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ORIGINAL ARTICLE

FimH Adhesin among *Enterobacter* spp. Isolates and its Relation to Biofilm Formation and Antimicrobial Resistance Pattern

[1]Mabrouk M Ghonaim, [1]Amira H Elkhyat, [2]Sally M El-Hefnawy, [1]Elham A Hossam Eldeen
[1]Departments of Microbiology and Immunology, [2]Biochemistry, Faculty of Medicine, Menoufia University, Shebin Elkom, Egypt

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Corresponding Author:
Elham Ayman Hossam Eldeen
Departments of Microbiology and Immunology, Menoufia University, Shebin Elkom, Egypt
Tel: +2- 01065564838
hossameldeenelham@gmail.com

ABSTRACT

Background: *Enterobacter spp., which are able to carry a number of antibiotic-resistance genes, can cause a variety of infections especially in immune-compromised individuals and patients from intensive care units (ICUs).* ***Objectives:*** *To assess the prevalence of Enterobacter spp. in Menoufia University Hospitals, and to investigate the relation between antimicrobial susceptibility patterns and biofilm production.* ***Methodology:*** *A total 296 clinical samples from patients admitted to Menoufia University Hospitals. Enterobacter spp. were identified by standard microbiological methods and Vitek-2 system. All Enterobacter isolates antibiogram was tested by the modified Kirby Bauer disk diffusion method, and for extended-spectrum β-lactamases and metallo-β-lactamase production. Biofilm production was detected by congo red agar, modified congo red agar methods and PCR.* ***Results:*** *Enterobacter spp. represented 17.3% of all the collected nosocomial isolates. Vitek-2 system showed that the predominant spp. was Enterobacter aerogenes (44%). Enterobacter isolates were resistant to amoxicillin (100%), doxycycline (82%) and gentamycin (76%). The rates of resistance to ceftriaxone, cefoxitin, cefepime, and amikacin were 64%, 72%, 60% and 70% respectively. Half of Enterobacter isolates were sensitive to piperacillin/tazobactam, meropenem, ciprofloxacin, ofloxacin and norfloxacin while 84% were sensitive to chloramphenicol. Production of ESβLs and MβL was found among 28% and 22% of isolates respectively. Biofilm production was found among 50% by CRA method and 56% by MCRA method, while conventional PCR showed fimH gene among 58% of Enterobacter isolates.* ***Conclusion:*** *Enterobacter spp. are serious nosocomial pathogens as they can produce ESβLs and carbapenemase, and produce biofilm that is related to their antimicrobial resistance. Therefore, their adequate prevention and control is imperative.*

INTRODUCTION

Nosocomial infections are form health problems because they reduce the quality of life in patients, increase the length of hospitalization, costs, and mortality and morbidity rates [1]. Hospitalized patients have high tendency to infections with resistant microorganisms due to presence of chronic diseases, heavy use of broad-spectrum antibiotics and the more frequent exposure to invasive procedures.[2]

Enterobacter spp. are important opportunistic nosocomial pathogens responsible for a soft tissue, surgical wound, urinary, respiratory, and gastrointestinal tract infections [3]. *They* tend to contaminate various medical hospital devices, surgical equipment and operative cleaning solutions.[4]

High rates of antibiotic resistance among *Enterobacter* spp., have been reported especially in ICU infections [5]. Emergence of antimicrobial-resistance has serious consequences on the treatment of infectious diseases[6]. The mechanisms of antibiotic resistance among *Enterobacter isolates* may be plasmid-mediated AmpC β-lactamases (pAmpC), extended-spectrum β-lactamases (ESBLs), carbapenemases, and metallo β-lactamases) MβLs).[7]

Biofilm is formed of complex, sessile communities of microbes attached to a surface or buried firmly in an extracellular matrix as aggregates. The biofilm matrix makes the bacteria tolerant to harsh conditions and resistant to antibacterials.[8] Biofilms have major role in antibiotic resistance, as they play a role in antibiotic trapping and impairment and plasmid exchange**.** Therefore, they can lead to persistent infections of many pathogenic microbes. Moreover, they are important in indwelling medical device, dental plaque, and upper respiratory and urogenital tract infections. [9]

In this study, we aimed to detect biofilm-producing *Enterobacter* strains phenotypically and genotypically (*fimH* gene) and to assess the relation between ability of biofilm production and antimicrobial drug resistance.

