

Original Article

Antimicrobial Resistance Pattern of *Enterobacter* Species Isolated from Different Clinical Specimens in a Tertiary Care Hospital of Bangladesh

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Abstract

The present study was undertaken to determine the antimicrobial resistance pattern of *Enterobacter* species to guide the clinician in selecting the best antimicrobial agent for an individual patient. A total of 50 clinical isolates of *Enterobacter* species were collected from different clinical specimens at the microbiology laboratory of BSMMU between August, 2018 and September, 2019. The two main species of *Enterobacter*, *E.cloacae* and *E.aerogenes* were identified by biochemical tests. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method and reported according to CLSI guidelines. Majority (56%) of the isolated *Enterobacter* were *E.cloacae*, 40% were *E.aerogenes* and 4% were other species. The *Enterobacter* isolates showed relatively high resistance rates to the cephalosporins including cefoxitin (82%), cefixime (62%), ceftazidime (46%) and ceftriaxone (46%). Resistance to the carbapenems and aminoglycosides was relatively low. The high resistance rates of *Enterobacter* species to multiple antibiotics makes it necessary for antimicrobial susceptibility testing to be conducted prior to antibiotic prescription.

Key words: *Enterobacter*, antimicrobial susceptibility, *E.cloacae*, *E.aerogenes*

Introduction

Enterobacter species are gram negative, facultative anaerobic rods belonging to the family Enterobacteriaceae. Strains belonging to the genus *Enterobacter* are important opportunistic nosocomial pathogens. Although there are several species of *Enterobacter* that can cause human disease, *E.cloacae* and *E.aerogenes* account for majority of the *Enterobacter* related infections.^{1,2} *Enterobacter* have an intrinsic resistance to ampicillin and narrow spectrum cephalosporins. These bacteria possess inducible chromosomally encoded betalactamases and through plasmid mediated resistance, are becoming resistant to many classes of antibiotics, including third generation cephalosporins and carbapenems.^{3,4,5} Resistance of *Enterobacter* spp. to 3rd generation cephalosporins is most typically caused by overproduction of AmpC beta lactamase that is induced by third generation cephalosporins. These factors pose a challenge in treating *Enterobacter* infections specially in the hospital setting.

This study was aimed to determine the antimicrobial resistance pattern of *Enterobacter* species isolated from different clinical specimens. Also, common species of *Enterobacter* causing various infections was identified. This would provide important information regarding the empiric therapy of *Enterobacter* infections and also reduce treatment failure in hospitalized patients.

Materials and Methods

Over a period of one year (August 2018 to September 2019), a total of 50 *Enterobacter* isolates were collected from different samples of urine, blood, wound swab, pus, CSF, tracheal aspirate and peritoneal fluid in the laboratory of Microbiology department of Bangabandhu Sheikh Mujib Medical University, Dhaka.

Antimicrobial susceptibility testing was carried out on the *Enterobacter* isolates by Kirby Bauer disc diffusion method⁶ using Mueller Hinton agar and commercially available antibiotic discs (Oxoid Ltd, UK). The antibiotics used were mecillinam (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), cefoxitin (30 µg), cotrimoxazole (1.25/23.75 µg), nitrofurantoin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), gentamicin meropenem (10 µg), aztreonam (30 µg), cefoxitin (30µg),

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cefixime (30 µg), piperacillin-tazobactam (100/10 µg), tigecycline (15 µg), colistin (10 µg) and polymyxin B (200 µg) was used. The disc content and the zone of inhibition was used as recommended by the Clinical Laboratory Standards Institute (CLSI, 2019).⁷ The zone diameters for colistin, polymyxin B and Tigecycline are not included in CLSI guideline, 2019. In case of tigecycline, zone of inhibition was calculated using European Committee on Antimicrobial Susceptibility testing EUCAST 2016 criteria.⁸ The disc zone diameters were interpreted according to the CLSI 2007 guidelines for colistin (resistant ≤10 mm and susceptible ≥11 mm) and polymyxin B (resistant ≤11 mm and susceptible ≥12mm). Susceptibility testing for Tigecycline, Polymyxin B and colistin was done for isolates that showed resistance to all the antimicrobial agents included in CLSI guideline, 2019. *E.coli* ATCC 25922 was used for quality control.

