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Prevalence of Extended-Spectrum Beta-Lactamase Producing *Enterobacteriaece* from various Clinical Isolates in Al-ansar Hospital, Medina, Saudi Arabia

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Extended spectrum beta-lactamases (ESBLs) producing *Enterobacteriaceae*. The enzyme is an increasing problem in hospitals worldwide. Therefore, it is imperative to quantify the problem, and reinforce guidelines promoting appropriate antibiotic use. The purpose of this study was to evaluate the incidence of ESBL and to analyze their antibiotic susceptibility in *Enterobacteriacea*e isolate from different sources Al-ansar hospital, in Medina, Saudi Arabia. A total of 283 were examined by direct microscopy (Gram stain and methylene blue) and parallel cultured in selective and non-selective media, therefore obtained between January and June 2014. All isolates were identified and tested for susceptibility by the VITEK II system (bioMérieux, Marcy l'Etoile, France) using the card for Gram-negative strains (GN cards) and AST-N291. Antibiotic susceptibility testing to beta-lactam/bate-lactamase inhibitor, cephalosporins, aminoglycosides, and carbapenems were performed by disk diffusion method. As for the identity of recovered 101 *Enterobacteriaceae* ESBL produce isolates, including *Escherichia coli* (57%), *Klebsiella pneumoniae* (40%), and*Proteus mirabilis* (3%). ESBL phenotype was seen in 14 (40%) of tracheal secretion isolates, 43(39%) from urine, 3(37%) from pus, and 13(36%) from sputum. wound and blood specimens were also encountered at a frequency of 31% and 29%, respectively. *E.coli* was most resistance to cotrimaxazole (68%), nitrofurantoin (50%), amoxicillin/ clavulanate (51%), piperacillin/tazobactam and ciprofloxacin (40% and 33%, respectively). *K. pneumoniae* was sensitive to imipenum (97%), meropenem (97%), amikacin (93%), and gentamicin (70%). *P.mirabilis*, *E. cloacae*, *P. stuartii, and S. marcescens*, strains were sensitive to amikacin, impenem, and meropenem antibiotics. Three isolates had reduced susceptibility to carbapenems. This study showed the existence of important ESBLs among the *Enterobacteriaceae* isolated from inpatients and outpatient. Continuous surveillance of ESBLs, in bacteria isolated from these patients have an important clinical impact, since, it can often provide valuable information for effective infection control measures and for the choice of appropriate antimicrobial therapy. The empiric use of third and fourth generation cephalosporins should be curtailed, as cephalosporin use was associated with an increased risk of ESBL production.

Key words: Extended spectrum beta-lactamases (ESBLs), *Enterobacteriaceae*, *Escherichia coli*, *Klebsiella pneumonia*, antibiotic.

Resistance to ß-lactam antibiotic agents, especially extended spectrum cephalosporins and other antibiotic agents among various clinical isolates of Gram-negative bacteria is on the rise worldwide[1]. beta-lactam antibiotics are among the most frequently used antibiotics for empirical therapy.

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The first description of extended spectrum beta-lactamases (ESBLs) in 1983, production of beta-lactamase is one of the strategies adopted by bacteria to develop resistance to beta-lactam class of antibiotics. ESBLs are enzymes that mediated resistance to extended-spectrum cephalosporins (e.g. ceftriaxone, cefotaxime, and ceftazidime) by hydrolysis of these antibiotics and which are inhibited by beta-lactamase inhibitors such as

clavulanic acid[1]. The plasmid-mediated resistance against cephalosporins can spread among related and unrelated Gram-negative bacteria. These new beta-lactamases are derived from mutation in older ß-lactamase like (TEM-1, TEM-2, and SHV-1) which confer resistance to penicillins but not to expanded- spectrum cephalosporins. TEM-1 beta-lactamase spread worldwide in different species of bacteria[1,3].

