Microbiology Research International
Vol. 8(2), pp. 29-34, April 2020
DOI: 10.30918/MRI.82.20.012
ISSN: 2354-2128
Full Length Research Paper

Determination of prevalence and antimicrobial sensitivity pattern of extended spectrum beta-lactamase producing *Enterobacteriaceae* from various clinical specimens in Medinah during Hajj

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Accepted 21 February, 2020

ABSTRACT

The spread of extended spectrum beta lactamase (ESBL) producing *Enterobacteriaceae* has dramatically increased and become a major public health concern worldwide. The main purpose of the study was to determine antibiotic resistance patterns among ESBL producing *Enterobacteriaceae* isolated from clinical specimen of pilgrims during Hajj in Medinah, Saudi Arabia. A total of 69 consecutive non-duplicate isolate *Enterobacteriaceae* were examined by cultured in selective and non-selective media, therefore between June and august 2019. All isolates were identified and tested for susceptibility by the VITAK II. Detection of ESBL was carried out by double disk synergy test technique. Of 69 of *Enterobacteriaceae* isolates, 3 isolates were associated with multidrug resistance (MDR) phenotype. All isolates showed variable resistance levels to all antibiotics used here expect to colistin, where they were all colistin-sensitive. Sensitivity of 98% was observed to amikacin. 12 out of 69 *E. coli* isolates were positive for ESBLs by phenotypic methods. 16 out of 69 *Klebsiella pneumoniae* isolated were ESBL producers. Most of the ESBLs were from urine (45%). The main risk factors for ESBL in these children were previous exposure to antimicrobials and prolonged hospital stay and female gender (68%). No resistance was recorded for the following combinations of antibiotic: amikacin plus nitrofurantoin, amikacin plus piperacillin-tazobactam. Carbapenems are the most sensitive and reliable treatment options for infections caused by ESBL producing *Enterobacteriaceae*. Amikacin plus piperacillin-tazobactam are good alternatives. Our study documented the high antimicrobial resistance of ESBL producing *Enterobacteriaceae* to many first line antibiotics currently used to treatment patients, and this implies the need to continuously revise the local guidelines used for optimal empirical therapy for patients.

Keywords: ESBL, *Enterobacteriaceae,* antibiotic resistance.

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INTRODUCTION

Extended spectrum beta lactamase (ESBL) enzymes are characterized by their ability to hydrolyze various antibiotics, including third generation cephalosporins (e.g. cefotaxime, ceftriaxone and ceftazidime) and monobactams (e.g. aztreonam) but not the cephamycins (e.g. cefoxitin and cefotetan) and carbapenems (e.g. imipenem, meropenem and ertapenem). The spread of ESBL producing *Enterobacteriaceae* has dramatically increased worldwide. There are at least 200 different types of ESBL enzymes. The first plasmid-mediated beta-lactamase in Gram-negative, temoniera-1 (TEM-1) was discovered in Athens, Greece in 1965 (Morrissey et al., 2013; Flokas et al., 2017; Jamali et al., 2017). Most ESBL can be divided into three groups: temoniera (TEM), sulfhydryl variable (SHV), and Cefotaximases (CTX-M) types. The TEM-1 β-lactamase has spread worldwide and is now found in different species of members of *Enterobacteriaceae*, *Neisseria gonorrhoeae*,

Pseudomonas aeruginosa and *Haemophilus influenzae* (Morrissey et al., 2013).

Sulfhydryl variable-2 (SHV-2) is an efficiently hydrolyzed cefotaxime and to a lesser extent ceftazidime. These non-TEM and non-SHV plasmid-mediated class A ESBLs have been reported as cefotaximases (CTX-M).

These enzymes hydrolyze cephalothin better than benzylpenicillin and they preferentially hydrolyze cefotaxime over ceftazidime. Five different clusters of CTX-Ms (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) have been recognized on the basis of their amino acid sequences (http://www.lahey.org/studies/webt.stm). The CTX-M enzymes have been identified predominantly from the community as a cause of urinary tract infections (Morrissey et al., 2013; Flokas et al., 2017).