Two species of *Enterobacter*, *E.cloacae* and *E.aerogenes* were identified using several biochemical tests including sugar fermentation test for Adonitol, D-sorbitol, L-rhamnose and Esculin and by two decarboxylation reactions- Arginine dihydrolase test and Lysine decarboxylase test. Species other than these two were categorized as others.

Results

Figure I showed the distribution of isolated *Enterobacter* into different species. Out of 50 *Enterobacter* isolates, 28 (54%) were *E.cloacae* and 20 (42%) were *E. aerogenes*. Two isolates (4%) were categorized as others.

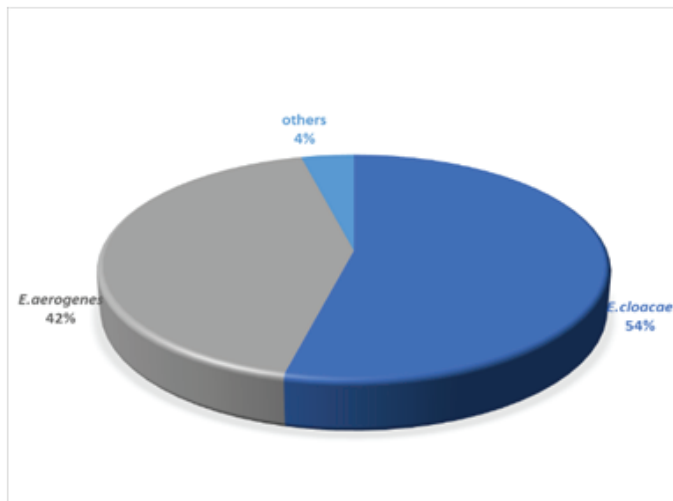


Figure-I: Distribution of *Enterobacter* isolates into different species

Table I: showed the distribution of *Enterobacter* isolated from different type of samples. Out of 50 *Enterobacter* isolates, 34 (68%) were isolated from urine, 7 (14%) from blood, 4 (8%) from wound swab and 1(2%) isolate each from pus, sputum, tracheal aspirate, CSF and peritoneal fluid. Majority of *Enterobacter* were isolated from urine.

Table-I: Distribution of *Enterobacter* spp. isolates among different clinical specimens (n=50)

Species	Sample								Total
	Urine	Wound swab	Blood	Pus	Sputum	Tracheal aspirate	CSF	peritoneal fluid	
<i>E.cloacae</i>	21	2	5	00	00	00	00	00	28
<i>E.aerogenes</i>	12	1	2	1	1	1	1	1	20
Others	1	1	00	00	00	00	00	00	02
Total	34	4	7	1	1	1	1	1	50

Table-II: Antimicrobial resistance pattern of *Enterobacter* spp. isolates

Antimicrobial agent	<i>E.cloacae</i> n=28 (%)	<i>E.aerogenes</i> n=20 (%)	Total n=50 (%)
Ceftazidime	10 (35.7)	12 (60)	23 (46)
Cefuroxime	12 (42.9)	14 (70)	27 (54)
Cefoxitin	22 (78.6)	17 (85)	41 (82)
Cefixime	15 (53.6)	15 (75)	31 (62)
Ceftriaxone	10 (35.7)	12 (60)	23 (46)
Co-trimoxazole	8 (28.6)	9 (45)	18 (36)
Ciprofloxacin	8 (28.6)	9 (45)	18 (36)
Nalidixic acid	11 (39.3)	12 (60)	25 (50)
Nitrofurantoin	15 (53.6)	07 (35)	22 (44)
(urinary isolates only, n=34)			
Gentamicin	09 (32.1)	05 (25)	15 (30)
Amikacin	06 (21.4)	07 (35)	14 (28)
Aztreonam	09 (32.1)	10 (50)	20 (40)
Meropenem	4 (14.3)	06 (30)	11 (22)
Netilmicin	6 (21.4)	7 (35)	14 (28)
Piperacillin-tazobactam	7 (25)	7 (35)	15 (30)
Ticarcillin-clavulanate	14 (50)	14 (70)	30 (60)
Colistin (n=10)	0	2 (20)	2 (20)
Polymyxin B (n=10)	0	2 (20)	2 (20)
Tigecycline (n=10)	0	0	0