Nosocomial infections caused by ESBL-producing Gram-negative bacteria have also been reported[4], which are mainly the result of extensive and inappropriate use of third-generation cephalosporins (ceftazidime, cefotaxime, and ceftriaxone). They can be found in a variety of *Enterobacteriaceae* species; however, majority of the ESBL producing strains are *Escherichia coli*, *Klebsiella species*. They have also been found in other genera of the *Enterobacteriaceae* including *Citrobacter*, *Enterobacter*, *Proteus*, *Serratia*, and *Salmonella*[3].

Confirmatory test susceptibilities to cefotaxime and ceftazidime alone those with clavulonate are compared using disk diffusion, increase significantly more than or equal to five mm increase in a zone diameter[7]. The treatment of choice for ESBL-positive isolates includes carbapenem and b-lactam/b-lactamase inhibitors.

Recently, high prevalence of antibiotic resistant bacteria and a trend of increase resistance under continued antibiotic. The major risk factors implicated are long-term exposure to antibiotics, nursing home residency, prolonged ICU stay, and catheterization[2].

This study was designed in Al-ansar hospital of Saudi Arabia to prevalence data on the occurrence of ESBL producing *Enterobacteriaceae* and analyse their antibiotic susceptibility pattern.

MATERIAL AND METHODS

Specimens and identification

During the study period from January to June 2014, two hundred eighty-seven specimens non-repeat culture were collected from inpatient and outpatient, Al-ansar, Medina. These were isolated from different sources including urine, sputum, tracheal aspiration, pus, and blood. Cultures were processed using standard microbiological methods. The specimens received were initially cultured on blood agar (Hi-Media, Mumbai, India) and MacConkey agar (Hi-Media, Mumbai, India). The blood culture bottles were incubated in the Bactec 9120 system as recommended by the manufacturer for seven days. When growth is detected in a blood culture bottle three drops of blood were sample with a sterile syringe and were inoculated onto blood agar, chocolate agar and Macconkey agar (Hi-Media, Mumbai, India). Urine samples were cultured on blood agar, and CLED agar (Hi-Media, Mumbai, India). The samples were incubated at 37ºC under aerobic conditions for 24-48h[5]. Identification of bacteria was carried out based on Gram staining, methylene blue, standard biochemical tests and VITEK II compact System (bioMérieux, Marcy l'Etoile, France) using GN-291cards for Gram negative bacteria.

Antibiotic susceptibility testing

After identification, all isolates were subcultured on Muller Hinton agar (MHA) (Hi-Media, Mumbai, India). The organisms were suspended in saline to turbidity 0.5 McFarland standard prepared from the pure colonies was inoculated on Muller Hinton agar plates, and left for 15 min to allow moisture absorption at room temperature before applying the multi-disk on the agar. The agar plates were then incubated at 35°C for 18-24 h. *E. coli* (ATCC 25923) was used as controls. The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [6] and also by VITEK II (bioMérieux, Marcy l'Etoile, France)compact System using AST-GN291 card.

The antibiotics disks were as follows (µg/disc): Amoxicillin/clavulanate(20/10), piperacillin/tazobactam (100/10), ceftazidime (30), cefotaxime (30), ceftriaxone (30), cefepime (30), amikacin (30), gentamicin (10) ciprofloxacin (5), trimethoprim-sulfamethoxazole (cotrimaxazole) (1.25/23.75), meropenem (10), imipenem (10), colistin (50), and nitrofurantoin for *in vitro* susceptibility of bacterial isolates to these antibiotics.

ESBL detection

CLSI guidelines to identify ESBL producing isolates using standard disk diffusion technique were followed, *i.e.*, diameter zone of ≤ 22mm with ceftazidime, ≤ 27mm with aztreonam, ≤

27mm with cefotaxime, and ≤ 25mm with ceftriaxone were considered potential ESBL produces and further proceeded for confirmation[6]. These tests were checked for quality using standard control ESBL negative strain of *E. coli* ATCC 25923.

