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Current antibiotic resistance of *Enterobacteriaceae* isolated at Avicenna military hospital of Marrakesh

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Abstract

Enterobacteriaceae are bacteria found in the digestive system, known for causing infections and displaying antibiotic resistance. The World Health Organization (WHO) identifies strains resistant to third-generation cephalosporins and carbapenems as priority pathogens. This study, conducted at Avicenna Military Hospital of Marrakech, aimed to assess the epidemiological and antibiotic resistance profiles of *Enterobacteriaceae* over a two-year period (May 2020 - April 2022) to inform updated antibiotic use recommendations.

This retrospective study analyzed 1,626 positive bacterial isolates from various hospital departments, including medical, surgical, intensive care, emergency units, and external samples. Of these, 1,163 (71.5%) were *Enterobacteriaceae*, predominantly *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*, primarily isolated from urine, pus, and blood cultures. Antibiotic resistance was assessed, revealing that 614 (52.8%) of *Enterobacteriaceae* were extended-spectrum beta-lactamase producers (ESBL-E), and 115 (9.9%) were carbapenemase-producing *Enterobacteriaceae* (CPE).

The study's findings align with existing literature, showing a higher prevalence of *Enterobacteriaceae* in males and frequent isolation of *Escherichia coli*. The rise in multidrug-resistant *Enterobacteriaceae*, particularly ESBL-E and CPE, poses risks such as therapeutic failure, increased morbidity and mortality, and significant economic impacts. To address this, the study emphasizes the importance of early detection, infection control measures, responsible antibiotic prescribing, and collaborative efforts across hospital departments to reduce the spread of resistant strains.

Keywords: *Enterobacteriaceae*; Antibiotic Resistance; Epidemiology

1. Introduction

Enterobacteriaceae are a diverse family of Gram-negative bacteria, including about 30 genera and over 100 species, commonly found in the digestive system and the environment. Some members of this family are part of the normal gut flora, while others can cause infections of varying severity due to their different pathogenic mechanisms. These bacteria are a significant concern in clinical settings, responsible for over 80% of the bacteria isolated in laboratories [1].

Antibiotic resistance within *Enterobacteriaceae* is a growing public health issue, exacerbated by the excessive and repeated use of antibiotics in both humans and animals. This misuse has increased selective pressure, leading to the emergence of drug-resistant strains and rendering many treatments ineffective. Notably, *Escherichia coli* and *Klebsiella pneumoniae* have developed resistance to critical antibiotics, such as third-generation cephalosporins and carbapenems, which has led the World Health Organization (WHO) to classify these resistant strains as priority pathogens in 2017 [2, 3].

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The spread of antibiotic resistance in *Enterobacteriaceae* is driven by various genetic mechanisms, including mutations and horizontal gene transfer between bacteria. Identifying and understanding both natural and acquired resistance mechanisms are essential for accurately interpreting antibiograms and developing effective treatment strategies. The complexity of this task has increased with the emergence of new resistance mechanisms, such as carbapenemase production, and the identification of environmental species in clinical bacteriology [4].

This study aims to establish the epidemiological and antibiotic resistance profiles of *Enterobacteriaceae* isolated at the Avicenne Military Hospital of Marrakech over a two-year period, from May 1, 2020, to April 30, 2022. By evaluating the evolutionary kinetics of these bacteria and comparing them with findings from other studies, the study seeks to update current knowledge and provide recommendations for the prudent use of antibiotics

2. Material and methods

This research is a retrospective descriptive study conducted to analyze the antibiotic resistance profiles of *Enterobacteriaceae* isolated at the Avicenne Military Hospital in Marrakech. The study was carried out in the microbiology laboratory of the Avicenne Military Hospital in Marrakech, spanning a two-year period from May 1, 2020, to April 30, 2022.

Samples were collected from various departments within the hospital, including surgical units and medical departments. Additionally, samples came from the Emergency Department, Intensive Care Unit, and outpatient services.

The specimens included central and peripheral venous catheters, urine samples, vaginal swabs, pus, blood cultures, bronchoalveolar lavage fluids, sputum, ascitic fluids, biopsies, pleural fluids, semen cultures, and osteosynthesis materials.

All diagnostic bacteriological samples received at the microbiology laboratory were included, except for duplicate samples from the same patient showing identical sensitivity profiles and samples collected during epidemiological investigations.

Bacterial identification was based on morphological, cultural, biochemical, and antigenic characteristics. The laboratory employed automated broth microdilution antibiograms using the *BD Phoenix*® system for precise identification of bacterial strains and their antibiotic susceptibility. Additionally, the standard disk diffusion method on Mueller-Hinton agar was used, following the CASFM 2022 guidelines.

