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Phenotypical Detection of Extended Spectrum Beta-Lactamases Producing *Escherichia coli*, from Clinical Isolates in Riyadh Region, Al-Kharj City, Kingdom of Saudi Arabia

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Abstract: ***Background and objective: the aim of the present study was to identify the phenotypical detection of extended spectrum beta-lactamases, (ESBL) Phenomenon among Escherichiacoli isolated from clinical samples in King Khalid Hospital and Price Sultan Center, Alkharj City, Riyadh Region, Kingdom of Saudi Arabia. Materials and Method: A total of 174 isolates of Escherichia coli were isolated by standard microbiological method, and identified by BD Phoenix system (Becton Dickinson, USA), full automated microbiological machine confirmed by API 20 E (BioMérieux, Marcy L'Etoile, France). The antimicrobial susceptibility pattern of the isolates was determined by BD Phoenix system confirmed by E. test (Epsilometer test), (Oxioid, UK) according to CLSI guideline (2008). The Extended spectrum beta-lactamases (ESBL) producing Escherichia coli were screened by BD Phoenix system confirmed by double disk diffusion method. p-value < 0.05 was counted as statistically significant. Result: more than forty two percent was found to be positive for ESBL producers and 57.5% as non-ESBL producers, urine samples were the most frequent in this study, males were more prevalent to ESBL producers than females, high resistant of ESBL producers was observed among different family of antibiotics including, pipracillin (76.3%), ampicillin (76.3%), tetracycline (62.7%), ceftriaxone (60.0%), cefuroxime (55.0%), trimethoprime / sulphamexazole (54.8%), azitronam (54.1%) and amoxicillin / Clavulanic acid (53.6%). Meropenes, imipenem, amikacin and tigycyline were the most active against ESBL producers. Conclusion: The prevalence of ESBL producers in this study was high compared with others studies performed in Saudi Arabia, so it requires sound infection control measures, antimicrobial management and measures to detect and control their spread should be considered.***

1. Introduction

The ESBLs are enzyme that mediate resistance to extended - spectrum third generation cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotenan) or carbapenems carbapenems (e.g., meropenems or imipenems) (CDC, 2010). In *Enterobacteriaceae* production of ESBLs constitutes the most common resistance mechanism tobeta-lactam antibiotics (Rossolini and Mantengoli, 2008). ESBLs commonly encoded on large transferable plasmids which also encode resistance to other antibiotics classes, therefore often express high levels of co- resistance to aminoglycoside, quinolones, beta-lactam / beta-lactm inhibitor combinationand concurrently from strain to strain (Hirakataa *et al*., 2005). Many new β-lactam antibiotics have been developed during the last 20 years specifically designed to be resistant to the hydrolytic action of β-lactamases. However, with each new class that has been used to treat patients, new β-lactamases emerged that cause resistance to that class of drug. One of these new classes was the oxyimino- cepholosporins, which became widely used for the treatment of serious infections due to Gram – negative bacteria in the 1980s. Resistance to expanded – spectrum β - lactam antibiotic due to β- lactamase emerged quickly. The first of these enzymes capable of hydrolyzing the newer β-lactams, SHV-2, was found in single strain of *Klebsiellaozaenae* isolated in Germany. Because of their increased spectrum of activity, especially against the oxyimino – cephalesporins, these enzymes were called extended – spectrum β - lactamases (Kliebe *etal*., 1985).the European Commission have recognized the importance of studying the emergence and determinants of acquired anti-microbial resistance and the need to devise appropriate strategies for their control. (WHO, 2000 and CDC, 2000), In particular, the Extended - Spectrum Beta - Lactamase (ESBL)-producing *Escherichia coli* are emerging worldwide. (Picozzi, *et al* 2013; Briongos *et al*, 2012 and Lu *et al*, 2012).

