ORIGINAL ARTICLE

Antimicrobial Resistance Pattern and Frequency of Multiple-Drug Resistant *Enterobacter* Spp. at A Tertiary Care Hospital in Southwest of Iran

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Abstract:

Background: Strains belonging to *Enterobacter* genus are important opportunistic nosocomial pathogens. Resistance to broad-spectrum antibiotics often complicates the treatment of *Enterobacter* infections. *Aim and Objectives*: The present study was aimed to determine the frequency and antibiotic resistance patterns of *Enterobacter* spp. obtained from a specialized Women's and Children's hospital. *Material and Methods:* This retrospective study was conducted during September 2015 to July 2016 in Shiraz, Iran. *Enterobacter* isolates were obtained from various clinical specimens and were identified using standard microbiological procedure. Antimicrobial susceptibility patterns were determined in accordance with CLSI recommendation. *Results:* Out of 61 *Enterobacter* spp., majority of isolates were obtained from blood 11 (18%) and followed by urine and eye specimens (11.5%). Antibiotic susceptibility revealed that all isolates were resistance to amikacin, and mostly resistance to cefotaxim and gentamycin with 90.2% and 75.4%, respectively. The highest sensitivity was toward polymyxin B 96.7%, co-trimoxazole 65.6% and imipenem 57.4%. Overall, the rate of Multiple Drug Resistant (MDR) isolates were 91.8% (n=56). Of MDR isolates, 39.2% of isolates were resistance to at least 3 antibiotics. *Conclusion:* Despite the significant role of MDR isolates in occurrence of infections, several locally available antibiotics still can have promising effects on outcome of *Enterobacter* infections in pregnant women and neonates.

Keywords: *Enterobacter*, Women, Children, Nosocomial infections, Antibiotic Susceptibility

Introduction:

Enterobacter species are Gram negative, facultative anaerobic rods, belonging to the family of Enterobacteriaceae which are the part of the gastrointestinal tract microbiota [1]. Strains belonging to this genus are important opportunistic nosocomial pathogens responsible for a variety of infections including skin, soft tissue, urinary tract, pneumonia, surgical wounds and gastrointestinal tract infections [2, 3].

Although there are several species of *Enterobacter* that can cause human disease, *Enterobacter cloacae* and *Enterobacter aerogenes* accounts for the majority of *Enterobacter* related nosocomial infections [1, 4]. Resistance to broad-spectrum cephalosporins often complicates the treatment of *Enterobacter* infections which is mediated by chromosomal AmpC cephalosporinase [5]. *Enterobacter* isolates encoded inducible AmpC ß-lactamases may initially show *in vitro* susceptibility to cephalosporins, but emerge resistance during third-generation cephalosporin therapy [6].

Enterobacter spp. showed up as an important pathogen in the Neonatal Intensive Care Unit (NICU) [7-9]. *E. cloacae* is an emerging drug

resistant nosocomial pathogen, which sometime cause respiratory infection, surgical site infections, Urinary Tract Infections (UTIs), sepsis, particularly at neonatal unit [10]. Colonization of mother by Extended Spectrum Beta Lactamases (ESBLs) producing *Enterobacter* isolate may characterize a source of transmission to neonate and associated with higher mortality rate [11].

The monitoring of antibiotic resistance patterns is a rational way to reduce the risk of antibiotic treatment failure in vulnerable populations, such as neonates and pregnant women [12, 13]. Therefore, the present study aimed to determine the frequency and antibiotic resistance patterns of *Enterobacter* spp. among various clinical samples obtained from a specialized Women's and Children's hospital.

Materials and Methods

Study Design and Samples

This retrospective cross-sectional study was performed during 11 months period from September 2015 to July 2016, at Hazrat Zainab hospital affiliated to Shiraz University of Medical Sciences, Shiraz, Iran. *Enterobacter* spp. isolated from different clinical samples such as wound, blood, urine, Bronchoalveolar Lavage (BAL), Endotracheal Tube (ETT), body fluids, eye etc. Hazrat Zainab hospitals is a 335 beds specialized Women's and Children's hospital.