In Saudi Arabia, Al-Garni et al. (2018) found 30.5% prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in the Taif region. In a recent study using PCR amplification, predominantly identified the CTX-M genotype among ESBL-producing *E. coli* isolates (Yasir et al., 2018). In addition, the CTX-M-15 gene was commonly found in uropathogenic *E. coli* isolates in Riyadh (Alqasim et al., 2018). However, variation exists in antimicrobial resistance rates and associated genes depending on the geographic area of the study (Alqasim et al., 2018; Matsumura et al., 2015). In Jordan, Al-Jamei et al. (2019) found 62% ESBL-producing *E. coli* in urinary tract infections.

Goals of this study were to determining the antimicrobial susceptibility patterns of a collection of clinical *Enterobacteriaceae* virous isolates, to phenotypically assess the ESBL carriage of these isolates. This study is important for providing clinicians with information required to facilitate the effective treatment and management of patients.

MATERIALS AND METHODS

This descriptive–analytic study was performed on 1208 specimens referred to Ohud Hospital, Medinah, Saudi Arabia, who agreed to participate in this study. The Ohud hospital is a 300 beds facility hospital with all general and subspecialty medical services. The hospital provides primary, secondary care services for Saudi and pilgrims patients. It also provides tertiary care services to all Saudi citizens on referral bases.

Sampling

Various clinical specimens including pus, urine, blood, CSF, ear swab, conjunctival swab, and semen that were received for routine culture and susceptibility testing in the clinical microbiology laboratory at Ohud Hospital, during a period of 3 months were studied.

Ethical approval

Ethical approval was obtained from the Institutional Review Board (IRB) at general directorate of health affairs in Medinah (IRB Number: 330).

Data collection

All samples were cultured onto blood agar, chocolate agar and Macconkey agar (Second Advance Medical Company, Saudi Arabia) and incubated at 37°C for 24 h. Midstream clean catch urine samples showing significant bacterial growth, of $>10^5$ colony-forming units (CFU/ml) with a single type of bacteria, were considered positive for UTIs. Isolates were identified by VITEK II system (bioMerieux, Marcy l'Etoile, France) using the card for Gram-negative strains (GN cards) and AST-N291 and AST-N292 (Lee et al., 2006; Maslikowska et al., 2016) and Antibiotic susceptibility testing to beta-lactam/bate-lactamase inhibitor, cephalosporins, aminoglycosides and carbapenems were performed by disk method. Samples that were tested manually or against only one of the AST-cards or to different AST-cards were excluded, that is, only samples that were tested against both AST cards were included in the study. Quality control was ensured by testing *E. coli* ATCC 25922 in every batch. The minimal inhibitory concentration (MIC) interpretive standards for *Enterobacteriaceae* were adopted from the CLSI guideline 2018 (Maslikowska et al., 2016; http://www.lahey.org/studies/webt.stm; Biehl et al., 2016) for the following groups of antibiotics:

- Group I: penicillins (ampicillin), beta-lactames/beta- lactamase-inhibitor combinations (amoxicillin/clavulanic acid and piperacillin/tazobactam), 3rd and 4th generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone, and cefepime);
- Group II: carbapenems (imipenem, meropenem);
- Group III: fluoroquinolones (ciprofloxacin);
- Group IV: aminoglycosides (amikacin and gentamicin);
- Group V: folate synthesis pathway inhibitors (sulfamethoxazole/trimethoprim);
- Group VII: lipopeptides (colistin);
- tigecycline
- nitrofurantoin (only urine)

Multidrug resistant (MDR) was defined as resistance to imipenem and meropenem plus 3 or more different antibiotic classes, including: at least 2 beta-lactames (penicillin, betalactames/beta-lactamase-inhibitor combinations, 3rd- and 4th-generation cephalosporins); amikacin or gentamicin; ciprofloxacin; or sulfamethoxazole/ trimethoprim.

Prodrug resistant (PDR) was defined as resistance to all tested antibiotics or only susceptible to colistin. Tigecycline was not included in this definition since no agreed breakpoints for tigecycline have been approved by the CLSI 2018 guideline (Afridi and Farooqi, 2012; Dangelo et al., 2016).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) for all isolates was carried out on Mueller–Hinton agar (Second Advance Medical Company, Saudi Arabia) using disk diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (Afridi and Farooqi, 2012; Dangelo et al., 2016) using a panel of 8 commercially available antibiotics (Master Group, Italy). In this method, inoculums containing test organism of 0.5 McFarland turbidity were streaked onto a Mueller Hinton agar plate with the help of swab sticks. *E. coli* ATCC 25922 was used as a control strain.

