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Extended-spectrum beta-lactamases in *Escherichia coli* Isolates from Urine Samples of Patients in Al-Bashir Hospital

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Abstract

Urinary tract infections (UTIs) are serious health affecting problems worldwide. UTIs cause more than 8.1 million visits to health care providers each year. About 60% of women and 12% of men will have at least one UTI during their lifetime. The extended-spectrum beta-lactamases (ESPL) has limited the use of multiple types of Antibiotics. This research will be carried out in Al-Bashir Hospital. The aim of this study was to identify the prevalence of ESBL in *Escherichia coli* isolates in the hospital by VITEK. 319 isolate were positive from EBSL from a total of 564. This suggest that a 56% of the patients who has been hospitalized in the time of the study may develop severe urinary tract infections caused by strains of *Escherichia coli* who have developed resistance to multiple types of antibiotics.

Keywords: urinary tract infections; prevalence; *E. coli*; antibiotic resistance; ESBL.

Introduction

Infection rates vary from nation to nation; Urinary tract infections (UTIs) are serious health affecting problems worldwide. *E. coli, K. pneumoniae, P. aeruginosa, S. saprophyticus, S. aureus* and *Proteus mirabilis* are most common bacteria causing UTIs in human beings.

The *E. coli* accounts for approximately 85% of community acquired UTIs and 50% of hospital acquired UTIs. 5 Different factors like age, gender, immunosuppression and urological instruments may affect prevalence of UTIs. 6 Catheter-associated UTIs are one of the most dangerous health risks contributing 34% of all health care associated infections. The emergence of extended-spectrum beta-lactamases (ESPL) has threatened the empirical use of cephalosporins and ciprofloxacin. Microorganisms use various mechanisms to develop drug resistance such as recombination of foreign DNA in bacterial chromosome, horizontal gene transfer and alteration in genetic material. β-lactamases are hydrolytic enzymes which cleave the β-lactam ring and are the primary mechanism of conferring bacterial resistance to β-lactam antibiotics, such as penicillins and cephalosporins. Resistance pattern of microorganisms vary from country to country, state to state, large hospital to small hospital and hospital to community. Currently, over 150 ESBLs have been described in a worldwide distribution In Jordan, the problem of antibiotic resistance is compounding because of overuse and misuse of antibiotics. There is no systematic national surveillance of antibiotic resistance and insufficient data is available to quantify the problem. ESBLs are associated with increased morbidity and mortality rates, prolonged hospital stays, and increased costs.

Methods

The study was conducted over a period of 6 months (October 2018 to March 2019) in the Department of Microbiology, Al-Bashir hospital.

Urine sample: were collected according to standard procedures and transported without delay.

Bacterial Culture: All samples were inoculated onto blood agar and MacConkey agar and incubated aerobically at 37°C (manual test). Bacterial pathogens were identified as per the standard protocol (manual and automated). All *Escherichia coli* isolates were subjected to routine antimicrobial susceptibility testing.

Automated identification of the bacteria: a pure colonies of the isolated bacteria were isolated and prepared to be read on VITEK by re-suspension in 3 ml of normal saline with a MIC value of 0.5-0.63 McF. A total of 564 *E. coli* I were isolated from urine samples for 492 patients (some patients have more than one sample during 6 months). In the screen test for ESBL production, 319 were positive in 269 patients, 236 were negative in 214 patients and 9 unknown in 9 patients (Table 1). Prevalence of ESBL producers among patients. It was found out that there was no statistically significant association among sex of the patients with ESBL producing organisms ESBLs.

Sucuptiplity testing: The standard methods using culture and antimicrobial susceptibility testing. Automated Identification (ID) cards and accurate susceptibility test (AST) cards are used to determine the identity and the sensitivity of the bacteria to various antibiotics, respectively.

Stastical analysis: was carried out using ANOVA.

Ethical Considerations: Ethical approval was obtained from the Ethical committee of the institute and informed consent was obtained from patients.

Results

A total of 564 *E. coli* I were isolated from urine samples for 492 patients (some patients have more than one sample during 6 months). In the screen test for ESBL production, 319 were positive in 269 patients, 236 were negative in 214 patients and 9 unknown in 9 patients (Table 1).

Prevalence of ESBL producers among patients. It was found out that there was no statistically significant association among sex of the patients with ESBL producing organisms ESBLs.

