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Enterobacter cloacae complex: An emerging pathogen with increasing rates of antibiotic resistance in a Tunisian hospital

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ABSTRACT

Background: *Enterobacter cloacae* is an opportunistic pathogen responsible for various diseases that may be difficult to handle due to the emergence of resistant strains. Our study aimed to describe the epidemiological profile of *E. cloacae* isolates and to establish an inventory of their antibiotic resistance patterns. **Methods:** A retrospective study, conducted at the Microbiology Laboratory in the Fattouma Bourguiba University Hospital, in Monastir, over a decade. All strains of *E. cloacae* gathered during the study period were included. Bacterial identification was performed by conventional methods. Antibiotic susceptibility study was based on the CA-SFM/EUCAST. **Results:** A total of 1343 strains of *E. cloacae* were collected mainly from the adult population. Strains were mainly gathered from Surgical Departments. Most samples were skin and soft tissue infections. Over a quarter of the strains were considered multidrug-resistant bacteria showing resistance to cephalosporins at 26.2% and to carbapenems at 6.2% of the strains. Carbapenemase-producing strains originated mainly from the General Surgery Department and Surgical Intensive Care Unit Department in 30% and 21% respectively. **Conclusion:** The prevalence of *E. cloacae* is increasing. The emergence of resistance to cephalosporins and carbapenems seems alarming, requiring more effort to limit its emergence.

Introduction

Enterobacter cloacae complex (ECC) is an important *Enterobacteriaceae* widely encountered in the environment [1]. It can be found in soil, water, plants, or food products. In healthcare facilities, *E. cloacae* can persist on inanimate surfaces such as washbasins, antiseptic solutions, or even incubators [1].

ECC presents a wide variety of virulence factors that enable the bacteria to integrate and adapt to the host environment, and to express its infectious potential, such as slime production and secretion of exotoxins [2].

Since the antibiotic discovery, the medical field has been revolutionized, and millions of lives have been saved. However, the rapid emergence of multi-drug-resistant bacteria is emerging as a global health threat [3].

The World Health Organization (WHO) estimated that more than 4 million deaths in 2019 are attributable to antimicrobial resistance (AMR) [4]. ECC is ranked as the third bacteria among *Enterobacteriaceae* responsible for clinical infections [5]. In Tunisia, ECC is emerging as a multidrug-resistant bacteria (MDR), particularly in healthcare-associated infections. Few recent

Tunisian studies have described the antibiotic resistance patterns of ECC strains in our region.

There is a need to provide updated information on the epidemiology and the prevalence of *E. cloacae* in hospital settings. It is also important to study antimicrobial susceptibility patterns of ECC within Fattouma Bourguiba University Hospital of Monastir.

Materials and methods:

A retrospective study, conducted over 10 years (from 1st January 2013 to 31st December 2022). All *E. cloacae* strains isolated in the University Hospital of Fattouma Bourguiba in Monastir, have been included. Clinical samples from both inpatients (including surgical, medical, pediatrics, emergency, and critical care departments) as well as outpatients were gathered.

Our target population covered all patients presenting a documented infection by *E. cloacae*. Data were gathered mainly from the laboratory records that accompanied the samples. For every infected subject, we evaluated the following parameters: Age, gender, corresponding ward, specimen type, and antimicrobial susceptibility profile.

Sample types were various, depending on clinical symptoms and suspected infection sites, such as urine, blood, body fluid (Cerebrospinal, peritoneal, pleural, or articular), skin, and soft tissue (wound or abscess), or biomedical devices. All samples gathered at the laboratory of microbiology were analyzed following standard protocols established by the French Society of Microbiology. *Enterobacter cloacae* were identified using conventional microbiology methods. Antimicrobial susceptibility testing was conducted using the disc diffusion tests on Mueller-Hinton agar. For colistin susceptibility, minimal inhibitory concentrations were determined by the standard broth microdilution method. For results interpretation, it was performed according to the CA-SFM/ EUCAST criteria (for the corresponding study year). Based on the available data, *E. cloacae* isolates categorized as "Susceptible, Increased exposure" to a molecule were considered resistant in our study.