METHODOLOGY

Collection of samples and identification of *Enterobacter* isolates:

This study was performed in Medical Microbiology and Immunology Department, Faculty of Medicine, Menoufia University. All the selected patients were subjected to full history taking and thorough clinical examination. The study protocol was approved by the Local Ethics Committee of Menoufia University.

Clinical samples [10]

- **Sputum samples**
- **Purulent secretions** (1-10 ml) form intubated patients.
- **Venous blood samples (10ml)** from each adult patient and 2–5 ml from infants and children and inoculated into blood culture bottles.
- **Mid-stream urine samples (**10–20 ml) were collected from un-catheterized patients. For catheterized patients, 5–10 ml samples were collected after discarding the first few drops of urine.
- **Pus** by a sterile cotton swab from infected wounds.

Identification of bacterial isolates:

A total of 296 clinical samples (76 sputum, 122 urine, 62 pus and discharge from wounds or soft tissue, 23 blood and 13 burn swabs**)** were received from the infected patients before antibiotics administration. All the specimens were cultured on different media (Oxoid, UK). The growing *Enterobacter* isolates were identified by standard methods, and VITEK 2 System. Confirmed *Enterobacter* isolates were suspended in nutrient broth supplemented with 16% glycerol and stored frozen at -80°C.[11]

Antimicrobial susceptibility testing:

Disk diffusion method:

Antimicrobial susceptibility testing for *Enterobacter* isolates was performed using Kirby-Bauer disk diffusion method against different antimicrobial agents (Oxoid) as recommended by CLSI, 2018. [12] The tested antimicrobials included amoxicillin (AML, 20µg), amoxicillin/clavulanate(AMC, 20µ/10 µg), piperacillin/tazobactam (TZP, 100/10µg), cefoxitin (FOX, 30µg), cefepime (FEP, 30µg), cefotaxime (CTX, 30µg), cefotaxime/clavulanate (CTC, 30/10µg), ceftriaxone (CRO, 30µg), ceftazidime (CAZ, 30µg), ceftazidime/clavulanate (CTZ, 30/10 µg), imipenem (IPM, 10µg), meropenem (MEM, 10µg), ertapenem (ETP, 10µg), aztreonam (ATM, 30µg), gentamicin (CN, l0µg), amikacin (AK, 30µg), tobramycin (TOB, 10µg), doxycycline (DO, 30µg), norfloxacin (NOR, 10µg), ciprofloxacin (CIP, 5µg), ofloxacin (OFX, 5µg), trimethoprim-sulfamethoxazole (TMP/SMX, l,25µg-23.75µg), and chloramphenicol (C, 30µg).

Screening and phenotypic confirmation of ESBLs *Enterobacter* producers:

Screening for ESBLs producers:

It was performed according to (CLSI, 2018). Ceftazidime (30µg), cefotaxime (30 µg), and ceftriaxone (30µg) were used. If a zone diameter of less than 22 mm for ceftazidime, less than 27 mm for cefotaxime, and less than 25 mm for ceftriaxone was recorded, the strain was considered to suspicious for ESβL production.[12]

Phenotypic confirmation for ESBLs production by cephalosporin/clavulanate combination disks:

All *Enterobacter* strains were subjected to confirmation (CLSI, 2018). The ceftazidime (30 µg) and cefotaxime (30 µg) disks alone and in combination with clavulanic acid (ceftazidime+clavulanic acid, 30/10 µg disks, cefotaxime+clavulanic acid, 30/10 µg) were applied onto a plate of Muller-Hinton agar, which was inoculated with the test strain. An increase of at least 5 mm in zone diameter for antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone denoted ESBLs-producing strains.[13]

