RESEARCH ARTICLE

THE PREVALENCE AND PHENOTYPIC CHARACTERIZATION OF EXTENDED-SPECTRUM β-LACTAMASES-PRODUCING *ESCHERICHIA COLI* STRAINS ISOLATES RECOVERED FROM TERTIARY HOSPITALS IN SANA'A CITY, YEMEN

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ABSTRACT

The emergence of ESBL producing *Escherichia coli* in clinical isolates is posing a serious threat for treating nosocomial infections. The aim of the study was to determine the frequency of extended spectrum β-lactamase (ESBL) producing Escherichia *coli* isolated from clinical specimens in several teaching and general hospitals in Sana'a city, Yemen, and to compare the phenotypic methods used for the characterization of ESBL producing strains. This cross sectional observational study was conducted from 1st July to 28th of August 2017, at the Department of Medical microbiology, Faculty of Medicine and Health Sciences, University of Sciences and Technology in Sana'a city, Yemen. A total number of 3500 various clinical samples were analyzed during the study period. *Escherichia coli* were identified using API 20E system and ESBL detection was carried out using double-disk synergy test (DDST) and CLSI confirmatory test. *Escherichia coli* were isolated from 100 samples, out of which 63 (63%) were ESBL producers and 37 (37%) were non-ESBL producers. The gender distribution of ESBL producing *Escherichia coli* was 35/45 (77.8%) in males and 28/55 (50.9%) in females. Highest frequency of ESBL producing *Escherichia coli* was detected in sputum (100%), wounds (83.3%) and urine (65.7%) samples. Comparison of DDST and CLSI confirmatory test showed that 65 (65%) isolates were characterized by DDST and 63 (63%) using CLSI confirmatory test. All ESBL-positive isolates were susceptible to imipenem, indicating that this agent is the best drug for treating serious infections caused by ESBL-producing *E. coli*. In conclusion, the present study shows moderately high frequency of ESBL producing *Escherichia coli* among patients suffering from sepsis in tertiary hospitals in Sana'a city. DDST was found to be less efficient in ESBL detection as compared to CLSI confirmatory test.

Keywords: Characterization of ESBL, CLSI confirmatory test, Double-disk synergy test (DDST), ESBL producing *Escherichia coli*.

INTRODUCTION

The difficulties seen in fighting infections caused by drug resistant organisms, especially those that have acquired resistance to beta-lactams, such as broad-spectrum cephalosporins, have resulted from the extensive use of broad-spectrum antibacterial agents. One important resistance mechanism to the beta-lactams, including new cephalosporins, is due to the destruction of the antibiotics by extended-spectrum beta-lactamases (ESBLs)[1]. Extended-spectrum β-lactamases (ESBLs) are plasmid encoded enzymes that hydrolyze β-lactam ring and cause resistance to β-lactam antibiotics which include third-generation cephalosporins such as ceftriaxone, ceftazidime, cefotaxime and the monobactam such as aztreonam[2]. The most common ESBLs are derived from widespread broad-spectrum β-lactamases TEM and SHV. Bacterial strains expressing these β-

lactamases are presenting great therapeutic challenges. In recent years there has been a significant increase in incidence and prevalence of ESBL producing bacteria[3]. In addition, *Enterobacteriaceae* mainly *Escherichia coli* and *Klebsiella* species are one of the most bacteria causes sepsis in human[1,2]. High resistance rates to antimicrobials used in the treatment of *E. coli* infections have been reported worldwide, particularly in developing countries including Yemen[4]. Genes responsible for ESBLs production arise by point mutation at the active site of the earlier β-lactamases (e.g. TEM-1 and SHV-1 enzymes), and they are usually plasmid mediated[5,6]. In addition, ESBLs-positive Gram-negative bacteria often carry genes that confer high levels of resistance to many other antibiotics (e.g. fluoroquinolones and aminoglycosides)[7,8]. The epidemiology of antibiotic resistant bacteria, however, varies due to various factors e.g. region, medical field type of infections, and time periods[8,9]. Therefore, identification and determination of antimicrobial susceptibility of bacterial pathogens in the local healthcare setting may aid the clinicians in selecting the appropriate antimicrobial agent (s) to treat infected patients. The aim of the study was to determine the frequency of extended spectrum β-lactamase (ESBL) producing *Escherichia coli* isolated from clinical specimens in several teaching and general hospitals in Sana'a city, Yemen, and to compare the phenotypic methods used for the characterization of ESBL producing strains.