Intermediate sensitive was considered resistant as MIC of drug was not evaluated

Discussion

Enterobacter are normal flora of the human gastrointestinal tract and are also found as commensals in the environment. In recent years, *Enterobacter* has turned out to be an important agent of nosocomial infections. In this study, out of isolated 50 *Enterobacter*, *E.cloacae* (54%) and *E.aerogenes* (42%) were most common. Only 2% were other species. This finding is similar to studies done by others. A study in Iran reported 77.1% isolated *Enterobacter* as *E.cloacae* and 22.8% isolates as *E.aerogenes*.⁹ Another study in China reported 68.2% blood isolates were *E.cloacae* and 26.4% blood isolates were *E.aerogenes*.¹⁰

Most of the *Enterobacter* isolates (68%) were obtained from urine in this study. This finding is similar to the findings reported in a review article.¹¹ Another study in Iran reported 11.5% *Enterobacter* were isolated from urine. In this study, 7(14%) *Enterobacter* isolates were obtained from blood. The study in Iran reported 18% *Enterobacter* isolates were obtained from blood.¹²

In case of antimicrobial resistance, high level of resistance were detected to cefoxitin, cefixime, cefuroxime, ceftazidime and ceftriaxone (82%, 62%, 54%, 46% and 46% respectively) in this study. Similar results were reported by a study in Nepal for ceftazidime, cefixime and ceftriaxone (55.8%, 61.8% and 58.8% respectively).¹³ This may be due to the production of β -lactamases, which may be encoded either chromosomally or on plasmids.

In the present study, 36% of *Enterobacter* isolates were resistant to co-trimoxazole and ciprofloxacin. A study in Nepal reported similar rate of resistance for these agents (40% and 44.1% respectively).¹⁴ In this study, resistance of *Enterobacter* to gentamicin, amikacin and piperacillin-tazobactam was 30%, 28% and 30% respectively. A study in Nepal reported resistance rate of *Enterobacter* to amikacin and piperacillin-tazobactam was 35.2% and 23.5% respectively.¹³ This may be due to widespread use of broadspectrum antibiotics in our hospitals. Resistance of *Enterobacter* to meropenem in this study was found 22%. This finding differs with the studies of Nepal which reported meropenem resistance 8.8% and imipenem resistance was 10.5%.^{9,13} Higher percentage of resistance in this study may be due to higher rate of use of meropenem in our hospitals. In this study, majority of urinary *Enterobacter* spp. (64.7%) isolates were resistant to nitrofurantoin. A study in Iran reported 57.1% urinary *Enterobacter* isolates were resistant to nitrofurantoin.¹² Another study in Nepal reported 55.5% urinary isolates were resistant to nitrofurantoin.¹³ This may be due to injudicial prescription of nitrofurantoin in UTI without doing the sensitivity testing in our country.

Resistance rate of *E.aerogenes* to most of the antibiotics was higher than *E.cloacae* isolates, in this study. Resistance rate of *E.aerogenes* to cefuroxime, ceftazidime and ceftriaxone were 70%, 60% and 60% respectively whereas for *E.cloacae* the resistance rates were 42.9%, 35.7% and 35.7% respectively. Urinary *E.cloacae* isolates were more resistant (53.6%) to nitrofurantoin than *E.aerogenes* (35%). Resistance rate of *E.cloacae* and *E.aerogenes* to gentamicin was 32.1% and 25% respectively. *E.cloacae* showed no resistance to colistin and polymyxin B. These findings differ with the study which reported resistance rates of *E.cloacae* to antimicrobial agents were higher than *E.aerogenes*.¹⁰ The main differences included resistance rates to ciprofloxacin (63.3% versus 18.8%) and aminoglycosides (41.8% versus 18.8% against amikacin). There were no significant differences in the resistance rates to third generation cephalosporins. Since the study included only patients with bacteremia and was performed in a single tertiary care hospital, the result may not be applicable to other institutions.

In this study, the most effective antibiotic was tigecycline. None of the isolates were resistant to tigecycline. Only 2(4%) isolates were resistant to colistin and polymyxin B. This may be attributed to the uncommon use of these drugs in empiric therapy of infections. Thus colistin, polymyxin B and Tigecycline can be good therapeutic options for multidrug resistant *Enterobacter* infections.

Conclusion

Enterobacter seems to be emerged with increasing resistance to multiple antibiotics. Extended survey should be launched in larger hospitals of our country to determine the true prevalence of *Enterobacter* causing nosocomial infections. Regular monitoring of antimicrobial resistance of *Enterobacter* should be done and Infection control program for prevention of nosocomial infection should be practiced in all the hospitals of our country.

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