2.1. Antibiotic Sensitivity Testing

- Detection of ESBL-Producing *Enterobacteriaceae*: Automated systems flagged potential ESBL-producing strains, which were further confirmed using phenotypic methods such as the synergy test on Mueller-Hinton agar and the combined disk method. These tests involved placing antibiotic-impregnated disks to observe enhanced inhibition zones indicative of ESBL production.

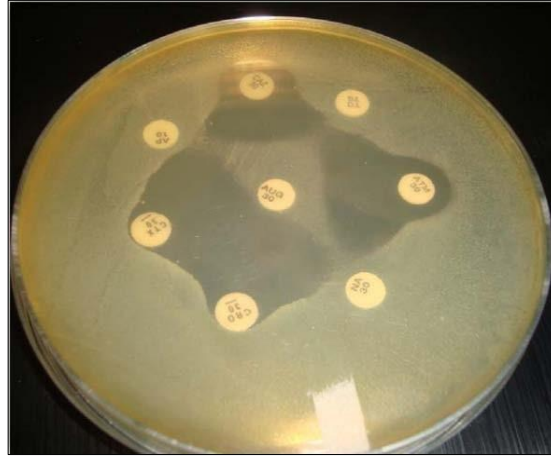


Figure 1 Positive synergy test with a "champagne cork" appearance (Bacteriology Laboratory of AMH)

- Detection of Carbapenem Resistance: For detecting carbapenem-resistant *Enterobacteriaceae* (CRE), phenotypic methods such as the modified Hodge test and inhibitor-based tests (using boronic acid, dipicolinic acid, and EDTA) were employed. These tests detected various carbapenemase types, including KPC, metallo- β -lactamases, and AmpC. The study also used molecular techniques like PCR (GenXpert Cepheid) to confirm carbapenemase production and detect specific resistance genes such as OXA-48, NDM, VIM, and KPC.

These combined methodologies ensured comprehensive detection and analysis of multidrug-resistant bacteria in the hospital setting, highlighting the importance of integrating both phenotypic and molecular techniques for accurate diagnosis and effective infection control.

2.2. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM SPSS Statistics, Armonk, NY, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

3. Results

Over a two-year period (May 1, 2020, to April 30, 2022), 1,626 positive isolates were collected at the laboratory of Military hospital of Marrakech, out of which 1,163 (71.5%) were identified as *Enterobacteriaceae*. The distribution of these bacteria showed a slight predominance in men (53.1%) compared to women (46.9%), with a sex ratio of 1.13.

In terms of the sources of isolation, most *Enterobacteriaceae* were found in samples collected externally (40.6%), followed by samples from medical services (19.6%), surgery services (17.3%), the intensive care unit (ICU) (11.4%), and emergency services (10.7%). Urine cultures (CBEU) accounted for the majority (71.1%) of the isolated *Enterobacteriaceae*, followed by pus samples (17.3%), blood cultures (3.7%), and smaller proportions from bronchoalveolar lavage fluids, sputum, biopsies, central catheters, and various other sources. The distribution of positive isolates varied by hospital unit: external samples were overwhelmingly from CBEU (96.62%), while ICU samples had a more heterogeneous distribution, including CBEU (21.33%), blood cultures, bronchoalveolar lavage fluids (both 20.67%), and diverse suppurations (15.33%).

Overall, *Escherichia coli* was the most frequently isolated species (n=708), followed by *Klebsiella pneumoniae* (n=239) and *Enterobacter cloacae* (n=79). Other less common species included *Proteus mirabilis* (n=31), *Klebsiella oxytoca* (n=26), *Morganella morganii* (n=24), and smaller numbers of *Citrobacter*, *Serratia*, *Providencia*, and *Salmonella* species (n=24). The global antibiotic resistance profile of *Enterobacteriaceae* isolated at the Avicenne Military Hospital of Marrakech shows a high resistance to Amoxicillin (78.61%) and Ticarcillin (78.29%), with moderate resistance to Amoxicillin-Clavulanic acid (61.03%), Trimethoprim-Sulfamethoxazole (40.60%), and Ciprofloxacin (39.40%). Lower resistance rates were observed for Nitrofurantoin (19.48%), Gentamicin (16.39%), Fosfomycin (9.08%), Imipenem (6.75%), Amikacin (4.70%), and Vancomycin (2.56%).

Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) were found in 614 isolates, with *Escherichia coli* as the dominant species (55.37%), followed by *Klebsiella pneumoniae* (28.34%) and *Enterobacter cloacae* (6.84%). The majority of ESBL-E came from CBEU (96.72%), and similar trends were observed across external samples, emergency services, and ICU. Figure II represents the antibiotic resistance profiles of ESBL-producing *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*.

