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Prevalence and Characterization of Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in Brack-Alshati, Fezzan, Libya

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Abstract

Background: Extended spectrum β-lactamase ESBL-producing Enterobacteriaceae are increasing worldwide. However, there is sparse data on their prevalence and nature in Libya. A prospective study was performed to:

a. Assess the prevalence of ESBL-producing Enterobacteriaceae among gastrointestinal and urinary tract infection patients and healthy individuals, and

b. Determine the antibiotics sensitivity profiles and β-lactamase production of the isolates.

Methods: 307 individuals were voluntarily enrolled in this study; 201 patients and 106 healthy individuals. 106 stool samples were collected from the healthy group and 80 stool and 121 urine samples were from patients. Enterobacteriaceae isolates were tested for ESBL production by the double disk synergy test, and antibiotic susceptibility testing was performed using the disk diffusion method.

Results: Of the total of 333 bacterial isolates, 208 were from patients and 125 from the healthy group. Patients yielded 149 *E. coli* (71.6%), 35 *Klebsiella pneumoniae* (16.8%), 6 *Klebsiella oxytoca* (2.9%), 6 *Proteus mirabilis* (2.9%), 5 *Enterobacter cloacae* (2.4%), 4 *Citrobacter freundii* (1.9%), 2 *Enterobacter agglomerans* (0.96%) and 1 *Salmonella typhi* (0.48%). On the other hand, 91 *E. coli* (72.8%), 24 *K. pneumoniae* (19.2%), 5 *Ent. cloacae* (4%) and *C. freundii* (4%) isolates were from the healthy group. In total, only 15 isolates (4.5%) produced ESBL of which, 12 (5.7%) were from patients and 3 (2.4%) from the healthy group. 8 out of 149 (5.36%) *E. coli*, and 4 out of 35 (11.4%) *K. pneumoniae* produced ESBLs from patients' isolates compared to a single *E. coli* (1.09%) and 2 *K. pneumoniae* (8.3%) from the healthy group isolates.

Conclusions: The prevalence of ESBL-producing Enterobacteriaceae strains was 4.5% among isolates from healthy and patients stool and urine samples in the targeted area in Libya. ESBL strains were more prevalent in patients' samples (5.7%) than healthy controls (2.4%). Their antibiotic sensitivity profile showed more resistance in patients' isolates than the healthy volunteers.

Keywords: *Extended β-lactamase-producing; Enterobacteriaceae; E. coli; K. Pneumoniae; Antibiotic resistance; Libya*

Abbreviations: AML: Ampicillin; AMP: Amoxicillin; TIC: Ticarcillin; KF: Cephalothin; FOX: Cefoxitin; CFP: Cefoperazone; CTX: Cefotaxime; CAZ: Ceftazidime; CRO: Ceftriaxone; IPM: Imipenem; ATM: Aztreonam; TZP: Piperacillin/Tazobactam; AMC: Amoxicillin/Clavulanic acid (augmentin); CIP: Ciprofloxacin; NA: Nalidixic acid; F30: Nitrofurantion; C30: Chloramphenicol; TE: Tetracycline; SXT: Trimethoprim/Sulfamethoxazole; CN: Gentamicin

Introduction

The increase in antimicrobial resistance of pathogenic bacteria is a major problem worldwide. Extended spectrum β-lactamases (ESBLs) are plasmid-encoded enzymes that are produced most often by *Klebsiella* and *E. coli* species. ESBLs are the most important factor contributing to Gram negative bacilli resistance to broad-spectrum β-lactam antibiotics such as cefotaxime, ceftriaxone, ceftazidime, aztreonam and cefpodoxime [1]. These β-lactamases are easily transferable among bacterial species. They are mostly of the KPC, VIM, IMP, NDM, and OXA-48 types. Their current extensive spread worldwide in Enterobacteriaceae is of great importance and concern. Infections caused by these bacteria have limited treatment options and have been associated with high mortality rates. The prevalence of carbapenemases in the Mediterranean region is particularly high constituting one of the most important reservoirs [2]. The first ESBL was reported in *Klebsiella pneumoniae* in 1983 [3]. The majority of ESBLs isolated from clinical samples have been SHV or TEM types of TEM-1, and 2, and SHV-1 [4]. More than 40 of these extended spectrum enzymes (named TEM-1, -2, -3 and SHV-1, -2, etc) have been discovered since [4]. These ESBLs have enhanced stability in the presence of β-lactam antibiotics and are positively selected through point mutations in the TEM and SHV β-lactamase genes [5,6]. In recent years, β-lactamases that hydrolyze carbapenems have also been discovered, such as; the *Klebsiella pneumoniae* carbapenemase (KPC) and metallo-β-lactamases (MBLs), such as the New Delhi metallo-β-lactamase (NDM) [7,8].

