Egyptian Journal of Medical Microbiology Volume 28 / No.4 / October 2019 33-41 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Detection of Siderophore Virulence Gene and Antibiotic Susceptibility in *Enterobacter* spp. Isolated from Hospital and Community Acquired Infected Patients, Egypt

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Key words:
Enterobacter, Prevalence, Resistance, Siderophores

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ABSTRACT

Background: *Enterobacter spp. has been reported as an important opportunistic, multi resistant bacterial pathogen and incriminated in nosocomial infections.* ***Objectives:*** *This study aimed to determine the prevalence of Enterobacter spp. & their antibiotic susceptibility profiles and to detect the ability of Enterobacter spp. to produce siderophore-mediated strategy for iron acquisition.* ***Methodology:*** *Enterobacter spp. were identified and confirmed by standard microbiological methods and Vitek- 2 system. Their Antibiotic susceptibility profiles were determined by the modified Kirby Bauer disk diffusion method. Also, extended-spectrum β-lactamases (ESβL) and metallo-β-lactamase production were tested by combined disc diffusion method. Irp2 Virulence gene was detected by conventional polymerase chain reaction (PCR).* ***Results:*** *A total of 50 Enterobacter isolates were collected in this study from different samples from hospital (Group1), community acquired infected patients (Group 2) and from the feces of healthy volunteers (Group 3). E.cloacae was the most predominant enterobacter species (54.0%). The highest isolation rate of Enterobacter spp. was from sputum samples. About 75%, 70% and 62.5% were ESβL producers, and about 62.5%, 50% and 37.5% were MβL producers among the three studied groups. Irp2 gene was detected in 65.5% of HAI group, 40% of CAI group and 25% of carriers.* ***Conclusion:*** *The combination between multi-drug resistance and siderophores' virulence genes in Enterobacter species is worrying, since prevalence of these opportunistic pathogens causing nosocomial infections is increasing.*

INTRODUCTION

Enterobacter spp. have been recognized as important pathogens and responsible for several infections including bacteremia, lower respiratory tract infections, skin and soft-tissue infections, urinary tract infections (UTIs), endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, central nervous system (CNS) infections and ophthalmic infections [1].

Enterobacter spp. as a major nosocomial pathogen can exhibit resistance to a variety of antimicrobials chemotherapy and it is one of the members of the ESKAPE group of pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aerugenosa and Enterobacter species)*, which the main bacterial infections are found in humans [2].

Some *Enterobacter* spp. can produce ESBLs enzymes; these enzymes make bacteria resistant to most beta lactam antibiotics, such as penicillins, cephalosporins, monobactam and aztreonam [3]. So, carbapenem are often used to treat serious infections caused by MDR *Enterobacter* spp. However, the emergence of carbapenem-resistant *Enterobacter* spp. is a serious public health concern as it limits the therapeutic options for bacterial infections [4].

Pathogenicity in *Enterobacter* is related to several virulence factors, such as fimbria, adhesins, polysaccharide capsule and siderophores (iron acquisition systems), that allow them to overcome innate host immunity and to sustain tissue damage and invasion [5]. Iron is an essential element required for the function of many proteins and enzymes involved in diverse biological processes including oxygen transport, gene regulation, and nitrogen fixation. During colonization of the host, pathogens must overcome host iron sequestration to establish infections through; various iron transport systems, intracellular iron stores, redox stress resistance systems, and iron responsive regulatory elements to control the expression of genes involved in diverse cellular functions [6].

The ability of *Enterobacter* spp. to compete for iron in the host organism is of paramount importance in the establishment of infection, which drove bacterial cells to developed iron assimilation systems through the production of low molecular weight iron chelates, called siderophores [7].

Enterobacter can produce enterobactin, aerobactin and yersiniabactin. Each of these siderophores may

perform different roles in cell metabolism, with yersiniabactin being more required in iron limited environment while enterobactin functions when iron supply is not limited [5].

The aim of this study was to determine the prevalence of Enterobacter infection in both hospital and community acquired infections as well as in healthy individual, to estimate the susceptibility of *Enterobacter* isolates to different antimicrobial agents, to detect the ability of *Enterobacter* spp.to produce siderophores virulence gene by conventional PCR and to determine the correlation between the presence of siderophore and antimicrobial drug resistance.

