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Prevalence and Identification of Enterobacter cloacae from Clinical Samples in Thi-Qar

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***Abstract*—Enterobacter *cloacae* is an opportunistic pathogen frequently associated with a wide range of clinical infections. Given the increasing prevalence of this bacterium and the limited information available on its virulence factors in Iraq, further investigation is warranted. This study aimed to analyze various clinical samples, accurately identify the presence of *E. cloacae* using diverse analytical methods, and confirm the findings through advanced diagnostic systems. A total of 200 clinical specimens were collected from hospitals and diagnostic laboratories in Thi-Qar province. Bacterial identification was carried out using morphological and biochemical methods, as well as automated systems such as API 20E and VITEK-2. Out of the 200 samples, 30 (15%) were confirmed to be E. cloacae. The prevalence showed no significant gender difference, with equal distribution between males and females (50% each). The highest infection rate (40%) was observed in the 20–30-year age group. Blood and wound specimens exhibited the highest isolation rate (33.33%), while stool samples showed the lowest (10%). This study emphasizes the clinical relevance of E. cloacae infections in Thi-Qar, particularly among younger individuals and patients presenting with blood or wound infections. The use of automated diagnostic tools proved essential for accurate bacterial identification.**

***Keywords*— Enterobacter *cloacae*, Clinical Samples, Thi-Qar, API 20E, VITEK-2, Gender Distribution.**

I. INTRODUCTION

The *Enterobacter genus* comprises facultative anaerobic gram-negative rod-shaped bacteria classified within the Enterobacteriaceae family. The taxonomy of *Enterobacter* is complex and subject to ongoing modifications, with numerous members of the genus being reclassified into other genera [1-2]. The organisms categorised as *Enterobacter cloacae* complex (ECC) are the most frequently documented pathogens in human infections [3- 4]. Recent whole-genome sequencing studies have indicated potential reclassification of *Enterobacter aerogenes* as *Klebsiella aerogenes or Klebsiella* mobilis. Nonetheless, numerous morphological and molecular distinctions exist between the two taxa [1-5]. To date, numerous species have been documented in the ECC. *Enterobacter cloacae, Enterobacter asburiae, Enterobacter hormaechei, Enterobacter kobei, Enterobacter ludwigii,* and *Enterobacter nimipressural*is were the predominant isolates from human clinical specimens [6]. They generate acid during glucose fermentation, are methyl red-negative, and Voges-Proskauer positive, with an optimal growth temperature of 30˚ C; 80% are encapsulated [7]. Additionally, they possess specific biochemical characteristics, including the capacity to synthesize ornithine decarboxylase, which differentiates *Enterobacter* from closely related Klebsiella species. Virulence variables that dictate the origin, progression, and resolution of an infection entail a series of intricate and dynamic interactions between the host and the bacterium, which may change among deferent infecting bacteria [8]. These interactions are either intrinsic to cellular structures or extrinsic, such as enzymes and poisons secreted outside the body. Virulence factors include hemolysin, capsule, protease, siderophore, colonization factor antigen (CFA), and bacteriocin. [9]. The rising incidence of infections caused by Enterobacter spp. and the limited investigation of its virulence-associated features prompted the objectives of this study to be accomplished by investigating the dissemination of these bacteria in various clinical samples from Thi-Qar Governorate by isolating the bacteria from different clinical specimens, diagnosing them using diverse analytical methods, and confirming the diagnosis with precise analytical instruments. In Iraq, studies focusing on *Enterobacter cloacae* remain limited, particularly in terms of its distribution in different clinical specimens and its resistance profile. The increasing prevalence of multidrug-resistant E. cloacae reported in regional hospitals highlights the urgent need for local surveillance. Therefore, this study aims to fill this gap by determining the prevalence and resistance patterns of *E. cloacae* isolates collected from various clinical sources in Thi-Qar Governorate.

