followed by Klebsiella sp (10.5%), Proteus mirabilis (4%), Pseudomonas aeruginosa (2%), other Gram-negative bacilli (0.9%) and others (0.5%). The study by Ahmanach and al [14], and Slimi and al [15], in 2019, showed that Escherichia coli predominates, followed by Klebsiella pneumoniae, while Proteus mirabilis is in third place, followed by Enterobacter cloacae, with similar rates of isolation. In Tunisia, Péan and al [16], showed that Escherichia coli was also in the first ranking, but with a higher percentage (81.7 %), followed by Klebsiella pneumoniae with a rate of 3.7 %. This study found that more than half (53.95%) of the infections detected were urinary tract infections diagnosed by urine cytobacteriological tests (UCTs), they are more frequently found in women than in men, with a female/male sex ratio of 1.8, this can be explained by the fact that the periurethral glands have no antibacterial activity contrary to prostatic fluid, plus the high rate of samples from women. followed by surgical site infections with a percentage of 14.10% detected during pus analysis, then bacteremia with a percentage of 5.89% detected in blood cultures, then respiratory infections with 5.27% diagnosed during pleural fluid analysis, followed by gastrointestinal infections with 4.65% and meningitis with 2.63%. Several studies have reported similar results, such as:

The study by Awa Ndir and al in 2015 [17], at a pediatric hospital in Senegal showed that bacteremia came first with 38% of cases. followed by urinary tract infections (36%), surgical site infections (13%), respiratory infections (9%) and meningitis (4%). The study by Sira Alice Dioman in 2008 [18], at the Point G University Hospital in Mali on the epidemiology of ESBLE showed that urinary tract infections were in the majority with a percentage of 64.4%, followed by respiratory infections with 21.12%, then septicemia with 7%, gastrointestinal infections with 5.3% and other types of infection with 2.18%. These results are in agreement with the study by Bouzeraa and Berrhil in 2018 [19], at the Military University Hospital of Constantine, on the determination of enterobacteria isolated in the intensive care unit, which found a predominance of Enterobacteriaceae in urinary tract infections with a percentage of 30.1%. The studies by Chekroud and al in 2017 [20], and Ould Baba ali et al [21], in 2019 also showed that urinary tract infections are the most prevalent, mainly caused by Escherichia coli species, with percentages of 58.23% and 43.14% respectively.

The study of the sensitivity of enterobacteria to the antibiotics tested revealed a several points: A high level of resistance in all the antibiotics tested, with the exception of amikacin, imipenem and meropenem, and the emergence of resistance to first-, second- and third-generation cephalosporins in hospitals. These levels of resistance are worrying and alarming. This situation is the result of pressure from the massive prescription and often abusive use of large spectrum antibiotics (beta-lactam antibiotics, fluoroquinolones, etc.), whether prescribed by doctors or self-medicated. The plasmid determinism of these acquired resistances also favours their dissemination. The results of the antibiotic resistance of the Enterobacteriaceae strains isolated showed that 41.86% of the strains (270) were resistant to betalactam antibiotics, including 104 strains (16%) resistant to carbapenems through the production of carbapenemase (EPC), and 58.4% of strains are resistant to first-, second- and third-generation cephalosporins. While only 26.25% of strains are resistant to aminoglycosides, 51% are resistant to fluoroquinolones, 37% are resistant to polymyxins, while resistance varies between 76% and 92% for penicillin A and carboxypenicilin groups.

However, Meropenem, Imipenem and Amikacin remain the most effective antibiotics for treating these enterobacterial infections. we noted that nosocomial strains are more resistant than community strains, with significant p-values of less than 0.05 for the most of antibiotics tested. The Escherichia coli species is the most resistant to betalactam

antibiotics, with 121 ESBL-producing strains, 62 of which are resistant to carbapenems. It is responsible for the majority of urinary tract infections, while 25.92% of Klebsiella pneumoniae strains are resistant to betalactam antibiotics, 11.48% of which are resistant to carbapenems. Several studies have reported comparable rates of resistance, such as that of Imane Benhiba and al [22], in 2013 at the Ibn Tofail University Hospital in Marrakech. The survey showed that 16% of enterobacteria produced extended-spectrum beta-lactam antibiotics (ESBL). Resistance to imipenem was recorded in 23 strains of enterobacteria: Enterobacter cloacae (13 strains), Klebseilla pneumoniae (4 strains), Echirichia coli (3 strains) and Citrobacter freundii (3 strains). Only amikacin showed a low level of resistance (17%). Acquired resistance of E. coli to amoxicillin by penicillinase production was 67%. No resistance was recorded for colistin.

