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Prevalence of enterobacteria producing carbapenemases and BLSE in the adult resuscitation environment at Hassan ii hospital in Fes

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Abstract

Enterobacteriaceae producing carbapenemases (EPCs) and extended-spectrum betalactamases (E-BLSEs) are an emerging threat. The aim of this study is to specify the epidemiological profile of E-BLSEs and EPCs, determine their prevalence in patients hospitalized in the adult intensive care unit of the CHU Hassan II hospital center, and describe their current antibiotic resistance profile for better patient management based on local data.

Our study is retrospective, carried out at the microbiology laboratory of CHU HASSAN II in Fez over a one-year period from January 1, 2022 to December 31, 2022, and concerns all E-BLSE and EPC strains isolated from all urine, pus, blood cultures and respiratory samples taken from adult intensive care units at CHU Hassan II in Fez.

Of the 1,792 bacteriological samples processed during this period, 352 were positive for Gram-negative bacilli (GNB), including 71 BMR, giving an overall prevalence of 20.17%.

During our study, we retained 71 BMR, including carbapenemase-producing enterobacteria with 30.98% of cases (22 strains) and 49 strains of E-BLSE B (64.01%).

The overall prevalence of EPCs among enterobacteria was 6.25%. The blaOX-48 gene predominated at (45.45%), followed by bla-NDM a (31.81%).and finally the two genes with a (22.72%).

The overall prevalence of E-BLSE was 14%. Among these E-BLSE, *Escherichia coli* constituted the majority (59.18%) of isolates, followed by *Klebsiella pneumoniae* with a rate of (32.65%). Then Enterobacter cloacae with a rate of (8.16%).

All EPC enterobacteria isolated showed resistance to quinolones. However, the sensitivity of our strains to amikacin was 98%, while colistin showed a sensitivity rate of 100%.

This study has shown that the prevalence of EBLSE and EPC in the adult intensive care setting is significant. We need to continue our efforts in early detection of these BMRs in hospitals to control their spread both in the hospital environment and in the community.

Keywords: Enterobacteria; Carbapenemases; OXA-48; NDM; Antibiotic resistance

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1 Introduction

Enterobacteriaceae belong to a family of Gram-negative enteric bacteria that constitutes one of the most important families of bacteria. They are commensal bacteria of the digestive flora, and can be responsible for serious infections. In their wild state, these bacteria are normally sensitive, but they can acquire mutations that make them multi-resistant to many of the antibiotics to which they are normally susceptible.

In fact, BMR are Multi-resistant Bacteria, including the most commonly observed extended-spectrum betalactamase (ESBL)-producing Enterobacteriaceae and carbapenemase (CPE)-producing Enterobacteriaceae.

E-BLSEs are broad-spectrum enzymes produced by Enterobacteriaceae, giving them resistance to virtually all b-lactam antibiotics, except cephamycins, and carbapenems [1], while carbapenemases are enzymes capable of hydrolyzing most βlactam antibiotics, including carbapenems, with variable efficacy.

Carbapenems are a class of antibiotics that includes Ertapenem, Imipenem and meropenem. They are among the most potent antibiotics and are often considered an ultimate therapeutic option for combating infections caused by multidrug-resistant bacteria, particularly those linked to Gram-negative bacillus species Resistance to these antibiotics considerably restricts the choice of available therapeutic options [1].

According to Paterson and Bonomo, 2005 Rawat and Nair, 2010, Enterobacteriaceae resistance to betalactams and carbapenems is due to: either the production of carbapenemases, or the production of extended-spectrum betalactamases (ESBLs) inactivating first-, second- and third-generation cephalosporins, Associated with a decrease in membrane permeability [1].

The increasing spread of antibiotic-resistant bacteria is a major problem for public health, as highlighted by several studies such as Bradford (2001), Ahoyo et al. (2007) and Rastogi et al. (2012) [24-25].

This increased resistance to antibiotics leads to a significant increase in morbidity and mortality rates linked to infectious diseases, as indicated in a study conducted by Hailaji et al. (2016) [26].