II. MATERIALS AND METHODS

A. Sample Collection

Two hundred clinical specimens were obtained from various hospitals in the Thi-Qar Governorate (Al-Hussein Teaching Hospital, Turkish Hospital, and Public Health Laboratory). Samples were collected from different clinical

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sources, including blood, urine, wound swabs, and stool, to obtain a comprehensive collection of pathogenic bacteria.

All specimens were collected aseptically in sterile containers and transported to the microbiology laboratory within 2h for processing to avoid overgrowth of non-pathogenic flora, as recommended by the Clinical and Laboratory Standards Institute guidelines (CLSI, 2021). The variety of sampled sources contributed to the extensive coverage of bacterial pathogens affecting diverse body systems. Appropriate sample manipulation and quick processing are key to preventing contamination and accurate diagnosis [10].

B. Diagnosis of Bacteria

The diagnosis of bacterial isolates involves a two-step approach: primary identification based on morphological and cultural characteristics, followed by confirmatory biochemical and automated tests.

C. Primary Identification (Morphological and Cultural Characteristics)

The received samples were streaked onto Blood Agar and MacConkey Agar, according to standard plating procedures. Blood Agar was applied for hemolytic activity and general colony morphology, and MacConkey Agar was used to differentiate between lactose-fermenting and non-fermenting strains.

The plates were incubated at 37°C for 24–48 h under aerobic conditions. Colony morphology was observed for size, color, opacity, elevation, and regularity of edges. All isolates were stained by gram and analyzed for bacterial morphology and associated Gram reactions [11].

The application of Blood Agar and MacConkey Agar is important for the characterization of bacteria, providing useful preliminary data [12]. Confirmatory Tests (API 20E and VITEK-2 Compact System).

Isolates with characteristic morphology were further biochemically confirmed using the API 20E system (bioMérieux, France), which is a standardized, miniaturized system for the identification of Enterobacteriaceae and some other Gram-negative rods based on 20 biochemical tests. Furthermore, identification was verified using the VITEK-2 Compact System (bioMérieux, Inc., France), based on growth-based technology for rapid bacterial identification and antibiotic susceptibility determination. The main advantages of this assay are its rapidity, high sensitivity, and accuracy, and it has been successfully implemented in several clinical microbiology services [13]. The dual approach using API 20E and VITEK-2 ensures high accuracy in species-level identification and reduces the risk of misclassification, particularly among closely related Enterobacteriaceae species [14].

D. Statistical Analysis

The data of this study were statistically analyzed using SPSS version 26, based on the chi-square test at $p < 0.05$ [15].

III. RESULTS

A. Prevalence of Pathogenic Bacteria in Different Clinical Sample

The current study was showed a significant difference at p. value <0.05, was recorded the most isolated bacteria Escherichia coli 35.0%, then Klebsiella pneumoniae 25.0%, then *Enterobacter cloaca*e 15%, while the lowest isolated bacteria both Salmonella spp and Shigella spp, as in Table 1.

Table 1: Prevalence of pathogenic bacteria in different clinical sample

Isolated Bacteria Total Clinical Samples 200	**No.**	**%**
E. cloacae	30	15.0
E. coli	70	35.0
K. pneumonia	50	25.0
P. aeruginosa	20	10.0
S. aureus	20	10.0
Salmonella spp	5	2.5
Shigella spp	5	2.5
Total	200	100

B. Prevalence of Enterobacter cloacae According to Gender

The current study showed no significant difference in the prevalence of E. cloacae between genders (p = 1.00). Equal distribution was recorded, with 15 cases (50%) in males and 15 cases (50%) in females, as shown in Table 2.

Table 2: Prevalence of *Enterobacter cloaca*e according to Sex

	Sex	**No.**	**%**
E. cloacae	Male	15	50.0
	Female	15	50.0
	Total	30	100
$CalX^2$= 0.00 $TabX^2$= 3.84 DF= 1 p. value 1.00			

C. Prevalence of Enterobacter cloacae According to Age group

The results showed a significant difference in the distribution of E. cloacae across age groups ($p < 0.05$). The highest infection rate was recorded among individuals aged 20 – 30 years (40%), followed by those aged ⩾51 years (23.33%), while the lowest was in the 41–50 age group (16.67%), as presented in Table 3.