The study by Hajar ben Abdallah and al [13], in 2005 at the Fattouma Bourguiba University Hospital in Monastir showed that Escherichia coli and Proteus mirabilis were more resistant to amoxicillin (61% and 71%), the association of amoxicillin and clavulanic acid (46.4% and 45.5%) and cotrimoxazole (39.6 and 26%). For Klebsiella sp strains, resistance to Cefotaxime and to the combination of amoxicillin and clavulanic acid was 42% and 20% respectively. The survey revealed an increase in resistance among enterobacteria to ofloxacin, from 11.9% to 17.6%, and to ciprofloxacin, from 9.1% to 14.5%. The study by Lahlou Amine and al [12], in 2009 at the Moulay-Ismail military hospital in Meknes showed that the frequency of overall resistance of hospital and community strains of enterobacteria to amoxicillin, amoxicillin combined with beta-lactamase inhibitors, quinolones, fluoroquinolones, sulphamethoxazole + trimethoprim and nitrofurans is very high. Third-generation cephalosporins and aminoglycosides continue to have good activity. The overall prevalence of extended-spectrum beta-lactamase (ESBL) production is observed in 9% of enterobacteria. Resistance to third-generation cephalosporins (C3G) and carbapenems by Enterobacteriaceae is a major public health problem worldwide. broad-spectrum betalactam antibiotics are used specifically in the treatment of potentially fatal resistant infections caused by multi-resistant bacteria [23]. The mechanism of resistance to C3Gs and carbapenems is the acquisition of genes encoding extended-spectrum betalactamases (ESBLs) and carbapenemases respectively. which makes treatment very difficult and threatens patients' lives [24].

The distribution of enterobacteria contamination in hospital wards shows that intensive care, emergency and urology departments are the most contaminated by enterobacteria resistant to beta-lactams and including carbapenems, with the isolation of 61 resistant strains in intensive care units, 40 strains in emergency and 33 resistant strains in urology responsible for urinary tract infections, This may be caused by the workload and the nature of the pathologies received by these departments, which encourages cross-contamination and the dissemination of germs in the environment with the formation of biofilms. The study by Lagha and al [25]. in 2015 which looked at antibiotic resistance in extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, concerning the distribution of strains by department, they found different results from those obtained in our study, with a predominance of enterobacteria in the intensive care unit (27%), then the internal medicine unit (20%), followed by the gynecology and pediatrics units (7% and 3% respectively). The results obtained show that there is a significant increase in the resistance of enterobacteria isolated in hospitals to most of the antibiotics tested, this situation has very harmful repercussions for patients with co-morbidities, and with immune failure leading to death. A good therapeutic strategy, supported by regular monitoring of antibiotic resistance, is essential to stop the emergence and spread of these multi-resistant bacteria.

Table 1: Type and Percentage of Enterobacteria Species Isolated

Bacterial species	**Number**	**Percentage**	**Nosocomial (N)**	**Community (C)**
Escherichia coli	375	58,13%	295 (78,66%)	80 (21,33%)
Klebsiella pneumoniae	141	21,86%	120 (85,10%)	21(14,81%)
Enterobacter cloacae	59	9,14%	43 (72,88%)	16 (27,11%)
Proteus vulgaris	45	6,97%	39 (86,66%)	6 (13,33%)
Serratia rubidaea	25	3,87%	20 (80%)	5 (20%)
Total	645	100%	N+C=100%	