METHODOLOGY

This study was performed during the period from December 2017 to June 2019 in Medical Microbiology and Immunology Department, Faculty of Medicine, Menofia University. The study protocol was approved by the Local Ethics Committee of Menofia University.

Bacterial strains:

A total of 50 *Enterobacter* isolates were isolated in this study; 32 from hospitalized patients (group I), 10 from community acquired patients (group II) and 8 from the feces of healthy volunteers (carrier group III). These strains were identified as recommended by Brenner [8] and species identification was performed using VITEK-2 system (BioMérieux, Hazelwood, MO).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done using Kirby-Bauer disk diffusion method against different antimicrobial agents on Mueller–Hinton agar (Oxoid) and results were interpreted according to CLSI [9] guidelines. The tested antimicrobials included amoxicillin (AML, 20µg), amoxicillin/clavulanate (AMC, 20µg/10µg), piperacillin/tazobactam (TZP, 100 µg /10µg), ceftazidime (CAZ, 30µg), cefepime (FEP, 30µg), ceftriaxone (CRO, 30µg), cefotaxime (CTX, 30µg), cefeprazone (CAZ, 30µg), cefoxitin (FOX, 30µg), aztreonam (ATM, 30µg), ofloxacin (OFX, 5µg), norfloxacin (NOR, 10µg), amikacin (AK, 30µg), tobramycin (TOB, 10µg), doxycycline (DO, 30µg),trimethoprim-sulfamethoxazole (TMP/SMX, l,25µg- 23.75µg), chloramphenicol (C, 30µg), imipenem (IPM, 10µg), meropenem (MEM, 10µg) and ertapenem (ETP, 10µg)

Detection of Extended-Spectrum Beta-Lactamases production by screening and confirmatory methods

Forty three *Enterobacter* isolates were suspicious to be ESβL producer by the screening disc diffusion test (they had zone diameter less than 22 mm for ceftazidime, less than 27 mm for cefotaxime, and less than 25 mm for ceftriaxone), confirmation to be ESβL producer was done using combined disc diffusion test (CDT). Ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg) were placed on Mueller Hinton agar and incubated aerobically at 37°C for 18-24 h. Organism was considered as ESβLs-producer if there was a ≥5 mm increase in diameter of ceftazidime/clavulanic acid disk than that of ceftazidime disk alone [9].

Detection of Carpabenemases (MβLs**) production by screening and confirmatory methods**

- Twenty eight *Enterobacter* isolates were suspicious to be MβL producer by the screening disc diffusion test. It is a simple test for reduced susceptibility to one or more carabapenem (ertapenem or imipenem) plus one or more of the indicator cephalosporin (cefeprazone, ceftriaxone and ceftazidime) and average diameters of zones of inhibition were measured and interpreted according to CLSI[9] guidelines**.**
- These strains were confirmed for MβLs production using imipinum-EDTA combined disc test. An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a MHA plate. Two 10 µg imipenem disks were placed on the agar 15 mm apart. After incubating overnight at 37°C, increase of zone size of more than 7 mm in the disk potentiated with the EDTA was interpreted as positive for MβLs production[10].

Detection of Siderophore virulence gene *(irp2*) by PCR

DNA extraction*:*

Enterobacter DNA were extracted from 1 ml of overnight cultures in Tryptic Soy Broth (BD-Difco) using the gene JET™ genomic DNA purification kit (ThermoFisher Scientific, UK)following the manufacturer's instructions. The DNA concentration was quantified by spectrophotometer analysis (Gene Quant II; Pharmacia). The following **primer sequence** of *irp2 gene*: *irp2* (F): ATT TCT GGC GCA CCA (R): GCG CCG GGT ATT ACG GAC TTC (size, 952 bp) was used[11].

Amplification reaction were prepared in a total volume of 50 µl; consisting of 25 µl Green PCR Master Mix (Promega, Madison, USA), 1µM forward primer, 1µM reverse primer, 1 ml DNA template and 22µL nuclease-free water. Conventional PCR program was performed in a thermocycler (Applied Biosystems, Singapore) as follow: initial denaturation (94°C for 3 min), followed by 35 cycles [DNA denaturation (94°C for 45 sec), primer annealing (57°C for 45 sec), and primer extension (72°C for 1 min)], followed by final extension at 72°C for 5 min. The amplified DNA was electrophoresed using 2% agarose gel (Fermentas, Lithuania) stained with ethidium bromide (Sigma, USA), and the bands (952 bp) were visualized and photographed [11].