Results:

During the study period, we collected a total of 1343 *E. cloacae* strains representing an average of 134 isolates per year in the Fattouma Bourguiba University Hospital of Monastir. A descriptive analysis revealed some key findings.

Prevalence of *E. cloacae* in the region of Monastir

Sé pou our laboratory, *E. cloacae* represented an overall prevalence of 3.5% among 38407 isolated bacteria. From 2013 to 2022, isolation rates of this species decreased between 2020 and 2021, while *E. cloacae* were significantly the most prevalent in 2022 by 4.4% as illustrated in the following **table 1**.

Characteristics of the infected patients

The median age was 44 years. The distribution of infected patients revealed that adults aged between 45 and 65 were the most susceptible to *E. cloacae* (positivity rate of 31%). The majority of isolates came from males (60.2%) with a sex ratio of 1,5.

Distribution of isolates according to the department

The proportion of *E. cloacae* strains among inpatients was significantly higher, particularly in surgical departments (41.2%), followed by intensive care units (ICU) (18.1%). **Table 2** shows the distribution of strains according to the housing department.

Distribution according to sample type

E. cloacae may cause a wide variety of clinical infections: It was mainly involved in skin and soft tissue infections by 42.3% as it was isolated in 569 sample cultures. Urinary tract infections were in the second row (28%). We also registered 150 cases of documented bloodstream infections by *E. cloacae* representing a ratio of 11.2% (Table 3).

Antimicrobial resistance pattern

For β -lactams, the highest resistance rates were observed for penicillin (ticarcillin and piperacillin) by 32%. Around a quarter of our isolates (26%) were resistant to third-generation cephalosporins (3GC). Overall resistance to carbapenems was found to be 6.2%. For the rest of the antibiotics, resistance levels for cefepime, ciprofloxacin, and gentamicin were respectively 17.3%, 16.4%, and 17.1% (Table 4).

Multi-drug-resistant *E. cloacae* by carbapenem resistance:

Among 1227 tested isolates, 76 *E. cloacae* strains (6.2%) were non-susceptible to at least one carbapenem. For a better reflection of the kinetics of carbapenem-resistant *E. cloacae* (CREC), we defined the following parameters:

- **The ratio:** Quotient of CREC strains among the entirety of *E. cloacae* isolates per year.
- **The prevalence:** Quotient of CREC strains among the total of Gram-negative bacilli acquiring resistance to carbapenems.

Analyzing the incidence rate of ECC infections over 10 years, a significant increase was observed since 2017 from 5.3% to 25.3% (**Figure 1**). Surgical departments harbored 38 carbapenem-resistant strains corresponding to 50%, followed by

ICU wards at 34.2% (**Table 5**). These multi-drug-resistant strains grew mostly from urine cultures at 48.7% and skin and soft tissue infections (18.4%) (**Table 6**). Furthermore, high levels of resistance regarding other antimicrobial agents were seen amid CREC reaching 68% for gentamicin and 75% for ciprofloxacin, restricting the therapeutic options (**Table 7**).

Table 1. Prevalence of *E. cloacae* per year.

Year	Number of <i>E. cloacae</i> isolates	Total of isolated species	Prevalence (%)	P value
2013	141	4432	3.2	0.2
2014	157	4153	3.8	0.3
2015	123	3119	3.9	0.2
2016	123	3313	3.7	0.5
2017	134	3900	3.4	0.8
2018	157	4105	3.8	0.2
2019	146	4750	3.1	0.1
2020	101	3488	2.9	0.07
2021	94	3358	2.8	0.08
2022	167	3789	4.4	0.005
Total	1343	38407	3.5	

Table 2. Distribution of *E. cloacae* isolates according to the housing hospital.