Double disc synergy assay

Synergy between a disc of third generation cephalosporin such as cefotaxime, ceftriaxone or ceftazidime (30 µg) andceftazidime/clavulanic acid (30/10 µg) disc was detected. MHA plates were prepared and inoculated with standardized inoculums of the bacteria (0.5 McFarland standard) to form a lawn culture. A 30 µg disc of each third generation cephalosporin antibiotic was placed on the agar at a distance of 15 mm (centre to centre) from a ceftazidime/clavulanic acid disc. *K. pneumonia* ATCC 700603 was used as the positive control. ESBL production was interpreted as positive if the inhibition zone around the test antibiotic disc increased toward the ceftazidime/clavulanic acid disc.

RESULTS

Of the 283 non-repetitive *Entero bacteriaceae* isolates that were included in the study. The subjects age varied from 19 years to 64 years. The majority of ESBL isolates were identified in 46-55 age group (29/101;27%), followed by the 36-45 age group (24/101;22%) whereas the age group 15-25 years (15/101;14) showed the lowest frequency of ESBL isolates (Fig. 1). There were 65 males (65/283; 24%) and 36 females (36/283; 13%) with a male to female ratio 1.8:1.

The maximum number of ESBL isolates were isolated from Male Medical Ward (MMW) 35/101 (34%), followed by Intensive Care Units (ICUs) 33/101(33%), Female Medical Ward (FMW) 15/101 (15%), Outpatient 9/101 (9%), Male Surgical Ward (MSW) 5/101 (5%), Female Surgical Ward (FSW) 4/101 (4%) (Fig. 2).

ESBL production was observed in101 (38%) isolates by double disk synergy and amongst these 58%, 40%, 3% isolates were *E. coli, K.pneumoniae*, and *P. mirabilis*, respectively (Table 1). The urinary tract infection was the major source of ESBL-producing isolates (43/101; 43%). Urine samples yielded *E. coli* (24/43; 49%); followed by *K. pneumoniae* (16/43; 26%). Also, wound swab samples revealed *E. coli* as a predominant isolates (18/24; 75%), while the predominant species isolated from the tracheal aspiration samples was *K. pneumoniae* (9/16; 56%) (Table 2&3).

Table 4 shows the antibiotic resistance of *Enterobacteriaceae*. For *P. mirabilis, E. cloacae, P. stuartii, and S. marcescens* strains were sensitive to amikacin, imipenem, and meropenem antibiotics. *E. cloacae* and *P. stuartii* were resistant to amoxicillin/clavulanate, piperacillin/tazobactam and ciprofloxacin. .*E. coli*

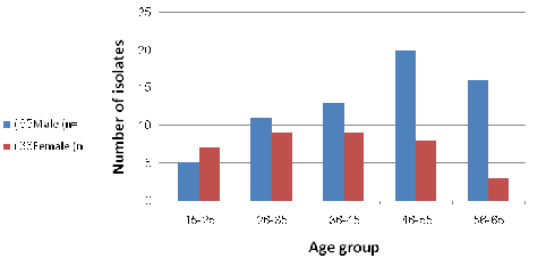


Fig. 1. Distribution of *Enterobacteriaece* isolates from different age groups

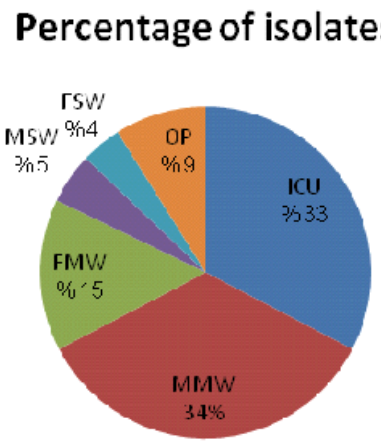
Table 1. *Enterobacteriaece* species

Species	Total number of isolates	ESBL producer
E. coli	140	58
K. pneumoniae	105	40
E. cloacae	8	-
P. mirabilis	13	3
P. stuartii	9	-
S. marcescens	8	-
Total	283	101

Table 2. Distribution of ESBL producer clinical samples

Species	Total number of isolates	ESBL producer
Urine	112	43(43)
Wound	78	24(24)
Sputum	36	13(13)
Tracheal secretion	35	14(14)
Blood	14	4(4)
Pus	8	3(3)
Total	283	101

was most resistance to cotrimaxazole (68%), nitrofurantoin (50%), amoxicillin/clavulanate (51%), piperacillin/tazobactam and ciprofloxacin (40% and 33%, respectively). *E. coli* was sensitive to imipenem (93%), amikacin (98%), and meropenem (92%). *K. pneumoniae* was sensitive to imipenem (97%), meropenem (97%), amikacin (93%), and gentamicin (70%). Three isolates had reduced susceptibility to carbapenems (Table 4).



