Original Article

Identification of Imipenem and Fosfomycin Resistance Genes in Enterobacter cloacae from Various Samples at a Tertiary Care Hospital in Bangladesh

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Abstract

Background: *Enterobacter cloacae is a significant pathogen that has been isolated from numerous clinical infections. Multidrug resistant (MDR) E. cloacae strains are increasing worldwide and limiting therapeutic options. This study's objective was to detect the fosfomycin and imipenem resistance genes among E. cloacae isolated from the patients of a tertiary care hospital in Bangladesh.* ***Materials and Methods:*** *This was a descriptive observational study conducted in the department of Microbiology, Dhaka Medical College, in collaboration with in-patient departments of Dhaka Medical College Hospital (DMCH) from January 2022 to December 2022. A total of 382 patients of different age and sex was selected by purposive sampling for this study. Wound swabs and pus, urine, blood and endotracheal aspirate were collected from the selected patients. The antimicrobial resistance pattern was determined for all isolated E. cloacae strains by disc diffusion method. Imipenem resistance gene (blaKPC) and fosfomycin resistance genes (fosA3 and fosC2) were detected by PCR using specific primers from Fosfomycin resistant and Imipenem resistant E. cloacae strain.* ***Results:*** *Among 382 samples, 247 (64.66%) were culture positive. Organisms were isolated and identified by culture, gram staining, and biochemical tests, of which 31 isolates were E. cloacae. Most of the isolates showed resistance to β-lactam inhibitors, the extended spectrum of cephalosporins, fluoroquinolones, amikacin, gentamicin, and carbapenems. However, the least resistant drug was tigecycline (35.48%). The imipenem resistance gene blaKPC was detected in 7 out of 22 imipenem-resistant E. cloacae isolates (31.82%). Additionally, fosfomycin resistance genes fosA3 and fosC2 were identified in 6 (30%) and 2 (10%) isolates, respectively, among the 20 fosfomycin-resistant E. cloacae samples.* ***Conclusion:*** *There is an ongoing need for surveillance programs to develop effective treatment strategies to combat antimicrobial resistance (AMR). It is essential to understand the prevalence of these resistance genes and the underlying factors contributing to their emergence to mitigate the burden of AMR.*

Keywords: Antimicrobial resistance (AMR), Enterobacter cloacae, bla-KPC, fosA3, fosC2.
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Introduction

The family *Enterobacteriaceae* includes the genus Enterobacter, which is a widespread group of Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacteria[1]. Among Enterobacteriaceae isolated from hospital-acquired infection, after *Escherichia coli* and *Klebsiella species*, *Enterobacter species* are listed as the third most common isolate[2]. Although several species of *Enterobacter* can cause human disease, *E. cloacae* and *E. aerogenes* account for the majority of *Enterobacter*-related infections[3].

Enterobacter spp. is increasingly associated with multidrug resistance, including resistance to the last resort carbapenems[4]. Among the two epidemics of carbapenem-resistant bacteria in the United States, the second is the epidemic caused by carbapenem-resistant *E. cloacae* (CR-Ecl)[5]. Fosfomycin is one of the few antibiotics available to treat infections caused by carbapenem-resistant Enterobacteriaceae (CRE)[6]. Although fosfomycin-inactivating enzymes currently play a limited to moderate role in the development and spread of fosfomycin resistance, their presence on transferable plasmids could become a major contributor of resistance dissemination in the future[7].

Due to the rising clinical significance of *E. cloacae*, it is critical to investigate the genetic mechanisms underlying their resistance patterns. This study specifically focuses on detecting the presence of the resistance genes responsible for imipenem and fosfomycin resistance in carbapenem-resistant and fosfomycin resistant *E. cloacae* strains isolated from clinical settings.

Materials and Methods

This descriptive observational study was conducted in the department of Microbiology, Dhaka Medical College in collaboration with inpatient departments

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of Dhaka Medical College Hospital (DMCH) during the period from January 2022 to December 2022. Ethical approval was gained from the Ethical Review Committee (ERC) of DMC (Ref: DMC/ECC/2022/34).