Screening and phenotypic confirmation of MβLs production:

Enterobacter isolates were tested against imipenem and meropenem (Oxoid) by disk diffusion method. The average diameters of zones of inhibition were measured and interpreted (CLSI, 2018). Suspected metallo β-lactamases were confirmed by imipenem/ ethylene diamine tetra-acetic acid (EDTA) combined disk test. The presence of an expanded growth inhibition zone between two discs or increase of zone size more than 7mm in imipenem/EDTA disk than imipenem disk alone was considered as MβL-positive.[14]

Detection of biofilm formation:

Phenotypic detection of biofilm formation:

Production of biofilm was studied by cultivation of *Enterobacter* isolates on CRA comprising brain heart infusion broth (BHI) 37 g/L (Oxoid, UK) , sucrose 50 g/L (Sigma), Congo red dye 0.8 g/L (Sigma), agar 10 g/L and 1000 ml water. Comparative growth on MCRA was determined. The modifications include changing the concentration of Congo red dye and sucrose, omission of glucose (Sigma) and replacement of BHIA by an alternative agar, blood base agar-2 (Oxoid, UK). Inoculated agar was incubated for 48 h at 37°C and subsequently 2-4 days at room temperature.[15]

Detection of biofilm-associated gene (fimH) by conventional PCR:

- DNA extraction: Bacterial DNA was extracted and purified using the gene JET™ genomic DNA purification kit (ThermoFisher Scientific, UK).
- Primer sequence of *fimH:*
  F (TACTGCTGATGGGCTGGTC), and
  R (GCCGGAGAGGTAATACCCC) primers[16]

were shipped and received in a lyophilized state (Invitrogen by Thermo Fisher, UK). The volume of nuclease-free H_2O added to the lyophilized primer was determined by reading the number of nmol of primers in the tube and multiplied by 10 to make a 100 µmol/l primer stock.

- Conventional PCR program was performed in a thermal cycler (Applied Biosystems, Singapore) that consisted of an initial denaturation (94°C for 2 min), followed by 40 cycles [DNA denaturation (94°C for 40 sec), primer annealing (50°C for 40 sec), and primer extension (72°C for 1 min)], followed by final extension at 72°C for 5 min[16]. The amplified DNA was electrophoresed using 2% agarose gel (Fermentas, Lithuania) stained with ethidium bromide (Sigma, USA), and the bands (640 bp) were visualized and photographed (Samsung, WB30F,Korea).

Statistical analysis

Computer SPSS program version 20 was used. The results were expressed as ranges and mean± SD. Chisquare test was done and p value <0.05 was considered as significant.

RESULTS

A total of 284/296 specimens (95.9%) showed positive cultures (279 showed single growth and 5 showed mixed growth (2 isolates for each). The most frequent Gram-negative isolates were *E. coli* (35.4%). *Enterobacter* spp. were 19.6%.[Table 1].

Table 1: Distribution of the isolated organisms among different departments

Isolated organisms	Total No.(%)	Departments					
		ICUs No.(%)	Urology No.(%)	Surgery No.(%)	Pediatrics No.(%)	Burn Unit No.(%)	Internal medicine No.(%)
Gram-positive cocci							
-*Staph. aureus*	16(72.7%)	9(56.2%)	2(12.5%)	1(6.3%)	1(6.3%)	3(18.7%)	-
-CNA	6(27.3%)	3(50%)	-	3(50%)	--	--	-
Gram-negative bacilli							
-*E. coli*	92(35.4%)	23(25%)	44(47.8%)	4(4.4%)	6(6.5%)	-	15(16.3%)
-*Klebseilla* spp.	74(28.5%)	36(48.6%)	6(8.1%)	15(20.3%)	13(17.6%)	2(2.7%)	2(2.7%)
- *Enterobacter*	50(19.2%)	27(54%)	9(18%)	6(12%)	5(10%)	1(2%)	2(4%)
-Pseudomonas	34(13.1%)	16(47.1%)	-	7(20.6%)	1(2.9%)	7(20.6%)	3(8.8%)
-*Proteus* spp.	10(3.8%)	3(30%)	-	--	7(70%)	-	-
Fungi							
-*Candida* spp.	7 (2.4%)	3(42.9%)	-	1(14.3%)	2(28.5%)	-	1(14.3%)
Total	289(100%)	120(41.5%)	61(21.1%)	37(12.8%)	35(12.1%)	13(4.5%)	23(8%)