Table 1. The Distribution of E-coli from the obtained samples from Al-Bashir Hospital

	Total number	Females	Males
Patients with *E. coli*	492	363	129
ESBL positive *E. coli* isolates	319	214 (67%)	105 (33%)
ESBL negative *E. coli* isolates	236	79 (34%)	157 (66%)
Unknown	9	-	-

**Number of isolates (564) are greater than the number of patients (492) due to readmission of some patients.

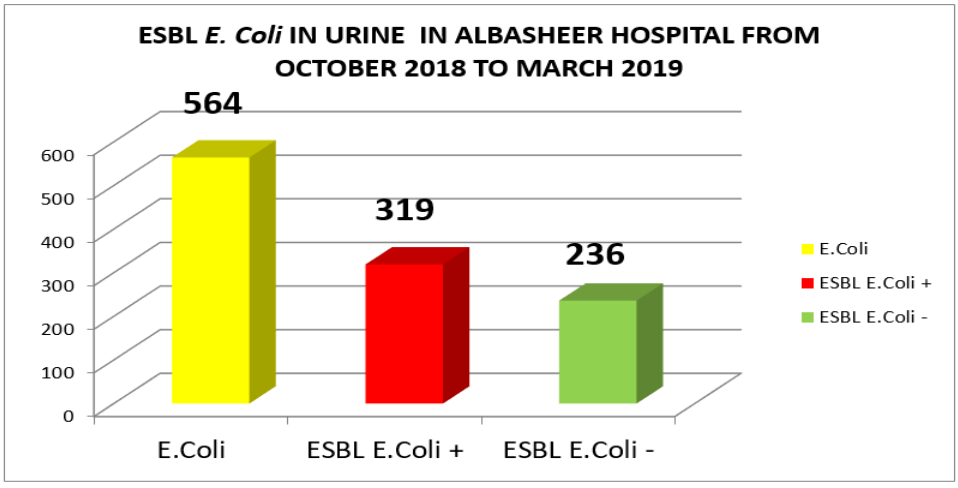

Figure 1: Represent ESBL E. Coli isolated from urine samples .

Code	Antibiotic n	Site of infe	Breakpoint	Number	%R	%I	%S
ESBL	ESBL			1882	54.9		45.1
PEN_NM	Penicillin G		S<=.125 R	736	80.4	0	19.6
AMP_NM	Ampicillin		S<=8 R>	3835	82.6	2	15.4
PIP_NM	Piperacillin		S<=16 R:	243	81.5	3.3	14.8
TIC_NM	Ticarcillin			8	0	0	0
OXA_NM	Oxacillin		S<=.25 R:	509	88.8	0	11.2
AMC_NM	Amoxicillin/Clavulanic		S<=8 R>	3673	32.1	27	40.9
SAM_NM	Ampicillin/Sulbactam		S<=8 R>	471	83.9	7.6	8.5
TCC_NM	Ticarcillin/Clavulanic a		S<=16 R:	168	77.4	9.5	13.1
TZP_NM	Piperacillin/Tazobacta		S<=16 R:	3432	19.1	7.3	72.7
CZO_NM	Cefazolin		S<=2 R>	631	96.5	0	3.5
CXM_NM	Cefuroxim(	Oral	S<=8 R>	112	76.8	8	15.2
CAZ_NM	Ceftazidime		S<=4 R>	5024	42.4	3.1	54.6
CRO_NM	Ceftriaxone		S<=1 R>	1278	81.7	2.1	16.2
CTX_NM	Cefotaxime		S<=1 R>	2646	54.9	1.5	43.6
FEP_NM	Cefepime		S<=2 R>	3816	31.1	9.6	59.3
FOX_NM	Cefoxitin		S<=8 R>	496	88.5	0	11.5
CFM_NM	Cefixime		S<=1 R>	260	84.6	2.3	13.1
CXA_NM	Cefuroxime axetil		S<=4 R>	112	76.8	8.9	14.3
ATM_NM	Aztreonam		S<=4 R>	848	69.6	1.9	28.5
ETP_NM	Ertapenem		S<=.5 R>	2256	3.5	0.6	95.8
IPM_NM	Imipenem		S<=1 R>	3446	15.4	1.6	83.1
MEM_NM	Meropenem		S<=1 R>	3818	16.8	0.7	82.5
AMK_NM	Amikacin		S<=16 R:	3886	2.6	2.1	94.4
GEN_NM	Gentamicin		S<=4 R>	4324	27.8	2.2	70
STH_NM	Streptomycin-High		S<=1024 F	203	0	0	100
TOB_NM	Tobramycin		S<=4 R>	639	34.9	6.1	55.1
RIF_NM	Rifampin		S<=1 R>	509	8.1	1.8	90.2
CIP_NM	Ciprofloxacin		S<=1 R>	6191	36.1	4.8	59.1
LVX_NM	Levofloxacin		S<=2 R>	1612	45.9	4.1	50
NOR_NM	Norfloxacin		S<=4 R>	2599	27.4	2.3	70.3
SXT_NM	Trimethoprim/Sulfame		S<=2 R>	3983	53.1	0	46.9
TMP_NM	Trimethoprim		S<=8 R>	113	69	0	31
FOS_NM	Fosfomycin		S<=64 R:	2591	6.1	2	92
CLI_NM	Clindamycin		S<=.5 R>	875	44.9	3.5	51.5
COL_NM	Colistin		S<=2 R>	369	3.3	7.3	89.4
ERY_NM	Erythromycin		S<=.5 R>	712	61	7	32
NIT_NM	Nitrofurantoin		S<=32 R:	3482	12.1	17	70.9
LNZ_NM	Linezolid		S<=4 R>	740	1.6	0.3	98.1
VAN_NM	Vancomycin		S<=4 R>	891	7.3	0.4	92.3
CHL_NM	Chloramphenicol		S<=8 R>	263	50.2	10.6	39.2
MNO_NM	Minocycline		S<=4 R>	228	43	21.5	32
TCY_NM	Tetracycline		S<=4 R>	960	57.6	0.1	42.2
TGC_NM	Tigecycline			2049	0	0	0
AMB_NM	Amphotericin B			24	0	0	0