According to the national epidemiological report published by Santé publique France at the end of December 2015, there has been an increase in the number of EPC-related episodes [3].

In this context the microbiology department of the Hassan II University Hospital in Fez, Morocco, conducted a study to assess the microbiological and molecular characteristics, as well as the antibiotic resistance profile of these strains, with the aim of improving their management.

2 Material and methods

This is a retrospective, descriptive, observational study over a one-year period from January 1, 2022 to December 31, 2022, including all strains of carbapenemase- or ESBL-producing Enterobacteriaceae isolated from patients hospitalized in the adult intensive care unit of CHU Hassan II in Fez. Data were collected from microbiology laboratory registers, and included all types of samples (blood cultures, catheters, respiratory samples, pus, punctures and urine).

The microbiology laboratory at the Centre Hospitalier Universitaire Hassan II in Fez uses a calibrated 10 µl loop to evenly distribute urine samples for enumeration, while respiratory samples are fluidized and subjected to successive dilutions before being star-stacked. Blood samples are placed directly into blood culture bottles, where they are incubated in the Bactec system until bacteria are detected. They are then inoculated onto blood agar plates for culture. Other types of sample are inoculated using the dial technique. The choice of culture media depends on the type of sample and the bacteria suspected, and includes ordinary, selective and enriched agars, adapted to each situation

Bacterial strains were identified on the basis of their cultural and biochemical characteristics (API Galleries) or by automated identification on Becton Dickinson's Phoenix 100. Antibiotic susceptibility was determined by a standard antibiogram performed by swabbing using the Mueller-Hinton agar diffusion method, and interpretation was made in accordance with the recommendations of the Antibiogram Committee of the Société Française de Microbiologie du CA-SFM 2022. [5].

The detection of extended-spectrum beta-lactamase (ESBL) production in our laboratory was carried out using the double-disk synergy test described by Olonitola et al. (2007). The principle is based on the use of a disk of amoxicillin-clavulanic acid (AMC) and a disk of third-generation C3G cephalosporin (ceftazidime, ceftriazone or cefotaxime) on an agar medium previously inoculated with the strain to be tested. The test is considered positive if a "champagne cork" image appears between the AMC and C3G discs.

For any carbapenemase-producing strain, we used a rapid visual multiplex immunochromatographic method that detects one or more of the five common carbapenemase enzyme types OXA-48, NDM, KPC, IMP, VIM on bacterial colonies.

The information collected was recorded and analyzed using Microsoft Excel software. Qualitative characteristics were represented in terms of frequencies and percentages, while quantitative characteristics were presented as averages.

The following antibiotics were used: imipenem, meropenem, tigecycline, colistimethate sodium (colistin), tigecycline.

3 Results

During the study period, 1792 samples were received at the microbiology laboratory of the Hassan II University Hospital, Fez, from the adult intensive care unit. Of these samples, 352 were positive for Gram-negative bacilli, including 71 BMR, 22.04%. The M/F sex ratio was 1.61, with an average age of 38,

Among the 352 BGN, the most frequent species were *Escherichia coli*, which accounted for 178 (50.56%) of isolates, followed by *Klebsiella pneumoniae* 136 (38.63%), Enterobacter cloacae 17 (4.8%) *Serratia marcescens* 12 (3.4%) and Proteus mirabilis 9 (2.55%) Table I.

In our study, we identified a total of 71 strains of multi-resistant bacteria (MRB), corresponding to an overall prevalence of 20.17%. Of these, EPCs accounted for 30.98% (22 strains), while extended-spectrum bacteria (ESBLs) were present in 64.01% of cases (49 strains).

The prevalence of betalactamase-producing Enterobacteriaceae among Gram-negative bacilli (GNB) was 13.92%, while that of carbapenemase-producing Enterobacteriaceae was 6.25%.

