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ORIGINAL ARTICLE

SFO-1 and *DHA-1* genes Detection in β-lactamases-producing *Klebsiella pneumoniae* Causing Blood Stream Infections Acquired in Intensive Care Units in Mansoura University Hospitals

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SFO-1, DHA-1, β-lactamases, Klebsiella pneumoniae, blood stream infections

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ABSTRACT

Objectives: *The study aimed to detect SFO-1 and DHA-1 genes and to differentiate AmpC with overlapped ESBL isolates. Also, it identified the risk factors aiding in emergence the ESBL producing K.pneumoniae causing blood stream infections in ICUs of Mansoura University Hospitals (MUHs).* ***Methodology:*** *This is a prospective study that enrolled 520 blood samples. Double disk synergy test (DDST) and Modified three-dimensional test were performed. Genotypic detection of SFO-1, Class A ESBL, and DHA-1, AmpC β-lactamase was done.* ***Results:*** *520 septicemic patients were enrolled with significant correlation for adults' acquisition of infection. The main bacteria isolates causing nosocomial infection in septicemic patients admitted to ICU were S. aureus, E. coli and K. pneumoniae. A significant difference in distribution of ESBL and AmpC β-lactamases was detected with significant association between impenem resistance and prevalence of ESBL in those patients. There was a low-occurrence of ESBL SFO-1 and DHA-1 detected in clinical samples. Surgical intervention and CVC were the significant risk factors for presence of ESBL but previous antibiotics and hospital stay were non-significant effectors for presence of ESBL.* ***Conclusion:*** *Though SFO-1 is a low-prevalence ESBL, it has been taken by a plasmid with many other multiple resistance determinants including many related genes, and go together with by a large DHA-1- plasmid.*

INTRODUCTION

Several factors contributed in dissemination of antibiotic resistance, comprising improper antibiotic usage, and deficiency of new antimicrobial agents[1].

β-lactamase production is the most common mechanism of β-lactam resistance in Gram-negative bacteria[2]. ESBLs can hydrolyze oxyimino-cephalosporins, as aztreonam and 3rd-generation cephalosporins, but clavulanic acid can inhibit it[1]. β-lactamases are classified by two methods. First; Ambler classification that differentiates beta-lactamases into four classes constructed on the protein homology of enzymes, β-lactamases of class A, C, and D are serine β-lactamase and class B enzymes are metallo-β-lactamases. Second; the Bush-Medeiros-Jacoby classification system that categorizes β-lactamases depending on the functional properties of enzymes[3].

Plasmid mediated AmpCs were reported for the first time in 1989[4]. Based on differences in the amino acid sequences, seven enzyme families are currently described: CMY, FOX, ACC, LAT, MIR, ACT, MOX and DHA[5].

The plasmid mediated AmpC enzymes are usually constitutively produced and confer resistance to penicillins, broad-spectrum cephalosporins, cefamycins and variably to aztreonam, and usually remain sensitive to cefepime and carbapenems[5]. They often occur together with other resistance genes[6].

The appearance of DHA-1-type pAmpC *K.pneumoniae* has been described in some European[7] and many Far -Eastern countries[8].

K.pneumoniae that produce SFO-1 enzyme were isolated in China having a big DHA-1-bearing IncFII plasmid (this enzyme is an ESBL). The plasmid had the gene of qnrB4[9].

SFO-1 is class A ESBL. Production of SFO-1 enzyme shows important hydrolytic action towards cefotaxime, but it shows no obvious action towards imipenem and cephamycins. It is powerfully suppressed by the action of the clavulanic acid, ESBLs. On the other hand, this enzyme is an inducible β-lactamase which is controlled by the action of the regulator AmpR-type which is inversely placed upstream of AmpA [10].

Finding of *blaSFO-1* was reported to present with blaDHA-1, armA and other resistance determinants in clinical strains of *K.pneumoniae* that produce ESBL [11].

The current study aimed to assess the epidemiology of the plasmid-mediated (ESBL) of SFO-1 and its co-

production for DHA-1 β-lactamases in clinical isolates of *K. pneumoniae* that produce ESBL from blood stream infections acquired in intensive care units of Mansoura University Hospitals (MUHs), Egypt and determining the potential risk factors that increase the type of infection to provide proper knowledge and efficient plan to control and treat that threat.