Bacterial Identification

The isolates were recognized by standard microbiological techniques consist of reaction with Triple Sugar Iron agar, Simmons' citrate agar, Christensen's urea agar, Indole test, Methyl red and Voges-Proskauer tests. The strains that confirmed as *Enterobacter* were stored in Tripticase Soy Broth (TSB) with 15% glycerol at -70 C for long preservation.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibilities testing was carried out on all isolates by standard disk diffusion method on Mueller-Hinton agar medium (Merck, Germany) as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. The followed antibiotics disks (HiMedia, India) including, polymyxin B (300 units), Co-trimoxazole (1.25/23.75 µg), Amikacin (30 µg), Imipenem (10 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Ceftazidime (30 µg) were used. Moreover, additional antibiotics used for urine samples were Nalidixic acid (30 µg), Ampicillin (10 µg) and Nitrofurantoin (300 µg). *Escherichia coli* ATCC 25922 strain was used as quality control purposes. Intermediate isolates in result were accounted as resistant.

Statistical Analysis

Analysis was performed by using SPSS™ software, version 21.0 (IBM Corp., USA). The results are presented as descriptive statistics in terms of relative frequency. Values were expressed as the mean ± standard deviation (continuous variables) or percentages of the group (categorical variables).

Results:

Of 61 *Enterobacter* spp. investigated in this study, 11 (18%) cases belonged to adults and 50 (82%) belonged to neonates. The majority of *Enterobacter* isolates were obtained from blood 11 (18%) and followed by urine (7) and eye (7) specimens each with 11.5%.

The antibiotic susceptibility results revealed that all isolates were resistance to amikacin, and then mostly resistance to cefotaxim and gentamicin with 90.2% and 75.4%, respectively. On the other hand, the highest sensitivity was toward polymyxin B (96.7%), co-trimoxazole (65.6%) and imipenem (57.4%).

As one of the most common source of *Enterobacter* infection, all isolates obtained from the urine specimens showed the full susceptibility toward polymyxin B and imipenem. Moreover, co-trimoxazole and ciprofloxacin showed promising effect for urine isolates with 85.7% and 71.4% sensitivity, respectively. Despite the low frequency of BAL and ETT specimens, obtained isolates showed a remarkable resistance rate to tested antibiotics specially gentamycin (Table 1).

Overall, the rate of Multiple Drug Resistant (MDR) isolates were 91.8% (n=56) among studied *Enterobacter* isolates. Of MDR isolates, 39.2% of isolates were resistance to at least 3 antibiotics, 37.3% to 5 antibiotics, 12.4% to 6 antibiotics, 7.1% to 4 antibiotics and 3.6% to 7 antibiotics. The full MDR pattern of *Enterobacter* isolates are showed in Table 2.

Table 1: Multiple Drug Resistant (MDR) Pattern of Studied Enterobacter Isolates

MDR No.	MDR Pattern	Total Number (Percentage)
3	AN, GM, CAZ	22 (39.2)
4	AN, CP, GM, CAZ	2 (3.6)
	SXT, AN, GM, CAZ	1 (1.8)
	AN, GM, AM, FM	1 (1.8)
5	AN,IPM, CP, GM, CAZ	10 (17.8)
	SXT, AN, IPM, CP, CAZ	8 (14.2)
	SXT, AN, CP, GM, CAZ	1 (1.78)
	SXT, AN,IPM, GM, CAZ	1 (1.8)
	AN, CP, CAZ, NA, AM	1 (1.8)
6	SXT, AN, IPM, CP, GM, CAZ	5 (8.9)
	SXT, AN, IPM, CP, GM, CAZ	1 (1.8)
	AN, GM, CAZ, NA, AM, FM	1 (1.8)
7	SXT, CP, GM, CAZ, NA, AM, FM	1 (1.8)
	PB, SXT, AN, IPM, CP, GM, CAZ	1 (1.8)
Total		56 (91.8)

PB: Polymyxin B; SXT: Co-trimoxazole; AN: Amikacin; IPM: Imipenem; CP: Ciprofloxacin; GM: Gentamicin; CAZ: Ceftazidime; NA: Nalidixic Acid; AM: Ampicillin; FM, Nitrofurantoin

