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Original Research Article

Prevalence of multidrug-resistant urinary isolates of *Enterobacter cloacae* – A three year retrospective analysis

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Abstract

Introduction: Urinary tract infections have emerged as both community acquired and health care associated infection that has caused global concern worldwide. ESKAPE pathogens have ability to evade antibiotics through various antimicrobial resistance mechanism. These MDR pathogens has increasingly been implicated in causing Urinary tract infections. Multidrug resistant *Enterobacter cloacae* have emerged as significant pathogen in causing majority of healthcare associated infections.
Aims and Objective: The main aim of the present study is to investigate the prevalence of multidrug-resistant *Enterobacter cloacae* as a significant pathogen causing urinary tract infection and to determine antibiotic-resistant pattern of *Enterobacter cloacae* among patient attending tertiary care hospital, Vadodara. And to identify MDRO and ESBL producing isolates.
Materials and Methods: This retrospective observational study was carried out at tertiary hospital, Vadodara for period of 3 years after obtaining ethical approval from institutional committee. A total of 1400 patients showing signs and symptoms of urinary tract were included in the present study.
Result: A total of 1400 urine samples were collected from patients having symptoms of urinary tract infections. A total of 195 *Enterobacter cloacae* isolates were obtained from 1400 samples. The urinary catheterization was significant risk factor associated with Enterobacter cloacae infection with p-value 0.001348. In our study there were 100 ESBL producers (51.2%) out of 195 total isolates. In our study prevalence among female patient (59%) was more as compared with male patient (41%). According to age group highest prevalence was among age group of 21-40 years (38.4%).
Out of total 195 *Enterobacter cloacae* isolates, 135 were multidrug resistant. *Enterobacter cloacae* showed highest resistance to ampicillin and third and fourth generation cephalosporins. *Enterobacter cloacae* showed least resistance to Fosfomycin (64.10%) which is drug of choice in our study.
Conclusion: The present study concluded that Urinary catheterization and Diabetes mellitus were significant risk factors leading to urinary tract infections. The current study implicates that proper surveillance system has to be established at rural based hospitals in order to tract emergence of multi drug resistant pathogens and for better patient outcome. And proper infection control measures should be implicated to reduce transmission of multidrug resistant pathogens.

Keywords: Enterobacter cloacae, ESBL, MDRO, Fosfomycin

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1. Introduction

Urinary tract infections have emerged as both community acquired and health care associated infection that has caused global concern worldwide.[1] After COVID -19 pandemic due to antibiotic overuse and misuse significant decrease in antimicrobial susceptibilities was noted. The decrease in susceptibility to various antibiotic such as piperacillin-tazobactam, carbapenems, and colistin is concerning, especially coinciding with the overuse of antibiotics during the COVID-19 pandemic. Such carbapenem resistant MDR Enterobacterals are leading to severe healthcare-associated infections especially urinary tract infection.[2,3]

ESKAPE pathogens have emerged as a significant public health concern worldwide due to acquisition of multidrug resistant gene. It causes spectrum of healthcare associated infections, out of which most common is urinary tract infections. ESKAPE pathogens include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae,*

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Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species.[4,5,9]

Enterobacter cloacae are Gram negative facultative anaerobic bacteria. It is actively motile due to presence of peritrichous flagella. It belongs to Enterobacteriaceae family and shows positive catalase test and negative oxidase test.[6,7] There are currently 22 species of Enterobacter but main pathogenic species include *Enterobacter cloacae, Enterobacter aerogenes,* and *Enterobacter sakazakii.*[7] *Enterobacter ssp.* is primarily associated with various healthcare -related infection and less commonly with community acquired infection such as urinary tract infection, ventilator associated pneumonia, surgical site infections, osteomyelitis and endocarditis.