The prevalence of ESBLs is variable geographically from place to place. In sub-Saharan Africa, the median prevalence of resistance to third – generation cephalosporins ranged from 0% to 47% (Leopold et al., 2014).Theodor Bilharz Research Institute, Cairo, Egypt reported that a total of 16% of all isolates, 19% of *E. coli* and 14% of *K. pneumonae* were ESBL-producers (Fam *et al*., 2011).In Latin America,

ESBL producing Enterobacteriaceae is also rising. Rates in *E. coli* were as high as 41% in 2009 in Mexico. In 2014, resistance of *K.pneumonae* isolates to third generation cephalosporins is a maker of ESBL Production ranged from 19% in Peru to 87% in Bolovia (PAHO, 2014). In the Arabian Peninsula, reported ESBL detection rate ranged from 8.9% to 36% in data from the Kingdom of Saudi Arabia (Kader and Angamuthu, 2005) (Baby, 2002) and (31.7%) in Kuwait (Mokaddas *et al*., 2008) The highest prevalence rate of 41% is from the United Arab Emirates (Al-Zarouni *et al*., 2008). Therefore, this study was performed to estimate ESBLs, producing *Escherichia coli* from clinical isolates in King Khalid Hospital and Prince Sultan Center, Alkharj City, Riyadh Region, Kingdom of Saudi Arabia.

2. Materials and method

Study design:
This study is a cross-sectional hospital based study.

Study area and duration:
This study was carried out during the period from October 2016 to July 2017, in King Khalid Hospital and Prince Sultan Center - Al Kharj, Riyadh region, KSA

Inclusion criteria:
All patients (both males and females of different ages) suffering from different symptoms suspected for *E. coli* infections, hospitalized or out patients, at the period of this study, attending to a study area, were included in this study. Any *E. coli* isolate that show resistance or moderate resistance to third generation cephalosporins antibiotics were selected in this study.

Exclusion criteria:
Isolates other than *E. coli* were excluded from this study.

Ethical consideration:
Ethical clearance for this study was obtained by Ministry of Health, and King Fahad Medical City, Saudi Arabia, complete information regarding risk factors are handed to all subjects under Study, maintaining confidentiality of information obtained from subjects under study.

Data analysis:
Data were recorded and analyzed. The collected data were analyzed using Statistical Package for Social Science (SPSS), Chi square test was used, and a p-value of < 0.05 was counted significant.

Isolation of *E. coli*
A total of 174 *E. coli* isolates (*E. coli*, $n = 174$) were obtained from King Khalid Hospital and Prince Sultan Center - Al Kharj City, Riyadh region, KSA, from hospitalized Patients and out patients,. The collected samples contained 89 isolates from urine, 16 from peritoneal fluid, 7 from sputum, 8 from ETT aspirates, 15 from abscess, 18 from wound swab, 5 from umbilical fluid, 9 from blood, 1 from stool, and one from semen. *E. coli* ATCC 25922 and K. *pneumonia* ATCC 700603 were used as quality controls. Isolation were done by using standard microbiological method.

Identification of isolates
All isolates preliminarily identified as Gram- negative, then identification were done by using BD Phoenix™ Microbiology System, (Becton Dickinson, USA), confirmed by using API 20 E (BioMérieux, Marcy L'Etoile, France), the result were interpreted according to the Manufactory recommendation. *E coli* ATCC 25922 were used as quality controls. Then all isolates were stored at -80°C in 15% glycerol (v/v) in tryptic soy broth.

Antimicrobial Susceptibility Testing
All identified *Escherichia coli* were tested for their antimicrobial susceptibility testing byusing BD Phoenix Automated Microbiology System instrument and MIC was detected according to the CLSI, 2016 recommendation. Confirmed by E-test (Epsilometer test), (Oxioid, UK) according to the CLSI guidelines (CLSI, 2008). The following antibiotics were used, amikacin (30mg), penicillin (10mg), cefoxtin (30mg), cefepime (30mg), cefotaxime (30mg), ceftazidime (30mg), cefuroxime (30mg), ciprofloxacin (5mg), ceftriaxone (30mg), gentamycin (10mg), imipenem (10mg), meropenem (10mg), levofloxacin (10mg), nitrofurantoin (300mg), piperacillin (100mg), tobramycin (10mg), tetracycline (30mg), tigecycline (30mg), trimethoprim / sulfamethoxazole (1.25/23/75mg), amoxillin- clavulanic acid (20/10mg), (Table 1).

Table 1: Antibiotics Susceptibility Patterns, break points CLSI/2016, M100S, 26th ed.