Statistical analysis

The data collected was tabulated and analyzed using SPSS program version 22.0. The results were expressed as ranges and mean ± SD. Chi-square and Fischer exact tests were done. P value <0.05 was considered as significant.

RESULTS

Out of 494 specimens taken; *S .aureus* was the most frequent isolated organism among the studied groups 141/494 (28.5%). *Enterobacter* spp. represented 50/494 (10.1%); 12.4% (32/285) from hospital acquired infections, 7.3% (10/138) from community acquired infections and 8.2% (8/98) from carriers. [Table1].

Table 1: Culture results of the studied groups (n=494)

Isolated bacteria (n=494)	Group I (HAI)		Group II (CAI)		Group III (Carriers)		Total	
	No.	%	No.	%	No.	%	No.	%
S. aureus	65	25.2	43	31.1	33	33.7	141	28.5
Streptococcus pneumonie	0	0.0	0	0.0	8	8.2	8	1.6
***Enterococcus* species**	0	0.0	0	0.0	5	5.1	5	1.0
Coagulase -ve staphylococci	40	15.5	29	21.0	0	0.0	69	14.0
***Klebsiella* species**	41	15.9	19	13.8	8	8.2	68	13.8
E. coli	38	14.7	13	9.4	27	27.5	78	15.8
P. aeroginosa	18	7.0	9	6.5	2	2.0	29	5.9
***Enterobacter* spp.**	**32**	**12.4**	**10**	**7.3**	**8**	**8.2**	**50**	**10.1**
Proteus	24	9.3	15	10.9	7	7.1	46	9.3
Total	**258**	**(52.2)**	**138**	**(27.9)**	**98**	**(19.8)**	**494**	**100**

Enterobacter species were more frequently isolated from sputum samples (40.6%) followed by urine and pus samples (21.9%) in group I, From pus samples (50%) followed by sputum (30%) and urine (20%) in group II [Table2].

Table 2: Distribution of *Enterobacter* isolates according to the type of specimen among the studied groups.

Sample	Group I (HAI) (n=32)		Group II (CAI) (n=10)		Fisher's exact test and P value	Group III (Carriers) (n=8)		Total	
	No.	%	No.	%		No.	%	No.	%
Blood	5	15.6	0	0.0	Test=1.77 P=0.18	0	0.0	5	10.0
Pus	7	21.9	5	50.0	Test=2.95 P=0.09	0	0.0	12	24.0
Sputum	13	40.6	3	30.0	Test=0.36 P=0.55	0	0.0	16	32.0
Stool	0	0.00	0	0.0	-	8	100.0	8	16.0
Urine	7	21.9	2	20.0	Test=0.02 P=0.89	0	0.0	9	18.0

E.cloacae was the most predominant *enterobacter* species (54.0%) followed by *E.areogenes* (34.0%), *E.agglomerans* (8%) and *E.sakazakii* (4%) among the 3 studied groups [figure 1]

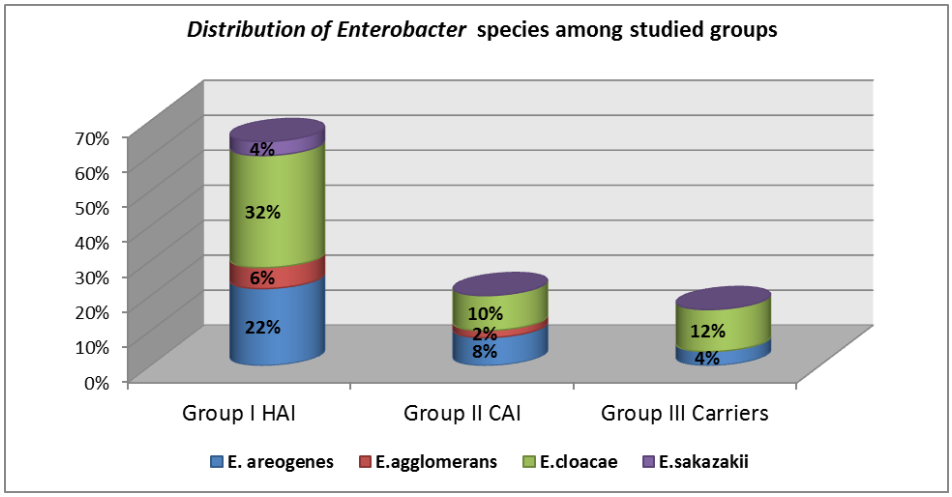

Fig. 1: Identification of *Enterobacter* species using VITEK-2 system

Hospital acquired *Enterobacter* isolates were highly resistant to amoxicillin (93.8%), followed by ceftazidime, cefepime, ceftriaxone, cefoxitin and amoxicillin/clavulanic acid (90% for each). On the other hand their sensitivity to amika**ci**n, choloramphinicol and tigecycline were (58.3%), (78.1%) and (71.9%) respectively.