Out of 50 *Enterobacter* isolates, 24 (48%), 13 (26%), 9 (18%), 3 (6%) and 1 (2%) were isolated from urine, sputum, pus, blood, and burn swabs respectively. The highest isolation rate of *Enterobacter* spp. was from ICU (54%) and the lowest from burns (2%). [Table 2]. Vitek2 system results showed that *Enterobacter aerogenes* was the predominant *Enterobacter* spp. (44%) [Figure 1].

Table 2: *Enterobacter* isolates according to type of sample among Hospital Departments

Sample	The studied departments					
	ICUs No. (%)	Urology No. (%)	Surgery No. (%)	Pediatrics No. (%)	Burn unit No (%)	Internal Medicine No. (%)
Sputum	8(61.5)	-	-	4(30.8)	-	1(7.7)
Urine	14(58.3)	9(37.5)	-	-	-	1(4.2)
Pus and wounds or	3(33.3)	-	5(55.6)	1(11.1)	-	-
Blood	2(66.7)	-	1(33.3)	-	-	-
Burn swab	-	-	-	-	1(100)	-
Total No. (%)	27(54)	9(18)	6(12)	5(10)	1(2)	2(4)

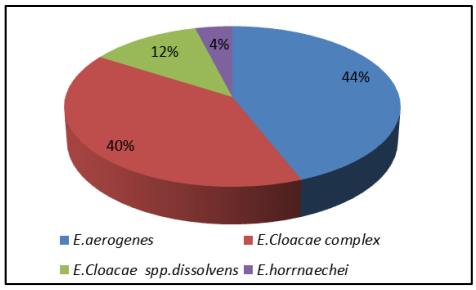

Fig. 1: Species identification of *Enterobacter* spp. By Vitek2 system

Enterobacter isolates were highly resistant to amoxicillin (100%), tetracycline (82%), gentamycin (76%), trimethoprim/sulfamethoxazole (74%), cefoxitin (72%), amoxicillin/clavulanic acid (72%), ceftazidime (70%) and amikacin (70%).On the other hand, *Enterobacter* isolates were highly sensitive to chloramphenicol (84%) [Table 3].

About 76%, 56% and 16% of *Enterobacter* isolates were MDR, XDR and PDR respectively.

Table 3: Antimicrobial susceptibility pattern of *Enterobacter* isolates by disk diffusion method

Antimicrobial agent	Disk content (µg)	*Enterobacter* No =50					
		Sensitive		Intermediate		Resistant	
		No	%	No	%	No	%
Amoxicillin (AML)	20 µg	0	0	0	0	50	100
Amoxicillin/clavulanic acid (AMC)	20/10 µg	14	28	0	0	36	72
Piperacillin/tazobactam (TZP)	100/10µg	29	58	0	0	21	42
Cefoxitin (FOX)	30 µg	14	28	0	0	36	72
Ceftazidime CAZ)	30 µg	15	30	0	0	35	70
Cefotaxime (CTX)	30 µg	19	38	0	0	31	62
Ceftriaxone (CRO)	30 µg	18	36	0	0	32	64
Cefepime (FEP)	30µg	20	40	0	0	30	60
Aztreonam (ATM)	30 µg	15	30	0	0	35	70
Ertapenem (ETP)	10 µg	24	48	0	0	26	52
Imipenem (IPM)	10 µg	22	44	2	4	26	52
Meropenem (MEM)	10 µg	27	54	0	0	23	46
Amikacin (AK)	30 µg	14	28	1	2	35	70
Gentamicin (CN)	10 µg	12	24	0	0	38	76
Tobramycin (TOB)	10 µg	16	32	0	0	34	68
Ciprofloxacin (CIP)	5 µg	27	54	0	0	23	46
Ofloxacin (OFX)	5 µg	28	56	0	0	22	44
Norfloxacin (NOR)	10 µg	28	56	0	0	22	44
Doxycycline (DO)	30 µg	7	14	2	4	41	82
Trimethoprim/Sulfamethoxazole (Co-trimexazole) (TMP)	1.25/23.75 µg	13	26	0	0	37	74
Chloramphenicol (C)	30 µg	42	84	0	0	8	16