ICU, intensive care unit; MMW, male medical ward; FMW, female medical ward; MSW, male surgical ward; FSW, female surgical ward; OP, Out-patient.

Fig. 2. Distribution of *Enterobacteriaceae* isolates from different wards

DISCUSSION

The major challenge to infection control is the prevention of the emergence and spread of ESBL-producing *Entero bacteriaceae*. Therapeutic options for treatment of infections involving ESBLs have also become increasingly limited. The efficacy of extended spectrum cephalosporins is compromised while co-resistance to cotrimaxazole, aminoglycosides and fluoroquinolones has been reported[8]. It is necessary to know their prevalence in a hospital setting so as to enable the clinician to select early appropriate antibiotic regimens to reduce average length of stay in a hospital there by reducing healthcare costs and to formulate an effective antibiotic policy[9].

Out of the overall 283 *Entero bacteriaceae* were assessed for ESBL phenotype, ESBL was detected in 101 (36%) of isolates. These

Table 3. ESBL pattern of *Enterobacteriaeces* in different source specimen.

Species	Urine	Wound	Tracheal aspiration	Sputum	Blood culture	Pus	Total
E. coli	24	18	5	9	2		58
K. pneumonia	16	6	9	4	2	3	40
P. mirabilis	3						3
Total	43	24	14	13	4	3	101

Table 4. Resistant Pattern of ESBL for clinical isolates of *Enterobacteriaece*.

		Antibiotics resistance													
Organisms	Number of isolates	AUG	P/T	CRO	CAZ	CXT	CPE	CUR	GM	AK	IMP	MER	CIP	FD	SXT
E. coli	140	51	40	41	41	41	41	47	33	2	7	2	35	50	68
K. pneumoniae	105	63	47	38	38	38	38	50	30	7	3	3	63	46	57
*E. cloacae**	8	100	100	-	-	-	-	-	50	0	0	0	100	-	86
P. mirabilis	13	100	100	23	23	23	23	38	33	0	0	0	67	33	67
P. stuartii	9	100	100	33	22	22	22	66	50	0	0	0	100	-	86
*S. marcescens**	8	90	100	-	-	-	-	-	33	0	0	0	40	50	60

AUG; Amoxicillin/clavulanate, P/T; Piperacillin/tazobactam, CRO; Cefotriaxone, CAZ; Ceftazidime, CXT; Cefotaxime, CPE; Cefepime, CUR; Cefuroxime, GM; Gentamicin, AK; Amikacin, IMP; Imipenem, MER; Meropenem, CIP; Ciprofloxacin, FD; Nitrofurantoin, and SXT; Cotrimoxazole.
* SPICE organisms

findings are consistent with those previously reported from other countries as USA (10), Canada (11), China (12), Italy (13), India (14,15,16 &17), United Arab Emirates (18), Jordan (19), Egypt (20, 21), as well as Saudi Arabia (22,23).

ESBL isolates were from the intensive care unit are likely to have higher use of invasive devices such as urinary and vascular catheters. Nosocomial infections caused by ESBL producing pathogens are associated with risk factors such as old age (>45 years), previous antibiotic use, prolonged hospitalization, inadequate antibiotic therapy, and presence of invasive devices (5,10). Our result revealed that *E. coli* (39%) and followed by *K. pneumoniae* (27%) were the predominant isolates inpatient and outpatient of Al-ansar Hospital. Similar findings have been observed in Chain and United Arab Emirate (5& 12). The present study indicates that *E. coli* is still the most common cause of urinary tract infection. This finding is consistent with the other studies from Italy[6].