Study population and sample collection*:* A total of 382 samples of different ages and genders according to inclusion and exclusion criteria were selected by purposive sampling. Three categories of patients of different ages and sex were included in this study, namely:

- Samples including urine, wound swabs, pus, and blood were collected from adult patients presenting with symptoms indicative of infection.
- Endotracheal aspirate (ETA) samples were collected from adult patients having suspected clinical infections and mechanical ventilation for more than 48 hours in intensive care unit (ICU).
- Urine, blood, wound swab and pus samples received from patients in the microbiology department for culture and sensitivity.

Microbiological methods: The organisms were isolated from the specimen by inoculation and culture on blood agar and MacConkey agar media. The identification of the organisms was carried out using colony morphology, Gram staining, and a series of standard biochemical tests. Samples with a significant colony count were only included for analysis. Among the culture-positive samples, *Enterobacter* sp. was successfully identified through a specific biochemical test. Subsequently E. cloacae were distinguished from E. *aerogenes* with precision. Susceptibility to antimicrobial agents of *E. cloacae* was determined by modified Kirby-Bauer disc diffusion technique using Mueller-Hinton agar media and zones of inhibitions were interpreted according to Clinical Laboratory Standard Institute guideline (CLSI, 2021). Fosfomycin and imipenem-resistant *E. cloacae* were tested for the detection of fosA3, fosC2 and blaKPC. Polymerase chain reaction (PCR) was performed using specific primers to identify imipenem resistance and fosfomycin resistance genes.

Data management and analysis: The collected data were checked, verified and edited daily. The data was coded, entered a computer, and analyzed using the SPSS statistical software. Pearson's chi-square test was applied to assess the association between specimen type and gene detection. A p-value of less than 0.05 was considered statistically significant.

Results

A total of 382 samples were collected from different age and sex according to inclusion and exclusion criteria from inpatient department of Dhaka Medical College Hospital. Out of the 382 samples, 247 (64.66%) were culture positive. Figure-1 shows distribution of different *Enterobacter* species identified by Lysine Decarboxylase (LDC) test. Among these, 31 (83.78%) were *E. cloacae* and 6 (16.22%) were *E. aerogenes*. Among the 31 *E. cloacae* samples,18 (58 %) was from urine samples, 6 (19%) from wound swab and pus, 4 (13%) from Endo Tracheal Aspirates (ETA), 3 (10%) from blood (Figure-2).

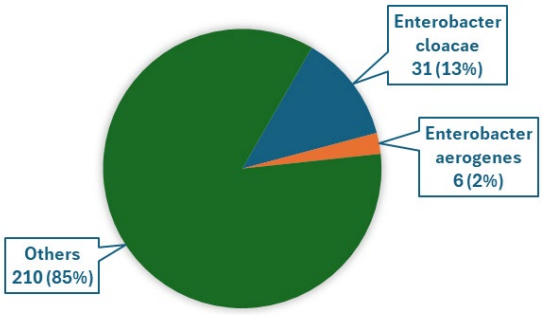

Figure-1: Distribution of *E. cloacae* and *E. aerogenes* in culture positive sample (n=247)

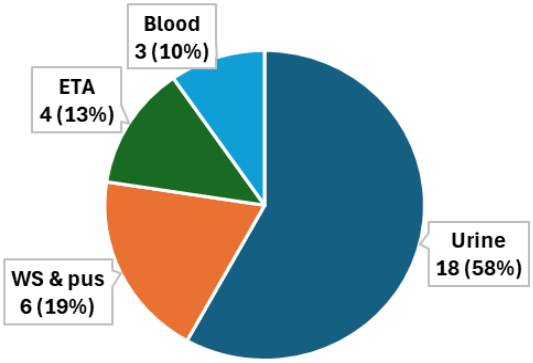

Figure-2: Distribution of *E. cloacae* among different samples (n=31)

Table-I: Antibiotic resistance pattern of isolated *Enterobacter cloacae* (n=31)