Table 2: Distribution of samples according to the nature of the patient's biological fluid		
Type of samples	**Number**	**Percentage**
Urine	348	53,95 %
Pus	91	14,10 %
Blood culture	38	5,89 %
Pleural fluid	34	5,27 %
Stools	30	4,65 %
Vaginal swab	28	4,34 %
Ascites fluid	25	3,87 %
Catheter	20	3,10 %
Cerebrospinal fluid (CSF)	17	2,63 %
Other effusion fluids	14	2,17 %
Total	645	100 %

Table 3: Percentage of resistance in Enterobacteriaceae strains isolated			
Antibiotics	**Nosocomial Strains N= 517**	**Community Strains N= 128**	**P value**
Amoxicillin	92%	81%	0,001
Amoxicillin + clavulanic acid	71%	67%	0,06
Ticarcillin	87%	76%	0,02
Cefalotin	94%	79%	< 0,002
Cefuroxime	70%	52%	0,02
Ceftazidime	76%	68%	0,0001
Céfotaxime	45%	24%	<0,0001
Nalidixic acid	61%	50%	0,02
Norfloxacin	58%	54%	0,12
Ciprofloxacin	45%	38%	0,08
Gentamicin	43%	24%	0,0002
Amikacin	21%	17%	0,11
Sulfamethoxazole+ trimethoprim	58%	53%	0,142
Nitrofurans	52%	46%	0,09
Chloramphenicol	34%	25	0,01
Colistin	41%	33%	0,002
Imipenem	17%	12%	0,01
Meropenem	13%	09%	0,07

Table 4: Distribution of Enterobacteriaceae strains by resistance profile and department				
Department	**ESBLE-producing strains**		**Non ESBL-producing strains**	**Total**
	Carbapenemase-producing strains EPC	Other ESBL-producing strains		
Intensive care	30 (28,84%)	31 (18,67%)	16 (4,26%)	77 (11,93%)
Emergency	18 (17,30%)	22 (13,25%)	18 (4,80%)	58 (8,99%)
Urology	13 (12,50%)	20 (12,04%)	20 (5,33%)	53 (8,21%)
Paediatrics	10 (9,61%)	18 (10,84%)	23 (6,13%)	51(7,90%)
Traumatology	9 (8,65%)	17 (10,24%)	29 (7,73%)	55 (8,52%)
Medicine	7 (6,73%)	16 (9,63%)	36 (9,60%)	59 (9,14%)
Haemodialysis	6 (5,76%)	14 (8,43%)	41 (10,93%)	61 (9,45%)
Maternity	6 (5,76%)	13 (7,83%)	60(16,00%)	78 (12,09%)
Gastrology	4 (3,84%)	8 (4,81%)	62 (16,53%)	74 (11,47%)
Pneumology	1(0,96%)	7 (4,21%)	70 (18,66%)	79 (12,24%)
Total	104 (100%)	166 (100%)	375 (100%)	645 (100%)

Table 5: Percentage of isolated Enterobacteriaceae according to ESBL production, by species			
Bacterial species	**ESBLE+**	**ESBLE-**	**Number**
Escherichia coli	121 (44,81%)	224 (59,73%)	375 (58,13%)
Klebsiella pneumoniae	70 (25,92%)	71 (18,93%)	141 (21,86%)
Enterobacter cloacae	40 (14,81%)	19 (5,06%)	59 (9,14%)
Proteus vulgaris	23 (8,51%)	22 (5,86%)	45 (6,97%)
Serratia rubidaea	16 (5,92%)	39 (10,40%)	25 (3,87%)
Total	(270) 100%	375 (100%)	645 (100%)

4. Conclusions

Enterobacteriaceae producing ESBL and carbapenemase are currently emerging worldwide. These infections are associated with high mortality and lethality rates, high costs and major treatment problems due to the limited number of therapeutic alternatives. The aim of our study was to establish an epidemiological profile of ESBL-producing Enterobacteriaceae isolated from the biological fluids of hospitalized patients, and to assess their level of antibiotic resistance. The results show the isolation of five bacterial species, predominantly by the Escherichia coli and Klebsiella Pneumoniae, with percentages of 44.81% and 25.92% respectively. The intensive care and emergency departments were the most contaminated, with percentages of 47.51% and 30.55%. The results of the antibiotic resistance of isolated enterobacteria strains showed that 41.86% of strains (270) were resistant to betalactam antibiotics, of which 104 strains (16%) were resistant to carbapenems 58.4% of strains were resistant to first-, second- and third-generation cephalosporins. While only 26.25% of strains are resistant to aminoglycosides. More than half (53.95%) of the infections detected during the study were urinary tract infections, caused by Escherichia coli species in 91% of cases.