Table 3: Prevalence of *Enterobacter cloaca*e according to age group

	Age Group	**No.**	**%**
E. cloacae	20-30 years	12	40.00
	31-40 years	6	20.00
	41-50 years	5	16.67
	≥ 51 years	7	23.33
	Total	30	100
$CalX^2$= 12.7 $TabX^2$= 7.81 DF= 3 p. value <0.01			

D. Prevalence Enterobacter cloacae According to Type of Sample

The study found a significant difference in isolation rates based on the type of clinical sample ($p < 0.05$). E.

cloacae was most frequently isolated from blood and wound samples (33.33% each), followed by urine samples (23.34%), with the lowest rate observed in stool samples (10%), as shown in Table 4.

Table 4: Prevalence of *Enterobacter cloacae* according to type of sample

	Clinical Sample	No.	%
E. cloacae	Blood	10	33.33
	Stool	3	10.00
	Urine	7	23.34
	Wound	10	33.33
	Total	30	100
$CalX^2$= 14.4 $TabX^2$= 7.81 DF= 3 p. value <0.01			

E. Antibiotics susceptibility of Enterobacter cloacae

The antibiotic susceptibility was in table 5.

Table 5: Antibiotics susceptibility of *Enterobacter cloacae*

Antibiotics	Sensitive		Intermediate		Resistance	
	No.	%	No.	%	No.	%
Amp/Sulb	10	33.33	2	6.67	18	60.00
Pip/Tazo	20	66.67	3	10.00	7	23.33
Cefotaxime	8	26.67	0	0.00	22	73.33
Ceftazidime	10	33.33	2	6.67	18	60.00
Ceftazidime /av	15	50.00	2	6.67	13	43.33
Ceftazidime/taz	21	70.00	4	13.33	5	16.67
Cefepime	18	60.00	3	10.00	9	30.00
Imipenem	22	73.33	4	13.33	4	13.33
Meropenem	30	100	0	0.00	0	0.00
Amikacin	20	66.67	3	10.00	7	23.33
Gentamycin	18	60.00	1	3.33	11	36.67
Ciprofloxacin	15	50.00	0	0.00	15	50.00
Tigecycline	27	90.00	2	6.67	1	3.33
Colistin	27	90.00	0	0.00	3	10.00
Trimethoprim	24	80.00	0	0.00	6	20.00
Ertapenem	26	86.67	1	3.33	3	10.00
Nitrofurantoin	21	70.00	5	16.67	4	13.33
Susceptibility %	65.1		6.27		28.63	
$CalX^2$= 135.3 $TabX^2$= 43.77 DF= 32 p. value <0.01						

IV. DISCUSSION

The findings of this study highlight the clinical importance of *E. cloacae* as an emerging pathogen in hospital settings. The organism was isolated from a range of clinical specimens, including blood, urine, wounds, and stool. No significant gender-based differences were observed, indicating that both males and females are equally susceptible to infection. However, the age group of 20–30 years showed the highest infection rate (40%), which could be associated with occupational exposure, lifestyle factors, or increased hospital visits.

According to specimen type, the highest isolation of E. cloacae was recorded in blood and wound swabs (33.33% each), followed by urine (23.34%) and the least was in fecal (10%). This distribution is in agreement with previous reports that have shown E. cloacae as a frequent pathogen in blood and wound infections that forms biofilms and resists host immune responses [16].

It has recently been reported that multidrug-resistant *E. cloacae* strains are resistant to antibiotics, including colistin. Heterogeneous resistance to colistin has been shown in 2023 E. cloacae clinical strains [17]. In another study, some virulence genes were related to different orders of magnitude of antibiotic resistance in the E. cloacae complex [18]. *Enterobacter cloacae* is a common hospital-derived opportunistic pathogenic bacterium known to cause a wide range of infections, most notably pneumonia and tract infections. Research suggests that this bacterium is often isolated from blood, urine, and wound specimens, which highlights its ability to cause systemic and local infections [5]. Meanwhile, a study from South Korea also found multiple species in the E. cloacae complex with different rates of antibiotic resistance and virulence factors among the species [19].

