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Research Article

Extended-Spectrum Beta-Lactamase- and Carbapenemase-Producing *Enterobacteriaceae* among Libyan Children

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Abstract

Introduction: Extended-spectrum β-lactamases (ESBLs), AmpC type, carbapenem resistant *Enterobacteriaceae* (CRE), are important mechanisms of resistance among *Enterobacteriaceae*. The aim of this study was to investigate the prevalence of ESBL, AmpC and CRE among *Enterobacteriaceae* isolates recovered from pediatric patients in Tripoli, Libya.

Methods: This cross-sectional study was carried out in Tripoli Children Hospital (TCH), a total of 915 Gram negative bacteria isolates were evaluated for susceptibility to a panel of antimicrobials and were analyzed phenotypically for the ESBL, AmpC type and CRE using chromagen media, E-test and combination disc test.

Results: The predominant organisms were *Escherichia coli* (56.8%) and *Klebsiella* spp. (21.4%). The overall prevalence of ESBL producing *Enterobacteriaceae* was 24.5% (224/915). Out of 224, *Enterobacteriaceae* proved ESBL producer, *Klebsiella* spp. (54%) and *E. coli* (34.4%) were the leading ESBL producers. ESBL-producers were more often resistant to major classes of antibiotics compared with non-ESBL producers, significantly high resistance rates ($P < 0.001$) were observed for ceftriaxone, cefepime, and ceftazidime (87.5 - 95.9%) among ESBL producers compared to non-ESBL producers (7.2 - 13.5%). MDR was documented for 50/224 (22.3%) of ESBL producers and was significantly higher ($P < 0.0001$) among ESBLs compared with non-ESBL producer isolates. Phenotypic detection of AmpC revealed 60/915 (6.6%) isolates as potential AmpC β-lactamase producers, *E. coli* exhibited a lower level of AmpC (8.3%) compared with *Klebsiella* spp. (56.6%). The overall prevalence of CRE was 9% (83/915). Carbapenemase-producing organisms in this study were as follows: *Klebsiella* spp. (44.6%); *Acinetobacter* spp. (24%); *Pseudomonas* spp. (9.6%).

Conclusion: This study revealed that the prevalence of ESBL, AmpC, CRE and MDR *Enterobacteriaceae* isolates in Children hospital was within acceptable frequency.

Keywords: *Enterobacteriaceae; ESBL; AmpC; CRE; MDR; Children; Libya*

Introduction

In the past decade, there has been a significant increase in the prevalence of various types of antibiotic-resistant bacteria, especially Gram negative bacteria (GNB), which include extended-spectrum β-lactamase (ESBL), carbapenem resistant *Enterobacteriaceae* (CRE),

and multidrug-resistant (MDR) non-fermentative organisms (e.g. *Acinetobacter baumannii*) [1]. They have been associated with expanded patient mortality, delayed length of hospitalization, and increased clinic-related expenses [2,3]. Reports show that GNB cause 70% of hospital associated infections, and up to 50% of those contact serious infections from these resistant GNB may die [4,5].

ESBL producing GNB has worldwide distributions with varying degree of prevalence in the community as well as hospitals [6-9]. For the pediatric population, blood stream infections and urinary tract infections due to *Enterobacteriaceae* resistant to ESBL are an emerging problem [10]. This alerts clinical microbiologists to identify these ESBL producing organisms parallel to antimicrobial susceptibility testing even in resource-limited settings by applying simple screening and confirmatory methods. Data obtained from such methods are so valuable to develop appropriate institutional-based drug therapy guideline [9,11]. Though various phenotypic ESBL detection methods have been described, implementation of highly sensitive and specific methods in resource-limited areas is challenging yet [11].