Antimicrobial Drugs	Resistant n (%)
Amikacin	24 (77.42)
Amoxiclav	24 (77.42)
Aztreonam	20 (64.52)
Piperacillin/tazobactam	22 (70.97)
Ceftazidime	28 (90.32)
Ceftriaxone	29 (93.55)
Ciprofloxacin	26 (83.87)
Nitrofurantoin	14 (77.78)
Gentamicin	24 (77.42)
Colistin	19 (61.29)
Imipenem	22 (70.97)
Fosfomycin	20 (64.52)
Tigecycline	11 (35.48)

Table-I shows an antimicrobial susceptibility pattern of the isolated *E. cloacae* which represents results as resistant. Among 31 isolated *E. cloacae*, the highest proportion of organisms 93.55% were resistant to ceftriaxone, 35.48% showed lowest resistance to tigecycline. Out of these, 20 (64.52%) showed resistance to fosfomycin, and 22 (70.96%) were resistant to imipenem.

Table-II: Detection of *bla*-KPC gene among Imipenem resistant *Enterobacter cloacae* by PCR in different samples (n=22)

Samples	Gene *bla*-KPC positive, n (%)	Total	p- value
Urine	5 (38.46)	13	χ^2=2.29 p= 0.515
Wound swab & Pus	2 (40.00)	5	
ETA	0 (0.00)	3	
Blood	0 (0.00)	1	
Total	**7 (31.82)**	**22 (100%)**	

Table-II shows that a total of 22 fosfomycin-resistant *Enterobacter cloacae* isolates were screened for the presence of the *bla*-KPC gene using PCR. The gene was detected in 7 (31.82%) of the isolates. Among different clinical samples, bla-KPC was most frequently detected in isolates from urine (38.46%), followed by wound swab and pus samples (40.00%). No bla-KPC gene was detected in isolates from endotracheal aspirates or blood samples. A chi-square test was performed to evaluate the association between sample type and the detection of the bla-KPC gene. The result showed no statistically significant association (p=0.515), which indicates that the distribution of bla-KPC among different specimen types was not significantly different.

Table-III: Detection of *fos*A3, *fosC*2 genes among Fosfomycin resistant *Enterobacter cloacae* by PCR in different samples (n=20)

Samples	*fos*A3, n (%)	*fos*C2, n (%)	Total	p-value
Urine	3 (25.00)	1 (8.33)	12	***fos*A3:** χ^2=1.35 p=0.717 ***fos*C2:** χ^2= 1.48 p= 0.687
Wound swab & Pus	2 (50.00)	1 (25.00)	4	
ETA	1 (33.33)	0 (0.00)	3	
Blood	0 (0.00)	0 (0.00)	1	
Total	**6 (30.00)**	**2 (10.00)**	**20 (100%)**	

Out of 20 fosfomycin-resistant *E. cloacae* isolates, the fosA3 gene was detected in (6; 30.0%) isolates, whereas the fosC2 gene was detected in (2; 10.0%) isolates. Among different specimen types, the highest detection rate of $fosA_3$ was observed in isolates from wound swab and pus samples (2; 50.0%), followed by endotracheal aspirate (1; 33.3%) and urine (3; 25.0%). For the *fos*C2 gene, detection was limited to only two sample types: urine 1 (8.33%) and wound swab/pus 1 (25.0%). Pearson's chi-square test revealed no statistically significant association for both genes. These results indicate that the distribution of *fos*A3 and *fos*C2 genes among different clinical samples were not significantly different (Table-III).

