Original Article

Prevalence of Extended-spectrum beta-lactamases-producing Escherichia coli and Klebsiella pneumonia from patients attending Tripoli University Hospital, Tripoli, Libya

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Abstract

Prevalence of extended-spectrum β-lactamases (ESBLs)- producing E. coli and K. pneumoniae has been increased globally. The study aimed to detect the prevalence of ESBL-producing E. coli and K. pneumoniae isolated from clinical specimens in Tripoli university hospital (TUH) and to evaluate their antimicrobial resistance profile. We retrospectively reviewed the recorded cultures results in the microbiology laboratory, TUH during the first half of the year 2021. 77% of E. coli and 72.5% K. pneumonia were multidrug resistant. ESBL phenotype detected in 20% of K. pneumonia and 17.5% E. coli. ESBLs were isolated more frequently from the surgical ward (32.4%). ESBLs show high resistance to beta-lactam and other antibiotics including TMP/SMZ, and aminoglycosides. Our study showed a considerably high prevalence of ESBLs positive strains of E. coli and k. pneumoniae.

Introduction

resistant (MDR)-Enterobacteriaceae are a common cause of healthcare-associated and community-acquired infections all over the world. They carry the utmost risk to health authorities due to their rapid growing resistance and the lack of developing new antibiotics against gram-negative bacteria.[1] The increasing use of extended-spectrum cephalosporin over the last decades complicate the problem leading to difficulty in finding appropriate treatment and increasing mortality and morbidity.

[2,3]More than half of all globally used antibiotics belong to Beta-lactam antibiotics. They are used to treat a broad spectrum of gram-positive and gram-negative bacteria. [3] The main resistance mechanism for ß-lactam antibiotics is ß –lactamase enzyme-mediated hydrolysis employed by many Gram-negative bacteria, in particular the enterobacteriaceae. [4] Extended spectrum β- lactamases (ESBLs) confer resistance to an extended range of β-lactam antibiotics including third and fourth generation cephalosporin's and aztreonam, but not to cephamycins and carbapenems.[3] ESBLs are blocked by beta lactamase inhibitors such as clavulanate, sulbactam or tazobactam.[3]

The worldwide emergence and rapid spread of extended-spectrum β-lactamase (ESBL)-producing E. coli (ESBL-EC) and Klebsiella pneumoniae (ESBL-KP) is of particular concern. [5] Despite a larger reservoir of E. coli in hospitals, K. pneumonia is more successful in disseminating, and is the most abundant producer [6, 7].

The prevalence of ESBLs producers is diverse. The studies in the Middle East and Asia revealed a higher prevalence of ESBLs than in others parts of the world. [3, 8, 9] Several risk factors are responsible for colonization with ESBLs producers. Among the most important are prolonged hospitalization, severe illnesses and surgery. [9] Irrational use of antibiotics is another significant risk factor. An association was detected between cephalosporin and aztreonam usage and increased rate of ESBLs isolation. [6] Furthermore, the inappropriate use of antibiotics in animal husbandry has been linked to the increase of MDR. Food-producing animals are a potential source for

transmission of ESBL -producing bacteria to humans. [10, 11, 12] in addition, over the past few years evidence that international travel to highly endemic areas, Asia, the Middle East, and Africa, represents a risk factor for ESBL carriage. [13, 14]

ESBL-producing Enterobacteriaceae have been classified by world health organization (WHO) as priority one (Critical) pathogens for developing new antibiotics.[15] ESBL genes are carried on highly mobile plasmids which also harbor genes to other classes of antibiotics including aminoglycosides and fluoroquinolones. [16]

Currently, carbapenems are regarded as the preferred agents for treatment of infections due to ESBL-producing organisms in critically ill patients. However, carbapenems resistance is increasing making the treatment options for ESBLs-producing strains more restricted. carbapenems alternatives can be considered in selected cases but the optimal conditions for the use of carbapenem-sparing agents is still a point of conflict. [17-20]

In Libya, there are diagnostic challenges of ESBL-producing organisms in most of our hospitals and health care worker are not aware of the problem of MDR microorganism including the ESBLs. ESBLs have been detected in clinical isolates in E. coli, Klebsiella and pseudomonas spp. by limited number of studies, information on ESBLs prevalence is still limited. [21- 26]

Clearly there is a need to have detailed information on ESBLs occurrence in Libya, including bacterial species, their antimicrobial susceptibility patterns in order to describe appropriate antimicrobial

therapy aiming to decrease their further spread. This study aimed to detect the prevalence of ESBLs -producing strains among multidrug resistant E. coli and K. pneumoniae from clinical isolates in TUH and to evaluate their antimicrobial resistance/susceptibility profile.