AmpC production is one of the mechanisms of resistance to β-lactams in enterobacteria, conferring resistance to all β-lactams except fourth-generation cephalosporins and carbapenems, and is typically associated with MDR [12]. Treatment options are severely limited because AmpC is often associated with other multiple resistance genes, such as those of resistance to quinolones as well as other β-lactamase genes [12,13]. However, due to recent emergence and spread of CRE throughout the world, clinical utility of this group of antibiotics is under threat [14]. Production of carbapenemases that are capable of hydrolyzing the carbapenems and loss of outer membrane proteins are major mechanisms through which *Enterobacteriaceae* develop resistance against this group of drugs [14,15]. The growing incidence of carbapenemase-producing strains is therefore, another major concern especially among under-resourced countries [16]. In Libya, only a few reports on CRE and AmpC production in *Enterobacteriaceae* strains were published [17-20]. The aim of this study was to investigate the prevalence of ESBL, AmpC and CRE among *Enterobacteriaceae* isolates recovered from pediatric patients in Tripoli, Libya.

Material and Methods

Collection of specimen

This cross-sectional study was carried out in Tripoli Children Hospital (TCH), during the period July 2013 to April 2014. The TCH has 335 beds and receives nearly 6,000 patients (children < 15 years) per year. Specimens were collected from different anatomical sites including (urine, stool, sputum, cerebrospinal fluid, blood), swabs (wound exudates, ear, throat, rectal, axilla, nasal), endotracheal tube tip, central line tube, urine catheter, execration in nasogastric tube. All specimens were taken as part of the clinical workup was included in this laboratory-based surveillance study. Demographic data, age, gender of the patient, in/out patients, department, and type of specimen were recorded. The consent of the patients or that of their guardians was obtained before specimen was collected. In this investigation, specimens were collected under approved ethical standards and the study was reviewed and approved by the Faculty of Medicine, University of Tripoli and National Centre for Disease Control.

Identification and Antibiotic Susceptibility Testing of Isolates

All specimens were cultured on different media by standard bacteriological procedures. Isolated organisms were identified at the species level and tested for their susceptibility to a variety of antimicrobial agents using the BD Phoenix Automated Microbiology System (PAMS, MSBD Biosciences, Sparks Md, USA) according to the manufacturer's instructions. The system uses combination panels for identification (ID) and antimicrobial susceptibility testing (AST) of bacteria. These include the Phoenix NMIC/ID Panels intended for *in-vitro* rapid ID and AST by minimal inhibitory concentration (MIC) of Gram-negative aerobic and facultative anaerobic bacteria from pure culture belonging to the *Enterobacteriaceae* and non-*Enterobacteriaceae* families. Reading and interpretation of panels were also performed according to the manufacturer's instructions.

Phenotypic detection of ESBL and AmpC

Confirmatory tests for ESBL production were performed with all of the isolates initially identified by the Phoenix system. Phenotypic confirmation of ESBLs was done using ESBL chromogen media (Liofilchem, Italy) and E-test strips containing ceftazidime, and

ceftazidime-clavulanate was used to determine the MIC ratio according to the manufacturer's instructions (Liofilchem, Italy) performed on Mueller Hinton agar. All isolates were screened and interpreted for ESBL phenotype according to the criteria of the Clinical and Laboratory Standards Institute [21]. Then, all isolates were initially screened for cefoxitin resistant strains using automated system, then subjected to phenotypic screening for AmpC production using two methods: ESBL and AmpC screen disc kit test (combination disc test [CDT] discs containing cefotaxime alone and in combination with clavulanic acid, cloxacillin and both of these inhibitors are applied) and AmpC E-test (cefotetan/cefotetan+cloxacillin), the AmpC E-test consists of a strip containing cefotetan on one end and cefotetan-cloxacillin on the other end. The results were interpreted and displayed in accordance with manufacturer's instructions (Liofilchem, Italy) and EUCAST guidelines for detection of resistance mechanisms was implemented, version 5.0 [22]. MDR was defined as showing resistance to three or more different classes of antibiotics such as fluoroquinolones, aminoglycosides, and cephalosporins [23]. Reference strain of *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603 were used as controls.

Phenotypic detection of CRE

Carbapenem resistance determinants were studied phenotypically using two different techniques: chromogenic culture media, this screening medium (Chromatic CRE) used for detection carbapenem-resistant *Enterobacteriaceae* and non-fermentative Gram negative bacilli (Liofilchem, Italy) and metallo-β-lactamase (MBL) E-test (Liofilchem, Italy) according to manufacturer's instructions and as previously described [24].