Department	<i>E. cloacae</i> isolates (n) (%)	Total of positive cultures	Prevalence (%)
Surgical Departments	553 (41.2%)	9487	5.8
Intensive care unit (ICU)	244 (18.1%)	3833	6.4
Departments of Medicine	198 (14.7%)	9519	2.1
Pediatric Department	69 (5.1%)	2623	2.6
Emergency Department	133 (10%)	5531	2.4
Outdoor patients	102 (7.6%)	3361	3
Other regional hospitals	44 (3.2%)	1876	2.3
Total	1343 (100%)	36230	3.7

Table 3. Distribution of *E. cloacae* isolates according to sample type.

Sample type	<i>E. cloacae</i> isolates (n) (%)	Total of positive cultures	Prevalence (%)
skin and soft tissue infections	569 (42.3%)	10137	5.6
Urine	376 (28%)	16144	2.3
Blood cultures	150 (11.2%)	3270	4.6
Respiratory	115 (8.6%)	3676	3.1
Biomedical devices	50 (3.7%)	1264	4
Body fluids	45 (3.6%)	972	4.6
Other*	38 (2.7%)	767	4.9
Total	1343 (100%)	36230	3.7

*: Ear swabs or drainage cultures [n=23, (1.7%)], vaginal swabs [n=4, (0.3 %)], eye swabs [n=5, (0.4%)], rectal multi-drug resistant (MDR) bacteria screening [n=4, (0.3%)], biopsy [n=2, (0.1%)].

Table 4. Antimicrobial susceptibility pattern of *E. cloacae*.

Antimicrobial agent	Non-susceptible <i>E. cloacae</i> isolates (n) (%)
Ticarcillin (N=1284)	412 (32.1%)
Piperacillin (N=1194)	390 (32.7%)
Ticarcillin-clavulanic acid (N=1220)	353 (28.9%)
Piperacillin-tazobactam (N=1243)	247 (19.8%)
Cefotaxime (N=1104)	283 (25.6%)
Ceftazidime (N=1185)	307 (26%)
Cefepime (N=359)	62 (17.3%)
Ertapenem (N=1226)	76 (6.2%)
Imipenem (N=1163)	46 (4%)
Meropenem (N=235)	9 (3.8%)
Colistin (N=521)	4 (0.7%)
Amikacin (N=1230)	35 (2.8%)
Gentamicin (N=854)	146 (17.1%)
Ciprofloxacin (N=1162)	190 (16.4%)
Levofloxacin (N= 566)	85 (15%)
Cotrimoxazole (N=309)	72 (23.3%)
Tigecycline (N=201)	25 (12.4%)
Nitrofurantoin (N=258)	176 (68.2%)
Fosfomycin (N=221)	16 (7.2%)
Chloramphenicol (N=409)	42 (10.3%)

*N=number of tested isolates

Table 5. Distribution of carbapenem-resistant *E. cloacae* isolates by department.

Department	Carbapenem resistant <i>E. cloacae</i> isolates (n) (%)	Total of carbapenem resistant bacteria	Prevalence (%)
Surgery departments:	38 (50%)	286	13.3
Digestive surgery	23 (30%)	120	19
Orthopedic surgery	10 (13.2%)	52	19
Other	5 (6.6%)	61	8.2
Intensive care units:	26 (34.2%)	371	7
Anesthesia-resuscitation	16 (21%)	237	6.7
Medical reanimation	10 (13.2%)	121	8.3
Departments of Medicine	8 (10.5%)	137	5.8
Pediatric Department	2 (2.6%)	46	4.3
Emergency Ward	1 (1.3%)	27	3.7
Outdoor patients	1 (1.3%)	80	1.6
Total	76	947	8