ESBL-producing Enterobacteriaceae have been responsible for numerous outbreaks of infections throughout the world and pose challenging infection control measures [9]. Urinary tract and blood stream infections are some examples. Intravascular and urinary catheters, emergency intraabdominal surgery, gastrostomy or jejunostomy tube, gastrointestinal colonization, length of hospital or intensive care unit stay, prior antibiotics, prior nursing home stay, severity of illness, and ventilator assistance are some risk factors for acquiring an infection with ESBL producing strains [10,11]. The frequency of ESBL-producing bacteria differs significantly by population geographic location [12-15].

Although the study of ESBL-producing Enterobacteriaceae in Libya was previously attempted [16,17], however, none has attempted to investigated samples from rural areas. Therefore, the goal of this study was to investigate the prevalence of ESBL-producing Enterobacteriaceae among gastrointestinal and urinary tract infection patients and healthy individuals in the southern Brack-Alshati area, Fezzan, Libya. Their antibiotic sensitivity profile to a panel of antibiotics employing double disk synergy and disk diffusion test, and β-lactamase production along with other isolates was determined.

Materials and Methods

This study voluntarily enrolled 307 individuals comprising 201 patients-aging 7 months to 95 years attending Brack General Hospital and other private clinics in this area and 106 healthy controls aging 19 to 36 years. The samples were collected in the period from October 2010 to April 2011. The male to female ratio was 1 : 2 among patients and controls. Healthy controls provided 106 stool samples and 80 stools and 121 urine samples were collected from the patients. An informed consent was obtained from all participants or their custodians prior the participant enrollment and the study was approved by our local committee for Medical and Research Ethics.

All samples were inoculated onto MacConkey agar plates (Mast Diagnostic, Mast Group LTD, Merseyside, UK). Additionally, the stool samples were inoculated onto Xylose Lysine Deoxycholate (XLD; Oxoid Limited, Hants, England). All plates were incubated at 37°C for 24 hours in air. The suspected colonies were streaked onto fresh MacConkey agar plates for purity and later identified using standard microbiological methods- Gram staining, oxidase test, IMVIC and carbohydrate fermentation tests. Isolates identified as Enterobacteriaceae were finally preserved on nutrient agar slants in individual screw capped bottles at 4°C.

The antibiotic susceptibility testing was performed using the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines [18]. *E. coli* ATCC 25922 was used as a control. Commercially available antibiotic discs (Oxoid Limited, Basingstoke, Hampshire, England) were used for antibiotics susceptibility testing including that included Ampicillin (AML, 10 µg), Amoxicillin (AMP, 10 µg), Ticarcillin (TIC, 75 µg), Amoxicillin/Clavulanic acid (Augmentin) (AMC, 20/10 µg), Piperacillin/Tazobactam

(TZP, 100/10 µg), Cephalothin (KF, 30 µg), Cefoperazone (CFP, 5 µg), Cefoxitin (FOX, 30 µg), Cefotaxime (CTX, 30 µg), Ceftriaxone (CRO, 30 µg), Ceftazidime (CAZ, 30 µg), Imipenem (IPM, 30 µg), Aztreonam (ATM, 30 µg), Gentamicin (CN, 10 µg), Tetracycline (TE, 30 µg), Ciprofloxacin (CIP, 5 µg), Nalidixic acid (NA, 30 µg), Chloramphenicol (C, 30 µg), Nitrofurantion (F, 300 µg), and Trimethoprim/Sulfamethoxazole (SXT, 1.25/23.75 µg).

The one-minute β-lactamase test was performed as described [19] to test for β-lactamase production by the bacterial isolates using the double disk synergy technique modified by Lautenbach, *et al.* [13]. Briefly, a single colony from an overnight blood agar culture was suspended in sterile 0.9% normal saline solution in a sterile tube. The turbidity of the test bacteria was adjusted by dilution to obtain 0.5 McFarland standards. A cotton swab was soaked in the suspension tube and the test bacteria were spread on Mueller-Hinton agar plates (Oxoid Limited, Hants, England). The disks containing the standard ceftazidime, ceftriaxone, cefotaxime, and aztreonam at 30 µg were placed 25 mm apart (center to center) from amoxicillin/clavulanic acid 20/10 µg. After an incubation period of 18 hours at 35°C, the zone of inhibition between Ceftazidime, Ceftriaxone, cefotaxime or aztreonam and amoxicillin/clavulanic acid indicated the presence of an ESBL-producing isolate. *E. coli* ATCC 25922 was used as negative control.

Results

A total of 333 bacterial isolates were isolated from patients and healthy individuals. Of these, 208 strains were isolated from patients and 125 were isolated from the healthy volunteers. Out of the 208 bacterial strains isolated from patients, 149 were *Escherichia coli* (71.6%), 35 were *Klebsiella pneumoniae* (16.8%), 6 were *Klebsiella oxytoca* (2.9%), 6 were *Proteus mirabilis* (2.9%), 5 were *Enterobacter cloacae* (2.4%), 4 were *Citrobacter freundii* (1.9%), 2 were *Enterobacter agglomerans* (0.96%) and 1 was *Salmonella typhi* (0.48%). On the other hand, of the total 125 isolates from the healthy volunteers, 91 were *E. coli* (72.8%); 24 were *K. pneumoniae* (19.2%); 5 were *Ent. cloacae* (4%) and 5 were *C. freundii* (4%).