METHODOLOGY

This is a prospective study that enrolled 520 blood samples from patients admitted in ICUs from May 2017 to May 2019 at Mansoura University Hospital, Mansoura, Egypt. The patients were fulfilling the criteria of hospital-acquired infection. A complete history and clinical examination were conducted. Approval for the current study protocol was constructed from the Mansoura faculty review board. Consent was undertaken from the study members.

The studied patients age was divided into two main groups: paediatric group (below 18 years) and adult group (above 18 years). Paediatric patients group was subdivided into neonates (0 to 28 days), infants (more than 1to 24 months), young children (more than 2 to 6 years), children (more than 6 to12 years) and adolescents (more than 12 to18 years) [12], while the adult group was subdivided into young adults (more than 18 to 40), middle adults or middle age (more than 40 to 65) and late adults or old age (above 65)[13].

Samples collection and processing:

Venous blood was drawn under complete aseptic condition. The blood samples were processed by direct Gram stain film examination, culturing on blood culture media then sub culturing on blood and MacConkey's agars. Colonies were identified by colonial morphology, Gram-stained films and biochemical reactions using API 20E (*Biomerieux SA, France*) [14]. Antibiotic sensitivity test was done by disk diffusion method according to CLSI (2017).

Phenotypic detection of ESBL-producing Klebsiella pneumoniae and AmpC β- lactamases

Double disk synergy test (DDST)[15]: it was performed for detection of ESBL**.** All *K.pneumoniae* isolates that showed resistance to any of the β-lactam drugs in routine sensitivity tests were examined for the presence of ESBL as shown in (figure 1).

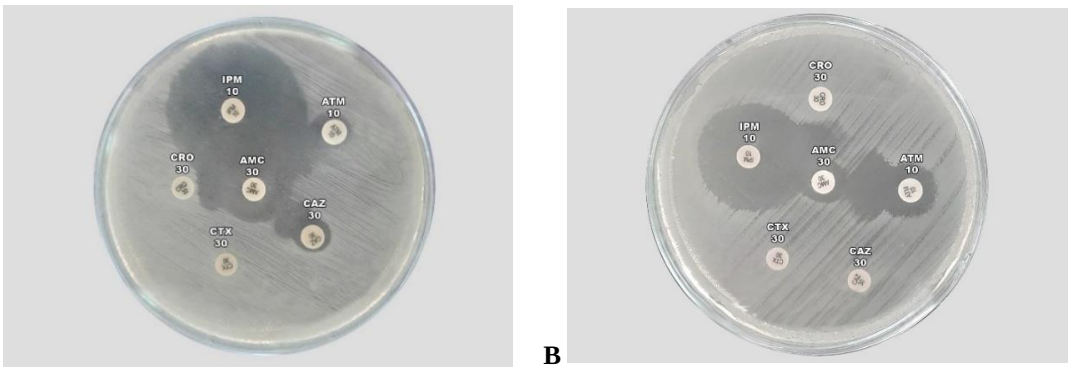

Fig. 1: The double disk synergy test. In plate (A) inhibition zones of aztreonam and ceftazidime are larger towards co-amoxiclav disk. In plate (B) inhibition zone of aztreonam is larger towards co- amoxiclav disk.
ATM= Azteronam, AMC=Co-amoxiclav, CAZ= Ceftazidime, CTX= Cefotaxime, CRO= Ceftriaxone, IPM= Imipenem

Modified three-dimensional test was done for detection of AmpC production[16] as shown in figure 2.

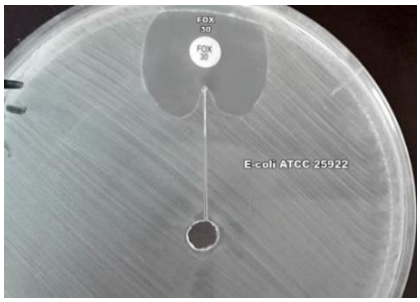

Fig. 2: Modified three-dimensional test. The plate is positive for AmpC producer showing clear distortion (arrow) of zone of FOX (Cefoxitin).

Genotypic detection of SFO-1, Class A ESBL, and DHA-1, AmpC β-lactamase

Plasmid DNA extraction (Gene JETTM Plasmid Miniprep Kit) (Thermo Fisher Scientific, US). The Procedure was performed according to manufacturer's instructions.