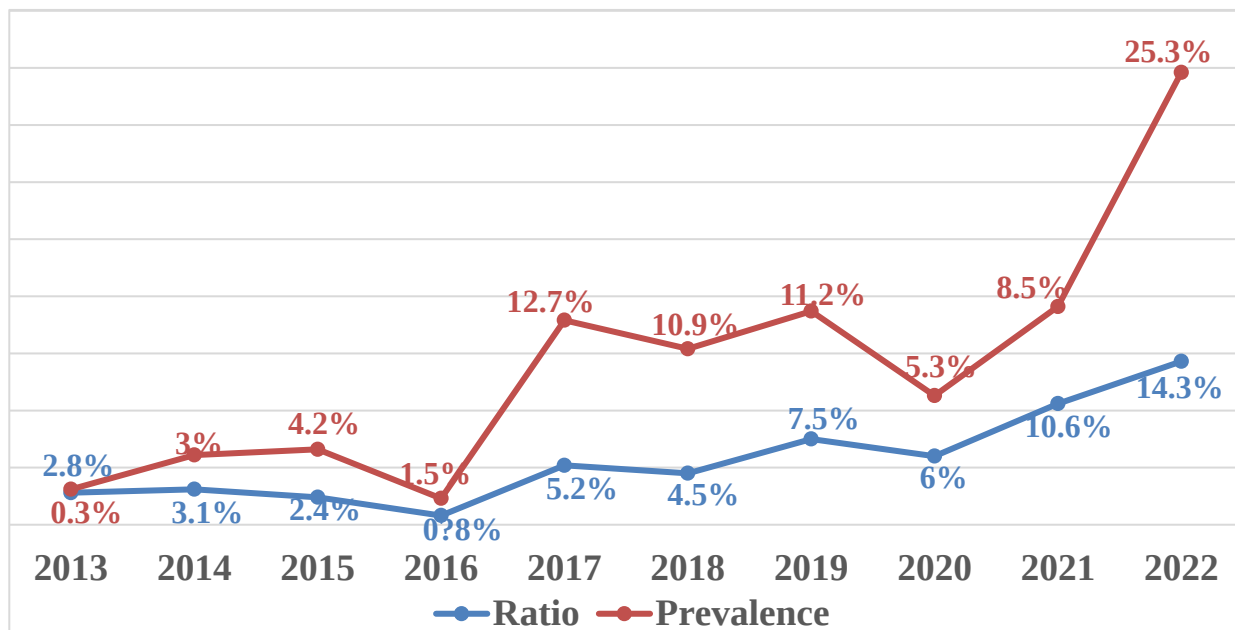
Table 6. Distribution of carbapenem-resistant *E. cloacae* strains according to sample type.

Specimen type	CREC isolates (n) (%)	Number of carbapenem-resistant samples	Prevalence (%)
Urine	37 (48.7%)	249	14.8
skin and soft tissue infections	14 (18.4%)	228	6.1
Respiratory	10 (13.2%)	206	4.8
Blood cultures	8 (10.5%)	111	7.2
Biomedical device	5 (6.6%)	108	4.6
Body fluids	1 (1.3%)	14	7.1
Biopsy	1 (1.3%)	31	3.2
Total	76	947	8

*N=number of tested isolates

Table 7. Associated resistance to other antimicrobials of CREC.

Antimicrobial agent	Number of resistant <i>E. cloacae</i> isolates (n)	Percentage (%)
Gentamicin (N=50)	34	68%
Amikacin (N=72)	11	15.3%
Ciprofloxacin (N=68)	51	75%
Colistin (N=50)	3	6%
Cotrimoxazole (N=66)	39	59%

Figure 1. kinetics of carbapenem-resistant *E. cloacae* isolates each year.

Discussion

Enterobacter cloacae species have great genetic plasticity and a major pathogenic role [6]. The dynamic process of this microorganism facing environmental changes allows it to acquire, under stressful conditions, virulence factors to survive [6]. Being an opportunistic agent, some pathovars of *E. cloacae* can transform into specific pathogens involved in various infections [6].

This microorganism represented 3.5% of bacterial pathogens isolated in our laboratory during the study period. It was isolated mainly from urine and skin and soft tissue infection cultures. The Orthopedic Surgery Department was on top of the list as the major department housing 175 isolates (13.2%), followed by the General Surgery Department with 162 *E. cloacae* strains (12%). A significant rise in multi-drug resistant strains has been noted since 2017.