SUBJECTS AND METHODS

An active surveillance cross sectional study was conducted at the Department of Medical Microbiology, Faculty of Medicine and Health Sciences, University of Sciences and Technology in Sana'a city, Yemen. The population of the study included in-patients admitted to three tertiary hospitals in Sana'a city: namely: University of Sciences and Technology Hospital, Al-Jumhori hospital, and Al-Kuwait hospital, from 1st July to 28th of August 2017. A total number of 3500 various clinical samples were analyzed during the study period. The samples were cultured on solid media as Blood, Chocolate and MacConkey agar. Cystine Lysine Electrolyte Deficient Medium (CLED) was used only for urine culture samples. *Escherichia coli* were identified by colonial morphology, Gram's stain, catalase test, oxidase test and API 20E system (bioMerieux). A seven digit number generated on the basis of various biochemical reactions of API 20E system was checked by API 20E software to confirm *Escherichia coli*[10].

Antimicrobial Drug Susceptibility Testing
A bacterial suspension of *Escherichia coli* was made according to the 0.5 McFarland turbidity standard and an even lawn of bacteria was made on the Mueller Hinton agar petri plate (90mm). The screening for ESBL *Escherichia coli* was performed using ceftazidime (30 μg) disk and ceftazidime resistant strains were considered as screen positives. DDST was performed by using disks containing amoxicillin/ clavulanate on Mueller-Hinton agar plate at a 20 mm distance from the indicator drugs;

ceftazidime (30μg) and cefotaxime (30μg). ESBL production was seen by the clavulanate mediated enhancement of the activity of the indicator drug as a keyhole effect[11].

The CLSI confirmatory tests were performed using disks of ceftazidime (30μg) and cefotaxime (30μg) alone and in combination with ceftazidime-clavulanate (30/10 μg) and cefotaxime-clavulanate (30/10μg). The CLSI confirmatory test was considered positive when the inhibition zone produced by the disks in combination clavulanate increased ≥5 mm than the disks without the clavulanate. The results of double disk diffusion test and CLSI test were compared.

Ethical Consideration
Ethical clearance for the study was taken from the Faculty of Medicine and Health Sciences, University of Sciences and Technology Research Review Committee. A written permission was also taken from the administrative Managers of the included hospitals.

Data analysis
The analysis of data was done by Epi Info version 6 statistical program (CDC, Atlanta, USA), where the chi-square (χ^2) and probability value (*p*) was calculated for the test of significance.

RESULTS

The detailed results of this study are presented in 3 Tables. *Escherichia coli* were isolated from 100 culture positive samples, out of which 63 (63%) were ESBL producers and 37 (37%) were non-ESBL producers (Table 2). The frequency of ESBL producing *Escherichia coli* in male and female patients was 35/45 (77.7%) (p=0.005) and 28/55 (51%), respectively. Occurrence of ESBL producing *Escherichia coli* was found to be highest in the sputum samples 6/6 (100%) (p=0.05) followed by the wound samples 5/6 (83.3%) (0.05), while in urine samples occurrence of ESBL producing *Escherichia coli* was 44/67 (65.7%) (Table 1). Antibiotic sensitivity test result for isolated *Escherichia coli* is presented in Table 3. All ESBL-positive isolates were susceptible to imipenem, indicating that this agent is the best drug for treating serious infections caused by ESBL-producing *E. coli*.