METHODS

An institutional- based cross-sectional descriptive study was conducted through retrospective analysis of culture result in the central laboratory in the Tripoli University hospital (TUH) from January 2021 to June 2021. Patients' demographic data, department and specimen types were recorded. Only one positive culture per patient was included in the study and repeated positive cultures from the same patient were excluded from the analysis. The number of isolates that were ESBL-producers were included and their antimicrobial resistance pattern were analyzed.

The identification of the isolates, we analyzed, were accomplished by VITEK® 2 (bioMérieux, France) automated system. Both a gram-negative identification card and an antimicrobial susceptibility testing card (AST-GN75, 22) panels were inoculated with a bacterial colony suspension prepared in 0.45% saline equal to the turbidity of a 0.5 McFarland standard. Confirmatory ESBL test substrates were included in wells of the AST-GN75, AST-GN22 panel. The test outcome was read as either ESBL positive or ESBL negative.

Data Analysis

Data were entered and analyzed using SPSS version 20 (Statistical Package for Social Science; release 20.0) Simple frequency was applied to see the prevalence of ESBLs and their distribution according to the departments. The antimicrobial resistance pattern among the ESBLs-producers was analyzed and compared with previous studies. Excel was used to draw all figures of

RESULT

Out of 1804 specimens, 951(52.7%) were from male patient and 853 (47.3%) were from female. The largest specimen size was from the pediatric age group 724(40.13%). More than 85% of the specimens were from the inpatient. The urine and blood were the most frequent specimens (37%, 30.4% respectively) as shown in Figure 1. Of the total cultures analyzed, 630 were positive yielding 750 isolates. Gram negative account for 60% (450/750). They were isolated more frequently from neonatal unit (22.4%), surgical wards (16.4%) and PICU (14.4%) as illustrated in Table 1.

Figure 1. Distribution of samples according to sex, age, patient 'setting and Clinical specimens.

NN=Neonatal unit, PMW= pediatric medical ward, PICU= pediatric intensive care unit, SW=surgical ward, SICU=surgical intensive care unit, MW=medical ward, MICU=medical intensive care unit, OPD= outpatient department, * Dialysis, nephrology, oncology, ** Surgical wound 127, others swabs (eye, ear, skin, vagina) 104,*** Central line (CL)48, Umbilicus venous catheter (UVC)22.
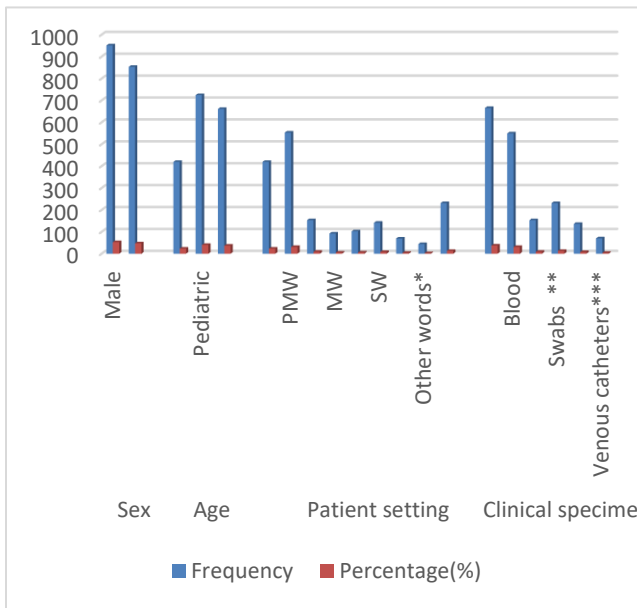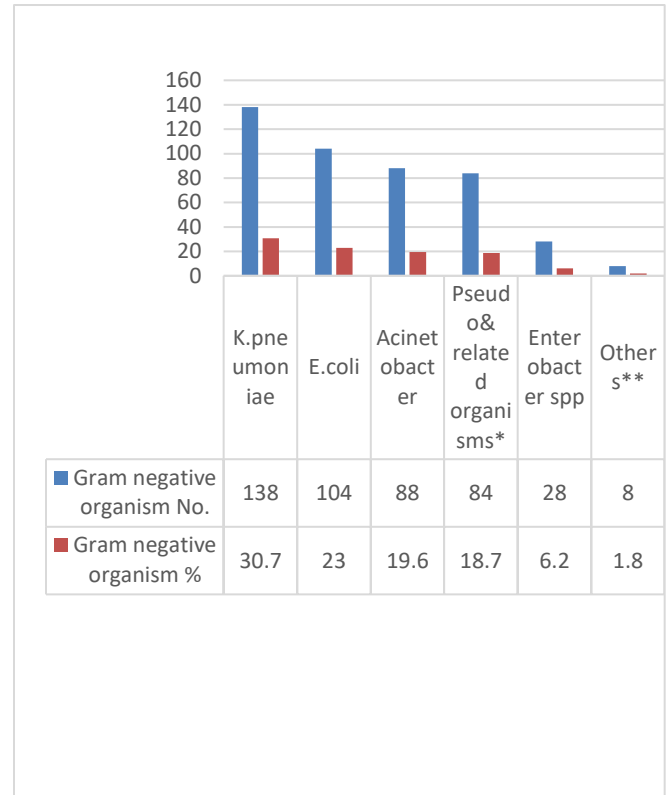
The K. pneumoniae was the most commonly isolated GNB (138) followed E. coli (104) and