Organism	Patients		Healthy Controls		Total	
	Isolates	ESBL (%)	Isolates	ESBL (%)	Isolates	ESBL (%)
E. coli	149	8 (5.4)	91	1 (1.1)	240	9 (3.8)
K. pneumoniae	35	4 (11.4)	24	2 (8.3)	59	6 (10.2)
K. oxytoca	6	0	0	0	6	0
P. mirabilis	6	0	0	0	6	0
Ent. cloacae	5	0	5	0	10	0
C. freundii	4	0	5	0	9	0
S. typhi	1	0	0	0	1	0
Ent. agglomerans	2	0	0	0	2	0
Total	**208**	**12 (5.8)**	**125**	**3 (2.4)**	**333**	**15 (4.5)**

Table 1: *Distribution of extended-spectrum β-lactamase producing and non-producing Enterobacteriaceae isolated from patients fecal and urine samples and fecal healthy control samples collected from Brack-Alshati, Fezzan, Libya.*

Out of the total 333 *Enterobacteriaceae* isolates from both patients and healthy controls, only 15 (4.5%) isolates were positive for ESBL as tested by the double disc synergy test. Among those 15 isolates, 12 (3.6%) isolates were from patients and 3 (0.9%) isolates were from healthy volunteers. In patients, 8 (5.36%) out of 149 *E. coli* strains, and 4 (11.4%) out of 35 *K. pneumoniae* strains were ESBL-producers. However, only a single (1.09%) *E. coli* strain out of 91 and 2 (8.33%) *K. pneumoniae* strains out of 24 isolated from healthy group produced ESBL. Of the ESBL-producing strains, 11 of patients' 12 isolates were from urine samples. Almost all the ESBL-producing isolates were consistently more resistant to a wide variety of antimicrobial agents than the non-ESBL-producers. The most

distinguishing feature of ESBL-producing *E. coli* and *K. pneumoniae* was their higher level of resistance to ampicillin, amoxicillin, ticarcillin, cephalothin compared to the non-ESBL-producing isolates. ESBL-producing E. coli isolates were also resistant to third generation cephalosporins such as ceftazidime, ceftriaxone, and cefotaxime with a range of 11-33%, while non-ESBLs *E. coli* isolates were sensitive to these drugs.

Antibiotic	*K. pneumoniae*		*E. coli*	
	% Resistance		% Resistance	
	ESBL	Non-ESBL	ESBL	Non-ESBL
Ampicillin	100	85	100	45
Amoxicillin	100	85	100	45
Ticarcillin	100	85	100	45
Cephalothin	83	15	100	42
Cefoxitin	67	15	22	16
Cefoperazone	17	0	22	3
Cefotaxime	50	0	33	0
Ceftazidime	50	0	11	0
Ceftriaxone	50	0	33	0
Imipenem	0	0	0	0
Aztreonam	17	0	0	0
Piperacillin/Tazobactam	0	4	22	0
Amoxicillin/clavulanic acid	67	4	33	6
Ciprofloxacin	17	4	44	10
Nalidixic acid	17	9	55	10
Nitrofurantion	50	25	11	3
Choramphenicol	50	11	33	10
Tetracycline	50	19	44	42
Trimethoprim/Sulphamethaxzole	33	23	33	36
Gentamicin	33	0	55	3

Table 2: *Comparison of resistance patterns of extended-spectrum β-lactamase-producing and non-producing K. pneumonia and E. coli isolated from patients fecal and urine samples and fecal healthy control samples collected from Brack-Alshati, Fezzan, Libya to antibiotics.*

The 15 ESBL-producing isolates showed variable resistance patterns. The resistance to cefotaxime, ceftriaxone, ceftazidime, and aztreonam were 40%, 40%, 26.7% and 6.7%, respectively. The correlation between the zone of inhibition recorded for the five antibiotics (ceftazidime, cefotaxime, ceftriaxone, aztreonam and augmentin) tested with the disk diffusion test and the zone of inhibition of the same antibiotics used above with the double disk synergy test (DDS, 25 mm) have revealed a positive correlation for both *E. coli* (r=95.2%, p=0.001) and *K. pneumoniae* (r=88.3%, p=0.001) as shown in Figure 1, suggesting equal validity for the two methods.

The results of β-lactamase testing revealed that 119 of the *E. coli* isolates (80%), 52 of *K. pneumonia* (88%), 9 of *Ent. cloacae* (90%), 5 of K. oxytoca (83.3%), 3 of *C. freundii* (33.3%), and 1 of *Ent. agglomerans* (50%) did not produced β-lactamase. Among *E. coli* isolates, 9 strains (3.8%) were positive for ESBL production. Similarly, among the *K. pneumoniae* isolates, six strains (10.2%) were positive for both.