Double disk synergy test

Double disk synergy test (DDST) using ceftazidime (30 µg),

ceftriaxone (30 µg), and amoxicillin/clavulanic acid (100/10 µg) disks (16) In this method, inoculums containing test organism of 0.5 McFarland turbidity were streaked onto a Mueller Hinton agar plate with the help of swab sticks. amoxicillin/clavulanic acid disk was placed in the center of the plate whereas disks containing ceftriaxone (30 µg) and ceftazidime (30 µg) were placed 20 mm from center to center from amoxicillin/clavulanic acid disk and plates were incubated overnight at 37°C. The test organism is considered to produce ESBL if the zone size around the test antibiotic disk increases toward the piperacillin-tazobactam disk (Figure1).

RESULTS

Over a period of two months, a total of 69 organisms of *Enterobacteriaceae* were isolated from different clinical samples (Table 1). More of the isolates were from female (47, 68%) than male patients (Table 1).

Of total isolates of *Enterobacteriaceae*, *E. coli* was predominant (52%) followed by *K. pneumoniae* (36%) (Table 2). *Enterobacteriaceae* resistance to various antibiotics groups is summarized in Table 3. *E. coli* was most resistant to ampicillin (86%), trimethoprim /sulfamethoxazole and amoxicillin/clavulanic acid (56%), piperacillin/tazobactam and cephalosporins (38 and 33%, respectively). *K. pneumoniae* was sensitive to amikacin and colistin (100%), tigecycline (98%), and gentamicin (4%). *P. mirabilis* and *E. cloacae* strains were sensitive to amikacin, imipenem, and meropenem antibiotics. Seven isolates had reduced susceptibility to carbapenems (Table 3). According to our definitions, three *Enterobacteriaceae* isolates were multidrug resistant (5%), while one isolate was pan-drug resistant (1.5%) (Table 4).

Figure 1. Double-disk synergy test: Organism showing enhanced zone of inhibition between ceftazidime and ceftriaxone and amoxicillin/clavulanic acid containing disk indicating extended-spectrum β-lactamase production.

Table 1. Distribution of *Enterobacteriaceae* isolates from different specimens, by patient's sex.

Specimen	Males		Females		Total	
	No	%	N	%	N	%
Blood	4	6	4	6	8	12
Urine	9	13	22	32	31	45
Swabs	5	7	16	23	21	30
Sputum	4	6	5	7	9	13
Total	22	32	47	68	69	100

Table 2. *Enterobacteriaceae* spp. isolated from various clinical samples.

Isolates	Number of isolates	Percentage
E. coli	36	52
K. pneumoniae	25	36
P. mirabilis	4	6
E. cloacae	4	6
Total	69	100

Table 3. Percentage resistance pattern of *Enterobacteriaceae* isolates from different clinical specimens.

Antibiotics \ Organisms	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Enterobacter cloacae*
Ampicillin	86	-	100	50
Amoxicillin/clavulanic acid	56	48	50	50
Cephatholin	33	68	50	75
Ceftazidime	33	68	50	50
Ciprofloxacin	11	44	25	25
Ceftriaxone	33	68	50	50
Cefotaxime	33	68	50	50
Cefepime	33	68	50	50
Gentamicin	14	4	0	25
Amikacin	0	2	0	0
Imipenem	3	28	0	0
Meropenem	0	28	0	0
Trimethoprim/sulfamethoxazole	56	56	25	75
Tigecycline	0	2	-	0
Piperacillin/tazobactam	38	32	0	25
Nitrofurantoin	11	12	-	0
Colistin	0	0	-	0

Table 4. Antimicrobial susceptibility of ESBL, multidrug resistant and Pandrug resistant *Enterobacteriaceae* isolates (n = 69) to various antibiotics tested.

Organisms	No. of isolates	Susceptibility (%)[c]
E.coil (ESBL)	12	17
K. pneumoniae (ESBL)	16	23
E. cloacae (ESBL)	2	3
Carbapenems drug resistant[a]	7	5
Multi drug resistant[a]	3	5
Pandrug resistant[b]	1	1.5

[a] Resistant to imipenem plus 3 or more different antibiotic classes; [b] Resistant to all tested antibiotics or only susceptible to colistin; [c] Percentage of total number of isolates tested (n = 69) susceptible to colistin only.