Figure 2. Gram negative bacteria frequency and percentage .

B.cepacia(12),S.maltophilia(4), P.aerugiosa(68), ** Proteus spp (4), C. freundii (3), S. marcescens (1)

	K.pneumoniae	E.coli	Acinetobacter	Pseudo& related organisms*	Enterobacter spp	Others**
Gram negative organism No.	138	104	88	84	28	8
Gram negative organism %	30.7	23	19.6	18.7	6.2	1.8

Acinetobacter species (88). The K. pneumoniae was isolates more frequently from neonatal unit (40) and pediatric intensive care unit (21) whereas E. coli was isolated more frequently from surgical ward (28) and pediatric medical ward (23) as demonstrate figure (2, 3). The differences in the distribution of microorganisms among different departments were statistically significant (p-value < 0.05).

Table 1. Culture positivity and distribution of microorganisms by department.

Department	Total specimens cultured n= 1804			
	No growth n= 1174(65.1%)	growth positive n= 630 (34.9%) 750 isolates		
		Grampositive n=233(31.1%)	Gram negative n= 450 (60%)	Candida spp. n=67(8.9)
NN(n=419)	261 (22.2)	55 (23.6)	101 (22.4%)	19 (28.3%)
PMW(n=553)	451 (38.4)	40 (17.2)	61 (13.6%)	6 (9%)
OPD(n=231)	180 (15.3)	20 (8.6)	29 (6.4)	8 (12%)
PICU(n=153)	76 (6.5)	24 (10.3)	65 (14.4%)	8 (12%)
SW(n=141)	47 (4)	40 (17.2)	74 (16.4%)	5 (7.4%)
MICU(n=102)	42 (3.6)	14 (6)	49 (11%)	11 (16.4%)
MW(n=92)	73 (6.2)	10 (4.3)	10 (2.2%)	1 (1.5%)
SICU(n=69)	31 (2.6)	10 (4.3)	45 (10%)	4 (6%)
Other department (n= 44)	13 (1.2)	20 (8.6)	16 (3.5%)	5 (7.4%)
Total	1174 (65.1)	233 (100)	450 (100)	67 (100)

Table 2 demonstrates the antimicrobial susceptibility to beta lactam; K. pneumoniae was more resistant to beta lactam than E. coli. A resistance rate of 56.5% and 45% to ceftazidime (CAZ) and ceftriaxone (CRO) respectively was recorded in K. pneumoniae. Resistance to cefepime (FEP), cefoxitin (FOX) and aztreonam (ATM) was 52.2%, 34.8% and 28.3% respectively. Resistance to carbapenems; reaches up to 41.3% for meropenem (MEM). On other hand, resistance of E. coli to CAZ and CRO was 35.6% and 27% respectively whereas resistance to FEP, FOX and ATM were significantly lower than that of K. pneumoniae (18.3%, 17.3% & 20.2% respectively. Additionally, E. coli show significantly lower resistance to

carbapenems with lowest resistance rate of 5.8% to ERT.