in the context of these alarming results, it would appear that it is necessary to continue and reinforce efforts to implement a good therapeutic strategy, updated by regular monitoring of local epidemiology and antibiotic resistance, is the most effective way of improving therapeutic management. In hospitals, the fight against these infections essentially involves strict compliance with asepsis and the rational use of antibiotics, oriented by sensitivity tests in order to preserve the therapeutic arsenal over the long term, at a time when the prospects for inventing new antibiotic molecules are virtually nil.

Acknowledgments

I would like to thank the hospital administration for authorizing me to carry out this study and also the provincial health delegation. my gratitude goes to the team at the provincial laboratory of epidemiology and environmental hygiene for their cooperation in carrying out the analysis of the samples

Financial Support

There is no source of funding for this study.

Conflicts of Interest

The authors declare that there is no conflict of interests.

Ethical approval

All ethical procedures have been respected and our study has been validated by a provincial ethics commission

References

[1] E. Aziz, M. aouane, R. Abdeljabbar. (2021). Microbiological Study of Surfaces in the Hospital Environment Case of the Provincial Hospital of Sidi Kacem, Morocco. *Indian Journal of Forensic Medicine & Toxicology*, *16*(1), 419–426.

[2] A. Ousmane, M. Abdoulaye, L. Mahaman, et al. (2019). Profil des germes uropathogènes communautaires isolés en milieu pédiatrique à Niamey au Niger. The journal of médical and Heath sciences, 20 : 87.

[3] O. Dziri, CA. Alonso, R. Dziri, et al, (2018). Metallo-β-lactamases and class D carbapenemases in South-East of Tunisia: implication of mobile genetic elements in their dissemination. Int J Antimicrob Agents, 52: 8717,

[4] E. Aziz, M. Aouane, & R. Abdeljabbar. (2022). Microbiological Analysis of Indoor Air in the Provincial Hospital of Sidi Kacem, Morocco. *Indian Journal of Forensic Medicine & Toxicology*, *16*(4), 216–222.

[5] S. Bouazza, N. Bouakka. (2016). Recherche des entérobactéries productrices de β-lactamases à spectre élargi dans la viande rouge. Mémoire de master en microbiologie appliquée à la sante et à l'environnement., Université de Tébessa.

[6] P. Riegel. (2003). Aspects bactériologiques des infections urinaires nosocomiales. Med Mal Infect ; 33 :255s- 65s.

[7] Société française de microbiologie (SFM), (2018). Comité de l'antibiogramme de la société française de microbiologie - European Committee on antimicrobial susceptibility testing (CASFM/EUCAST) - Recommandations 2018 – V.2.0 Septembre.

[8] S. Nijssen, A. Florijn, MJ. Bonten, et al, (2004). b-lactam susceptibilities and prevalence of ESBL-producing isolates among more than 5000 European Enterobacteriaceae isolates. Int J Antimicrob Agents. 24:585-91.

[9] R. Jones, M. Pfaller. (2003). Antimicrobial activity against strains of Escherichia coli and Klebsiella spp with resistance phenotypes consistent with an extended-spectrum b-lactamase in Europe. Clin Microbiol Infect, 9:708-12.

[10] G. Peirano, JD. Pitout. (2010). Molecular epidemiology of *Escherichia coli* producing CTXM ß-lactamases: the worldwide emergence of clone ST131. International Journal of Antimicrobial Agents ; 35 : 316-321.

[11] M.S. Nourhane, M. Lamia, (2022). Les infections à entérobactéries et leurs antibiorésistances au niveau de l'EPH d'El khroub, Constantine. Mémoire de Master. Faculté des Sciences de la Nature et de la Vie Département de biologie appliqué.