Among the 71 BMR identified, the most frequent species were: *Escherichia coli*, which accounted for 35 isolates (49.29%), closely followed by *Klebsiella pneumonia* with 30 isolates (42.25%). Next came *Enterobacter cloacae* with 5 isolates (7.04%), and *Proteus mirabilis* was the least frequent with just 1 isolate (1.40%).

In the double-disk synergy test, we found that 49 of the 71 BMR, equivalent to 64.01%, were ESBL. Among these strains, *Escherichia coli* was the most frequently isolated, accounting for 29 cases, or 59.18%. Next, *Klebsiella pneumoniae*was present in 16 cases, representing 32.65%, followed by *Enterobacter cloacae* with 4 cases, or 8.16% Table II.

Among the 71 BMR we identified, we noted the presence of 22 carbapenemase-producing strains. *Klebsiella pneumoniae* was the most frequent species, with 14 cases, representing 63.63% of the total. Next, *Escherichia coli*was present in 6 cases, i.e. 27.27%, followed by *Proteus mirabilis* with 1 case, i.e. 4.54%, and finally *Enterobacter cloacae* with also 1 case, i.e. 4.54% Table III.

For the 22 carbapenemase-producing Enterobacteriaceae strains, we carried out additional tests to confirm and characterize the phenotype. The OX48 phenotype was the most frequently observed, with 10 cases, representing 45.45% of the total. Next, the NDM phenotype was present in 7 cases, or 31.18%, while both genes were identified in 5 cases, equivalent to 22.72%.

Among the 14 *Klebsiella pneumoniae* cases, we found that 6 carried the blaNDM gene, representing 42.85% of these cases. In addition, 3 cases carried the blaOXA48 gene, equivalent to 21.42%, while 5 cases carried both genes simultaneously, or 35.71%. All 6 *Escherichia coli* strains carried the blaOXA48 gene. The *Proteus mirabilis* strain carried the blaNDM gene, while the Enterobacter cloacae strain carried the blaOXA48 gene.

The results of urine samples showed a high prevalence of BMR, with 34 positive cases among the 180 samples taken (BGN positive), corresponding to a BMR rate of 18.18%. For respiratory samples, including PDP, sputum and BAL, we identified 17 positive cases among 62 samples (BGN positive), equivalent to a BMR rate of 27.41%. For blood cultures,

we identified 9 positive cases among 44 samples (BGN positive), representing a BMR rate of 20.45%. In contrast, other types of samples, such as puncture fluids and pus samples, showed relatively low BMR rates. BMR rates by sample type are shown in Table IV.

All the ESBL Enterobacteriaceae we isolated showed resistance to aminopénicillines, amoxicillin-clavulanic acid and cephalosporins. In the majority of cases, these strains were also resistant to fluoroquinolones.

All carbapenemase-producing Enterobacteriaceae strains were resistant to the antibiotics imipenem, ertapenem, meropenem and quinolones.

However, the sensitivity of our strains to amikacin was 98%, while colistin showed a sensitivity rate of 100%.

Table 1 Number and percentage of enterobacteria strains isolated

Isolated strains	Number of total	Pourcentage d'isolat
Escherichia coli	178	50.56%
Klebsiella pneumonie	136	38.63%
Enterobacter clocae	17	4.8%
Serratia marcescens	12	3.4%
Proteus mirabilis	9	2.55%

Table 2 Prevalence of ESBL-producing strains of Enterobacteriaceae

Isolated strain	Number	% De positivité en BLSE
Escherichia coli	29	59.18%
Klebsiella pneumonia	16	32.65%
Enterobacter cloacae	04	8.16%

Table 3 Prevalence of EPC versus Enterobacteriaceae isolation by species

Species	EPC	prevalence
Escherichia coli	14	63.63%
Klebsiella pneumonia	06	27.27%
Enterobacte cloacae	01	4.54%
Proteus mirabilis	01	4.54%

Table 4 Prevalence of ESBL- and carbapenemase-producing strains of Enterobacteriaceae by type of sample

