Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum β-Lactamases Producing *Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* in Khartoum Sudan

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Abstract

Background: Resistance to broad-spectrum beta-lactams, mediated by extended-spectrum β lactamase enzymes (ESBL), is an increasing problem worldwide.

Objective: The aim of this study was to determine the prevalence and antimicrobial susceptibility pattern of extended spectrum β-lactamases producing *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* isolated from clinical specimens.

Methods: This is across sectional study. A total of 162 *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* strains were isolated and identified from patients suffering from bloodstream infections, urinary tract infections, ear infections and wound infections using conventional microbiology techniques. Isolated strains were tested for antimicrobial resistance using disc diffusion technique and ESBL-production was detected using modified double disk potentiation test.

The results: Out of 162 clinical isolates, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* represented 44.4%, 38.9%, and 16.7% respectively. 39.5% were confirmed as ESBLs producers. 0.6% *Escherichia coli* and 0.6% *Klebsiella pneumoniae* were found to be AmpC β lactamase positive and 4.3% strains were found to be producing both ESBL and AmpC β lactamase (Co-Producer).

Conclusion: The prevalence of ESBL is increasing and necessary steps to prevent the spread and emergence of resistance should be taken.

Key words: *Escherichia coli*; *Klebsiella pneumonia*; *Proteus mirabilis*; Extended-Spectrum-β lactamases (ESBL); Multidrug Resistant MDR Sudan.

{**Citation:** Rasha Mohammed Hassan, Mohammed Nafi Hammad. Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum β-Lactamases Producing *Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* in Khartoum Sudan. American Journal of Research Communication, 2016, 4(8): 60-66} www.usa-journals.com, ISSN: 2325-4076.

Introduction

In spite of great advances in the antimicrobial therapy and the early detection of risk factors, infectious diseases continue to be a major cause of mortality and morbidity worldwide [(1)]. The most common organisms responsible for these infections are multidrug resistant Gram-negative bacilli, particularly members of the family Enterobacteriacae [(2)]. Extended-spectrum β-lactamases (ESBL) are enzymes produced by many Gram-negative bacteria which have the ability to change the susceptibility of different antimicrobial agents [(3)]. The ESBL are plasmid-mediated enzymes with the capability to hydrolyze and inactivate broad spectrum of β-Lactam antimicrobials, including third-generation cephalosporins, penicillins and aztreonam; but are inhibited by clavulanic acid[(3,4)].The two most common plasmid mediated β-Lactamases are the TEM-1 and SHV-1 family mainly expressed in *Escherichia coli* and *Klebsiella pneumoniae*, respectively; that confer resistance to antimicrobials [(5)]. ESBL-producing organisms are often also able to reduce the susceptibility of other non-β-lactamase antimicrobial classes, such as aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, tetracycline and nitrofurantoin; thus, leaving a limited range of therapeutic agents [(6)]. The growing frequency of ESBL-producing bacteria in clinical settings is causing treatment failure and greater hospital costs due to infections caused by this bacterium [(7)]. The presence of ESBL in many *Escherichia coli* strains are of serious concern, since these organisms are the most common cause of different human infections [(4)]. ESBL are becoming a great challenge and an increasing problem for hospitals worldwide [(4,8)]. The Clinical and Laboratory Standards Institute (CLSI) recommends the detection of ESBL in Gram-negative bacteria by recognizing their decreased susceptibility to the third generation cephalosporins such as ceftazidime, cefotaxime and ceftriaxone [(6,9)]. Once an ESBL is suspected, it should be confirmed by standardized methods [(9)]. The determination of inhibition by clavulanic acid is a common criteria used in all phenotypic methods for the detection of ESBL [(6,9)]. Several methods have been developed to detect the presence of ESBL including double-disk synergy test (DDST) and double-disk diffusion test (DDDT), using cefotaxime and ceftazidime disks with or without clavulanic acid. The prevalence of ESBL among pathogenic bacteria varies geographically and in hospital settings, and is rapidly changing overtime [(11)]. AmpC β-lactamases are clinically important cephalosporinas encoded on the chromosomes of many of the Enterobacteriaceae, where they mediate resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and β-lactamase inhibitor-β-lactam combinations[(12)]. In many bacteria, AmpC enzymes are inducible and can be expressed at high levels by mutation. Overexpression confers resistance to broad-spectrum cephalosporins including cefotaxime, ceftazidime, and ceftriaxone. Transmissible plasmids have acquired genes for AmpC enzymes, which consequently can now appear in bacteria lacking or poorly expressing a chromosomal bla-AmpC gene, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* [(12)]. Resistance due to plasmid-mediated AmpC enzymes is less common than extended-spectrum β-lactamase production in most parts of the world but maybe both harder to detect and broader in spectrum. AmpC enzymes encoded by both chromosomal and plasmid genes are also evolving to hydrolyze broad-spectrum cephalosporins more efficiently. Techniques to identify AmpC β-lactamase-producing isolates are available, but are still evolving and are not yet optimized for the clinical laboratory, which probably now underestimates this resistance mechanism [(12)]. Carbapenems can usually be used to treat infections due to AmpC-producing bacteria, but carbapenem resistance can arise in some organisms by mutations that reduce influx (outer membrane porin loss) or enhance efflux (efflux pump activation) [(12)].The aim of this study was to determine the prevalence and antimicrobial susceptibility pattern of extended spectrum β-lactamases producing *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* isolated from clinical specimens.