Antibiotic family	Antibiotic	Disc content	MIC µg/ml according to CLSI., 2016		
			S	I	R
Betalactam	Ampicillin	10 µg	≤8	16	32≥
	Cefoxitin	30 µg	≤8	16	32≥
	Cefuroxime	30 µg	≤8	16	32≥
	Ceftazadime	30 µg	≤4	8	16≥
	Cefotaxime	30 µg	≤1	2	4≥
	Ceftriaxone	30 µg	≤1	2	4≥
	Cefepime	30 µg	≤2	-	16≥
	Imipenem	10 µg	≤1	2	4≥
	Meropenem	10 µg	≤1	2	4≥
	Aztreonam	30 µg	≤4	8	16≥
	Pipracillin	100 µg	≤16	32/64	128≥
Beta-lactam/ Betamases inhibitor	Amoxicillin-Clavulanic acid	20/10 µg	≤8/4	16/8	32≥
Aminogly cosides	Amikacin	30 µg	≤16	32	64≥
	Tobramycin	10 µg	≤4	8	16≥
	Gentamycin	10 µg	≤4	8	16≥
Fluoroq-uinolones	Ciprofloxacin	5 µg	≤1	2	4≥
	Levofloxacin	5 µg	≤2	4	8≥
Tetracyclines	Tetracycline	30 µg	≤4	8	16≥
	Tigecycline	30 µg	≤4	8	16≥
Nitrofurans	Nitrofurantoin	300 µg	≤32	64	128≥
Folate pathway inhibitors	Trimethoprime-sulfamexazole	1.25/ 23.75 µg	≤2/28		4/76≥

Phenotypic detection of ESBL production

Phoenix ESBL test
Phenotypic detection of ESBLs first done by the BD Phoenix system, the strains were tested, both in terms of identification and antimicrobial susceptibility, with the

NMIC/- 50 and NMIC/ID-70 BD Phoenix GN Combo panels with regard to ESBL detection, the panels differ in their cephalosporin profiles and the ranges of their MICs. The panels were inoculated and incubated according to the manufacturer's recommendations. BD Phoenix ESBLs screening test, inclusive in both panels, utilizes the growth response to selected cephalosporins (cefotaxime, ceftazidime, cefpodoxime, and ceftriaxone), with or without clavulanic acid, todetect the production of ESBLs. The results were analyzed with the integrated BDXpert system (Farbert *et al*., 2008).

Confirmatory test for ESBL

The Double Disc Synergy Test (DDST) was adopted to confirm the presence of ESBLs (Pal *et al*., 2009). A tube containing about 2 ml of sterile normal saline was inoculated with a pure culture growth until matching with 0.5 McFarland turbidity, the bacterial suspension were streaked onto Mueller Hinton agar plate by using sterile cotton swab. Using a sterile forceps, ceftazidime (30 µg) and ceftriaxone (30 µg) disc were gently placed on the agar at distance of 15 mm, center to center from a combination disc of amoxillin (20 µg) - clavulanic acid (10 µg), (MASTDICS, UK). The plates were then incubated for18-24 hours and extended - spectrum in the zone of inhibition was observed and interpreted Positive result of ESBL was interpreted as any isolates that has the zone around the test antibiotics disc increased towards the center the center disc of amoxicillin-clavulanic acid. The results were further interpreted using standard guidelines. A ≥ 5 mm increase in zone diameter for either antimicrobial agent compared to its zone when tested alone signifies positive result (CLSI, 2006).

3. Result

Isolation and identification

Isolation of 174 clinical samples of E. coli were done by standard microbiological method, identification of isolates was carried out by using BD phoenix 100 (USA). *E. coli* ATCC 22955 was used as quality control. Identification confirmed by API 20 E.

Antimicrobial Susceptibility Testing

All isolates (174) were subjected to antimicrobial susceptibility testing by BD phoenix Automated Microbiology System instrument, USA according to CLSI, 2016, confirmed by E. test (Epsilometer test), (Oxioid, UK), according to CLSI guidelines (CLSI, 2008). The sensitivity result indicate that more than seventy six percent (128/168) of all isolates were resistant to ampicillin, 45 (76.3%) to pipracillin, 90 (53.6%) to amoxicillin/Clavulanic acid, 73 (54.1%) resistant to aztreonam, 66 (39.3) were resistant to levofloxacin, 40 (23.8%) of isolates were resistant to gentamycin, 73 (43.5%) isolates were resistant to (ciprofloxacin), 66 (60.0%) isolates were resistant to ceftriaxone, 93 (55.4%) isolates were resistant to cefuroxime, 83 (49.4) isolates were resistant to ceftazidime, 19 (11.3%) isolates were resistant to cefoxitin, 26 (41.9%) isolates were resistant to cefotaxime and 87 (51.8%) of all isolates were resistant to cefepime (Table 2).There was statistically significant association (P-value < 0.001) between the previous antibiotics and ESBL producers and non - producers (Table 3), (Figure 1).