Results

A total of 915 GNB isolates were characterized, the majority (65%) were isolated from in-patients specimens primarily from urine (45.5%) and less frequently from tips (22.3%); swabs (16.7%); blood (5.1%); and others (10.4%). The ages of the patients were between 1 day to 14 years, 490 (53.6%) females and 425 (46.4%) males. The predominant organisms were *Escherichia coli* (56.8%), *Klebsiella* spp. (21.4%), *Pseudomonas* spp. (6.7%), *Enterobacter* spp. (5.2%), *Acinetobacter* spp. (4.5%), *Proteus* spp. (2.4%), *Citrobacter* spp. (1.2%), and others (1.8%). Table 1 shows the distribution of GNB isolated from different clinical specimens of pediatric patients.

Over all high resistant rates were observed among *Enterobacteriaceae* identified in this study to ampicillin (87.4%), amoxycillin-clavulanic acid (57%), and trimethoprim-sulfamethoxazole (34.4%). Moderate resistant was observed to ceftriaxone (35%), cefoxitin (31.7%), ceftazidime (30.9%) and aztreonam (27.9%). On the other hand, low resistance rates were detected for gentamicin (19.8%), piperacillin/tazobactam (16.2%), imipenem (9.4%), meropenem (7.6%), amikacin (6.3%), and colistin (3.6%).

Of the most common isolated GNB, *E. coli* strains showed low rates of resistance to β-lactams (6.6% - 73.6%), aminoglycosides (0.4 - 12%), fluoroquinolones (18.3 - 24.3%) and carbapenem (0-0.4%). MDR was observed in 116/915 (12.7%) of *Enterobacteriaceae* strains examined, MDR was mainly documented for *Acinetobacter baumannii* 36/41 (87.8%) (Table 1).

The results of antimicrobial susceptibility tests for ESBL producing strains (224/915, 24.5%) and non-ESBL producers (691/915, 75.5%) are summarized in table 2. ESBL producers were more often resistant to major classes of antibiotics compared with non-ESBL producers. Significantly high resistance rates ($P < 0.001$) were observed for ceftriaxone, cefepime, and ceftazidime (87.5 - 95.9%) among ESBL producers compared to non-ESBL producers (7.2 - 13.5%). High rates of resistance were also demonstrated to gentamicin (47.3%) and trimethoprim-sulfamethoxazole (42.8%). MDR was documented for 50/224 (22.3%) of ESBL producers, and was significantly higher ($P < 0.0001$) among ESBLs compared with non-ESBL producer isolates. The overall prevalence of ESBL producing *Enterobacteriaceae* was 24.5%. Out of 224 *Enterobacteriaceae* proved ESBL producer, *Klebsiella* spp. (54%), *E. coli* (34.4%), *Enterobacter* spp. (7.6%), *Proteus* (1.3%), *Citrobacter* (1.8%), and *Morganella* spp. (1%) were positive.