Polymerase Chain Reaction (PCR) was done. The forward and reverse primers were manufactured on request by (Biosearch Technologies, USA). The primers were selected according to; *BlaSFO-1* gene[13] and *BlaDHA-1* gene[17] as illustrated in table 1**.** The Procedure was done according to manufacturer's instructions.

Table 1: Forward and reverse primers sequences of SFO-1 and DHA-1 genes.

Gene	Forward primer (5'----------3')	Reverse primer (5'----------3')	Size (bp)	Ta (℃)
BlaSFO-1	ATTCAGCAGCAACTGTCCG	ACGCTTATCGCTGGGAAT	447	54
BlaDHA-1	AACTTTCACAGGTGTGCTGGGT	CCGTACGCATACTGGCTTTGC	405	64

bp=Size of PCR amplicon, Ta= annealing temperatures

Agarose gel electrophoresis of amplified plasmid DNA was done using DNA plasmid marker; #IB01300 marker 100bp DNA ladder (IBI Scientific, USA). The products of PCR were scanned by UV illuminator after electrophoresis on agarose gel. This showed the following:

***BlaSFO-1* gene in ESBLs-producing *K.pneumoniae* isolates:**

Bands represent *blaSFO-1* gene were read at expected amplicon size (447 bp), as shown in figure 3.

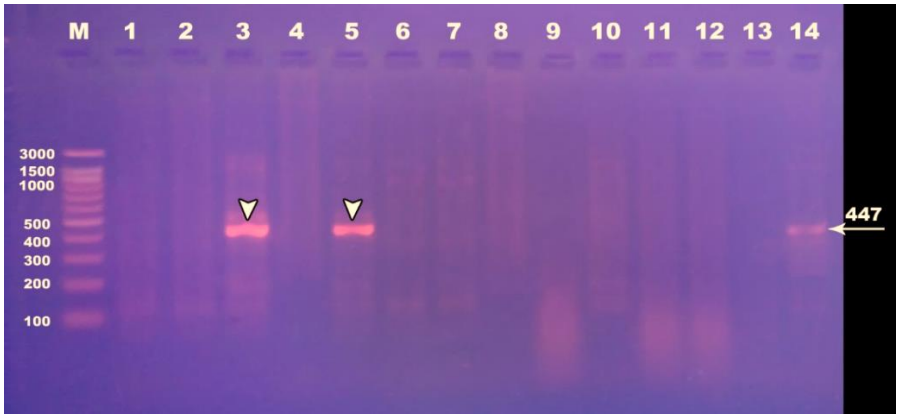

Fig. 3: *blaSFO-1* gene in ESBL-producing *K.pneumoniae* isolates by PCR.

BlaDHA-1* gene in AmpC β-lactamase-producing *K.pneumoniae* isolates*:

Bands represent blaDHA-1 gene were read at expected amplicon size (405 bp), as shown in figure 4.

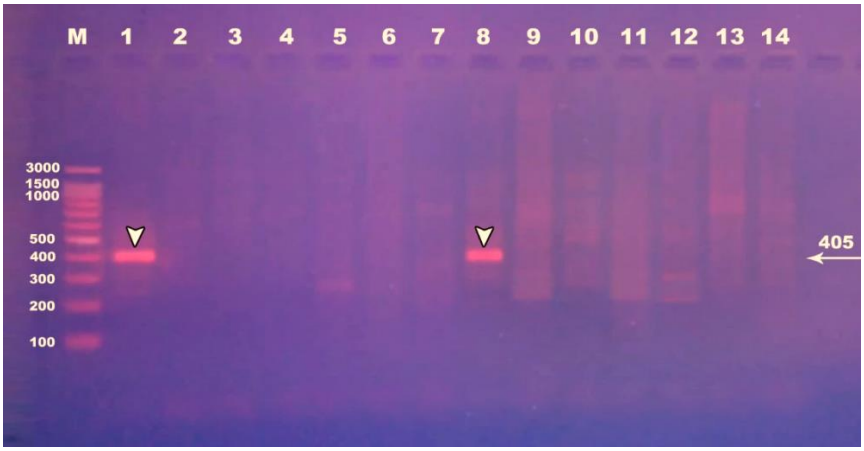

Fig. 4: *blaDHA-1* gene in AmpC β-lactamase producing *K.pneumoniae* isolates by PCR.