[12] L. Amine, M. Chegri, H. Lkassmi, (2009). Epidémiologie et résistance aux antibiotiques des entérobactéries isolées d'infections urinaires à

l'hôpital militaire Moulay-Ismail de Meknès. Science direct, 11 : 90-96.

[13] H. Ben Abdallah, O. Sahnoun, F. Ben Romdhane, et al (2008). Profil de sensibilité aux antibiotiques des entérobactéries uropathologènes isolées dans la région de Monastir. Review Tunisian Infectiology, 02 (02) : 5-8.

[14] S. Ahmanach, O. kaci, (2019). Etude de la résistance des bacilles à Gram négatif aux β-lactamines à partir de divers prélèvements pathologiques. Mémoire de master en Microbiologie appliquée. Bouira, Algérie : Université Akli Mohand Oulhadj, p81.

[15] C. Slimi, L. Boucefiane. (2019). Etude du profil épidémiologique des entérobactéries productrices de β-lactamases à spectre étendu (BLSE) isolées dans la région d'Ain Defla. Mémoire de master : Microbiologie appliquée. Algérie : Université djilali bounaama, p108

[16] Y. Péan, FW. Goldstein, F. De Bels. (2001). Évolution de la sensibilité et épidémiologie de la résistance des entérobactéries de ville au cours des enquêtes Vigil'Roc. Méd Mal Infect ; 31 : 609-621.

[17] A. Ndir, A. Diop, K. Roughyatou, et al (2016). Infections caused by extended-spectrum beta-lactamases producing Enterobacteriaceae: clinical and economic impact in patients hospitalized in 2 teaching hospitals in Dakar, Senegal. Antimicrobial Resistance and Infection Control, 5:13

[18] S. Dioman. (2008). Épidémiologie des entérobactéries productrices de beta-lactamases à spectre élargi au chu du point G. thèse de pharmacie. La Faculté de Médecine de Pharmacie et d'Odonto-Stomatologie, p 92.

[19] H. Brrehil H, A. Bouzeraa. (2018). Bactériologie des entérobactérie isolées au niveau du service de réanimation de l'hôpital militaire régional universitaire de Constantine (HMRUC). Mémoire de master.

[20] R. Chekroud, R. Fathi. (2017). Étude du profil bactériologique et de la sensibilité aux antibiotiques des entérobactéries responsables des infections urinaires. Mémoire de Master en Hygiène Hospitalière et Santé. Constantine, Algérie. Université des Frères Mentouri, p130.

[21] R. Ould Baba Ali, K. Taibi . (2019). Etude de l'antibiorésistance des Entérobactéries productrices de Beta-lactamases à spectre étendu isolées à l'Hôpital de Boufarik. Mémoire de Master : Microbiologie. Blida, Algérie. Université de Blida 1, p76.

[22] I. Benhiba, T. Bouzekraoui, J. zahidi, et al (2015). Epidemiology and antibiotic resistance Enterobacteriaceae urinary of adult's infections in Marrakech chu and commitment therapy. Uro'Andro. V,1 N° 4; 166-171.

[23] G. Mayoral, M. Ferreyra, A. Eden, et al (2009). Évolution de la résistance des entérobactéries aux céphalosporines de troisième génération de 2000 à 2008 au centre hospitalier de Perpignan France. Pathologie Biologie.07.032.

[24] D. Mkaouar, F. Mahjoubi, S. Mezghani, et al, (2008). Etude de la résistance des entérobactéries aux céphalosporines de troisième génération dans les hôpitaux de Sfax, Tunisie (1999–2005). Médecine et maladies infectieuses. 11 ;017, P ; 293-298.

[25] N. Lagha, (2015). Etude de la résistance aux antibiotiques des entérobactéries productrices de ß-lactamases à spectre étendu (BLSE) isolées de l'hôpital de laghouat. Thèse de Doctorat : Sciences. Tlemcen, Algérie : Université Abou bekr Belkaïd. P80.