Discussion

Enterobacter species are commonly found in the environment and are frequently present in the intestinal microbiota of humans and animals. They showed up as an important pathogen in the Neonatal Intensive Care Unit (NICU). The attributable mortality rates associated with *Enterobacter* infections range from 6% to 40%. Treatment of infection with *Enterobacter* spp. is challenging and broad resistance to third-generation cephalosporins, penicillin, and quinolones is an increasing problem[8-11]. In the present study out of 382 samples, 247 (64.66%) samples were culture positive, of which 37 (14.98%) were *Enterobacter* species. A National Nosocomial Infection Surveillance system (NNIS) in India showed that *Enterobacter* accounts for 5-11% of all Noso-positive acquired blood, wound, respiratory tract infection, and urinary tract infection[12,13]. Among 37 isolates of *Enterobacter species*, 31 (83.78%) were identified as *Enterobacter cloacae* and 6 (16.22%) were identified as *Enterobacter aerogenes* by biochemical tests. Furthermore, a study conducted in India by Sujatha, et al[14] reported that 77.94% of the isolates were *E. cloacae*, while 22.05% were *E. aerogenes*. This similarity in findings may be due to

the studies being carried out in the same geographic region.

In the present study, commonly used antibiotics were used on isolated *Enterobacter cloacae*. Imipenem resistance in *Enterobacter cloacae* was 70.96%. Imipenem resistance among these species shows significant variation across different regions of the world. Khajuria, *et al*[15] from India reported 53.8% resistance to imipenem for *Enterobacter spp.* Whereas Adwan, *et al*[16] from Palestine showed a resistance rate as low as 12.2%. The rising frequency of imipenem-resistant *Enterobacter* spp. in Bangladesh may be linked to the increased use of imipenem in clinical settings.

Among the 31 Isolated *Enterobacter cloacae*, 64.52% were resistant to fosfomycin in this study. A study in India by Gopichand, *et al*[17] reported that 36% *of Enterobacter spp.* were resistant to fosfomycin which is lower than the present study. This difference is probably influenced by variations in antibiotic use patterns, healthcare systems, and the prevalence of bacterial strains with resistance mechanisms. Among the fosfomycin resistant *Enterobacte*r *cloacae,* 30% were positive for *fos*A3, and 10% were positive for *fos*C2. A study in China by Hameed, *et al*[18] reported that 23.08% *of Enterobacter cloacae* isolates were positive for fosA3. The plasmid, pKP46 carries nine genes (fosA among them) conferred resistance to several antibiotics including penicillin's, cephalosporins, fosfomycin, aminoglycosides, quinolones[19]. The increasing resistance to fosfomycin among *Enterobacter cloacae* may be attributed to the presence of multidrug-resistant plasmids. Among imipenem resistant *Enterobacter cloacae*, 31.82% isolates were positive for *bla*KPC genes. According to a study conducted in North Dakota, USA, between December 2011 and December 2012, among 19 isolateed *E. cloacae*, 17 were positive for *bla*KPC[20]. The low prevalence may have been influenced by this study's small sample size.

This study highlights a worrying trend in antibiotic resistance among Enterobacter cloacae isolates. The rate of imipenem resistance found here (70.96%) is quite a bit higher than what's been reported in other countries like India and Palestine. This could be due to the frequent use - or possibly overuse - of imipenem in local healthcare settings, which might be driving up resistance. Similarly, the fosfomycin resistance rate (64.52%) is also higher than figures reported elsewhere, possibly because of differences in how antibiotics are used, infection control practices, or the types of resistant strains circulating in the region. What's especially concerning is that we found resistance genes like fosA3, fosC2, and bla-KPC in several isolates. These genes are often carried on plasmids, which means they can easily spread between bacteria. Overall, these findings show how important it is to keep monitoring resistance patterns and be more careful with antibiotic use to help slow the spread of these hard-to-treat infections.

Conclusion

Enterobacter cloacae are demonstrating resistance to critical antibiotics such as imipenem and fosfomycin. Several isolates of these bacteria carry resistance genes, including bla-KPC, fosA3, and fosC2. These genes can potentially transfer between different bacterial strains, making infections more challenging to treat. These findings highlight the urgent need for improved antibiotic stewardship and ongoing monitoring of resistance patterns. Raising awareness and taking proactive measures can significantly help in preventing antibiotic-resistant infections.

Conflict of interest

The authors declared that they have no conflict of interests.

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