Statistical analysis:

Data analysis was achieved using statistical software program (SPSS for Windows, version 21, USA). Chi-square test was performed. The association between the ESBLs and each of AmpC β- lactamases, antibiotics resistance, and SFO-1, and the association between AmpC β-lactamases and DHA-1 were studied using a univariate logistic regression analysis model. In this method, the dependent variable was the presence of ESBLs. Risk factors with a significant association at $p < 0.1$ (two-sided) were selected for further analysis using a multivariate logistic regression model. Regression coefficient (B), Wald, p-value, standard error (S.E.), odds ratio (OR), and a 95% confidence interval (95% CI) were standard for each evaluated risk factor. In all statistical analyses, the results were statistically significant at $p < 0.05$.

RESULTS

Study was carried out from May 2017 to May 2019 counting 520 blood samples from patients with hospital acquired blood stream infections that occurred in ICUs of Mansoura university Hospitals, Egypt. The demographic data of the enrolled septicemic patients were illustrated in table 2.

Table 2: Demographic Data of septicaemia patients admitted to ICU.

Variables		Study group (n = 520)	
		No.	**%**
Age			
Paediatrics		**148**	**28.46 %**
Neonates	0 -28 days	56	10.77 %
Infants	> 1 – 24 months	37	7.12 %
Young child	2 – 6 years	10	1.92 %
Child	6 – 12 years	27	5.19 %
Adolescent	12 – 18 years	18	3.46 %
Adults		**372**	**71.54 %**
Young adults	18 – 40 years	144	27.69 %
Middle adults	40 – 65 years	154	29.62 %
Old adults	Above 65 years	74	14.23 %
Gender			
Males		246	47.31 %
Females		274	52.69 %

The main isolation percentage of bacteria that cause hospital-acquired infection in septicemic patients admitted to ICU was recorded for *S.aureus* (27.69%), *E.coli* (24.42%), *K.pneumoniae* (21.54 %).

There was a significant difference ($P<0.001$) between ages of septicaemia patients. However, there was non-significant difference between males and females.

There was a significant difference ($P < 0.001$) in distribution of ESBLs detected. Seventy-three patients were positive ESBLs (65.2%), while 39 patients were negative ESBLs (34.8%). The median age of septicemic patients was 49. There was a significant difference ($P = 0.003$) between different gender of septicemic patients. The percentage of septicemic female patient with ESBLs- producing *K.pneumoniae* was 63 %, while that of male patients was 37 %. The frequency of AmpC β-lactamases detected in isolated *K.pneumoniae* showed significant ($P = 0.001$) difference in distribution of AmpC β-lactamases detected in isolated *K. pneumoniae.* In the studied septicemic patients, 24 patients were found to be positive AmpC (21.4 %), while 88 patients were negative AmpC (78.6 %).

There was a significant association ($P < 0.05$) between the presence of ESBLs and presence of AmpC β-lactamases in septicaemia patients admitted to ICU infected with *K. pneumoniae* (table 3).

Table 3: Association between ESBLs and antibiotics resistance in isolates from septicaemia patients admitted to ICU infected with *K. pneumoniae*