Enterobacter cloacae have inherited resistance to various antibiotic class like penicillin and fist & second generation of cephalosporins. This is due to acquisition of Amp C gene which codes for enzymes Amp C-type cephalosporinase which results in breakdown of these antibiotics. The genetic mutation of Amp D gene leads to increasing production of these enzymes, which leads to development of resistance to some third-generation cephalosporins and aztreonam.[10,11]

According to annual report of ICMR on antimicrobial resistance and surveillance Enterobacterales were the commonest isolates causing urinary tract infection (74.2%). According to 7 year survey data published by ICMR from year 2017 to 2023 there was increase in incidence of *Enterobacter cloacae* from 1.36% to 1.72%.[2,7]

The current need for present study is that it will help to trace emerging multidrug resistant organism which is leading to complicated urinary tract infection .This study was carried out at rural based tertiary care hospital where there is need for continuous surveillance for controlling spread of drug resistance and optimizing patient outcome. This retrospective study will provide an insight on how we can develop strategies for implementation of antimicrobial stewardship. This study will also guide clinicians in formulating antibiotic policy for our hospital setting.

2. Aims and Objectives

1. To evaluate various risk factors associated with urinary tract infection among both male and female patient
2. To compare isolation rate of *Enterobacter cloacae among* CAUTI and non CAUTI cases
3. To study ESBL producing *Enterobacter cloacae* isolates
4. To study various risk factors associated with MDRO isolates
5. To study antibiotic-resistant pattern of urinary isolates of *Enterobacter cloacae*

3. Materials and Methods

3.1. Study Design

This retrospective observational study was carried out at rural based tertiary hospital, Vadodara for period of 3 years after obtaining ethical approval from institutional committee (SVIEC/ON/MEDI/RP/AUG/2024/02). Total of 1400 patients showing signs and symptoms of urinary tract infection were enrolled in study.

Sample size calculation for prevalence study [$N= Z^2 P(1-P) /e^2$]
N= sample size
Z= 1.96 at 95% confidence interval
e= Margin of Error 5%

3.2. Selection criteria

All patient admitted to inpatient location were included in the present study. Patient fulfilling NHSN criteria were included in CAUTI cases. Urine samples of outpatient were excluded from study. All urine samples with delay in transportation were not included. The urine samples showing mixed bacterial contamination were excluded. UTI secondary to any other site of infection was not included.

3.3. Data collection

Various demographic data like age, gender, associated risk factors (diabetes mellitus, renal calculi, congenital anomalies, malignancy, liver disease, autoimmune disorder, prostatic enlargement etc.) were recorded. Also clinical manifestation, use of antibiotics and patient outcome was noted.

3.4 Microbiological analysis

3.4.1. Sample collection

In case of non-catheterized patient mid-stream urine sample was collected and in patient with indwelling urinary catheter approximately 10ml of urine sample was aspirated from catheter following aseptic precaution.

3.4.2. Processing

Using semi quantitative culture method all urine samples were inoculated on Mac Conkey agar and Hi Chrome agar. Hi Chrome agar was used as differential medium in which chromogenic substrate is cleaved specifically by glucosidase enzyme possessed by *Enterobacter* species resulting in formation of blue-green colonies. All culture plates were aerobically incubated at 37ºc for 24 hours. All pathogens were considered as per Kass concept of significant bacteriuria.

3.4.3. Bacterial identification

All *Enterobacter* isolates were identified based on colony morphology, gram staining characteristics and biochemical

reactions. The species level identification of Enterobacter species was done by automated Vitek 2 instrument.

3.4.4. Antibiotic susceptibility test

The antibiotic susceptibility testing of *Enterobacter cloacae* isolates was done by manual Kirby Bauer disk diffusion method. A bacterial suspension of 0.5 Mc Farland was prepared and then lawn culture done on Muller Hinton agar plates. The zone of inhibition was measured and sensitivity breakpoints were calculated using Clinical and Laboratory Standards Institute guidelines 2024 Further MIC of drug was obtained using Vitek 2 Compact which enhanced our reporting. The antibiotic included Ampicillin, Gentamicin, Amikacin, Meropenem, Imipenem**,** Piperacillin/Tazobactum, Ceftazidime, Cefotaxime, Cefepime, Amoxicillin/clavulanic acid, Ciprofloxacin, Norfloxacin, Nitrofurantoin, Fosfomycin and trimethoprim-sulphamethoxazole.