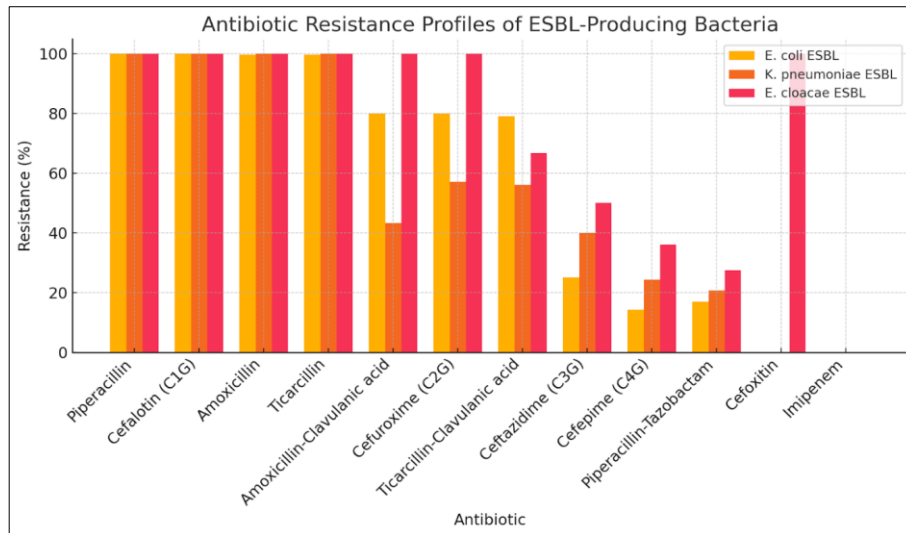


Figure 2 Antibiotic resistance profiles of ESBL-producing bacteria

Carbapenemase-producing *Enterobacteriaceae* (CPE) were less common, with *Klebsiella pneumoniae* being the most frequently isolated species (40.87%), followed by *Escherichia coli* (17.39%) and *Enterobacter cloacae* (15.65%). Most CPE-positive samples in external collections were from CBEU, while ICU samples were again more varied, with blood cultures (28.26%) and bronchoalveolar lavage fluids (23.91%) being the most common. CPE displayed high resistance levels, particularly to Piperacillin (98.78%), Cefalotin (98.11%), Amoxicillin (94.74%), Ticarcillin-Clavulanic acid (90.32%), Ticarcillin (89.11%), and Amoxicillin-Clavulanic acid (88.50%). Resistance to Aztreonam was 77.78%, to Ceftazidime 73.02%, to Cefepime 60.22%, and to Piperacillin-Tazobactam 58.72%. Carbapenem resistance was notable, with rates of 57.47% for Ertapenem, 53.57% for Imipenem, and 50% for Meropenem, while Amikacin resistance remained relatively low at 10%.

4. Discussion

Antibiotic resistance in *Enterobacteriaceae* is a growing global health concern. The World Health Organization (WHO) has identified strains resistant to third-generation cephalosporins and carbapenems as critical pathogens, underscoring the urgency of addressing their spread. Understanding these resistance trends is crucial for updating antibiotic use guidelines and combating the public health threat posed by resistant bacteria. In our study, the male-to-female ratio for *Enterobacteriaceae* isolation was 1.13, similar to studies by SY et al., and SARR et al. from Senegal, with ratios of 1.19 and 1.13, respectively [5,6]. This slight male predominance may be attributed to the male-dominant patient population of the military hospital. However, studies from other regions showed variable sex ratios, including a high male predominance in India (M/F = 2.84) [7] and female predominance in several studies with ratios ranging from 0.51 to 0.8 [8-9]. No significant correlation between gender and *Enterobacteriaceae* isolation was established. Most *Enterobacteriaceae* isolates originated from external samples (40.6%), followed by samples from medical (30.3%), surgical (17.3%), and ICU departments (11.4%). These results partially matched SARR's study in Senegal, where external samples contributed to 53% of isolates [6], and EDDAIR et al. study in Rabat, which showed a lower rate for external samples (33.8%) but a higher rate from medical services (45.9%) [10]. Studies by LAGHA et al., BENTABET et al., and FOULAL et al. reported significantly lower external sample contributions (0-8%) and higher isolation rates from ICU (27-47.3%) [8,13,14]. The observed variations may result from differences in hospital infrastructure and pre-hospital care protocols. Urine samples (CBEU) accounted for 71.1% of the isolates, followed by pus samples (17.3%) and blood cultures (3.7%). While CBEU dominance is consistent with other studies, including those by GUPTA et al. (50%), SARR et al. (56.2%), and others, our study reported a higher CBEU percentage [7,8,10,14]. Pus isolates ranked second across multiple studies, with percentages ranging from 13% to 22%, while blood cultures showed isolation rates of 3.7% to 7% [6,8,14,15].