ESβLs production was detected among 14/50 (28%) of *Enterobacter* isolates by cephalosporin/clavulanate combination disk, while MβL production was detected among 11/50 (22%) of *Enterobacter* isolates by Imipenem/EDTA combined disk test [Table 4]. Also, 9/11 (81.8%) of MβL-producing *Enterobacter* isolates were also ESβLs-positive [Figure 2].

Table 4: Screening and confirmatory methods used for detection of ESβLs and MβLs-producing *Enterobacter* isolates

Total isolates No=50	**ESβL production**								**χ2**	**p value**
	Screening test (disk diffusion test)				**Confirmatory test (combined disk test)**					
	Yes		**No**		**Yes**		**No**		**17.6**	**0.001**
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**		
	35	70	15	30	14	28	36	72		
Total isolates No=50	**MβLs production**								**χ2**	**p value**
	Screening test (disk diffusion method)				**Confirmatory test (inhibitor based method)**					
	Yes		**No**		**Yes**		**No**		**12.1**	**0.001**
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**		
	26	**52**	**24**	**48**	**11**	**22**	**39**	**78**		

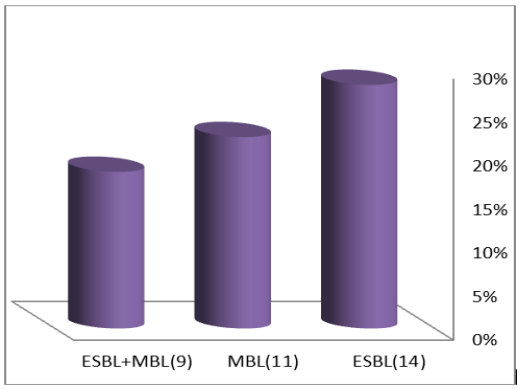

Fig. 2: Distribution of ESβLs and MβL among *Enterobacter* isolates

Biofilm production was detected among 25/50 (50%) of *Enterobacter* isolates by CRA method and among 27/50(56%) by MCRA method. On the other hand, 58% of *Enterobacter* isolates were positive for the *fimH* gene by conventional PCR [Figure 3] The *fimH* gene was detected among 20/27 (74.1%) of the biofilm-producing and only among 9/23 (39.1%) of the non-biofilm-producing *Enterobacter* isolates [Table 5].

Fig. 3: Agarose gel electrophoresis for the PCR amplified products of *Enterobacter fimH* gene
- Lane M: DNA molecular size marker (1000 bp).
- Lanes 2,3,6,7 & 8 were *fimH* gene-positive (640 bp).
- Lanes 1,4,5,9 & 10 were *fimH* gene-negative (640bp).

Table 5: Detection of biofilm formation by MCRA method in relation to *fimH* gene

***FimH* gene**	**Biofilm formation**			
	Biofilm-formation No=27		**Non-biofilm-formation No=23**	
	No	**%**	**No**	**%**
Positive (No=29)	20	74.1	9	39.1
Negative(No=21)	7	25.9	14	60.9
χ2	6.23			
P value	<0.05			

The *fimH* gene was detected among 10/14 (71.4%) of the ESβLs-producing *Enterobacter* isolates and among 8 (72.2%) of the Mβ-producing isolates [Table 6].