Community acquired *Enterobacter* isolates were highly resistant to amoxicillin (100%) followed by ceftriaxone, cefeprazone (80% for each) ceftazidime, cefepime, azetronam, On the other hand their sensitivity to tigecycline were (70%) amikacin, choloramphinicol, norfloxacin and tobramycin (60 %), ofloxacin and doxycycline (50 %).

Enterobacter isolated from carrier were highly resistant to amoxicillin, ceftazidime, cefepime, ceftriaxone, cefeprazone and cefoxitin (75%).On the other hand their sensitivity was 100% to tigecycline, 62.5% to doxycycline, meropenem and choloramphinicol, 50% to piperacillin/ tazobactam, ofloxacin, norfloxacin, amikacin, tobramycin, etrapenum and imipenem [Table 3].

Table 3: Results of antibiotic susceptibility tests of *Enterobacter* isolates by disk diffusion method.

Antibiotic	B Groups	*Enterobacter*(No. =50)			χ^2 *test and P value*
		S No. (%)	I No. (%)	R No. (%)	
Amoxicillin	**HAI** **CAI** **Carrier**	2 (6.3) 0 (0.0) 1 (12.5)	0(0.0) 0 (0.0) 1 (12.5)	30 (93.8) 10 (100) 6 (75.0)	Test = 6.75 P=0.15
Amoxicillin/ clavulanic acid	**HAI** **CAI** **Carrier**	3 (9.4) 1 (10.0) 2 (25.0)	0 (0.0) 2 (20.0) 1 (12.5)	29 (90.6) 7 (70.0) 5 (62.5)	Test =7.93 P=0.09
Piperacillin/ Tazobactam	**HAI** **CAI** **Carrier**	1 (3.1) 3 (30.0) 4 (50.0)	5 (15.6) 4 (40.0) 1 (12.5)	26 (81.3) 3 (30.0) 3 (37.5)	Test = 17.02 P=0.002*
Ceftazidime	**HAI** **CAI** **Carrier**	1(3.1) 3 (30.0) 1 (12.5)	2(6.3) 0(0.0) 1 (12.5)	29 (90.6) 7 (70.0) 6 (75.0)	Test = 7.21 P=0.12
Cefepime	**HAI** **CAI** **Carrier**	2(6.3) 1 (10.0) 2 (25.0)	1(3.1) 2 (20.0) 0 (0.0)	29 (90.6) 7 (70.0) 6 (75.0)	Test = 6.92 P=0.14
Ceftriaxone	**HAI** **CAI** **Carrier**	1 (3.1) 1 (10.0) 2 (25.0)	2(6.3) 1 (10.0) 0(0.0)	29 (90.6) 8 (80.0) 6 (75.0)	Test = 4.87 P=0.30