Table 2: Antibiotic Resistance Pattern of Enterobacter Isolates

Antibiotic Sample	PB	SXT	AN	IPM	CP	GM	CAZ	NA	AM	FM
Blood (11)	1	3	11	2	4	10	10	-	-	-
Eye (7)	0	1	7	2	2	6	7	-	-	-
Urine (7)	0	1	7	0	2	4	3	3	7	4
BAL (2)	0	0	2	2	2	2	2	-	-	-
ETT (2)	0	1	2	1	1	2	2	-	-	-
Wound (2)	1	1	2	0	0	0	1	-	-	-
Fluid (1)	0	0	1	0	0	1	1	-	-	-
Other (29)	0	14	29	19	19	21	29	0	0	0
Total (61)	2 (3.3)	21 (34.4)	61 (100)	26 (42.6)	30 (49.1)	46 (75.4)	55 (90.1)	3 (4.91)	7 (11.4)	4 (6.5)

(Data presented as Number (%);PB: Polymyxin B; SXT: Co-trimoxazole; AN: Amikacin; IPM: Imipenem; CP: Ciprofloxacin; GM: Gentamicin; CAZ: Ceftazidime; NA: Nalidixic Acid; AM: Ampicillin; FM, Nitrofurantoin

Discussion:

The resistance to multiple antimicrobial agents is common phenomenon among Enterobacteriaceae [15-17]. Therefore, the increasing knowledge about susceptibilities of these infectious agents has an important role to achieve success with antibiotics treatment [18]. Recently, there are many reports of outbreaks caused by MDR *Enterobacter*, especially in the neonatal setting [7, 9, 19]. We analyzed the antibiotic resistance and MDR pattern of *Enterobacter* spp. isolated from hospitalized patients.

In our study frequency of *Enterobacter* spp. in blood was remarkable compared to other specimens. There are some reports showing that *Enterobacter* is the most prevalent organism isolated from blood specimens in hospitalized patients, suggest that blood is one of the favorite sites of *Enterobacter* infections [18, 20, 21]. Moreover, *Enterobacter spp.* considered as a frequent cause of UTIs which have higher incidence among neonates and pediatric [22, 23]. According to our results several authors introduced *Enterobacter* as one of the most prevalent isolated microorganisms associated with UTIs among Iranian patients [24-26].

In the present study, *Enterobacter* isolates showed full resistance to amikacin. Acquired amino-glycoside resistance mainly occurs through the presence of aminoglycoside-modifying enzymes [27, 28]. Previous reports indicate to increasing rates of aminoglycosides resistant strains. Consistent to our findings, Alizadeh Taheri *et al*.'s study reported that 100% of clinical *Entrobacter* isolates were resistant to amikacin [29]. Moreover, recently high rates of aminoglycoside resistance observed in *Enterobacter* isolates

obtained from bloodstream infections in Nemazee hospital in Shiraz [30].

Our findings indicated that, polymixin B as effective antibiotic agent can be recommended used for MDR *Enterobacter* isolates. In our study, beside the polymyxin B, the lowest rate of antibiotic resistance among *Enterobacter* isolates belonged to co-trimoxazole, ciprofloxacin and imipenem, respectively. Despite of increasing fluoroquinolone and carbapenem resistance among Enterobacteriaceae [31-33], clinical studies emphasize on effectiveness of these agents [34, 35]. In our results, co-trimoxazole showed promising effect for tested isolates; while in several studies from Iran resistance to co-trimoxazole has been found as more than 50% of strains [25, 30, 34, 36]. However, *Enterobacter* spp. may be vary in antibiotic susceptibility pattern in different regions [35]. Many studies have examined the prevalence of MDR in Enterobacteriaceae [37, 38]. In the present study, frequency of MDR in *Enterobacter* isolates as one of the most common causes of Enterobacteriaceae related infections was remarkable. The majority of isolates resistant to at least 3 locally tested antibiotics, which was comparable with previous reports [34, 37, 38].

Retrospective studies often are not without limitations, and lack of detection ESBL isolates can be mentioned. Moreover, because of single-center base study, the prevalence of *Enterobacter* isolates and MDR pattern in our patients cannot be generalized to entire region.

Conclusion:

Findings of present study highlight the role of *Enterobacter* as one of the important cause of infection in pregnant women and neonates. Meanwhile, we showed the importance of MDR isolates in occurrence of infections; however, several locally available antibiotics still can have promising effects on outcome of *Enterobacter* infections. Future monitoring of antibiotic susceptibility among *Enterobacter* isolates can help in the prevention and control of nosocomial infections.

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