Table 6: Prevelance of *fimH* gene among ESβLs- and MβL-producing *Enterobacter* isolates (no=50)

FimH gene	Total	ESβL-production				MβL-production				χ^2	p value
		Yes		No		Yes		No			
		No	%	No	%	No	%	No	%		
FimH-positive	**29**	**10**	**71.4**	**19**	**52.7**	**8**	**72.7**	**21**	**53.8**	**2.3**	**>0.05**
FimH-negative	**21**	**4**	**28.6**	**17**	**47.3**	**3**	**27.3**	**18**	**46.2**		
Total	**50**	**14**	**28%**	**36**	**72%**	**11**	**22%**	**39**	**78%**		

Considering that the detection of the *fimH* gene by PCR as a standard gold test, the sensitivity, specificity, PPV, NPV and accuracy of CRA and MCRA method were 66% Vs 69%, 71% Vs 67%, 76% Vs 74%, 60% Vs 61% and 68% Vs 68% [Table 7].

Table 7: Sensitivity, specificity, PPV, NPP and accuracy of phenotypic methods in relation to PCR for detection of biofilm among 50 *Enterobacter* isolates

Methods	PCR		Sensitivity	Specificity	PPV	NPV	Accuracy
	+ve (No=29)	-ve (No=21)					
Congo red agar:							
– Positive (No=25)	19	6	66%	71%	76%	60%	68%
– Negative (No=25)	10	15					
Modified Congo red agar:							
– Positive (No=27)	20	7	69%	67%	74%	61%	68%
– Negative (No=23)	9	14					

PPV =positive predictive value NPV =negative predictive value

DISCUSSION

Enterobacter spp. belong to the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) which are resistant to antimicrobials[1718] and cause nosocomial infections throughout the world [19].

In this study, 50 *Enterobacter* spp. were isolated from 296 clinical samples (17.3%) collected from patients admitted to different departments, Menoufia University Hospitals. A similar rate (18.2%) was reported in Berlin[21], while higher rate (32%) was reported in Iran[22] and lower rate (5.7%) was reported in Egypt[23]. These differences may be due to regional differences in hygiene status and variable resistance to antibiotics [24].

Most of *Enterobacter* spp. were isolated from ICU (54%) and urine samples (48%). In agreement, 40.5% of *Enterobacter* spp. were isolated from ICU in Iran [25] *and* 51.5% of *Enterobacter* were isolated from urine samples in Brazil [26]. *Enterobacter* spp are able to survive in wet places, mechanical respiration equipment and pipes of difficult access to be washed and/or dried.[20]

This study revealed high rates of resistance to gentamycin (76%). Similar results were obtained in Iran [3]. Also, 62% were resistant to cefotaxime similar to that reported in Iraq [27]. Resistance to imipenem in this study was 52%, a finding similar (53.84%) to that demonstrated in India. [28] Interestingly, *Enterobacter* isolates were highly sensitive to chloramphenicol (84%), a result similar to that obtained in India [29].The higher levels of resistance to these antimicrobials is probably associated with possible production of plasmid-mediated ESBLs. Some of these plasmids in *Enterobacter* isolates may also carry genes that encode resistance to trimethoprime/sulfamethoxazole, quinolones and aminoglycosides [30].

CLSI recommends using multiple agents, including aztreonam, ceftazidime, and cefotaxime for ESBLs screening [15]. In this study, 28% of *Enterobacter* isolates were ESβL-producers by combined disk test using ceftazidime and cefotaxime with and without clavulanic acid. Similar results were obtained by *El-Hendi et al.*[31] (28.9%) and *Mahmoud et al.*[32] (33.3%) in Menoufia University Hospitals, Egypt. ESBLs and carbapenemases, which are often encoded by genes located on large plasmids, represent an emerging public health concern [33].

Regarding MβL production, our study showed that 11 (28%) of *Enterobacter* isolates were MβL-producers by inhibitor-based method. These results are consistent with that reported by *Khajuria et al.*[28] (25.71%) and

Mahmoud et al.[32] (20%). However, lower results (12.6%) were obtained by Biendo *et al*.in India [35].