DISCUSSION

The treatment of infectious diseases is fundamental matter for human health and the daily increase in bacterial resistance has raised patients' costs in recent years. The production of ESBLs is also a major risk to the use of the new generation of cephalosporins. In the last 20 years, the rate of ESBL production by *Enterobacteriaceae* has raised considerably[12,13,14]. Among *Enterobacteriaceae*, *E. coli* is one of the most important causative agents of nosocomial infections[15] and *E. coli* is one of the most isolated bacteria from in-patients in Sana'a hospitals; therefore, we selected this bacteria for our study. Occurrences of infection effected by extended spectrum beta-lactamase producing *E. coli* have been widely reported all over the world following the widespread use of the expanded spectrum cephalosporins[16,17-20]. In our study, phenotypic screening of ESBL showed that 63% of *E.*

coli isolates were positive for ESBL production. Based on this result, the prevalence of ESBL producing *E. coli* was high. The lower prevalence of ESBLs producing *E. coli* has been reported by a number of previous studies; for example in a study by Ramazanzadeh *et al.* revealed a 34.8% ESBL positive rate among strains of Gram-negative bacteria[21] and Mobasherizadeh *et al.* showed that among a total of 2035 consecutive clinical isolates identified as *E. coli* in Al-Zahra Hospital in Iran, 898 (44.1%) and 432 (21.2%), were ESBL producers for hospitalized and non-hospitalized patients, respectively[22]. However, the rate of ESBL producing *E. coli* in our study roughly similar to that reported by Bazzaz *et al.* [23] and by Jalalpoor and Mobasherizadeh in Iran which they showed that the prevalence of ESBL positive strains of *E. coli* was 59.2% and 58% respectively[23,24]. This varies in prevalence of ESBLs from region to region and from hospital to another is uncertain whether this is because of the differences in infection control practices between hospitals or to differences in the use of new cephalosporins[16]. Our study, has also demonstrated that the rates of ESBLs production in Yemen are similar to that of other countries in our area and the world, such as; India (57.1%), Turkey (57%) and published data from European countries, such as; France, Italy, the Netherlands, Germany, and Spain, as well as in the United States, Australia, Japan, Tanzania, Thailand and Pakistan, which showed a higher prevalence of ESBL-producing isolates as in the present study[22, 25-27].

Feizabadi *et al.*[1] found that the rates of resistance for ciprofloxacin, cefepime, ceftazidime, and cefotaxime were; 21.4%, 28%, 76% and 84.0%, respectively. The comparison of our study results with the above-mentioned study shows that antibiotic resistance to four of the previously mentioned antibiotics is higher in our study in which the rates of resistance for ciprofloxacin, cefepime, ceftazidime, and cefotaxime were; 47%, 91%, 87%, and 85%, respectively. In addition, a high resistance rate for first line drugs including; amoxicillin, and trimethoprim-sulfamethoxazole were found in our study (96% and 66% respectively) this result is similar to Mobasherizadeh *et al.* study, in which both non-hospitalized and hospitalized isolates of *E. coli* were more resistant to first line drugs including; amoxicillin, and trimethoprim-sulfamethoxazole[22]. Our result, which is comparable with other studies in developing countries, is due to the widespread use of these drugs because of their low cost and easy administration. Imipenem, amikacin and Nitrofurantoin were the most effective antibiotics against hospitalized ESBL-producing *E. coli* isolates in our study. All ESBL-positive isolates were susceptible to imipenem, indicating that this agent is the best drug for treating serious infections caused by ESBL-producing *E. coli*. Our result is similar to that reported by Gholipour *et al.* and Mobasherizadeh *et al.* in which all ESBL-positive isolates were susceptible to imipenem, and amikacin[22,28]. The lower rates observed with nitrofurantoin in our study (3%) may be due to less use of the drug in treating bacterial infections (including UTIs) in Yemen. Newer antibiotics like third generation cephalosporins (e.g. ceftriaxone and cefotaxime) and fluoroquinolones (e.g. ciprofloxacin and norfloxacin) have been more widely used in recent years in Yemen. This observation can be supported by the findings of the present investigation of high resistance rates to ciprofloxacin, cephalosporins such as ceftazidime, ceftriaxone, and cefepime among *E. coli* (Table 3).