3.4.5. Detection of ESBL (Extended-spectrum beta-lactamases)

ESBL producing bacteria are resistance to most of the beta-lactam antibiotics which includes penicillin, cephalosporins, and the monobactam drugs such as aztreonam.

In our study for phenotypic confirmation for presence of ESBL two antibiotic disk cefotaxime (30ug) and ceftazidime (30ug) disk with or without clavulanate (10ug) was used. According to CLSI guidelines all 4 drugs were kept on Mueller-Hinton Agar and difference of ≥5 mm between zone diameter of either of cephalosporin disks and their respective cephalosporin/clavulanate disk confirms the ESBL production. For further confirmation Aztreonam disks were used.

3.4.6. MDR (Multidrug resistant)

Multidrug resistant (MDR) isolates are defined as bacteria which are non-susceptible to at least one agent in three or more antimicrobial categories.

3.5 Statistical methods

All data was collected and organized in Microsoft Excel 2019 format and analysis was done using Epi Info statistical software. For analytical analysis of data Chi-square test was performed to establish the significant association between various risk factors. All the study data was presented in form of tables and graphs.

4. Results

A total of 1400 urine sample from patients who showed symptoms of urinary tract infections were included in the present study. Among the total 560 were male and 840 were female patients. Among all patients fever (70%) was most common clinical presentation followed by burning micturition (60.7%), dysuria (53.5%) and frequency (35.7%).

In our present study Urinary catheterization (30.7%) and Diabetes mellitus (25%) were significant risk factors. Among all predisposing factors, presence of renal calculi (15.3%) was third commonest risk factors. Among 560 male patient, 44 (3.1%) had prostatomegaly as risk factor.

Table 1: Evaluation of various risk factors

S.No.	Risk Factor	Total Number of patients (n=1400), %	Male patient (n=560)	Female patient (n=840)
1	Diabetes Mellitus	350 (25 %)	194	156
2	Renal calculi	215 (15.3%)	103	112
3	Congenital abnormality	12 (0.85%)	8	4
4	Malignancy	36 (2.5%)	22	14
5	Liver disease	65 (4.6%)	52	13
6	Autoimmune disorder	21 (1.5%)	6	15
7	Pregnancy	78 (5.5%)	-	78
8	Post Renal transplantation	08 (0.57%)	5	3
9	Prostatic Enlargement	44 (3.1%)	44	-
10	Urinary catheterization	430 (30.7%)	262	168

Out of total 1400 samples 840 were culture positive samples. Out of total 1400 patients 970 were non-CAUTI cases and 430 were CAUTI cases. A total of 195 *Enterobacter cloacae* isolates were obtained from 1400 samples. The urinary catheterization was significant risk factor associated with Enterobacter cloacae infection. The chi-square statistic is 10.275. The *p*-value is .001348. Significant at $p < .05$ (**Table 1**)

Table 2: Prevalence of *Enterobacter cloacae* among catheterized and non-catheterized urine sample

S.No.	Sample	Total	Culture positive	*Enterobacter cloacae isolates*	*P value*
1	Urine samples	1400	840	195	0.00134 Significant
2	Non CAUTI cases	970	545	85	
3	CAUTI cases	430	295	110	

Out of total 195 Enterobacter cloacae isolates, 80 isolates (41%) were from male patients and 115 isolates (59%) were from female patients. **Figure 1**

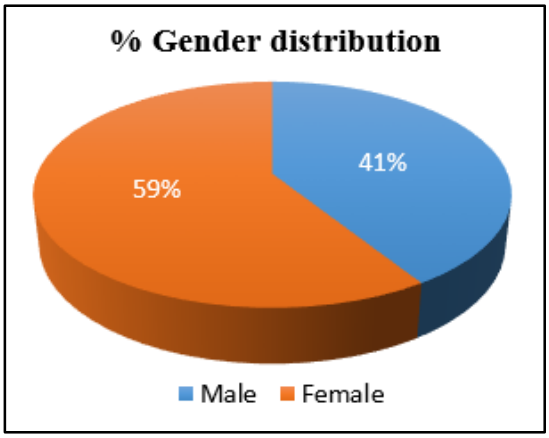

Figure 1: % Gender distribution

According to age wise distribution of *Enterobacter spp*. highest prevalence of 38.4% was observed among age group 21-40 years, followed by 28.2% in age group of 41-60 years, 20.5% in age less than 20 years and 12.8% in age more than 60 years as shown in (**Table 3**)