Antibiotic	B Groups	*Enterobacter*(No. =50)			χ^2*test and P value*
Cefeprazone	**HAI**	3 (9.4)	1(3.1)	28 (87.5)	Test = 2.14
	CAI	2 (20.0)	0 (0.0)	8 (80.0)	P=0.71
	Carrier	2 (25.0)	0(0.0)	6 (75.0)	
Cefoxitin	**HAI**	3 (9.4)	0(0.0)	29 (90.6)	Test = 12.11
	CAI	3 (30.0)	2 (20.0)	5 (50.0)	P=0.02*
	Carrier	2 (25.0)	0(0.0)	6 (75.0)	
Aztreonam	**HAI**	4 (12.5)	5(15.6)	23 (71.9)	Test =1.84
	CAI	2 (20.0)	1 (10.0)	7 (70.0)	P=0.76
	Carrier	2 (25.0)	2(25.0)	4 (50.0)	
Ofloxacin	**HAI**	11 (34.4)	5(15.6)	16 (50.0)	Test =1.65
	CAI	5 (50.0)	2 (20.0)	3 (30.0)	P=0.80
	Carrier	4 (50.0)	1(12.5)	3 (37.5)	
Norfloxacin	**HAI**	16 (50.0)	0(0.0)	16 (50.0)	Test =19.51
	CAI	6 (60.0)	4 (40.0)	0(0.0)	P=0.001**
	Carrier	4 (50.0)	3(37.5)	1 (12.5)	
Amikacin	**HAI**	18 (58.3)	2(6.3)	12 (37.5)	Test =2.30
	CAI	6 (60.0)	2 (20.0)	2 (20.0)	P=0.68
	Carrier	4 (50.0)	1(12.5)	3 (37.5)	
Tobramycin	**HAI**	12 (37.5)	9(28.1)	11 (34.4)	Test = 3.32
	CAI	6 (60.0)	3 (30.0)	1(10.0)	P=0.51
	Carrier	4 (50.0)	1(12.5)	3 (37.5)	
Doxycycline	**HAI**	7 (21.9)	5(15.6)	20 (62.5)	Test =12.06
	CAI	5 (50.0)	4 (40.0)	1 (10.0)	P=0.02*
	Carrier	5 (62.5)	1(12.5)	2 (25.0)	
Trimethoprim/	**HAI**	7 (21.9)	7(21.9)	18 (56.3)	Test = 2.94
Sulfamethoxazole	**CAI**	4 (40.0)	1 (10.0)	5(50.0)	P=0.57
(Co-trimexazole)	**Carrier**	2 (25.0)	3(37.5)	3 (37.5)	
Choloramphinicol	**HAI**	25 (78.1)	6(18.8)	1 (3.1)	Test = 4.95
	CAI	6 (60.0)	2 (20.0)	2(20.0)	P=0.29
	Carrier	5 (62.5)	1(12.5)	2 (25.0)	
Imepenem	**HAI**	7 (21.9)	6(18.8)	19 (59.4)	Test = 3.89
	CAI	3 (30.0)	1 (10.0)	6(40.0)	P=0.42
	Carrier	4 (50.0)	2(25.0)	2 (25.0)	
Etrapenum	**HAI**	7 (21.9)	6(18.8)	19 (59.4)	Test = 2.59
	CAI	3 (30.0)	2 (20.0)	5(50.0)	P=0.63
	Carrier	4 (50.0)	1(12.5)	3 (37.5)	
Meropenum	**HAI**	8 (25.0)	5(15.6)	19 (59.4)	Test =6.27
	CAI	3 (30.0)	3(30.0)	4(40.0)	P=0.18
	Carrier	5 (62.5)	0(0.0)	3 (37.5)	
Tigacycline	**HAI**	23 (71.9)	3 (9.4)	6 (18.7)	Test =3.02
	CAI	7 (70.0)	1 (10.0)	2 (20.0)	P=0.55
	Carrier	8 (100)	0 (0.0)	0 (0.0)	

About 90.6%, 80% and 75% of *Enterobacter* isolates among the three studied groups respectively were potential ESBL producer by screening method, while by confirmatory method; only 75%, 70% and 62.5% were ESβL producers without statistical significant difference. About 78.1%, 70% and 62.5% of were potential MβL producers by screening method. And by confirmatory method only 62.5%, 50% and 37.5% of isolates were MβL producers without statistical significant difference [table 4].

Table 4: ESBL and Carbapenamase (production among *Enterobacter* isolates of the studied groups:

	Group I HAI (No. =32)	Group II CAI (No. =10)	Group III Carrier (No. =8)	Fisher's exact test	P value
	No. (%)	No. (%)	No. (%)		
	ESBL production				
ESBL screening				Test= 0.82 Test=1.43	P1=0.58
Positive	29 90.6	8 80.0	6 75.0	Test=0.06	P2=0.56
Negative	3 9.4	2 20.0	2 25.0		P3=1.00
ESBL confirmatory					
Positive	24 75.0	7 70.0	5 62.5	Test=0.09 Test=0.50	P1=1.00
Negative	8 25.0	3 30.0	3 37.5	Test=0.11	P2=0.66
					P3=1.00
MβL production					
MβL screening					
Positive	25 78.1	7 70.0	5 62.5	Test=0.28 Test=0.83	P1=0.68
Negative	7 21.9	3 30.0	3 37.5	Test=0.11	P2=0.65
					P3=1.00
MβL confirmatory					
Positive	20 62.5	5 50.0	3 37.5	Test=0.49Test=1.64	P1=0.71
Negative	12 37.5	5 50.0	5 62.5	Test=0.28	P2=0.25
					P3=0.66

P1--HAI versus CAI P2--HAI versus Carrier P3--CAI versus Carrier

Irp2 virulence gene was detected among 21/32 (65.5%), 4/10(40%) and2/8(25%) of HAI, of CAI and of carriers respectively by conventional PCR technique [table5]. About 85.7%, 100% and 62.5% of ESBL and MβL producing *Enterobacter* isolates were siderophores producers had *Irp2* gene [figure 2 &table 6].