K. pneumoniae isolated from patients showed higher levels of resistance against almost all the antimicrobials tested compared to *K. pneumoniae* isolates from the healthy volunteers.

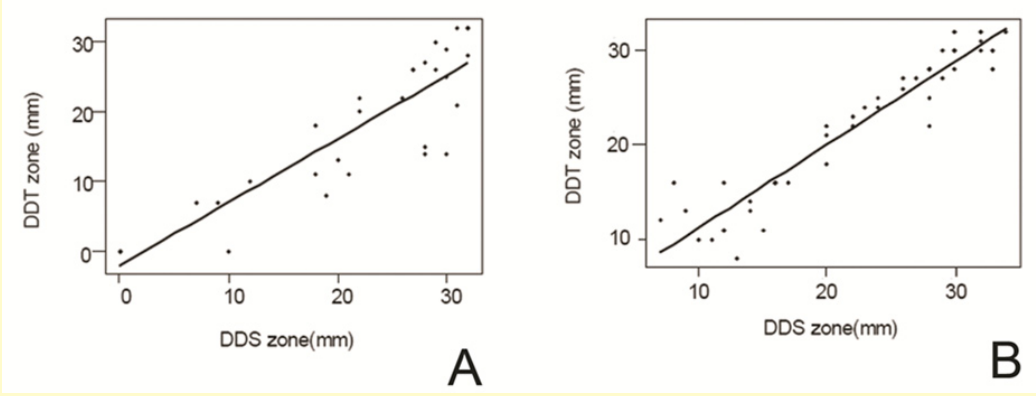

Figure 1: *Correlation of the result of the two methods: Double Disk Synergy (DDS) and Disk Diffusion Test (DDT)-for detection of β-lactamase production in K. pneumoniae (Panel A) and E. coli (Panel B) isolated from patients fecal and urine samples and fecal healthy control samples collected from Brack-Alshati, Fezzan, Libya (p = 0.00).*

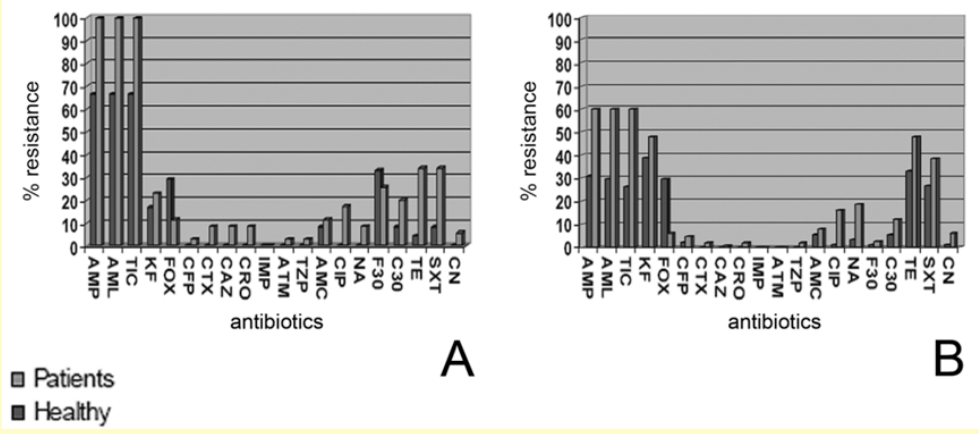

Figure 2: *Comparison of antibiotics resistance of K. pneumoniae (panel A) and E. coli (panel B) isolated from patients fecal and urine samples (light shaded bars) and fecal healthy control samples (dark shaded bars) collected from Brack-Alshati, Fezzan, Libya.*

All 39 (100%) *K. pneumoniae* isolates from patients were resistant to ampicillin, amoxicillin and ticarcillin. In contrast, only 67% of *K. pneumoniae* strains isolated from the healthy group were resistant to the same antibiotics ($p < 0.05$). A similar pattern was seen among *E. coli* isolates from patients that showed high level of resistance to the majority of the antimicrobials compared to *E. coli* isolates from the healthy group.

For instance, a range of 48-60% of isolates were resistant to the commonly used drugs including ampicillin, amoxicillin, ticarcillin, cephalothin and tetracycline compared with resistance rates for the healthy group ($p < 0.05$). Other antibiotics including cefoxitin, cefotaxime, ceftriaxone, augmentin, nalidixic acid and trimethoprim/sulphamethaxzole showed variable antimicrobial efficacy against isolates from both patients and healthy group with no statistical significance difference for each of *E. coli* and *K. pneumoniae*.