In addition, the sensitivity result indicated that more than seven percent (12/168) resistant to meropenem and six percent (10/168) resistant to imipenem with (P-value < 0.05) between ESBL producers and non – producers (Table 3). More over fifty four percent (92/168) isolates resistant to trimethoprime / sulfamexazole (Table 2) and 18 (30.5%) isolates resistant to tobramycin, with (p-value < 0.01) (Figure 1). Where more than sixty two percent (37/59) isolates resistant to tetracycline, 17 (10.1%) of isolates resistant to nitrofurantoin and one (0.6%) of all isolates resistant to tigecycline as lowest resistant antibiotic percent to all isolates in this study (Tables 2), it was concerned the drug of juice to ESBLs producer in this study, 167 (99.4%) off all isolates susceptible to tigecycline with p-value > 0.05 (Table 3), (Figure 1). In addition, imipenem and meropenem were concerned the second stage for treatment of ESBL producers with high percent of susceptibility rate 158 (94.0%) and 156 (92.9%), respectively.

Table 2: Antibiotic Susceptibility testing of *E. coli* isolates

Antibiotics	R	S	Total
AUG	90 (53.6%)	78 (46.4%)	168
AMP	128 (76.2%)	40 (23.8%)	168
CEP	87 (51.8%)	81 (48.2%)	168
CTX	26 (41.9%)	36 (58.1%)	72
CFX	19 (11.3%)	149 (88.7%)	168
CAZ	83 (49.4%)	85 (50.6%)	168
CXM	93 (55.4%)	75 (44.6%)	168
CIP	73 (43.5%)	95 (56.5%)	168
CRO	66 (60.0%)	44 (40.0%)	110
GM	40 (23.8%)	128 (76.2%)	168
IMI	10 (6.0%)	158 (94.0%)	168
LEV	66 (39.3%)	102 (60.7%)	168
MEM	12 (7.1%)	156 (92.9%)	168
NI	17 (10.1%)	151 (89.9%)	168
TRI/SMX	92 (54.8%)	76 (45.2%)	168
ATM	73 (54.1%)	62 (45.9%)	135
TIG	1 (.6%)	167 (99.4%)	168
TOB	18 (30.5%)	41 (69.5%)	59
TET	37 (62.7%)	22 (37.3%)	59
PIP	45 (76.3%)	14 (23.7%)	59

Where: R, resistance; S, susceptible; AUG, Amoxicillin – Clavulanic acid; MP, ampicillin; CEP, cefepime; CTX, cefotaxime; CFX, cefoxitin CAZ, ceftazidime; CXM, cefuroxime; CIP, ciprofloxacin; CRO, ceftriaxone; GM, gentamycin; IMI, imipenem; LEV, levofloxacin; MEM, meropenem; NI, nitrofurantoin; ATM, aztreonam; TIG, tigecycline; TRI / SMX, trimethoprime / sulfamexazole; TOB, tobramycin; TET, tetracycline; PIP, pipracillin.

Table 3: Antimicrobial resistance Pattern among ESBL producers and non-ESBL producers