Figure 2: Represent the susceptibility test of different antibiotics for ESBLE. Coli .

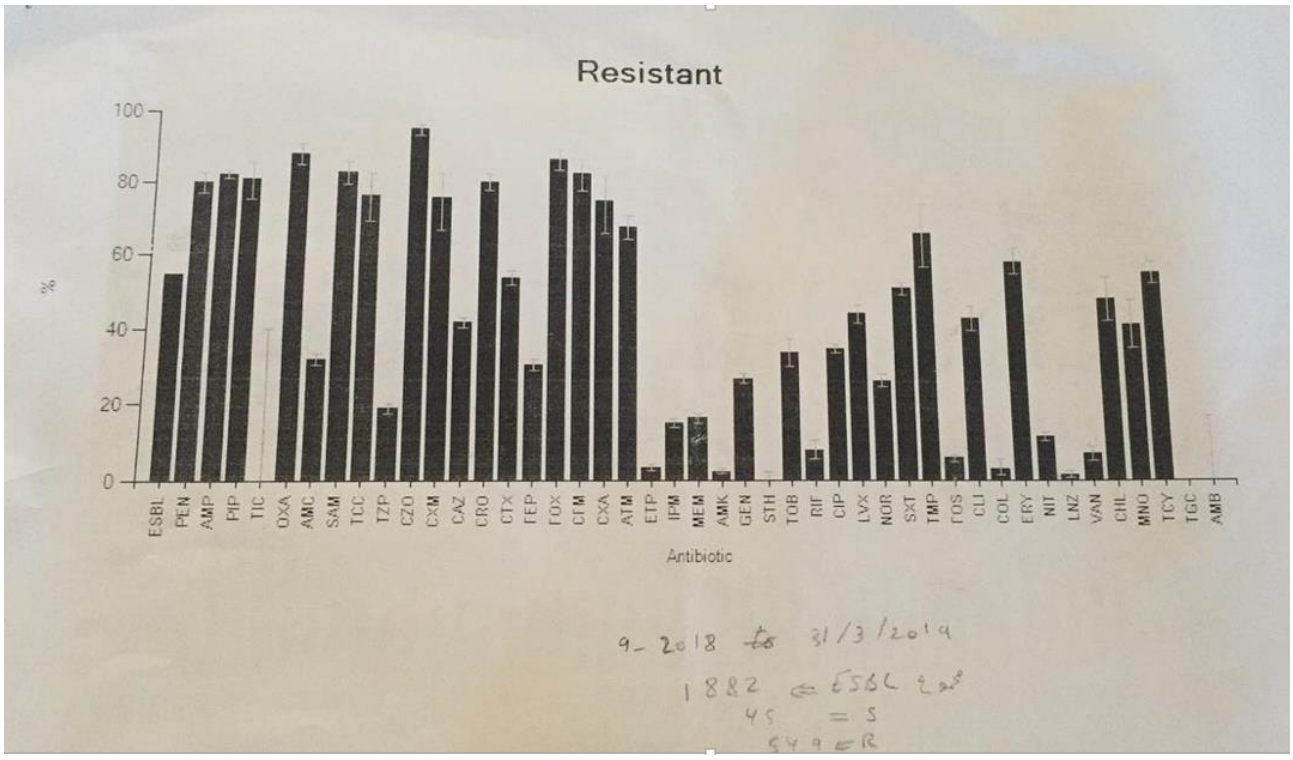

Figure 3: represent the susceptibility test of different antibiotics for ESBLE. Coli

Discussion

Antibiotic resistance is increasing at alarming levels and has emerged as a major public health in the world. β-lactams are among the commonly used classes of antibiotics. However, resistance to β-lactams has also semerged and production of β-lactamases is the most common cause of resistance to these drugs. To counter the effect of β-lactamases, penicillinase resistant penicillins and first generation cephalosporins were introduced the past.

This remained the major therapy for about 20 years, before the resistance due to β-lactamases produced by Gram negative bacteria became a serious problem. Subsequently, a group of β-lactamases hydrolyzed extended spectrucephalosporins and were named Extended Spectrum Beta- Lactamases (ESBL).

There is no consensus regarding the definition of ESBLs. ESBLs may be defined as a group of enzymes that are capable of conferring resistance to penicillins, first, second and third generation cephalosporins and aztreonam (but not cephamycins and carbapenems) and render them ineffective. ESBLs are transmissible β-lactamases which are inhibited by clavulanic acid, tazobactam or sulbactam, and which are encoded by genes that can be exchanged between bacteria. Majority of the ESBLs are found in *Klebsiella spp*. and *Escherichia coli* of the *Enterobacteriaceae* family.

Risk factors for infection with ESBL producing organisms are prolonged antibiotic usage, Effective and rational usage of antibiotics in hospital patients are important for prevention of development of antibiotic resistance.

ESBL producing strains remain undetected as they are difficult to detect by routine susceptibility testing methods and may show false susceptibility to antibiotics by routine methods. ESBL detection is important as knowledge about its prevalence is helpful to formulate infection control measures and to prevent their spread.