Discussion

The prevalence of Enterobacteriaceae strains producing ESBLs varies worldwide. The highest rates among *K. pneumoniae* isolates from clinical specimens were reported from Latin America (44%), Asia/Pacific Rim (22.4%), Europe (13.3%), and North America (7.5%). A similar trend was shown for *E. coli* in terms of specific geographical variation but at lower prevalence rates of 13.5%; 12%; 7.6% and 2.2%, respectively [12]. In our study, a much lower rate of 4.5% of all isolates-from both patients and healthy volunteers were ESBL producers. Such rate was higher considering patients' samples only (5.8% ESBL-producers). This is comparable to 6.3% reported by Bonfiglio in Italy [20]. A higher percentage of 13.4% among *E. coli* isolates from diarrhea stool samples of Northern Libyan children has also been recorded; while nine *E. coli* isolates (6.7%) demonstrated AmpC β-lactamases [17]. However, this prevalence is lower than those reported in other countries. For instance, it was reported that the prevalence was 55% in Jordan [21], 42% in Israel [22], 36% in Saudi Arabia [23], 38.5% in Egypt and 27.4% in Greece [24]. Countries of the Gulf Cooperation Council share a high prevalence of ESBL-and carbapenemase-producing Gram-negative bacilli, most of which are associated with nosocomial infections [25]. Infections with bacteria producing extended-spectrum beta-lactamases (ESBLs) are increasing across Africa too. ESBL-producing Enterobacteriaceae are significant causes of infections and antibiotic resistance at Korle-Bu Teaching Hospital in Accra, Ghana with ESBL expression rate of 49.3% in clinical samples [26].

Nonetheless, lower prevalence rates were also reported from other countries such as Denmark with less than 1% prevalence in the Copenhagen area [27]. Variability in the reported ESBL production prevalence could be attributed to a number of factors. For most of these types of studies, bacteria were isolated from ICUs patients and other hospitalized patients. Furthermore, studies looking for ESBL prevalence are usually performed on bacterial isolates from nosocomial infections. This in return may favour the finding of Enterobacteriaceae strains that produce ESBL and hence inflating the percentage of prevalence of ESBL-producing bacteria [12,28-30]. Antibiotic policies and protocols implemented at different geographical regions may also contribute to these differences in prevalence [6,9, 31,32]. A Saudi study reported 12.7% rate of ESBL expression among isolates from fecal specimens of healthy individuals and community outpatients. Of these, 87 (95.6%) were *E. coli* and 4 (4.4%) *Klebsiella pneumoniae* [30]. The community could be a reservoir of these ESBL-producing bacteria because rate of fecal carriage of ESBL-producers was very comparable among healthy persons (12.3%) and community outpatients (13.7%). The extensive usage of antibiotics, particularly non-prescription empirical usage, may correlate with greater prevalence due to positive selection. Moreover, environmental and genetic background susceptibility factors could have a significant impact on prevailing microbial ecology [33].

The prevalence of ESBL was greater among *K. pneumoniae* strains compared to *E. coli* in the present investigation. This is in agreement with previous studies [20-22,34-36]. Out of 125 isolates from the healthy volunteers, ESBL production was found only in 3 (2.4%) strains. Although a very low prevalence, healthy carriers of ESBL-producing bacteria may act as a reservoir for the spread of multi-drug resistant bacteria in the community. In general, strains of *E. coli* and *K. pneumoniae* that produced ESBLs were more resistant to the β-lactam and non-β-lactam antibiotics tested than non-ESBL expressing strains. Only imipenem was effective against both groups. This was in agreement with a number of other reported studies [24,37,38]. The susceptibility pattern for piperacillin/tazobactam against ESBL-producing bacteria has been extremely variable. In the present investigation, the sensitivity rates of both ESBL-and non-ESBL-producing strains of *K. pneumoniae* ranged from 94-100%. This is in agreement with the findings at Asia-Pacific region and South Africa medical centers, and other studies [3,39-41]. However, ESBL-producing E. coli strains have shown a greater resistance to

piperacillin/tazobactam (22%) than non-ESBL-producing *E. coli* strains (0%). In a Saudi study, prevalence of ESBL was 25.6% among isolates of *K. pneumoniae* and all ESBL-positive isolates were sensitive to imipenem and tigecycline. However, the resistance rate to gentamicin, amikacin, and ciprofloxacin was 87.3%, 10%, and 9.1%, respectively [42]. A hospital-based isolation would show higher prevalence of ESBL-production that was the case for a study conducted at Riyadh, Saudi Arabia showing sticking high rate of ESBL-producing *K. pneumoniae* isolates (55%) [43]. However, in the same city 20.4% of *E. coli* isolates were ESBL-producers [44]. While an Iranian clinical isolates of *E. coli* showed 33.3% were ESBL-producers [45]. In another clinical Enterobacteriaceae study in the Netherlands ESBL gene expression was evident in 81% of isolates [46].

Although, it has been reported that gentamicin can be used against ESBL-producing bacteria [41], we have found that 55% of ESBL-producing *E. coli* were resistant to gentamicin, compared with 3% of the non-ESBL-producing *E. coli*. Similarly, 33% of ESBL-producing *K. pneumoniae* were resistant to gentamicin, whereas, none of the non-ESBLs-producing isolates were resistant to the same drug. These findings are in agreement with other data reported from UK [47]. Nonetheless, another report [40], suggested that ESBL-producing strains may not necessarily be more susceptible to gentamicin. A Ghanaian study showed marked increase in minimum inhibitory concentrations of ESBL-producing Enterobacteriaceae isolated from clinical samples compared with other strains. The study found that 17% of ESBL-producers were resistant to two or more antibiotics (aminoglycosides, fluoroquinolones, sulfonamide, and carbapenems), whereas, only 3.2% of non-ESBL-producers were multidrug resistant [26].