Type of sample	BGN-positive samples	BMR-positive samples		BMR rate
		BLSE	EPC	
PDP	62	06	11	27.41%
KT	34	03	01	11.76%
BLOODCULTURE	44	05	04	20.45%
PUS	36	05	02	19.44%
ECBU	180	30	04	18.88%
TOTAL	352	71		20.17%

4 Discussion

Enterobacteriaceae producing extended-spectrum betalactamases and carbapenemases cause serious infections in weakened and immunocompromised patients. [6]. The results of this study reveal a high overall rate of BMR at a 22.04%, It is important to emphasize that this prevalence may present significant variations depending on countries and regions, as well as healthcare facilities. According to a study conducted in France in 2018, the prevalence of BMR in healthcare establishments was 14.8% [21].

During our study we found 352 BGN positives and the most isolated strains were essentially *Escherichia coli* with (50.56%) %, *Klebsiella pneumoniae* with (38.63%) %, *enterobacter cloacae* (4.8%), *Serratia marcescens* (3.4%) and P. mirabilis (2.55%), similar to the results found in Cameroon by Gangoué-Piéboji et al. (2005) [10], who found 61.02% *Escherichia coli* 41.16% *Klebsiella pneumoniae*. and 5.6% *enterobacter cloacae*.

In our study the ESBL rate among enterobacteria was 13. 5%, this observation is close to data in the literature, which show an increase in the frequency of ESBL-producing enterobacteria in recent years (Fouad et al. 2019) [23].

These extended-spectrum beta-lactamases are represented mainly by *Escherichia coli* with a rate of 59.18% and *Klebsiella pneumoniae* with a rate of 32.65%. Several studies have also highlighted the emergence of these two genera [23].

Urine samples contained more EBLSE than other samples. The same result was obtained in a study carried out in Saudi Arabia (Kader and Kumar, 2005), where urine samples contained more EBLSE than suppurations. [11]. In contrast to the results of the study carried out in Nepal by Jeny and Nabaraj in 2015, where enterobacteria were mainly identified in pus samples, followed by urine and blood samples, pus was the main site of enterobacteria collection with 148 cases out of 309, thus representing 47.90% of isolates, while urine samples collected 126 cases out of 309, or 40.78% of isolates. [12-13].

According to our study, the rate of carbapenemases observed in enterobacterial strains was 6.25%.

Klebsiella pneumonia dominated the EPC picture, with a prevalence of 22.53% (16 cases out of71BMR). This observation is in line with other studies carried out in different countries, which showed an increase in the incidence of *Klebsiella pneumonia*. According to Patel, the frequency of carbapenemases (EPCs) in *Klebsiella pneumoniae* strains was 26% [7-8-9].

In Tunisia, Mansour reported that the prevalence of carbapenemase-producing *Klebsiella pneumonia* was 13.2% in Mahdia's Tahar Sfar Hospital [14].

According to the literature, carbapenem resistance in enterobacteria is due to the production of transmissible carbapenemases of the KPC type, metallo-β-lactamase and OXA-48[1]. In our context, carbapenemase-producing enterobacteria were mainly of the NDM and OXA-48 types.

In our population, immunochromatographic typing showed the predominance of the OXA48 gene at 45.45%, followed by the NDM gene at 31.81%, and co-expression of these two genes was found in 22.72% of strains. The increase in the rate of NDM-type EPCs is worrying, given that this type is resistant to almost all available antibiotics.

In summary, the results of this study highlight a high prevalence of MRB, mainly marked by a significant distribution of enterobacteria resistant to third-generation cephalosporins. The prevalence of carbapenem-resistant Enterobacteriaceae is also a cause for concern, with a significant proportion of strains producing NDM- and OXA-48-type enzymes.

5 Conclusion

In summary, the results of this study revealed a high frequency of multi-resistant Gram-negative bacteria (MR-GNB) in the Hassan II Hospital in Fez. These results will contribute to improving our understanding of bacterial ecology and the efficacy of antibiotics against various pathogens in the intensive care units of the Hassan II Hospital in Fez.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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