Antibiotics	ESBL production		P. value
	ESBL producer	ESBL non-producer	
AK	4 (100.0%)	0 (0.0%)	P < 0.05
AUG	67 (74.4%)	23 (25.6%)	P < 0.001
AP	74 (57.4%)	55 (42.6%)	P < 0.001
CEP	72 (82.8%)	15 (17.2%)	P < 0.001
CTX	23 (88.5%)	3 (11.5%)	P < 0.001
CFX	12 (63.2%)	7 (36.8%)	P < 0.001
CAZ	72 (86.7%)	11 (13.3%)	P < 0.001
CXM	74 (79.6%)	19 (20.4%)	P < 0.001
CIP	48 (65.8%)	25 (34.2%)	P < 0.001
CRO	50 (75.8%)	16 (24.2%)	P < 0.001
GM	28 (70.0%)	12 (30.0%)	P < 0.001
IMI	1 (10.0%)	9 (90.0%)	P < 0.05
LEV	46 (69.7%)	20 (30.3%)	P < 0.001
MEM	2 (16.7%)	10 (83.3%)	P < 0.05
NI	11 (64.7%)	6 (35.3%)	P > 0.05
TRI/SM	49 (52.7%)	44 (47.3%)	P < 0.01
ATM	63 (86.3%)	10 (13.7%)	P < 0.001
TIG	1 (100.0%)	0 (0.0%)	P > 0.05
TOB	12 (66.7%)	6 (33.3%)	P < 0.01
TET	18 (48.6%)	19 (51.4%)	P > 0.05

Where AUG, Amoxicillin – Clavulanic acid; AMP, ampicillin; CEP, cefepime; CTX, cefotaxime; CFX, cefoxitin; CAZ, ceftazidime; CXM, cefuroxime; CIP, ciprofloxacin; CRO, ceftriaxone; GM, gentamycin; IMI, imipenem; LEV, levofloxacin; MEM, meropenem; NI, nitrofurantoin; ATM, aztreonam; TIG, tigecycline; TRI / SMX, trimethoprime / sulfamexazole; TOB, tobramycin; TET, tetracycline; PIP, pipracillin.

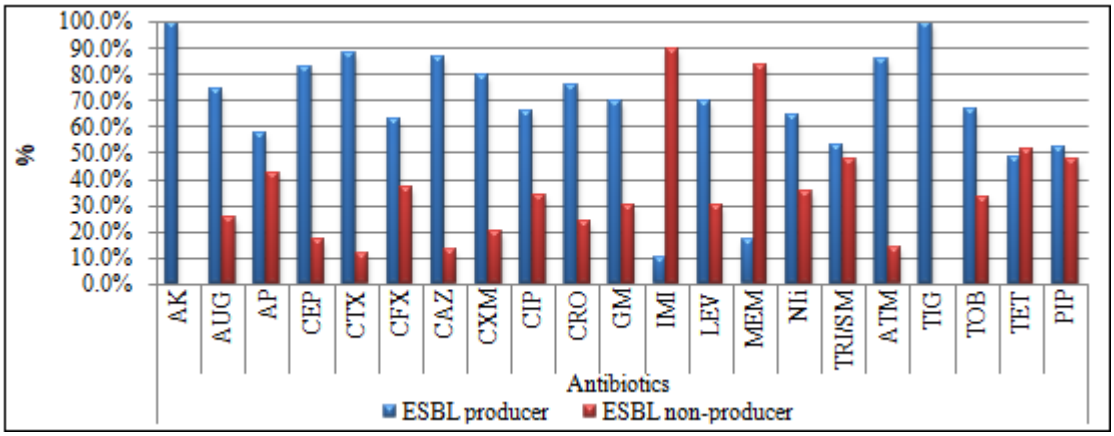

Figure 1: Distribution of antibiotic percentage among ESBL producers and non-ESBLs

Where AUG, Amoxicillin – Clavulanic acid; AMP, ampicillin; CEP, cefepime; CTX, cefotaxime; CFX, cefoxitin; CAZ, ceftazidime; CXM, cefuroxime; CIP, ciprofloxacin; CRO, ceftriaxone; GM, gentamycin; IMI, imipenem; LEV, levofloxacin; MEM, meropenem; NI, nitrofurantoin; ATM, aztreonam; TIG, tigecycline; TRI / SMX, trimethoprime / sulfamexazole; TOB, tobramycin; TET, tetracycline; PIP, pipracillin.

Phenotypic detection of ESBLs

The Phenotypic detection of ESBLs done by using phoenix system according to CLSI recommendation (CLSI, 2016), *E. coli* ATCC 25922 were used as quality control. Confirmed by Double Disc Synergy Test (figure 2). The results were showed 74 (42.5%) of isolates were ESBLs producers and 100 (57.5%) of isolates were non – ESBL producers.