Organisms Antibiotic	Total n = 915	*E. coli* n = 520 (56.8%)	*Kleb* n = 196 (21.4%)	*Pseu* n = 61 (6.7%)	*Enter* n = 48 (5.2%)	*Acineto* n = 41 (4.5%)	*Proteus* n = 22 (2.4%)	*Cit* n = 11 (1.2%)	Others n = 16 (1.8%)
Amikacin	6.3%	0.4%	5.8%	5%	0%	34.1%	9.1%	100%	31.3%
Gentamicin	19.8%	12%	40%	5%	33.3%	92.7%	36.4%	9.1%	43.8%
Ertapenem	16.3%	0.4%	16.3%	100%	6.2%	100%	4.5%	9.1%	12.5%
Imipenem	9.4%	0%	6.8%	23%	0%	92.7%	41%	100%	6.25%
Meropenem	7.6%	0%	9.5%	11.5%	0%	92.7%	0%	100%	6.25%
Cefoxitin	31.7%	5.8%	24.7%	100%	89.6%	100%	4.5%	81.8%	56.2%
Ceftazidim	30.9%	12.3%	62.1%	20%	29.2%	95.1%	0%	45.5%	18.8%
ceftriaxon	35%	13.9%	62.1%	95.1%	43.7%	95.1%	9.1%	27.3%	18.8%
Cefepime	26.1%	13.5%	62.1%	13.1%	29.2%	92.7%	9.1%	18.2%	12.5%
Azitronam	27.9%	13.9%	62.1%	26.2%	37.5%	100%	0%	36.4%	18.8%
Ampicillin	87.4%	73.6%	100%	100%	100%	100%	68.2%	90.9%	87.5%
Amoxa.-Clav.	57%	26.2%	65.3%	100%	100%	100%	9.1%	81.8%	43.8%
Piprac.-tazob.	16.2%	6.6%	34.7%	1.6%	20.8%	92.7%	0%	36.4%	6.25%
Colistin	3.9%	0%	0%	0%	0%	0%	100%	0%	87.5%
Trimeth.-Sul.	34.4%	38.5%	29%	95.1%	20.8%	95.1%	31.8%	54.5%	6.2%
Nitrofuran-ton	29%	3.9%	39%	100%	47.8%	100%	100%	95.1%	68.8%
Ciprofloxacin	25.5%	24.3%	22.1%	3.3%	14.6%	92.7%	41%	45.5%	12.5%
Levofloxacin	23.1%	18.3%	16.3%	0%	14.6%	92.7%	41%	45.5%	12.5%
MDR	116 (12.7%)	36 6.9%	42 22.1%	2 3.3%	0	36 87.8%	0%	0%	0%

Table 1: *Antibiotic resistance patterns of Enterobacteriaceae clinical isolates.*

Antibiotics	ESBL No. 224	Non-ESBL No. 691	P	OD
Amikacin	4.9%	6.7	P = 0.1219	2.5707
Gentamicin	47.3%	15.4	P < 0.0001	9.6658
Ertapenem	16%	15.8	P = 0.0018	3.0848
Imipenem	7.5%	9.5	P = 0.0601	2.4679
Meropenem	9.3%	7.4	P = 0.0050	3.8560
Cefoxitin	39.7%	21.4	P < 0.0001	5.8759
Ceftazidim	89.7%	10	P < 0.0001	27.7634
ceftriaxon	95.9%	13.5	P < 0.0001	21.1531
Cefepime	87.5%	7.2	P < 0.0001	33.9330
Azitronam	90%	10.2	P < 0.0001	27.7634
Ampicillin	100%	80.1	P < 0.0001	3.8560
Amoxa.-Clav.	94.1%	43.8	P < 0.0001	6.6604
Piprac.-tazob.	33.4%	10.1	P < 0.0001	10.1799
Colistin	0%	0	0	0
Trimeth.-Sul.	42.8%	35	P < 0.0001	3.7899
Nitrofuran-ton	32.5%	27.5	P < 0.0001	10.1799
Ciprofloxacin	39.2%	21.4	P < 0.0001	5.6088
Levofloxacin	33%	19.5	P < 0.0001	5.0900
MDR	50 22.3%	66 9.5%	P < 0.0001	7.0951

Table 2*: Antibiotic resistance patterns of ESBL and non-ESBL producers.*

Table 3 shows the detection of AmpC production using two tests. The CDT and E-test for all isolates revealed 60/915 (6.6%) isolates as potential AmpC β-lactamase producers, *E. coli* exhibited the lower level of AmpC (8.3%) compared with *Klebsiella* spp. (56.6%), *Enterobacter* spp. (7.6%), *Citrobacter* spp. (5%), *Serratia* spp. (3.3%) and *Morganella* spp. (3.3%). A 56/60 (93.3%) isolates were positive for both ESBLs and AmpC.