It is a well known fact that using the disk diffusion test as a single method for detection of ESBL expression is not adequate. Other investigators have reported similar observations [20,21,48-50]. ESBL-producers are able to hydrolyze extended-spectrum penicillins, cephalosporins and aztreonam. The minimal inhibitory concentration (MICs) of these antimicrobial agents may be within the sensitivity range. For this reason, CLSI guidelines recommends that ESBL-producers be reported as resistant to all penicillins and cephalosporins and aztreonam, even when they show sensitivity to these agents by such conventional tests [18]. A positive significant correlation between the two antimicrobial sensitivity methods was evident for *E. coli* and *K. pneumoniae*. Therefore, we and other investigators recommend the use of the double disk synergy in conjunction with the disk diffusion test for the detection of ESBL-producing bacteria in the laboratory setting [51].

According to the present results, strains isolated from patients were more resistant to all antibiotics tested in comparison to those isolated from healthy individuals. A similar observation was noted by Kunin and Liu who recorded high frequency of antibiotics use and resistance among patients presenting at emergency wards, clinics and the community in Taiwan [52], and in patients from the Gulf area [53]. However, there were few exceptions where *K. pneumoniae* isolates were more resistant to cefoxitin and nitrofurantion. *C. freundii* was more resistant to augmentin, while *Ent. cloacae* isolates were more resistant to ampicillin, amoxicillin, cefoxitin, cephalothin and nitrofurantion in the healthy group of individuals in the present study . The differences in the overall antibiotic resistance of *E. coli* and *K. pneumoniae* in patients and the healthy group were statistically significant ($p < 0.05$). Nearly 20% of ESBL-producing Enterobacteriaceae isolates were from community-associated intra-abdominal infections and isolates from hospital-associated intra-abdominal infections had more complicated pattern of β-lactamases combinations than isolates from the community from Asia-Pacific region [54].

Conclusion

Our results have shown that patients with gastrointestinal and urinary tract infections harboured more ESBL producing Enterobacteriaceae strains compared with healthy individuals in the community. Although the overall prevalence of ESBL producing bacteria presented in our study is relatively not higher in comparison with other figures reported worldwide. However, the bacterial strains isolated from the patients were more resistant to antibiotics generally used in compacting infections than strains isolated from the healthy volunteers. The high resistance rates to most of the antibiotics tested mandates increased surveillance and molecular characterization of these isolates. Additionally policies to reduce misuse of antibiotics are mandatory, and to stopping investing money in useless antibiotics.

Bibliography

1. Gniadkowski M. "Evaluation and epidemiology of extended-spectrum beta-lactamases (ESBLs) and ESBL-producing microorganisms". *Clinical Microbiology and Infection* 7.11 (2001): 597-608.
2. Nassima Djahmi., *et al*. "Epidemiology of carbapenemase-producing Enterobacteriaceae and *Acinetobacter baumannii* in Mediterranean countries". *BioMed Research International* 2014 (2014): 1-11.
3. Knothe H., *et al*. "Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*". *Infection* 11.6 (1983): 315-317.
4. Bradford PA. "Extended-spectrum β-lactamases in the 21st century: characterization, Epidemiology, and detection of this important resistance threat". *Clinical Microbiology Reviews* 14.4 (2001): 933-951.
5. Livermore DM. "Beta-lactamases in laboratory and clinical resistance". *Clinical Microbiology Reviews* 8.4 (1995): 557-584.
6. Bush Karen., *et al*. "A functional classification scheme for β- lactamases and its correlation with molecular structure". *Antimicrobial Agents and Chemotherapy* 39.6 (1995): 1211-1233.
7. Paterson DL. "Resistance in gram-negative bacteria: Enterobacteriaceae". *The American Journal of Medicine* 119.6 (2006): S20-S28.
8. Yong D., *et al*. "Characterization of a new metallo-beta-lactamase gene, *bla*NDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India". *Antimicrobial Agents and Chemotherapy* 53.12 (2009): 5046-5054.
9. Poulou A., *et al*. "Outbreak caused by an ertapenem-resistant, CTX-M-15-producing *Klebsiella pneumoniae* sequence type 101 clone carrying an OmpK36 porin variant". *Journal of Clinical Microbiology* 51.10 (2013): 3176-3182.
10. Rupp ME and Fey PD. "Extended spectrum beta-lactamase (ESBL) - producing Enterobacteriaceae considerations for diagnosis, prevention and drug treatment". *J Drugs* 63.4 (2003): 353-365.
11. Colonder,R. "Extended-spectrum beta-lactamases: A challenge for clinical microbiologists and infection control specialists". *American Journal of Infection Control* 33.2 (2005): 104-107.
12. Reinert RR., *et al*. "Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the in vitro activity of tigecycline". *Journal of Antimicrobial Chemotherapy* 60.5 (2007): 1018-1029.
13. Lautenbach E., *et al*. "Extended-spectrum β-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact on resistance of outcomes". *Clinical Infectious Diseases* 32.8 (2001): 1162-1171.
14. Coque TM., *et al.* "Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe". *Euro Surveillance* 13.47 (2008): 19044.
15. Paterson DL. "Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs)". *Clinical Microbiology and Infection* 6.9 (2000): 460-463.
16. Buzayan MM., *et al*. "Detection of extended spectrum β-lactamases among urinary *Escherichia coli* and *Klebsiella pneumoniae* from two centres". *Jamahiriya Medical Journal* 10 (2010): 10-16.
17. Ahmed SF., *et al*. "Fecal carriage of extended-spectrum β-lactamases and AmpC-producing *Escherichia coli* in a Libyan community". *Annals of Clinical Microbiology and Antimicrobials* 13.22 (2014): 1-8.
18. NCCLs (2002) Performance standards for antimicrobial disk susceptibility tests, Twelfth Informational Supplement, *NCCLs document M100-S12,* NCCLs, Wayne. PA.
19. Escamilla J. "A modified one-minute β-lactamases test". *Antimicrobial Agents and Chemotherapy* 9.1 (1976): 196-198.
20. Bonfiglio G., *et al*. "Epidemiology of bacterial resistance in gastrointestinal pathogens in a tropical area". *International Journal of Antimicrobial Agents* 20.5 (2002): 387-389.
21. Shehabi AA., *et al*. "High incidence of Klebsiella pneumoniae clinical isolates to extended-spectrum β- lactam drugs in intensive care units". *Diagnostic Microbiology and Infectious Disease* 36.1 (2000): 53-56.