E. cloacae strains represented 3.5% of all the isolated bacteria in our microbiology laboratory. Several studies worldwide revealed similar prevalence rates between 2% and 5.5% [7-9]. An American study focusing on injured soldiers during wars in Iraq and Pakistan showed a very high prevalence of *E. cloacae* between 2009 and 2014 reaching 18% [10].

The annual prevalence of this agent remained stable until 2020. It decreased reaching the lowest level of 2.8% between 2020 and 2021. This was probably due to the burden of the COVID-19 pandemic and the restricted hospital admission policies. The global outbreak of the SARS-CoV-2 virus led to worldwide lockdowns along with the application of strict disinfection procedures. These containment measures showed a significant and sustained outcome on pathogens transmission modes [11].

Our data revealed that approximately one-third (31%) of our infected population involved adults aged between 45 and 65 years for both genders. Elderly patients (>65 years old) represented 25.5% of the strains. A Colombian study reported similar findings [12]. It is also important to notice that the neonatal population was misrepresented in our research since Fattouma Bourguiba Medical Complex did not include a maternity department.

In our present study, *E. cloacae* was mostly isolated from indoor patients by 79.2%. Surgical wards and ICU departments harbored the majority of isolates by 41.2% and 18.1% respectively. Our results were in line with most studies revealing that *E. cloacae* is a pathogen acquired mainly in surgical environments [13].

It was difficult in our study to classify all the documented infections as community or healthcare-acquired due to the lack of data such as their medical history or recent admission in medical care facilities. Although this pathogen is usually found in healthcare settings, its pathogenicity is yet to be proved due to the lack of research unveiling it [5]. Such patients are generally fragile, having severe co-morbidities. They may undergo major surgeries and invasive procedures. All these factors can change the patient's microbiota, prompting bacteria to express virulence factors.

Amoxicillin-clavulanate is frequently prescribed in the post-operative context. This explains the vulnerability of patients in the surgical department to contract this pathogen since *E. cloacae* has a chromosomal cephalosporinase (*AmpC*) that confers intrinsic resistance to aminopenicillins and their combination with clavulanic acid [1].

E. cloacae was isolated from skin and soft tissue infections and urine culture in 42.3% (n=569) and 28% (n=376) respectively. According to the literature, this bacterium has a wide pathogenic potential. It can be responsible for bacteremia, endocarditis, septic arthritis, skin and soft tissue infections, lower respiratory infections, urinary tract infections, and intra-abdominal infections [14]. Skin and soft tissue infections remained predominant causing a major problem in low to middle-income countries [15]. Lack of hygiene, difficulties in water access, and overcrowding in such regions are the main predisposing factors [15].

An overall increase in antimicrobial resistance was observed when analyzing antimicrobial susceptibility trends. AMR is a natural phenomenon currently accelerated by several factors such as the overuse of antimicrobials. Moreover, the COVID-19 pandemic represented also a burden by the expansion of empiric antibiotics prescriptions for hospitalized patients, whether for prevention or management of secondary bacterial infections.

Our strains presented resistance to third-generation cephalosporins in 26.2%. Recent national data from the “L’Antibioreistance en Tunisie” network analyzed in 2022 revealed that 27.9% of isolates showed resistance to third-generation cephalosporins [16], a resistance rate slightly higher than ours. Disparities of AMR rates between different regions of the world were noted

where the highest levels reached 57.1% resistance in Ethiopia and 39% in France [17,18].

Additional studies are required to elucidate resistance mechanisms. It is crucial to pursue the molecular characterization of multi-drug resistance *Enterobacteriaceae*. Data established by other studies emphasizing that *bla*_{TEM-1}, *bla*_{CTX-M15}, and *bla*_{SHV} are the major genes implicated in third-generation cephalosporins resistance by producing Extended Spectrum β -lactamase (ESBL) [19]. Another mechanism of resistance is the hyperproduction of chromosomal *AmpC*.