In our study, we detected high rates of ESBL in *E. coli* (57%) and *K. pneumoniae* (40%) by screening test. This was in inagreement with the results obtained by Saurina et al., 2000, in a study also in USA (10). On other hand, other study in Saudi Arabia (22) reported that *E. coli* are becoming more common than ESBL-producing *Klebsiella* spp. In United Arab Emirate and in the Italy, the rates of ESBL positivity in *E. coli* and *K. pneumoniae* isolates were lower than those of our study[18, 13].

Resistance to antibiotics poses a serious and growing problem, because such resistant bacteria are becoming more difficult to treat. These findings also suggest other possibilities for our high resistance rates, such as inappropriate, uncontrolled empiric therapy or cross acquisition of resistance rather than the development of natural resistance. So the empirical and the indiscriminate use of antibiotics should be avoided and prompt infection control strategies in hospitals with special consideration in critical patient should be established in order to decrease the emergence and the spread of drug resistance among bacterial pathogens[24].

Treatment failures have been reported with the use of cephalosporins and beta-lactam/ beta-lactamase inhibitor combinations (amoxicillin with clavulanate and piperacillin with tazobactam) for infections caused by ESBL-producing isolates[25]. The level of resistance reported here for amoxicillin/clavulanate is much higher than that reported in other studies[24] and also much higher than the 25% resistance to piperacillin/tazobactam among ESBL isolates described in United Arab Emirates[18], suggesting that this beta-lactam/ beta-lactamase inhibitor combination may not be useful in our setting. Susceptibility to other antibiotic was follows: 97% for meropenem, 95% for amikacin, 95% for imipenem, 67% for gentamicin, and 52% for of ciprofloxacin.

CONCLUSION

It is very important for the clinical microbiology laboratories to have the ability to detect and report ESBLs production in clinical isolates of *Enterobacteriaceae*. Besides, monitoring and judicious usage of ESBL, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with ESBL. Amikacin and carbapenems remain the most effective drugs, but the presence of carbapenem-resistant ESBL-producing *Enterobacteriaceae* and occurrence of multidrug resistance are of concern. Infection control measures should be aggressively followed to prevent such infections among these high risk patients.

REFERENCES

1. Paterson DL, Bonomo RA . Extended spectrum ² lactamases: A clinical update. *Clin Microbiol Rev* 2005; **18**:657-686.
2. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum ² lactamases among enterobacteriaceae spp. isolated at a tertiary care institute. *Indian J Med Microbiol.* 2006; **24**: 208-211.
3. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *ClinMicrobiol Rev.* 2005;**18**: 657–686.
4. Abhilash KP, Veeraraghavan B, Abraham OC . Epidemiology and outcome of bacteremia caused by extended spectrum beta-lactamase (ESBL)-producing Escherichia coli and Klebsiella spp. in a tertiary care teaching hospital in south India. *J Assoc Physicians India.* 2010; **58**: 13-17.