22. Venezia SN., *et al*. "Occurrence and phenotypic characteristic of Extended-spectrum beta-lactamases among member of the family of Enterobacteriaceae at the Tel-Aviv Medical Center and evaluation of diagnostic tests". *Journal of Clinical Microbiology* 41.1 (2003): 155-158.
23. Babay HA. "Detection of extended-spectrum β-lactamases in members of the family enterobacteriaceae of a teaching hospital, Riyadh. Kingdom of Saudi Arabia, *Saudi Medical Journal* 23.2 (2002): 186-190.
24. Bouchillon SK., *et al*. "Determining incidence of extended-spectrum β-lactamase producing Enterobacteriaceae, vancomycin-resistant *Enterococcus faecium* and Methicillin- resistant *Staphylococcus aureus* in 38 Centers from 17 countries: the PEARLS study 2001-2002. *International Journal of Antimicrobial Agents* 24 (2004):119-124.
25. Zowawi HM., *et al*. "β-Lactamase Production in Key Gram-Negative Pathogen Isolates from the Arabian Peninsula". *Clinical Microbiology Reviews* 26.3 (2013): 361-380.
26. Obeng Nkrumah N., *et al*. "High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance". *The American Journal of Tropical Medicine and Hygiene* 89.5 (2013): 960-964.
27. Kjerulf A., *et al*. "The prevalence of ESBL-producing *E. coli* and *Klebsiella* strains in the Copenhagen area of Denmark". *APMIS* 116.2 (2008): 118-124.
28. Gardam M and Conly J. "Extended spectrum beta-lactam enterobacteriaceae: The nosocomial pathogen du jour? Where does the list end"? *The Canadian Journal of Infectious Diseases* 6 (2000): 1425.
29. Garcia JA., *et al.* "Antimicrobial resistance in gram-negative isolates from European-intensive care units: data from the meropenem yearly susceptibility test information collection (MYSTIC) program". *Journal of Chemotherapy* 14.1 (2002): 25-32.
30. Kader AA and Kumar AK. "Prevalence of extended-spectrum β-lactamase among multidrug resistant gram-negative isolates from a general hospital in Saudi Arabia". *Saudi Medical Journal* 25.5 (2004): 570-574.
31. Jones RN., *et al*. "Antimicrobial activity against trains of *Escherichia coli* and *Klebsiella spp*. with resistance phenotypes consistent with an extended-Spectrum β-lactamases in Europe". *Clinical Microbiology and Infection* 9.7 (2003): 708-712.
32. Chanawong A., *et al*. "Dissemination and evolution of new Plasmid-mediated extended-β-lactamases among Enterobacteriaceae isolates from the People's Republic of China". *Clinical Microbiology and Infection* 6 (2000): 77.
33. Kader AA and Kamath KA. "Faecal carriage of extended-spectrum beta-lactamase-producing bacteria in the community". *Eastern Mediterranean Health Journal* 15.6 (2009): 1365-1370.
34. Hadziyannis E., *et al*. "Screening and confirmatory testing for extended-spectrum beta- lactamase (ESBL) in *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* clinical isolates. *Diagnostic Microbiology and Infectious Disease* 36.2 (2000): 113-117.
35. Saurina G., *et al*. "Antimicrobial resistance in Enterobacteriaceae in Brooklyn, N.Y: epidemiology and relation to antibiotic usage patterns". *Journal of Antimicrobial Chemotherapy* 45.6 (2000): 895-898.
36. Bell JM., *et al*. "Prevalence of extended-spectrum beta-lactamase (ESBL) producing clinical isolates in the Asia-Pacific region and South Africa: Regional results from SENTRY antimicrobial surveillance program (1998-99)". *Diagnostic Microbiology and Infectious Disease* 42.3 (2002): 193-198.
37. Lee SH and Jeong SH. "Antibiotic susceptibility of bacterial strains isolated from patients with various infections". *Letters in Applied Microbiology* 34.3 (2002): 215-221.
38. Xiong Z., *et al*. "Investigation of extended-spectrum beta-lactamase in *Klebsiella pneumoniae* and *Escherichia coli* from China". *Diagnostic Microbiology and Infectious Disease* 44.2 (2002): 195-200.
39. El Kholy A., *et al*. "Antimicrobial resistance in Cairo, Egypt 1999-2000: a survey of five Hospitals. *Journal of Antimicrobial Chemotherapy* 51.3 (2003): 625-630.
40. Villanueva FD., *et al*. "Extended-spectrum beta-lactamases-production among *Escherichia coli* and *Klebsiella spp*. at the makati Medical Center: Tentative solutions". *Journal Microbiology Infectious Diseases* 32 (2003): 103-108.