Antibiotics	Extended Spectrum β-lactamase (ESBL)		P-value	Odds ratio	95 % CI
	Yes	No			
Amikacin			0.545	1.657	0.318 – 8.626
Resistant	67 (91.8 %)	37 (94.9 %)			
Sensitive	6 (8.2 %)	2 (5.1 %)			
Ampicillin			0.505	1.422	0.503 – 4.019
Resistant	58 (79.5 %)	33 (84.6 %)			
Sensitive	15 (20.5 %)	6 (15.4 %)			
Cefipime			0.476	1.552	0.460 – 5.243
Resistant	62 (84.9 %)	35 (89.7 %)			
Sensitive	11 (15.1 %)	4 (10.3 %)			
Aztreonam			0.291	0.500	0.135 – 1.846
Resistant	68 (93.2 %)	34 (87.2 %)			
Sensitive	5 (6.8 %)	5 (12.8 %)			
Cefotaxime			0.611	1.338	0.435 – 4.118
Resistant	61 (83.6 %)	34 (87.2 %)			
Sensitive	12 (16.4 %)	5 (12.8 %)			
Ceftazidim			0.393	1.614	0.534 – 4.871
Resistant	59 (80.8 %)	34 (87.2 %)			
Sensitive	14 (19.2 %)	5 (12.8 %)			
Ceftriaxone			0.145	2.600	0.693 – 9.749
Resistant	60 (82.2 %)	36 (92.3 %)			
Sensitive	13 (17.8 %)	3 (7.7 %)			
Ciprofloxacin			0.888	0.936	0.370 – 2.368
Resistant	57 (78.1 %)	30 (76.9 %)			
Sensitive	16 (21.9 %)	9 (23.1 %)			
Gentamicin			0.839	0.899	0.322 – 2.508
Resistant	61 (83.6 %)	32 (82.1 %)			
Sensitive	12 (16.4 %)	7 (17.9 %)			
Impenem			0.004	0.076	0.009 – 0.660
Resistant	72 (98.6 %)	33 (84.6 %)			
Sensitive	1 (1.4 %)	6 (15.4 %)			
Trimethoprim			0.328	0.628	0.246 – 1.602
Resistant	60 (82.2 %)	29 (74.4 %)			
Sensitive	13 (17.8 %)	10 (25.6 %)			
Levoloxacin			0.487	0.736	0.311 – 1.746
Resistant	55 (75.3 %)	27 (69.2 %)			
Sensitive	18 (24.7 %)	12 (30.8 %)			

As regards to Chi-square analysis, there was a significant association ($P < 0.05$) between impenem resistance and prevalence of ESBLs in those patients.

Studying the distribution of SFO-1 and DHA-1 detected in isolated *K.pneumoniae* (n = 112) concluded that there was a significant difference ($P < 0.001$) in distribution of SFO-1 and DHA-1. In the studied septicemic patients, 3 patients only were found to be positive SFO-1 (2.7 %), while 109 patients were negative SFO-1 (97.3 %). Furthermore, 2 patients only were found to be positive DHA-1 (1.8 %), while 110 patients were negative DHA-1 (98.2 %). Regarding Chi-square test, there was a non-significant association between the prevalence of ESBLs and prevalence SFO-1 in the targeted group ($P=0.199$). Also, there was a significant association ($P < 0.006$) between the presence of AmpC β-lactamases and presence of DHA-1.

Potential risk factors associated with ESBLs-producing *K.pneumoniae* isolates and the univariate analysis of ESBL were summarized in Table 4.

Table 4: Distribution of potential risk factors associated with ESBLs- producing *K.pneumoniae*

Risk factors	Extended Spectrum β-lactamase (ESBL)		p-value	Odds ratio	95 % CI
	Yes	No			
Previous antibiotics			0.104	2.023	0.859 – 4.766
Yes	43 (58.9 %)	29 (74.4 %)			
No	30 (41.1 %)	10 (25.6 %)			
Surgical intervention			0.010	3.070	1.280 – 7.365
Yes	38 (52.1 %)	30 (76.9 %)			
No	35 (47.9 %)	9 (23.1 %)			
Central venous catheter (CVC)			0.025	0.355	0.141 – 0.894
Yes	62 (84.9 %)	26 (66.7 %)			
No	11 (15.1 %)	13 (33.3 %)			
Hospital stay			0.621	0.789	0.307 – 2.023
≤ 7 days	55 (75.3 %)	31 (79.5 %)			
> 7 days	18 (24.7 %)	8 (20.5 %)			

DISCUSSION

The US Centres for Disease Control and Prevention (CDC) antibiotic resistance pressures report has classified *K.pneumoniae* as a serious drug resistance threat [18]. Epidemiologic and drug resistance analysis of ICU *K.pneumoniae* is important for hospital-acquired infections control and enabling the good choice and the usefulness of empirical therapy[19].

Along the research period, 520 septicemic patients' blood samples were recruited, of these, paediatric patients were 28.46 %, and adults were 71.54 %, with significant acquisition of infection in adults. The same results were obtained by Wang et al.[18]. Many authors consider old age as risk factor[20]. Studies reported no correlation found between age[21] and infection or with the gender[17, 21].

In our study, the main isolates that cause nosocomial infection in septicaemia patients were *S.aureus* (27.69%), *E.coli* (24.42%) and *K.pneumoniae* (21.54 %).