Resistance to expanded-spectrum cephalosporins, IPM resistance can occur in ESBL-producing *Enterobacter* isolates by carbapenemase production or by the loss of porins in the outer membrane.[34]. In the current study, it was found that 9/14 ESBLs-producing isolates were also MβL-producers (64.3%). Similar findings were previously reported by *Biendo et al.*[35] who found that 42% ESBL-producing *Enterobacter aerogenes* were also MβL-producers. Also, *Huang et al.* [33] in China showed that 26.1% of ESBLs-positive *Enterobacter* isolates, were MβL-positive.

In the present study, 54% and 50% of *Enterobacte*r spp. displayed a positive biofilm phenotypically on MCRA and CRA respectively. In agreement with our results, *Soares et al.*[37] reported that 54% of *Enterobacter* spp. strains were biofilm producers in Brazil. However, higher results (70.8%) were obtained by *Abdul-Razzaq et al.*[38]. In contrast, *Abdallah et al.*[39] found that 0% of *Enterobacter* isolates were able to produce biofilm on bacteria isolated from urinary tract infection in Ain Shams University. Type 1 fimbriae are important for adherence to surfaces and are responsible for specific interactions with mannose-containing receptors in many host tissues. This type of fimbriae recognize their receptor targets by *virtue* of organelle tip-located adhesins which mediates both bacterial adherence to and invasion of host cells, and contributes to the formation of intracellular bacterial biofilms by pathogen. [36]

In the current study, 29/50 (58%) *Enterobacter* isolates were *fimH* gene-positive. Lower (40%) and higher results (75%) were reported by *Hassan et al.*[16] and *Abdul Razzaq et al.*[38] respectively. Also, 20/27 (74.1%) of the biofilm-producing *Enterobacter* isolates were *fimH*-positive while 7/27 (25.9%) of the biofilm-producing *Enterobacter* isolates were *fimH*-negative with a significant difference ($p<0.05$). On the other hand, 9 (39.1%) of the non-biofilm-producing isolates had *fimH* gene while 14 (60.9%) of the non-biofilm-producing isolates were without this gene. Although type 1 fimbriae (*fimH*) is important adhesion factor for bacterial initial attachment to the biological surfaces, presence of this gene is not the only determinant for biofilm development and several environmental and genetic factors may be involved with expression of this gene [40].

Regarding the biofilm and antibiotic resistance, our study showed that all the biofilm-producing *Enterobacter* spp. were resistant to amoxicillin while, 26/27 (96.3%) of the isolates were resistant to doxycycline with a significant difference ($p<0.05$). A significantly ($p<0.05$) higher resistance among the biofilm-producing isolates were also seen with ceftriaxone, gentamicin and azetronam (88.9%, 85.2% and 81.5%). Similar results were obtained by *Soares et al.*,[37] who found that the biofilm-producing *Enterobacter* isolates were highly resistant to antimicrobials (100% to sulphonamides, 93% to cephalosporins and quinolones, 87% to macrolides, 80% to tetracycline and fluroquinolones). Failure of antimicrobial agents to treat the biofilm-producing bacteria may be attributed to tolerance to harsh conditions antibacterials by the biofilm matrix surrounding bacteria. Moreover, the available antibiotics are ineffective for treating these biofilm-related infections. Hence, it is critically important to design or screen anti-biofilm molecules that can effectively minimize and eradicate the biofilm-related infections [8].

CONCLUSION & RECOMMENDATIONS

Enterobacter is a frequent cause of nosocomial infections and *E. aerogenes* was the predominant species isolated from clinical samples. PCR was more reliable as compared to CRA and MCRA methods for detection of the biofilm-producing *Enterobacter* spp. There was a relation between biofilm production and antimicrobial resistance forming a serious threat to empiric therapy of *Enterobacter* isolates. Therefore, it is important to monitor resistance of these strains to antibiotics *and* to find a strategy to improve their prevention and control.

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