41. Schiappa DA., *et al*. "Ceftazidime-resistant *Klebsiella pneumoniae* and *Escherichia coli* blood stream infection: a case-control and molecular epidemiologic investigation". *The Journal of Infectious Diseases* 174.3 (1996): 529-536.
42. Tawfik AF., *et al*. "Prevalence and genetic characteristics of TEM, SHV, and CTX-M in clinical *Klebsiella pneumoniae* isolates from Saudi Arabia". *Microbial Drug Resistance* 17.3 (2011): 383-388.
43. Al-Agamy MH., *et al*. "Prevalence and molecular characterization of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in Riyadh, Saudi Arabia". *Annals of Saudi Medicine* 29.4 (2009): 253-257.
44. Al-Agamy MH., *et al*. "Molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* in Riyadh: emergence of CTX-M-15-producing *E. coli* ST131". *Annals of Clinical Microbiology* and Antimicrobials 13.4 (2014): doi: 10.1186/1476-0711-13-4.
45. Nakhaei Moghaddam M., *et al*. "Prevalence and Molecular Characterization of Plasmid-mediated Extended-Spectrum β-Lactamase Genes (balaTEM, blaCTX and blASHV) Among Urinary *Escherichia coli* Clinical Isolates in Mashhad, Iran". *Iranian Journal of Basic Medical Sciences* 15.3 (2012): 833-839.
46. Voets GM, *et al*. "Population distribution of Beta-lactamase conferring resistance to third-generation cephalosporins in human clinical Enterobacteriaceae in the Netherlands". *PLoS One* 7.12 (2012): e52102.
47. Elvy JA., *et al*. "Extended Spectrum β-Lactamase (ESBL) producing bacteria in a large UK NHS Hospital Trust". *Journal of Infection* 55 (2007): e59.
48. Shen D., *et al*. "Characterization of extended spectrum β-lactamases-producing *Klebsiella pneumoniae* from Beijing, China". *International Journal of Antimicrobial Agents* 18.2 (2001): 185-188.
49. Ho PL., *et al*. "Comparison of screening methods for detection of extended-spectrum β-lactamases and their Prevalence among *Escherichia coli* and *Klebsiella spp*. in Hong Kong". *APMIS* 108.3 (2000): 237-240.
50. Jacoby GA and Han P. "Detection of extended-spectrum β-lactamases in clinical isolates of *Klebsiella* and *Escherichia coli"*. *Journal of Clinical Microbiology* 34.4 (1996): 908-911.
51. Gualy Z., *et al*. "Ability of different methods to detect extended-spectrum β-lactamases in enterobacteriaceae". *Clinical Microbiology and Infection* 7 (2001): 394.
52. Kunin CM Liu YC. "Excessive use of antibiotics in the community associated with delayed admission and masked diagnosis of infectious diseases". *Journal of Microbiology, Immunology and Infection* 35.3 (2002): 141-146.
53. Khanfar HS., *et al*. "Extended spectrum beta-lactamases (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*: trends in the hospital and community settings". *Journal of Infection in Developing Countries* 3.4 (2009): 295-299.
54. Sheng WH., *et al*. "Distribution of extended-spectrum β-lactamases, AmpC β-lactamases, and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal infections in the Asia-Pacific region: results of the study for Monitoring Antimicrobial Resistance Trends (SMART)". *Antimicrobial Agents and Chemotherapy* 57.7 (2013): 2981-2988.

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