Figure 3. Gram-negative profile according to department
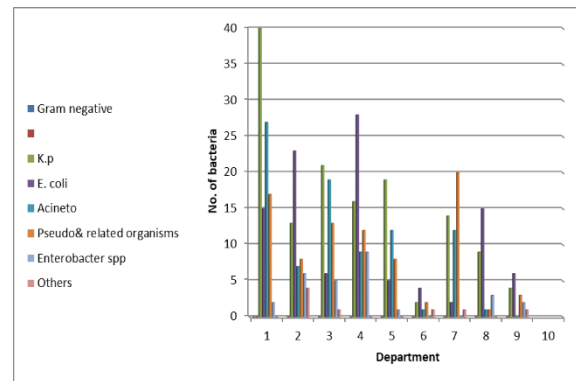
1=NN, 2= PMW,3=PICU, 4=SW, 5=SICU, 6=MW, 7=MICU, 8=OPD, 9=Other departments

Table 3 illustrate that eighty (77%) of the E. coli and 100 (72.5%) of the K. pneumoniae were multidrug resistant (non-susceptibility to at least one agent in three or more antimicrobial categories). (Table 3). Among these, ESBLs-producers were phenotypically identified in 14(17.5%) of the E. coli and 20(20%) of the K. pneumoniae. The difference in ESBLs production between E. coli and K. Pneumoniae were statistically insignificant (P-value > 0.05). More than 80% of the ESBLs- producers were isolated from the inpatients departments.

Table 2. Resistance pattern of K. pneumoniae and E. coli to beta-lactam antibiotics.

Antibiotics	Bacteria		Total 242
	k. pneumoniae (No.=138)	E.coli (No.=104)	
	No. %	No. %	No. %
CRO	62 45	28 27	90 37.2

CAZ	78 56.5	37 35.6	115 47.5
FOX	48 34.8	18 17.3	66 27.3
FEP	72 52.2	19 18.3	91 37.6
ATM	39 28.3	21 20.2	60 24.8
AMP	IR 0	35 33.7	35 14.5
AMS	46 33.3	18 17.3	64 26.4
IMP	26 18.8	9 8.7	35 14.5
ERT	34 24.6	6 5.8	40 16.5
MEM	57 41.3	15 14.4	72 29.8

Amp(10μg),AMS(10/10μg,),MEM(10μg),IPM(10μg),ERT(10μg),FEP(30μg),CRO(30μg),CAZ(30 μg),FOX(30μg), ATM (30 μg), IR=intrinsic resistance.

The differences in distribution of ESBLs-producer's between inpatients and outpatients and within departments were statistically significant (P-value < 0.05). Highest rate of ESBL-positive strains was from surgical ward (32.4%) and neonatal unit (14.7%). ESBL- E. coli was more prevalent in surgical ward than ESBL- K. pneumoniae (42.9% v/s 25%). The Surgical swabs were the major source of ESBLs-positive isolates in both E. coli (50%) and K. pneumoniae (30%) followed by urine as shown in Table 3.

The isolates demonstrate variable degree of susceptibility to anti-ESBLs antimicrobial. The

ESBLs- K. pneumoniae susceptibility was highest to minocycline (85%), amikacin and ertapenem (70% both) followed by meropenem (65%), Levofloxacin (60%). On other hand, resistance to TMP/SMZ and tobramycin among ESBL- K. pneumoniae was high (65%, 55% respectively). ESBL- E. coli, susceptibility was highest to ertapenem (85.7%), followed by meropenem and minocycline (78.6% both) and amikacin (71.4%). Resistance of ESBL- E. coli to TMP/SMZ was higher than that of ESBL-K. pneumoniae (71.4%) whereas resistance to gentamycin, tobramycin and ciprofloxacin were lower (35.7%) (21.4%). Generally, ESBL-K. pneumoniae was more resistant than ESBL- E. coli to most of the tested drugs with exception of TMP/SMZ and minocycline. 50% of ESBL-K. pneumoniae and 57% of ESBL-E. coli were resistant to cefoxitin as shown in table 4.