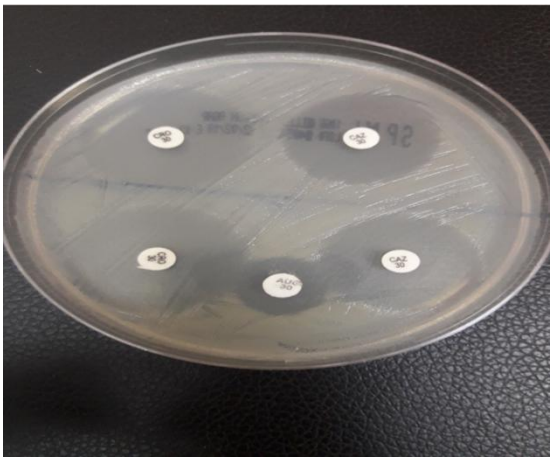

Figure 2: Double discs synergy test with CAZ = ceftazidime; AUG = amoxicillin + clavulanic acid and CRO=ceftriaxone

4. Discussion

Although research regarding ESBL producers made a high light and attention regionally and worldwide. Data and information regarding Alkharj City is still limited. Thus, the aim of this study is to detect the prevalence of ESBL producing *E. coli.*in Alkharj City.

In the present study, we found that the percentage of ESBL producers is more than forty - two isolates (74/174) were positive for ESBLs and more than fifty seven isolates (100/174) isolates were negative for ESBLs. Several studies in Saudi Arabia detected and characterized ESBLs genes. In study carried out by Babyay *et al*., 1999, at King Khalid University Hospital, Riyadh, Kindom of Saudi Arabia demonstrated Thirty six percent from isolates produced ESBLs. Among those 42% were *Klebsiella pneumoniae* and 20% were *Escherichia coli* and in our study ESBLs-producing *Escherichia coli* was higher than this study and were think this due to the fact that *Escherichia coli* have been an important source of transferable antibiotic resistance (Jarlier *et al*., 1988).

Mogahid *et al*., (2016) observed high resistance of ESBL producers among antibiotics belonging to different families including Aztereonam (95.3%), Cephalothin (95.3%), Ampicillin (95.3%), Ciprofloxacin (72.9%), trimethoprim-Sulpamethaxazole (71.8%), Norfloxacin (68.2%), Levofloxacin (60.0%), Amikacin (33.9%) and Gentamicin (24.7%). And this is same finding as our study when were reported high resistance of antibiotics belonging to different families among ESBL producers including aztereonam (86.3%), Levofloxacin (69.7%), Ciprofloxacin (65.8%) and ampicillin (57.4%), with P. value <0.001. Trimethoprim-Sulfamethaxazole (71.8%) with P. value < 0.01. also were reported in this study amikacin 4 (100%) and tigecycline 1 (100%), represent as a highest resistance rate of antibiotics among ESBL producers in this study with P. value < 0.05 and > 0.05, respectively.

In study carried out by Al-gamy *et al*., (2014) in Riyadh, Saudi Arabia, who reported imipenem, meropemen, colistin, fofsomycin and tigecycline are most active agents (susceptibility: 100%) and amikacin (27.63%) while our finding is same in the departure of Tigecycline (100%), imipenem (94%) and meropenem (92.9%), susceptible agents, but our findings were different in amikacin when were reported (100%) susceptible inthis study.

In Latin America, ESBL - producing *Enterobacteriaceae* was also rising. Rates in *E. coli* were as high as 41% in 2009 in Mexico (PAHO, 2014). While our finding was higher than this finding, More than forty-two isolates (74/174) were positive for ESBLs. In sub - Saharan Africa, the median prevalence of resistance to third – generation Cephalosporins ranged from 0 to 47 percent (Leopold *et al*., 2014), this finding was same as our study. In Theodor Bilharz Research Institute, Cairo, Egypt reported that a total of 16% of all isolates, 19% of *E. coli* and 14% of *K. pneumonae* were ESBL- producers (Fam *et al*., 2011), while our finding reported (42.52%) ESBL- producers. Were reported in this study ESBL- producers among hospitalized patients (39.65%) compared by (2.87%) of out patents. This finding was same finding with Storberg, (2014), who reported in North Africa ESBL prevalence from 12 to 99 percent in hospitals and 1% to 11% in communites. Storberg, (2014).

From this study, we can conduct that there is relatively proportion of ESBL producers in Al-Khaj City (42.52%) compared to others parts in Saudi Arabia, which will be considered as a major risk to the health authorities.

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