The comparison of DDST and CLSI confirmatory test showed that the higher numbers of positive isolates were detected by CLSI confirmatory test (100%) than the DDST (95.4%) (4.6% were false positive for ELSB) (Table 2). A study conducted by collecting 91 ESBL producers from 32 hospitals in Kinki area of Japan reported DDST positive for 97.80% of the isolates and was negative for only 2.19% of isolates.[29] Rao *et al.* used DDST and CLSI confirmatory test on 126 ESBL screen positive isolates. Their result of the DDST method detected 86.5% and the CLSI detected 73.8% of the cases[30]. Study conducted by Dechen *et al.* showed that ESBL producers can be detected by DDST and CLSI confirmatory test with equal efficacy. Their results showed 100% agreement in DDST and CLSI method for detection of ESBL producers[31]. Another study from India reported 135 screen positive ESBL producers. In this study the DDST showed positive results in 126 (93.3%) while CLSI in 135 (100%) cases[32]. These studies support the results of our study where CLSI confirmatory test is found to be better than DDST.

CONCLUSION

In conclusion, high frequency of ESBL producing *E. coli* was found at our study. CLSI confirmatory tests generated better results than DDST. Due to the wide spread of ESBL producing strains, it is important to maintain the active surveillance system at microbiological laboratories for early detection of ESBL producing organisms. Preventive measures to stop the colonel spread of the resistant strains could significantly reduce the risk of treatment failure and help in the generation of sound epidemiological data.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

No conflict of interest associated with this work.

AUTHOR'S CONTRIBUTION

This research work is part of PhD thesis. The candidate is the first author (MAA) who conducted the laboratory and field works; and wrote up the thesis. The corresponding author (HAA) supervised the laboratory and field works, revised and edited the thesis draft and the manuscript.

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Table 1: The association between ESBL *E. coli* isolates and sex and sites of infections

Factors	ESBL positive n=63		*P value*
	No	**%**	
Sex			
Male n=45	35	77.8	0.005
Female n=55	28	50.9	0.005
Site of infections (clinical specimens)			
Urine n=67	44	65.7	0.4
Wounds n=6	5	83.3	0.05
Sputum n=6	6	100	0.05
Pus n=9	4	44.4	0.26
HVS n=5	2	40	0.27
Blood n=4	2	50	0.58
Others specimens n=3	0	0	0.02

P value significant ≤0.05

Table 2: Comparison of DDST and CLSI detection of ESBL (n=100).

Tests	Positive isolates		Negative isolates	
	No	**%**	**No**	**%**
Double Disk Synergy test	65	65	35	35
CLSI confirmatory test	63	63	37	37

Table 3: The percentages of antimicrobial resistance detected among 100 multidrug-resistant *E. coli* isolates.

Antimicrobial agent (µg)	Number	Percentage
Amikacin (30µg)	6	6
Amoxicillin-Clavulanic Acid (30 µg)	96	96
Aztreonam (30 µg)	72	72
Cefepime (30 µg)	81	81
Cefepime-Clavulanic Acid (30µg)	91	91
Cefotaxime (30µg)	85	85
Ceftazidime (30µg)	87	87
Ceftazidime-ClavulanicAcid (30µg)	97	97
Ceftriaxone (10µg)	84	84
Ciprofloxacin (5 µg)	47	47
Gentamicin (10µg)	23	23
Imipeneme (10 µg)	0	0
Nitrofurantoin (300µg)	3	3
Norfloxacin (10 µg)	50	50
Trimethoprim-sulfamethoxazol (25µg)	66	66