Seventy-six strains of *E. cloacae* (6.2%) were resistant to carbapenems. According to Tunisian national data, resistance to ertapenem reached an overall rate of 14.3% [16].

Our findings were in line with several studies demonstrating a resistance rate varying from 1% in Italy to 18.4% in Iran [20,21]. Almost all CREC strains (n=74) were isolated from hospitalized patients by 97.4%. The highest resistance rates were recorded in surgical departments (50%). The spread of MDR clones was also obvious in ICU reaching 34.2%. A Chinese study also highlighted that the spread of such strains is an endemic hospital-related phenomenon [22].

In our study, CREC isolates grew essentially from urine samples at 48.7%, followed by skin and soft tissue infections (18.4%) and respiratory sample cultures (13.2%). *Enterobacter cloacae* may be a result of common-source epidemics [23]. Bacterial transmission can occur through contaminated surfaces. Caregivers may also play an important role as vectors from one patient to another. Carbapenem-resistant *Enterobacterales* can also arise from endogenous flora (Skin, oropharyngeal, or gut microbiota). A Brazilian study conducted in an adult intensive care unit proved that a patient out of six was colonized by a carbapenem-resistant *Enterobacterales* associated with a considerable risk of developing invasive infections [24]. In Tunisia, molecular characterization revealed an insidious rise of class D carbapenemase, OXA-48 [25]. These enzymes are difficult to detect by phenotypic methods, due to their low hydrolysis of carbapenems with no activity on some expanded-spectrum cephalosporins. Plasmids and mobile genetic elements can also confer resistance to other classes of antimicrobials by carrying additional resistance genes targeting fluoroquinolones or aminoglycosides [26].

Associated resistance rates among CREC strains to ciprofloxacin, gentamicin, and amikacin were 75%, 68%, and 15.3% respectively. Limited treatment options have a major impact, with serious clinical consequences such as increased mortality and longer hospital stays [27].

According to the guidelines of the European Committee of Antimicrobial Susceptibility Testing, *E. cloacae* has intrinsic heterogeneous resistance to colistin with a key mutation of the *mcr-1* gene [28]. Some promising antimicrobials have recently been ceftazidime-avibactam, ceftolozane-tazobactam, and cefiderocol, while others are still undergoing clinical trials. The goal of developing new therapies is to limit the molecular machinery enabling the global spread of MDR strains [29].

Our study represented some limitations consisting mainly of the lack of clinical features and the analysis of risk factors for patients prone to *E. cloacae* infections. Since it was a retrospective study, some data were non-recoverable along with a shortage of antimicrobial discs for susceptibility testing.

The strength of this study was the fact that it covered 10 years, serving as a base for the epidemiological status of *E. cloacae* infections in the region of Monastir.

limitations:

This study is one of the very few to focus on ECC, and the global understanding of bacterial resistance trends, particularly in Tunisia. Understanding these aspects is of major interest for public health and infection control. The major limitation of this study is the lack of clinical data; a medical record has recently been implemented in our hospital. Future studies will allow us to investigate the clinical risk factors for ECC infection.

Conclusions

Although the overall prevalence of *E. cloacae* was only 3.5%, such a pathogen is currently raising concern as it is associated with high levels of antimicrobial resistance, emphasized by the outbreak of the COVID-19 pandemic in 2020. The global spread of CREC strains is an emerging public health threat. Therefore, an adapted approach should be implemented to curb the dissemination of these resistant pathogens. It is necessary to engage several actors in an antimicrobial stewardship program to

influence policy development and improve practices.

List of abbreviations:

CREC: Carbapenem resistant *Enterobacter cloacae*

ECC: *Enterobacter cloacae* complex

ESBL: Extended spectrum betalactamase

ICU: Intensive care units

Conflicts of Interest.

The authors declare no conflicts of interest.

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None

Data availability

All data generated or analyzed during this study are included in this published article.

Authors' contribution

All authors made significant contributions to the work presented, including study design, data collection, analysis, and interpretation. They also contributed to the article's writing, revising, or critical evaluation, gave final approval for the version to be published.

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