Table 5: Prevelance of *irp2* virulence gene in *Enterobacter* isolates among studied groups:

	Group I HAI (No. =32)	Group II CAI (No. =10)	Group III Carrier (No. =8)	Fisher's exact test	P value
	No. (%)	No. (%)	No. (%)		
irp2 virulence gene				Test=2.08	P1=0.27
Positive	21 65.6	4 40.0	2 25.0	Test=4.32	P2=0.04*
Negative	11 34.4	6 60.0	6 75.0	Test=0.45	P3=0.64

P1--HAI versus CAI P2--HAI versus Carrier P3--CAI versus Carrier
***significant difference**

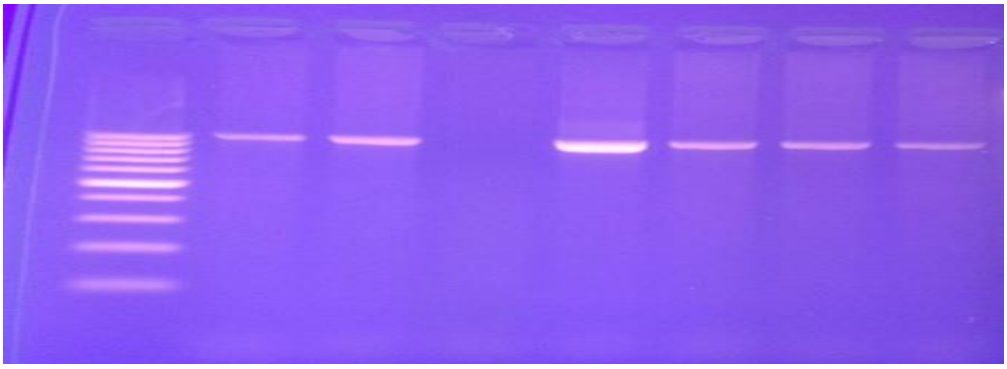

Fig.2: Agarose gel electrophoresis for the PCR amplified products of Enterobacter *irp2* virulence gene.
- Lane M: DNA molecular size marker (1000 bp).
- Lanes 2, 3, 5. 6, 7 & 8 were *irp2* gene-positive (952 bp).
- Lanes 3 was *irp2* gene-negative

Table 6: Association between acquiring *irp2* virulence gene and ESBL or MβL production among the three studied groups:

	Presence of *irp2* virulence gene among studied group		
	Group I HAI (No. =21)	**Group II CAI (No. =4)**	**Group III Carrier (No. =2)**
	No. (%)	**No. (%)**	**No. (%)**
ESBL by confirmatory test			
Positive	18 85.7	4 100	2 62.5
Negative	3 14.3	0 0.0	0 37.5
MβL by confirmatory test			
Positive	18 85.7	4 100	2 62.5
Negative	3 14.3	0 0.0	0 37.5

DISCUSSION

In the last decades, species of the genus Enterobacter have aroused greater concern, since they are increasingly associated with nosocomial infections, especially in immunocompromised patients[12].

In this study, *Enterobacter* spp. infection represented10.1%; 12.4% (32/258), 7.3% (10/138) and 8.2% (8/98) of them were from HAI, CAI and from carriers respectively. A slightly higher rates (18%) and (18.2%) were reported by Tohamy et al [13] in Egypt and Wang et al [14] in Germany respectively. On the other hand, a higher rate (32%) was reported by Abid [15] in Iraq.

The highest isolation of *Enterobacter* isolates were from sputum (32%) followed by pus (24%) and urine samples (18%). In agreement with us Renk et al [16] in Germany found that; most *Enterbacter* isolates were from sputum samples (30.5%). While, Uzunović et al [17] in Bosna found that the majority of *Enterobacter* isolates were from urine (60%) followed by wound samples (26.7%) and, Malekzadegan *et al* [18] in Iran found the majority of isolates were obtained from blood (18%) followed by urine and eye specimens (11.5% for both).