Isolates 915	ESBL n = 224 (24.5%)	AmpC n = 60 (6.6%)	CRE n = 83 (9%)
Klebsiella ssp.	121 (54%)	34 (56.6%)	37 (44.6%)
E. coli	77 (34.4%)	5 (8.3%)	7 (11.6%)
Enterobacter ssp.	17 (7.6%)	14 (23.3%)	6 (7.2%)
Proteus	3 (1.3%)	0	0
Citrobacter spp.	4 (1.8%)	3 (5%)	2 (2.4%)
Serratia spp.	0	2 (3.3%)	3 (3.6%)
Morganella spp.	2 (1%)	2 (3.3%)	0
A. baumanni	0	0	20 (24%)
Pseudomonas spp.	0	0	8 (9.6%)

Table 3: *Distribution of ESBL, AmpC and CRE among clinical isolates.*

Regardless of their ESBL result, all *Enterobacteriaceae* showed carbapenem (ertapenem, imipenem and/or meropenem)-resistant or carbapenem-intermediate *Enterobacteriaceae* were checked for the production of carbapenemase using two methods. The overall prevalence of CRE was 9% (83/915). Carbapenemase-producing organisms in this study were as follows: *Klebsiella* spp. (44.6%); *Acinetobacter* spp. (24%); *Pseudomonas* spp. (9.6%); *E. coli* (11.6%); *Enterobacter* spp. (7.2%); *Citrobacter* (2.4%) and *Serratia* ssp. (3.6%).

Discussion

ESBL producing *Enterobacteriaceae* infections are a growing threat to children. The treatment of pediatric MDR *Enterobacteriaceae* infections, including ESBLs and CRE will undoubtedly become more and more challenging, and heightened awareness of ESBL producing bacteria in children and the dedication of targeted resources for prevention and management of ESBL infections remain imperative. Their number is constantly increasing, especially in *E. coli* and *Klebsiella* which are involved in numerous healthcare infections and epidemics. In the present study, we investigated the incidence of ESBL, AmpC and CRE among *Enterobacteriaceae* clinical isolates from pediatric hospital in Libya. The overall prevalence of ESBL-producer *Enterobacteriaceae* was 24.5% which showed agreement with other studies conducted in North Africa: in Algerian hospitals, ESBL existed in 16.4 - 31.4%; while the prevalence ranged from 11.7 to 77.8% in hospitals and was 0.7 and 7.3% in two communities in Tunisia; and ESBLs were found in 11 - 42.9% of Egyptian samples in both hospitals and communities [25]. However, our result was higher compared to previous studies carried out in Libya 10 - 20.5% [20,26-28]. Increased incidence of ESBL infections is also associated with an increase in MDR strains (22.3%) compared with non-ESBL producers (9.5%) ($P < 0.0001$). This indicated that ESBL-producing *Enterobacteriaceae* are growing rapidly over time. *Klebsiella* spp. was the most frequent ESBL-positive *Enterobacteriaceae* (54%, 121/224) in line with previous local studies (21.7 - 88%) [17,20,27-30]. A similar trend was observed were *K. pneumonia* was the leading ESBL producer of *Enterobacteriaceae* clinical isolates obtained from Ethiopian children [31]. The global frequency of ESBL production in the *Klebsiella*, *Enterobacter* and *Serratia* group is estimated at 31.4%, their number is constantly increasing which is involved in numerous nosocomial infections and epidemics. Little has been published on the current situation in Libya, this study showed that the prevalence of ESBL for *Klebsiella*, *Enterobacter* and *Serratia* strains is 54%, 7.6%, and 0% respectively.

ESBLs usually present resistance to other classes of antibiotics, such as quinolones and trimethoprim/sulfamethoxazole [32-34]. Therefore, the broadest-spectrum antibiotic agents, carbapenems, are recommended to treat infections caused by ESBL-producing bac-

teria [32,35]. Most ESBL-producer *Enterobacteriaceae* showed significantly high resistance to aztreonam (90%), gentamicin (47.3%) and sulfamethoxazole-trimethoprim (42.8%) and ciprofloxacin (39.2%) compared to non-ESBL isolates ($P < 0.0001$). This result indicates that gentamicin the commonly used of these antibiotics in Libya especially for the treatment of infection caused by ESBL-producing strains may result in treatment failure in a significant proportion of cases. The choice of antibiotic agents effective against ESBLs-producing species is currently limited, which may cause serious therapeutic problems in the future. In 2009, Zorgani outlined all the challenges posed by these organisms which were considered a definite threat to the future of antimicrobial chemotherapy and must be seriously addressed by the laboratory, clinicians treating infected patients, and infection control professionals in Libya [36].