5. Ghadiri H, Vaez H, Khosravi S, Soleymani E . The Antibiotic Resistance Profiles of Bacterial Strains Isolated from Patients with Hospital-Acquired Bloodstream and Urinary Tract Infections. *Crit. Care. Res. Pract.* 2012; 790-797.
6. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility: Sixteenth informational supplement. Wayne, PA: CLSI. 2006; 100-S16.
7. Al-Agamy MHM, ShiblAM, Tawfik AF. Prevalence and molecular characterization of extended-spectrum ²-lactamase-producing Klebsiella pneumoniae in Riyadh, Saudi Arabia. *Ann. Saudi. Med.* 2009; **29**(4):253–257.
8. Hansotia JB, Agarwal V, Pathak AA, Saoji AM. Extended spectrum ² lactamase mediate resistance to third generation cephalosporins in Klebsiella pneumoneae in Nagpur, central India. *Indian J Med Res.*1997; **105**:160-5.
9. Sinha P, Sharma R, Rishi S, Sharma T, Sood S, Pathak D. Prevalence of extended spectrum beta lactamase and AmpC beta lactamase producers among Escherichia Coli isolates in a large tertiary care hospital in Jaipur. *Indian J PatholMicrobiol.* 2008; **51**(3): 367-369.
10. Saurina G, Quale GM, Manikal VM, Oydna E, Landman D. Antimicrobial resistance in enterobacteriaceae in Brooklyn NY: Epidemiology and relation to antibiotic usage patterns. *J Antimicrob Chemother.* 2000; **45**: 895-898.
11. Cordero L, Rau R, Taylor D, Avers LW. Enteric Gram negative bacilli blood stream infections: 17 years experience in a neonatal intensive care unit. *Am J Infect Control.* 2004; **32**:189-195.
12. Xiong Z, Zzhu D, Zhang Y, Wang F. Extended spectrum beta-lactamase in Klebsiella pneumoniae and Escherichia coli isolates. Zhongua Yi XueZaZhi. 2002; **82**: 1476-9.
13. Luzzaro F, Mezzatesta M, Mugnaioli C, Perilli M, Stefani S, Amicosante G, *et al*. Trends in production of Extended Spectrum B-Lactamases among Enterobacteria of medical interest: Report of the second Italian nationwide survey. *J ClinMicrobiol.* 2006; **44**: 1659-64.
14. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum ² lactamase producing Gram negative bacteria in a tertiary care hospital. *Indian J Med Res.* 2002; **115**: 153-7.
15. Manchanda V, Singh NP, Goyal R, Kumar A, Thukral SS. Phenotypic characteristics of clinical isolates of Klebsiella pneumoniae and evaluation of available techniques for detection of extended spectrum beta lactamases. *Indian J Med Res.* 2005; **122**: 330-337.
16. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. *Indian J Med Res*. 2004; **120**: 553-556.
17. Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended spectrum ²-lactamase producing Gram negative bacteria in septicaemic neonates in a tertiary care hospital. *J Med Microbiol.* 2003; **52**: 421-425.
18. Al-Zarouni M, Senok A, Rashid F, Al-Jesmi SM, Panigrahi D. Prevalence and Antimicrobial Susceptibility Pattern of Extended-Spectrum Beta Lactamase Producing *Enterobacteriaceae* in the United Arab Emirates. *Med PrincPract.* 2008; **17**: 32–36.
19. Nimri LF, Azaizeh BA.First Report of Multidrug-Resistant ESBL-Producing Urinary *Escherichia coli* in Jordan. *BritishMicrobiology Research Journal.* 2012; **2**(2), 71-81.
20. Khater ES, Sherif HW. Rapid Detection of Extended Spectrum '-lactamase (ESBL) Producing Strain of *Escherichia coli* in Urinary Tract Infections Patients in Benha University Hospital, Egypt. *British Microbiology Research Journal*. 2014; **4**(4): 443-453.
21. Gharib MA, Ouda NH, Saeed M, Hamed L, Abdel Rahman SM. Prevalence of Extended Spectrum Beta Lactamase Producers in an Egyptian Critical Care Center. *Egyptian Journal of Medical Microbiology.* 2009; **18**(4):139-146
22. Panhotra BR, Saxena AK, Al-Ghamdi AM (2004). Extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* hospital acquired bacteremia. Risk factors and clinical outcome. *Saudi Med J*. 2004; **25**: 1871-1876.
23. Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *ClinMicrobiol Rev.* 2001; **14**: 933-951.
24. Al-Ahmadey ZZ and Mohamed SA . Antimicrobial susceptibility pattern of bacterial isolates in the intensive care unit of Al-ansar hospital, Saudia Arabia. *European Journal of Advanced Research in Biological and Life Sciences.* 2014; **2**: 1-7.
25. Rupp ME, Fey PD . Extended Spectrum ²-Lactamase (ESBL)-Producing Entero-bacteriaceae Considerations for Diagnosis, Prevention and Drug Treatment. *Drugs.* 2003; **63**(4): 353-3.