In the current study, *E.cloacae* was the most predominant *Enterobacter* species (54.0%) followed by *E.areogenes* (34%). This was in agreement with AL-Tawfiq et al [19] in Saudi Arabia, Marcos et el [20] and Hussain et al [21] in Iraq who found that 60%, 71.9% and 89.3% of *Enterobacter* isolates were *E.cloacae*.

Hospital aquired *Enterobacter* isolates were highly resistant to Amoxicillin (93.8%) followed by Ceftazidime, Cefepime, ceftriaxone, Cefoxitin and Amoxicillin/clavulanic acid (90%), Cefeprazone (87.5%), Piperacillin/ tazobactam (83.5%) and Aztreonam (71.9%).On the other hand, 78.1%, 71.9% and 58.3% of them were susceptible to Choloramphinicol, Tigecycline and Amikacin respectively. This result matched with Uzunović et al[17] who found the resistance rates to amoxicillin, ceftazidime, ceftriaxone, cefotaxime, and cefepime 100.0%, 90.0%, 90.0%, 90.0% and 83.0% respectively. On the other hand, (37.5%) of the *Enterobacter* isolates were resistant to Amikacin, These results were correlated with Bunyan et al [22] who found 25% of *E. cloacae* resistance to Amikacin. Sensitivity to quinolones and aminoglycosides is an important finding, as they are the drug of choice for the treatment of infections caused by many Gram-negative rods [23].

The resistance rate to imipenem, meropenem, etrapenum, was 59.4% for each. This was in agreement with In Khajuria et al [24] in india and Biendo et al [25] in France who found 53.84% and 56.4% of *Enterobacter* spp. resistant to imipenem. On other hand Uzunović et al [17] in Bosna found that all isolates were susceptible to imipenem and meropenem,

In the present study, 75% of hospital aquired *Enterobacter* isolates were ESβL-producers. This result was in consistent with Adwan *et al* [26] in Iraq who found 80.5%, of *Enterobacter* isolates ESBL producer while high result was reported by Ramazanzadeh et al [3] in Iran who found 100% of *Enterobacter* isolates were ESβL-producers, and lower rate (28%) was reported by Ghoneim et al[27] in Egypt.

Regarding MβL production, 62.5% of hospital aquired *Enterobacter* isolates were MβL-producers. This was in agreement with Biendo et al[25] in France who found 66.6% of *Enterobacter* isolates was MβL-producer, while higher percent (86%) was reported by Abid et al [15] in Iraq and lower percents (25.7%), (22%) by Khajuria et al[24] and Ghoneim et al [27]. ESBLs and carbapenemases represent an emerging public health concern [28].

Enterobacter spp. from community and hospital acquired infections possess virulence factors important for the establishment of extra-intestinal infections [(11)]. In the current study, 65.6% of hospitalized *Enterobacter* isolates were siderophore producers by detection of *irp2* gene. In agreement with Bunyan et al [22] in Iraq who found 87% were siderophore producer. While, Hussain et al [21] in Iraq found all *Enterobacter* isolates were

siderophore producers. On the other hand, a lower result (23.1%) was reported by Šmarda et al [29].

The presence of *irp2* gene in normal microbiota indicated the ability of this gene to be mobilized, spread to community isolates and increasing the population risk to bacterial infection. This study revealed that 40% of community acquired *Enterobacter* isolates and 25% of carrier isolates were siderophore producers. Nearly similar result (44%) was observed in Brazil by Lopes et al[11], but they didn't find any sidrophore production among Enterobacter of the carrier group.

About 85.7%, 100% and 62.5% of ESBL and carbapenamase producing *Enterobacter* isolates among studied groups were siderophores producers had *Irp2* gene. Starlander et al [30] in Sweden found that, ESBL and carbapenamase producing *E.coli* were more siderophores producers (87%) and (60%) than non siderophores producers.

CONCLUSION & RECOMMENDATIONS

Presence of virulence gene responsible for synthesis of siderophore together with antibiotic resistance observed in this study impose significant therapeutic limitation on treatment of infection caused by Enterobacter. So, powerful infection control programs should be designed and put into action to prevent the dissemination of these resistant isolates throughout the hospitals.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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