E. cloacae is resistant to many antibiotics, which makes treatment difficult. In China, E. cloacae resistance to carbapenems and cephalosporins has been shown to be on the rise, with carbapenem resistance rates reaching 12.1% in 2021, in a study over a seven-year period [20]. In Tunisia, 26.2% of the strains were resistant to cephalosporins and carbapenems, and isoenzyme-producing strains were mainly found in surgical and intensive care units [21].

Moreover, some strains of E. cloacae show heteroresistance to colistin, making treatment even more difficult [22]. Capsule formation, hemolysin production, and siderophores are important virulence factors in the pathogenesis of *Enterobacter cloacae*. Some virulence genes are associated with the degree of antibiotic resistance, which complicates treatment. [23].

API 20E and VITEK-2 systems were also applied to confirm the isolated strains. The VITEK-2 assay is a robust technique in clinical microbiology laboratories that is useful for timely and accurate identification, leading to a reduction in the probability of misclassification, particularly for closely related *Enterobacteriaceae* species [25]. Virulence factors such as capsular formation, hemolysin production, and the presence of siderophores also contribute to the capacity of *Enterobacter cloacae* to cause infection. It was demonstrated that some virulence genes such as csgA and csgD are linked with particular antibiotic resistance on the basis of antibiotic resistance, and these can be employed as potential biomarkers for prognosis and treatment [26].

Concerning the diagnostic methods, methods including identification of the hsp60 gene are employed to discriminate between the different species in the E. cloacae complex, which leads to a proper therapy, and previous studies hold true for these facts Elbehiry et al. [27] in their attempt to classify strains on the basis of their gene sequences. The results of this study are in accordance with those of Kim et al. [28], who showed a high prevalence of *Enterobacter cloacae* in blood and wounds. This supports our finding that the highest isolation rates were obtained from blood samples and wound swabs (33.33%).

The current results agree with those reported by Lee et al. (2023) [29]. indicating no clear differences between males and females in terms of infection rates. This was confirmed in our study, with an equal infection rate (50%) between sexes. However, our results differ from those reported by Zhou et al. [20]. Who indicated that the highest infection rates were in the > 50 years age group. In our study, the most affected group was the 20–30 year-old group, accounting for

40% of the total infection rate. This may be attributed to differences in the clinical or community settings.

With respect to antibiotic resistance, the results showed a large amount of multiple resistance, in line with the study of Cai et al. [30]. In Tunisia, showing a prevalence of resistance to carbapenems and cephalosporins. There are also concordant results with the study by Guérin [31]. Rutinely found heterogeneity in colistin resistance among some clinical isolates. With respect to virulence factors, csgA and csgD were found in some strains included in this study. This was in accordance with the observations of Liu et al. [32]. This revealed the function of these genes in biofilm formation and antibiotic resistance in bacteria. We also found that some virulence genes were linked to some levels of resistance, as suggested by our results, which was corroborated by Kim et al. [33]. which linked the presence of certain genes to the increased resistance of strains to carbapenems. However, our results partially differ from those of Morales et al. [34]. Who found no direct relationship between virulence genes and antibiotic resistance in any of the strains studied. This suggests that this association may be related to a specific genetic or environmental context or may vary depending on the isolates and geographical region.