DISCUSSION

The percentage of ESBL producing *Enterobacteriaceae* in Hajj pilgrims admitted in Ohud Hospital was taken. The prevalence of ESBL in medical ward was 43%; however, the prevalence rate of ESBL among *E. coli* and *K. pneumoniae* isolated from different samples were 17 and 23%, respectively compared to prevalence of ESBL in the Sultanate of Oman in 2008 (13.3% for *E. coli* and 16.6% *K. pneumoniae*) and Europe (10.8% for *E. coli* and 13.6% for *K. pneumoniae*) (Matsumura et al., 2015; Al-Jamei et al., 2019).

More of the Enterobacteriaceae isolates identified in our study were more from female (68%) than male patients, in agreement previous studies (Biehl et al., 2016). In this regard, previous studies in Saudi Arabia showed that the prevalence rates of ESBL-producing were 6.5 and 10.3% in 2002 and 2004, respectively (Afridi and Farooqi, 2012). In the present study, isolates of *Enterobacteriaceae* showed ESBL resistance rate (34%) to third generations of cephalosporins. This observation was consistent with a number of studies implicating *Enterobacteriaceae* particularly *E. coli* in urinary tract infections, which are believed to be the most common infections all over the world among hospitalized patients, including in Saudi Arabia (Afridi and Farooqi, 2012; Dangelo et al., 2016; Azap et al., 2010). The most common source of *Enterobacteriaceae* isolates was urine (45%), followed by swab (30%), sputum (13%) and blood (12%) which is in agreement with previous findings from King Fahad Medical City (KFMC) hospital in a 2016 study and at Sultan Qaboos University Hospital, Sultanate of Oman (Al-Jamei et al., 2019; Biehl et al., 2016).

The *Enterobacteriaceae* resistance pattern across various sample origins was significantly different for quinolones, tetracyclines, gentamicin and sulfamethoxazole/trimethoprim. The different resistance pattern for *Enterobacteriaceae* from different sample sources is and further investigations are needed to elucidate the cause of these differences (Afridi and Farooqi, 2012).

The isolated ESBL-producing *Enterobacteriaceae* showed highest resistance to trimethoprim-sulfamethoxazole (56%), followed by amoxicillin/ clavulanic acid (48%), and then ciprofloxacin (44%). On the other hand, the carbapenems (imipenem and meropenem) were the most effective against the bacteria with a susceptibility result of 97 and 100%, respectively. The sensitivity to other drugs was: Amikacin (100%) followed by nitrofurantoin (91%) then piperacillin-tazobactam (62%). Other studies showed similar resistance results (Al Mously et al., 2016). El Bouamri et al. reported 75.1, 69.8 and 40.1% to each of trimethoprim-sulfamethoxazole, amoxicillin/clavulanic acid and ciprofloxacin respectively as well as (100%) sensitivity to imipenem and (97%) sensitivity to amikacin (Morrissey et al., 2013). Saltoglu et al. in 2015 mentioned that urinary ESBL-producing *E. coli* showed 100% sensitivity to carbapenems that is consistent with this study result and a higher 97.6% sensitivity to nitrofurantoin (Alqasim et al., 2018; El Bouamri et al., 2014).

A study from Medinah showed no resistance to tigecycline (3%), even at MIC 1.5 to 2.0 mg/L. Due to the wide range of resistance to tigecycline, there is a growing need for agreed breakpoints of susceptibility to be declared and accepted by CLSI, EUCAST, BSAC, FDA and other institutions. Colistin is still considered to be the most effective single antibiotic against MDR Enterobacteriaceae and is always kept as a last resort due to the growing rates of resistance to carbapenems in recent decades (Dangelo et al., 2016; Al Muharrmi et al., 2008; Alqasim et al., 2018; Saltoglu et al., 2015). Many of the isolates producing these enzymes were also resistant to quinolones, aminoglycosides and trimethoprim often due to plasmid co-expression of other resistance mechanisms. Our study also showed that carbapenems still remains the best treatment option for these highly resistant pathogens and piperacillin/tazobactam with amikacin.

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Citation: Ziab A, 2020. Determination of prevalence and antimicrobial sensitivity pattern of extended spectrum beta-lactamase producing *Enterobacteriaceae* from various clinical specimens in Medinah during Hajj. Microbiol Res Int, 8(2): 29-34.