Table 3. Multidrug resistance and distribution of ESBLs according to department and specimen type.

		K. pneumoniae n= 138 No. (%)	E. coli n= 104 No. (%)	Total n= 242 No. (%)
Non-MDR		38(27.5)	24(23)	62 (25.6)
MDR		100(72.5)	80(77)	180(74.4)
Non-ESBLs		80(80)	66(82.5)	146(81.1)
ESBL producers		20 (20)	14(17.5)	34(18.9)
Department Inpatien	NN	3(15)	2(14.3)	5(14.7)
	PICU	3 (15)	1(7.1)	4(11.8)
	MICU	1 (5)	ND	1(2.9)
	SICU	2 (10)	2(14.3)	4(11.8)
	SW	5 (25)	6(42.9)	11(32.4)
Outpatient	PMW	3 (15)	ND	3(8.8)
	OPD	3 (15)	3(21.4)	6(17.6)
	Total	20 (100)	14(100)	34(100)
Specimen	WS	6(30)	7(50)	13(38.2)
	Blood	5(25)	ND	5(14.7)
	Urine	5(25)	3(21.4)	8(23.5)
	ETT	4(20)	1(7.1)	5(14.7)
	Eye swab	ND	2(14.2)	2(5.9)
	CSF	ND	1(7.1)	1(2.9)
	Total	20(100)	14(100)	34(100)

Table 4 Resistance/susceptibility profile of ESBL-K. pneumoniae and ESBL- E. coli.

GN=gentamicin(10µg),TOB=tobramycin(10µg),AK=amikacin(30µg),CIP=ciprofloxacin(5µg),MIN=minocycline(30µg),CT=colistin(10µg),LEV=levofloxacin(5µg),TMP/SMZ=trimethoprim/sulfamethoxazole(1.25/23.75µg)

DISCUSSION

Remarkably there is high alarming percentage of MDR strains among the K. pneumoniae and E. coli isolated from different departments and specimens. In this study, MDR was detected in 77% of E. coli and 72.5% of K. pneumoniae. This is higher than that registered by previous study in

ESBL		GN	TOB	AK	MEM	ERT	CIP	LEV	TMP/SMZ	MIN	CT	Fox	AMS	CRO	CAZ
K.P n (%) 20	S	10 (50)	8 (40)	14 (70)	13 (65)	14 (70)	10 (50)	12 (60)	7 (35)	17 (85)	17 (85)	8 (40)	10 (50)	2 (10)	2 (10)
	R	8 (40)	11 (55)	6 (30)	4 (20)	3 (15)	9 (45)	7 (35)	13 (65)	1 (5)	3 (15)	10 (50)	6 (30)	13 (65)	10 (50)
	I	2 (10)	1 (5)	0	3 (8.6)	3 (15)	1 (5)	1 (5)	0	2 (10)	0	2 (10)	4 (20)	5 (25)	8 (40)
E. coli n (%) 14	S	8 (57.1)	9 (64.3)	10 (71.4)	11 (78.6)	12 (85.7)	9 (64.3)	9 (64.3)	4 (28.6)	11 (78.6)	11 (78.6)	6 (43)	8 (57.1)	3 (21.4)	4 (28.6)
	R	5 (35.7)	5 (35.7)	2 (14.3)	3 (21.4)	0	5 (35.7)	5 (35.7)	10 (71.4)	1 (7.1)	3 (21.4)	8 (57)	3 (21.4)	9 (64.3)	5 (35.7)
	I	1 (7.1)	0	2 (14.3)	0	2 (14.3)	0	0	0	2 (14.3)	0	0	3 (21.4)	2 (14.3)	5 (35.7)

Libya (33.2% and 42% for E. coli and K.pneumoniae respectively). In addition, resistance to third generation cephalosporin was higher than that recorded before by El-mohammady et al [24] for both microorganisms. This high rate is of concern taking in consideration that most of doctors in our hospitals describe third generation as an empirical therapy for critically ill patients.