Table 3: Age wise distribution of *Enterobacter cloacae* isolates

Age wise distribution (years)	Total urine samples (n=1400)	*Enterobacter cloacae isolates (n=195)*	% Isolation of *Enterobacter cloacae*
< 20	340	40	20.5%
21-40	445	75	38.4%
41-60	370	55	28.2%
>60	245	25	12.8%

Out of 195 *Enterobacter cloacae* isolates, total 100 isolates were ESBL (Extended-spectrum beta-lactamases) producers. Out of 100 ESBL producers, 20 were isolated from non-catheterized urine samples and 80 were isolated from catheterized urine sample. Urinary catheterization is not significantly associated with ESBL production (p value=0.0732).(**Table 4**)

Table 4: Total number of ESBL producers

S.No.	Type of sample	Total (n=1400)	*Enterobacter cloacae isolates* (n=195)	*ESBL producers* (n=100)
1	Non CAUTI cases	970	85	20
2	CAUTI cases	430	110	80

Out of total 195 *Enterobacter cloacae* isolates, 135 were multidrug resistant, which are non-susceptible to at least one drug in three or more antimicrobial class. Out of 135 MDRO isolates, 54 were male patients and 81 were female patients. Risk factors were evaluated among MDRO isolates as shown in **Table 5**. P value less than 0.05 were considered significant. Liver disease was significant risk factor associated with MDRO isolate of *Enterobacter cloacae.*

Table 5: Evaluation of risk factors among MDRO Isolates

S.No.	Risk Factor	Total Number of Male patients (n=80)	Male patient With MDRO isolates (n=54)	Male patient With non- MDRO isolates (n=26)	P value
1	Diabetes Mellitus	15	10	5	0.93
2	Renal calculi	5	3	2	0.71
3	Liver disease	12	5	7	0.038 Significant
4	Prostatic Enlargement	5	2	3	0.17
5	Urinary catheterization	18	12	6	0.93
S.No.	**Risk Factor**	**Total Number of Female patients (n=115)**	**Female patient With MDRO isolates (n=81)**	**Female patient With non- MDRO isolates (n=34)**	**P value**
1	Diabetes Mellitus	20	12	8	0.39
2	Renal calculi	12	8	4	0.76
3	Liver disease	6	2	4	0.04 Significant
4	Urinary catheterization	35	26	9	0.54

Enterobacter cloacae showed highest resistance to third and fourth generation cephalosporins. It showed resistant to following carbapenem group of drugs, Meropenem (74.3%) and Imipenem (72.8%). *Enterobacter cloacae* showed least resistance to Fosfomycin (64.1%). Antibiotic resistance pattern is demonstrated in **Figure 2**.