Al-Bashir Hospital, central and referral hospital in Jordan, is a 1500 bedded care producing *Escherichia coli* in urine cultures. Hence this study was undertaken to document the prevalence and resistance pattern of ESBL producing *Escherichia coli* and to help in implementing an effective antibiotic policy.

Infections by ESBL producing organisms have emerged as a major problem and the failure of therapy with broad spectrum antibiotics are creating serious problems.

Misuse/overuse of antimicrobials in urinary tract infections will not only be expensive but also cause unforeseen menace of drug resistance in future as patients are more susceptible to infection and colonization by various pathogens .The findings of this study emphasizes the need for a continuous surveillance in the patients to detect the resistant strains, strict guidelines for the antibiotic therapy and the implementation of infection control measures to reduce the increasing burden of antibiotic resistance. Knowledge of the resistance pattern of ESBL producing *Escherichia coli* in this geographical area will be helpful in formulating the antibiotic policy AL-Bashir hospital. It is recommended that along with conventional antibiogram, routine ESBL testing should be done.

Conclusion

The rapid noninvasive method allows a direct detection of the bacterium but less sensitive than biopsy method. Also false-negative results may occur following a new infection before the antibody level is sufficiently elevated.

The highest sensitivity was using tissue samples and antimicrobial susceptibility testing. Resistance to clarithromycin and metronidazole is mostly due to the use of these drugs for infectious diseases other than *H.*

pylori infection. This explains why those countries in northern Europe, which have a strict policy for antibiotic use, still have a low prevalence of resistance.

We recommended the alternative method to solve problem of clarithromycin resistance by using modified regimen which replace clarithromycin with levofloxacin 500 mg once daily in combination with amoxicillin daily plus a standard dose proton pump inhibitor twice daily: continue regimen for 10 to 14 days.

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