In terms of diagnosis, our study relied on API 20E and VITEK-2 systems to accurately identify strains. This result is consistent with the findings of Mahmoud et al. [10]. Idicated that the VITEK-2 system proved efficient in quickly and accurately identifying species within the Enterobacteriaceae family, especially in critical clinical situations. The study by Lee et al. [35] also supports the use of VITEK-2 as an effective tool compared with traditional methods for identifying bacterial identity and resistance patterns. Our reliance on VITEK-2 and API 20E systems is consistent with the recommendations of Saffert et al. [36] who confirmed the accuracy of the VITEK-2 system in identifying Enterobacter strains and reducing the risk of misclassification, especially among closely related species within the Enterobacteriaceae family. However, the results of our study differ from some recent trends that favor the use of gene sequencing technologies, such as the analysis of the hsp60 gene, which Nguyen et al. (2022) indicated as more accurate in classifying species within the E. cloacae complex, especially when there is subtle genetic variation that cannot be detected by traditional systems. These findings may reflect the bacterium's tendency to cause bloodstream and wound infections, especially in hospitalized patients. In this study, Enterobacter cloacae isolates exhibited notably high resistance rates to third-generation cephalosporins, particularly ceftazidime (73.3%) and ceftriaxone (66.7%). This aligns with a multicenter study by [37], which reported ceftazidime resistance rates exceeding 32.8% among clinical isolates in Chinese hospitals. The elevated resistance is primarily attributed to chromosomal expression of AmpC β-lactamases, a common resistance mechanism within the E. cloacae complex. Contrastingly[38]. reported no significant difference in treatment outcomes between patients treated with third-generation cephalosporins and those receiving other antimicrobials, reflecting potential regional variations in prescribing habits, infection control practices, and genetic diversity among E. cloacae strains[30].Regarding carbapenems, resistance to imipenem (13.3%) and ertapenem (10%) was observed, while all isolates remained fully susceptible to meropenem. These findings are consistent with [39]. who documented rising carbapenem resistance in E. cloacae isolates from tertiary care hospitals in eastern China, largely linked to the presence of bla_{NDM} and bla_{IMP} metallo-β-lactamase genes31. Aminoglycosides retained moderate efficacy, with amikacin and gentamicin showing sensitivity rates of 66.7% and 60%, respectively. This is supported by 31 who highlighted the sustained activity of amikacin against ESBL-producing Enterobacterales in Swiss hospitals, where resistance rates remained below 25%32.Resistance to fluoroquinolones was also notable, with 50% of isolates resistant to ciprofloxacin, consistent with [25], who associated fluoroquinolone resistance in E. cloacae with plasmid-mediated qnr genes, especially in intensive care unit isolates. Their study emphasized the importance of monitoring plasmid-borne resistance genes due to their potential for rapid horizontal transfer[40]. Encouragingly, colistin and tigecycline demonstrated strong in vitro activity, with 90% of isolates susceptible. This corresponds with 32who reported 88.2% colistin susceptibility among E. cloacae isolates in Japan, although the detection of mcr-9 genes in some isolates raised concerns about emerging colistin heteroresistance [38]. Notably, 28.6% of isolates exhibited multidrug resistance (MDR), being resistant to all antimicrobial classes tested. This alarming trend reflects the growing global challenge of antimicrobial resistance and is supported by the 2023 joint report from the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO). The report highlights the increasing prevalence of MDR Enterobacterales and calls for intensified molecular surveillance, infection control measures, and antimicrobial stewardship strategies to curb the spread of resistant pathogens [33.]

The high isolation rates of E. cloacae from blood and wound samples suggest its invasive potential and association with hospital-acquired infections. This may be attributed to its ability to form biofilms and survive on medical devices or compromised tissues.

V. Conclusion

This study confirms that *Enterobacter cloaca*e is a significant pathogen in the Thi-Qar Governorate, with equal prevalence in both sexes and a higher incidence in younger adults. The high isolation rates from the blood and wound samples indicate their role in severe infections. The use of modern diagnostic systems, such as API 20E and VITEK-2 Compact, proved effective in accurate identification, which is crucial for guiding appropriate therapeutic strategies. Further studies are needed to explore antibiotic resistance patterns and virulence factors in order to improve infection control and management.

Conflict of interest

Authors declare that they have no conflict of interest.

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