The occurrence, types and rate of dissemination of AmpC enzymes has increased worldwide, their early detection is crucial and critical [37]. In the present study, over all prevalence of *Enterobacteriaceae* carrying AmpC was 6.6% (60/915), *Klebsiella* spp. (56.6%, 34/60) was the most potential AmpC β-lactamase producer compared with *E. coli* (8.3%, 5/60). Prevalence of plasmid AmpC in Libya is not known, due to the limited number of epidemiological surveys. In Libya, only a few reports on AmpC production in *Enterobacteriaceae* strains were published [20,26]. Recently, Zorgani and colleagues detected plasmid-mediated AmpC genes in 7.9% of *K. pneumonia* and 4% of *E. coli* isolated from Tripoli Medical Centre and Children hospital, the gene encoding CMY enzyme was the most prevalent (66.6%) of AmpC positive isolates followed by MOX, DHA and EBC [17]. In our region, AmpC β-lactamases show marked variation in geographic distribution. In Algeria, two studies reported a prevalence of plasmid mediated AmpC β-lactamases of 1.6% and 2.18% [38,39]. Plasmid-mediated AmpC β-lactamases were detected in Tunis, Tunisia, in 78 isolates (0.59%) of *E. coli*, *K. pneumoniae*, and *P. mirabilis* [40]. These rates of prevalence was much lower than the result reported in Egypt by Wassef., *et al*. who reported that 26.9% harbored the plasmid mediated AmpC [41]. The result was equivocal with Fam., *et al.* from Egypt where AmpC prevalence was 28.3% [42].

Carbapenemase-producing *Enterobacteriaceae* have been steadily spreading worldwide during the last decade. According to recent data from the Centers for Disease Control and Prevention in the United States, the percentage of CRE increased from 1.2% in 2001 to 4.2% in 2011 [43]. The highest increase in proportion, from 1.6% to 10.4%, was observed for *Klebsiella* spp. during the same period [43]. In this study the occurrence of CRE was 9% among *Enterobacteriaceae* isolates, *Klebsiella* spp. was the leading carbapenemase producer (44.6%, 37/83) followed by *A. baumannii* (24%, 20/83) and *E. coli* (11.6%, 7/83), these isolates were autochthonous associated with MDR. *Klebsiella* spp. recorded the highest level of ESBL-producer, AmpC and CRE (54.1%, 56.6% and 44.6%, respectively) compared with other isolates investigated in this study. Many studies conducted in Libya revealed the presence of carbapenem resistant-encoding genes (OXA-23, OXA-48, NDM-1, VIM-2 and GES) contributed to antibiotic resistance in Libyan hospitals [19,44-46]. In the Mediterranean basin, during recent years, the emergence of CRE becomes an alarming problem. The prevalence of these CRE is variable across Mediterranean countries; a high prevalence can be found in Italy, Greece, Turkey, and Israel, whereas a low prevalence is still reported in Croatia, Slovenia, and Libya [47].

Children are particularly vulnerable in the MDR *Enterobacteriaceae* pandemic due to lack of broad-spectrum antibiotics approved for use in children. ESBL-producing strains frequently harbor co-resistance genes conferring resistance to aminoglycosides and fluoroquinolones, among others, limiting therapeutic options, especially with oral agents [48,49]. Fosfomycin has oral dosing for older children and is useful in the management of cystitis, this drug is not available in Libya, whereas colistin remains elusive and this study indicated that all isolates remained susceptible to colistin [7,48]. The use of β-lactam/β-lactamase inhibitor drugs in the management of infections by ESBL-producing bacteria is still controversial. Carbapenems remain the gold standard of treatment for serious pediatric ESBL infections [48,50].

Conclusion

This study revealed that the prevalence of ESBL, AmpC, CRE and MDR *Enterobacteriaceae* isolates in Libya was within acceptable frequency. Fastidious hygiene, patient isolation, cohorting, dedicated staff, and implementing antibiotic regimen policies were all encouraged to control dissemination of ESBL and CRE-producing *Enterobacteriaceae*.

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Declaration of Interest

The authors report no declarations of interest.

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