The rate of ESBLs positive strains among MDR E coli was in accordance with figures revealed by zorganiet al.[22] and Altayaret al.[21] whereas El-mohammady et al.[24] and Taher et al.[23] reported much lower rates of 6.7% and 5.3% respectively. For K. pneumoniae, our result was in accordance with previous studies in Libya [24, 25] and higher than that reported by Taher et al. [23]

Higher figures for both of the microorganisms were detected in Egypt reaching more than 75% [27-30]. In Africa, a higher rate (53.3%) of ESBL- K. pneumoniae and lower rate (9.4%) of E. coli were reported in Ethiopia. [31] In addition, higher rate of ESBLs-E. coli and K. pneumoniae were reported from Tanzania. [32, 33] Higher figures of ESBLs production for both microorganisms were detected in S. Arabia. [34, 35]

Resistance to CAZ and CRO in ESBL-K. pneumoniae and CRO in ESBL- E. coli were lower than reported before in Libya. [22] In our study we depend on the laboratory records that were incomplete and the result of susceptibility was not recorded for a large number of antibiotics.

In our study, susceptibility of ESBL- K. pneumoniae to meropenem was 65% and this is lower than rate recorded in Saudi Arabia (99%) and Egypt (100%). [36, 37] Nevertheless, susceptibility of ESBL-E. coli to ERT and MEM were lower than that reported in middle east countries [36].

The possibility of inducible AmpC co-producers should be considered among the

50% of ESBL-K. pneumoniae and 57% of ESBL-E. coli that were resistant to cefoxitin. The ESBLs are inhibited by beta-lactam/ beta lactamase inhibitors (BL-BLI) combination and susceptible to cefoxitin, on other hand, the AmpC producers are not inhibited by BL-BLI combination and are resistant to cefoxitin. Disc Approximation Assay should be used to confirm the presence of AmpC. [38] In the recorded results we reviewed there was no clue that the test for Amp C was done.

The susceptibility of ESBL- isolates to aminoglycosides was in accordance with result from Egypt and lower than that reported from Saudi Arabia. [34, 35, 36] Although noticeably high, resistance to fluoroquinolone in our isolates was lower than that reported from Egypt. [37] Resistance levels for the Trimethoprim/Sulfamethoxazole for both microorganisms were higher than recorded from that recorded in previous study. [34] The prevalence of quinolones and TMP/SMZ-resistant among ESBLs should be investigated for appropriate empirical treatment as both of them are commonly prescribed by doctors for treatment of urinary tract infections on empirical base.

High susceptibility rate to colistin recorded in both microorganisms but according to last updated CLSI guidelines, the MIC for colistin recorded by the automated system as VITEK® 2 are not reliable and colistin susceptibility testing should be accomplished by colistin broth disk elusion or broth microdilution or colistin agar testing. [39]

Our finding of elevated levels of resistance to non-beta lactam classes of antibiotics arises as the plasmids responsible for ESBLs production also incorporate genetic material coding for resistance to other antibiotics and these plasmids are frequently transferable between bacterial species.

Among numerous classical risk factors for acquisition of ESBL-producing enterobacteriaceae is the surgery. [40] In this study ESBL- K. pneumoniae and ESBLs-E. coli were isolated more frequently from surgical ward specifically from the wound swabs at a significant rate in accordance with other studies. [34,40]

Conclusion and Recommendations

High rate of MDR among K. pneumoniae and E. coli was detected in this study and prevalence of ESBLs among these MDR is high. Furthermore, the resistance of ESBLs to TMP/AMZ and other non-betalactam antibiotics is alarming yet resistance to carbapenems is not prevalent.

The health authority in Libya should set up a hospital surveillance system monitoring the level of drug resistance & implementing preventive measures including infection prevention & effective antimicrobial stewardship programs that guide the physicians in making appropriate prescribing practice in order to reduce the increasing trend of AMR. Upgrading the quality of the service provided by microbiology laboratory to resolve the present challenges in diagnosis of ESBL-producing organisms is crucial.

LIMITATION

The main limitation was the incomplete registration of the data.

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