Escherichia coli was the most frequently isolated species (60.9%), followed by *Klebsiella pneumoniae* (20.6%), *Enterobacter cloacae* (6.8%), and *Proteus mirabilis* (2.7%). These results align with other studies, including those by SY et al., EDDAIR et al., AKEL et al., and JANS et al., where *E. coli* isolation rates ranged between 48.6% and 63.3% [5,10-12]. *K. pneumoniae* was the second most common species across these studies, with rates between 12.8% and 31.3%, while *E. cloacae* showed comparable rates globally, ranging from 6% to 7.8%. *Proteus mirabilis* exhibited more variation, with isolation rates from 3.1% in Senegal to 9% in Belgium. For General Beta-Lactam Resistance, the study revealed high resistance rates to beta-lactams, with 78.61% resistance to Amoxicillin, 78.29% to Ticarcillin, and 61.03% to Amoxicillin-Clavulanic Acid (AMC). These rates align with other studies, particularly those from Rabat, Togo, and Cameroon [10,19,20].

Among the 614 ESBL-producing isolates, *E. coli* was predominant (55.37%), followed by *K. pneumoniae* (28.34%) and *E. cloacae* (6.84%). Similar results were observed in studies by SARR et al., BAUX-POMARES et al., GUILLET et al., and AJDAKAR et al. [6,16,17,18]. The prevalence of *K. pneumoniae* and *E. cloacae* showed some variations between studies but remained consistently high. All the isolates were resistant to Piperacillin and Cefalotin, and nearly 100% resistant to Amoxicillin, Ticarcillin, and AMC. Resistance to Ceftazidime (33.96%) and Cefepime (19.51%) was also observed, matching findings from studies by EDDAIR et al., and GUPTA et al. [7,10] (Table I).

K. pneumoniae was the most frequently isolated CPE (40.87%), followed by *E. coli* (17.39%) and *E. cloacae* (15.65%). These results align with other studies, including those by TIDRARINE et al., JANS et al., LAURENT et al., NANCY et al., and DORTET et al., which also reported *K. pneumoniae* as the dominant CPE species [65,72-75]. CPE isolates demonstrated high resistance to Piperacillin (98.78%) and Amoxicillin (94.74%), with 57.47% resistant to Ertapenem and 53.6% resistant to Imipenem, consistent with national and regional data [11,21,22].

It is crucial to combat the spread of multidrug-resistant Enterobacteriaceae, as antibiotic resistance poses a significant therapeutic challenge. Key strategies include improving adherence to standard precautions, implementing screening and isolation measures, and ensuring appropriate antibiotic management to reduce misuse.

Bacteriology labs play a vital role in detecting these bacteria early, particularly with selective media for third-generation cephalosporin-resistant strains. If carbapenem resistance is suspected, PCR testing should be performed. Any suspected or confirmed case in a hospitalized patient must be immediately reported for necessary containment measures. Key objectives include monitoring endemic multidrug-resistant bacteria and tracking bacterial ecology and resistance trends.

Table 1 Comparison of Resistance Rates of ESBL-Producing *Escherichia coli*

| Author (year) | Our study % | SY Sénégal 2021 [57] % | BAUX-POMARES France 2015 [61] % | BENAISSA Rabat 2021 [79] % | KAMGA Cameroun 2014 [80] % |
|-------------------------------|-------------|------------------------|---------------------------------|----------------------------|----------------------------|
| Pipéracillin | 100.0 | 77.6 | - | - | 76.0 |
| C1G | 100.0 | 56.0 | - | 98.0 | 60.0 |
| Amoxicillin | 99.7 | 82.7 | - | 100.0 | 93.2 |
| Ticarcillin | 99.7 | 82.3 | - | - | 90.3 |
| AMC | 81.6 | 35.3 | 79.0 | 82.0 | 84.5 |
| C2G | 80.0 | - | 98.0 | 99.0 | 69.9 |
| Ticarcillin - Clavulanic Acid | 79.1 | - | - | - | 76.0 |
| C3G | 25.0 | 27.3 | 78.0 | - | - |
| Pipéracillin-Tazobactam | 16.9 | - | 11.0 | 47.0 | - |
| C4G | 14.2 | 26.8 | - | - | - |
| Céfoxitin | 0.0 | 7.6 | 12.0 | 11.0 | - |
| Imipenem | 0.0 | 0.2 | 0.0 | 2.0 | - |

5. Conclusion

Our study, in line with existing literature, confirms the growing significance of multidrug-resistant *Enterobacteriaceae* due to their rising co-resistance to beta-lactams, carbapenems, and other antibiotics. This alarming trend raises concerns about potential therapeutic dead ends, increased morbidity and mortality, and economic burdens, as well as the spread of resistance across hospital departments. It is crucial to implement strict measures to limit the spread of multidrug-resistant strains and reduce their impact.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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