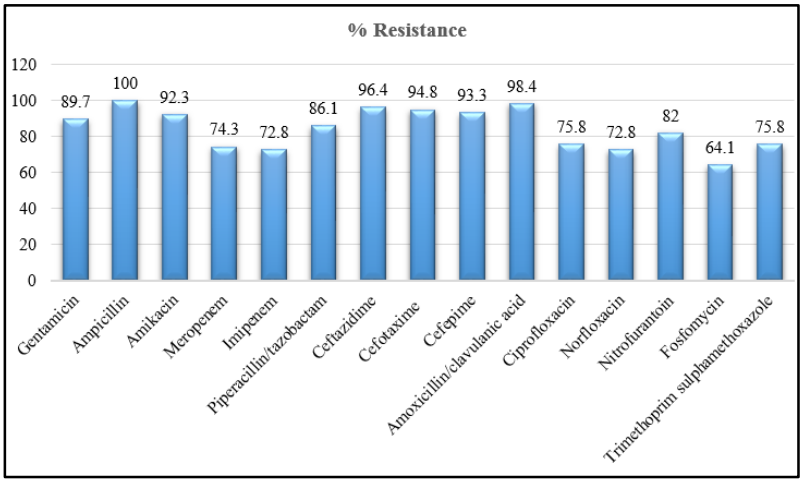

Figure 2: % Antibiotic resistant of *Enterobacter cloacae* isolates

5. Discussion

In our study out of total 1400 samples 840 were culture positive samples. Among the total 560 were male and 840 were female patients. Among all patients fever (70%) was most common clinical presentation followed by burning micturition (60.7%), dysuria (53.5%) and frequency (35.7%).

In our present study Urinary catheterization (30.7%) and Diabetes mellitus (25%) were significant risk factors .In a study by Anna Mary et al[18] Diabetes mellitus was the most common risk factor among both males (61%) and in females (76.3%). Prostatic enlargement (56.1%) was common factor which resulted in a complicated UTI among male patient.

Out of total 1400 samples 970 were non catheterized urine sample and 430 were catheterized urine sample. A total of 195 (13.9%) *Enterobacter cloacae* isolates were obtained from 1400 samples. *The urinary catheterization was significant risk factor associated with Enterobacter cloacae infection with p*-value 0.001348.

In a study conducted by Ayman et al. 2024 (12), 2300 urine samples were collected from patient showing urinary tract infection symptoms. Out of 2300 samples, 189 (8.21%) *E. cloacae* isolates were obtained. Out of 189 isolates, 82 (43.3%) were ESBL producers .In this study female patient accounted for 55.14% and male patients accounted for 46.345% and highest prevalence was among age group of 0-10 years (24.87%).

In our study prevalence of *E. cloacae* was 13.9% which is higher than study by Ayman et al. The difference in prevalence rate may be due to change in hospital environment and infection control practices. In our study there were 100 ESBL producers (51.2%) out of 195 isolates. In our study female patient accounted for 59% and male patient accounted for 41 %. In contradiction to study by Ayman et al, in our study prevalence was highest in age group of 21-40 years (38.4%).

Out of total 195 *Enterobacter cloacae* isolates, 135 were multidrug resistant, which are non-susceptible to at least one drug in three or more antimicrobial class. Out of 135 MDRO isolates, 54 were male patients and 81 were female patients. Among MDRO isolates liver disease was significant risk factor among male (P value=0.038) and female patient (P value=0.04)

Enterobacter cloacae showed highest resistance to ampicillin and third and fourth generation cephalosporins. It showed resistant to following carbapenem group of drugs, Meropenem (74.3%) and Imipenem (72.8%). *Enterobacter cloacae* showed least resistance to Fosfomycin (64.1%). In recent study carried out by Jain et al.2021[13] concluded that *E. cloacae* showed 100% resistance to ampicillin and amoxicillin-clavulanic acid. In study by Stock et al.[14] *E. cloacae* showed highest resistance to first and second generation cephalosporins. However, study by Dehkordi et al.[15] concluded that only 20% were susceptible to imipenem.

6. Conclusion

The present study concluded that Urinary catheterization and Diabetes mellitus were significant risk factors leading to urinary tract infections. There were 100 ESBL and 135 MDRO out of total 195 Enterobacter cloacae isolates. In our study we also found that Liver disease was significant risk factor for MDRO isolates, thus proper screening of such patients must be done. The current study implicates that proper surveillance system has to be established at rural based hospitals in order to tract emergence of multi drug resistant pathogens and for better patient outcome. And proper infection control measures should be implicated to reduce transmission of multidrug resistant pathogens.

7. Ethical Consideration

This study was approved by institute ethical approval committee with ref. no. SVIEC/ON/Medi/RP/Aug/24/2

8. Source of Funding

None

9. Conflict of Interest

None

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