Agaba et al.[22] reported *K.pneumoniae* (30%), *Acinetobacter* species (22%) and *S. aureus* (14%) were the most frequently isolated bacteria. Piruozi et al.[23] showed the most common isolated microorganisms were *S.epidermidis* (43.33%) and *E.coli* (16.66%). In Egypt, it was reported that the most common pathogens were *K.pneumoniae* (28.7%) and *Acinetobacter* species (13.7%)[29-33]. Others showed that in neonatal setting, *Klebsiella* species accounted for 33.3% of isolates, and were the most frequent isolation[24]. This difference in findings may be due to different criteria of patient selection, the case mix, severity of illness, type of ICU, stay length, rate of device utilization and criteria of discharge[25].

In this study, we found a significant difference between ages of septicaemia patients admitted to ICU infected with *K. pneumoniae*. On the other hand, there was non-significant difference between males and females' septicaemia. The same results were obtained by Tian et al.[26]. Also, Guo et al.[27] reported age as a risk factor and no significant difference regarding the gender.

In many Enterobacteriaceae, AmpC expression is low but induced under pressure selection of β-lactam exposure[28].

The present study findings showed a significant difference in distribution of ESBL and AmpC β-lactamase detected in isolated *K. pneumoniae*. Seventy-three patients were found to be positive ESBL (65.2 %), while 39 patients were negative ESBL. We found on the other hand 21.4 % of patients to be positive AmpC, while 78.6 % were negative.

The study demonstrated a significant association between impenem resistance and prevalence of ESBL. In contrast, there was no significant association between resistance to each of amikacin, ampicillin, cefipime, aztreonam, cefotaxime, ceftazidim, ceftriaxone, ciprofloxacin, gentamicin, trimethoprim and levofloxacin and prevalence of ESBL in septicemic patients. The same results obtained by Singh et al.[30]. Penicillin and cephalosporins showed a lesser degree of susceptibility, which may be due to unprecedented use and over the counter sale of these drugs. β-lactamase inhibitor combinations, aminoglycosides, norfloxacin and nitrofurantoin, showed a good susceptibility rates and these drugs may be recommended as a first-line of treatment of infections caused by ESBL *K.pneumoniae*[30].

Also, Goyal et al.[34] reported a higher rate of resistance to ciprofloxacin (93.8%), trimethoprim-sulfamethoxazole (79.1%), gentamicin (66.7%) while low resistance to amikacin (14.7%) among the ESBL isolates.

Our findings showed a significant difference in distribution of *blaSFO-1* and *blaDHA-1* detected in *K. pneumoniae*. Three patients only were found to be positive *blaSFO-1* (2.7 %), while 109 patients were negative *blaSFO-1* (97.3 %). Furthermore, 2 patients only were positive DHA-1 (1.8 %), while 110 patients

were *negative blaDHA-1* (98.2 %). Indicating the presence of *blaSFO-1* and *blaDHA-1* in the isolates is rare. The association between the prevalence of ESBL and prevalence *blaSFO-1* was a non-significant association. Zhou et al.[35] showed the same results.

Our study reported a significant association between the presence of AmpC β-lactamase and presence of DHA-1 in septicemic patients admitted to ICU infected with *K. pneumoniae*. Chang et al.[36] reported a clear predominance of DHA-1 with ESBL-producing *K.pneumoniae* isolates.

Also, it was found a significant association between the presence of ESBLs and presence of AmpC β-lactamases. Organisms over expressing AmpC are usually resistant to all beta lactam antimicrobials, except for cefepime, cefpirome and carbapenems [37].

As regard the risk factors, the present results revealed that surgical intervention and central venous catheter were the significant risk factors for presence of ESBL. However, previous antibiotics and hospital stay were non-significant effectors. Many studies reported the same results [38- 39] on contrary to Shanthi and Sekar[40].

CONCLUSION

Our findings showed a significant correlation in distribution of SFO-1 and DHA-1 detected in *K.pneumoniae* isolated from septicaemia patients admitted to ICU. But we found a low-prevalence ESBL SFO-1 and DHA-1 emerged in clinical isolates. Also the surgical intervention and CVC are the significant risk factors for presence of ESBL. However, previous antibiotics and hospital stay are non-significant effectors for presence of ESBL.

- 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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