Methods

A total of 162 clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolated from patients suffering from bloodstream infections, urinary tract infections, ear infections and wound infections from different Khartoum hospitals during the period of November 2015 and January 2016 were included in this study. Strains were isolated by inoculation of collected specimens on CLED, Brain Heart Infusion Broth or Blood agar and MacConkey agar (Depending on the specimen) after overnight incubation at 37°C. The isolates identified based on colony morphology, Gram's stain, KIA test, citrate utilization test, urease production test, indole production test and motility test, according to standard microbiological procedures. The isolates sub-cultured onto nutrient agar and incubated at 37°C for approximately 18 to 24 hours prior to testing. The antimicrobial susceptibility pattern of the isolated strains was determined by Kirby Bauer disc diffusion method on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials according to CLSI [(13)]. ESBLs were screened according to zone diameters described in CLSI guidelines; ceftazidime ≤ 22 mm, cefotaxime ≤ 27 mm, ceftriaxone ≤ 25 mm, aztreonam ≤ 27 mm, cefpodoxime ≤ 22 mm and were confirmed by modified double disk synergy test (KEYHOLE). This test was done by using a disc of augmentin (20µg amoxicillin + 10µg clavulanic acid) and discs of cefpodoxime (30µg), ceftazidime (30µg),cefotaxime (30µg) and cefepime (30µg); which were placed around augmentin disc keeping the distance of 16 to 20 mm from it. (Center to center). The organisms were considered to be producing ESBL when the zone of inhibition around any of these cephalosporin discs showed a clear-cut increase towards the augmentin disc [(11)]. AmpC beta lactamase was detected using cefepime and cefoxitin discs. The organisms were considered to be producing ESBL when strains are resistant to the cefoxitin susceptible to cefepime [(11)].

The Results

During the study period a total of 162 clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolated from different clinical specimens: 25 (15.5%) blood, 83 (51.2%), urine, 4 (02.4%) ear swab, and 50 (30.8%) wound swab, from different Khartoum hospitals during the period of November 2015 and January 2016 were included in this study. *Escherichia coli* accounted for 72 (44.4%), *Klebsiella pneumoniae* 63 (38.9%) and *Proteusmirabilis*27 (16.7%) (Table 1). Out of 162 isolates 64 (39.5%) strains were found to be ESBL producers. *Escherichia coli* accounted 32/72 (44.4%), *Klebsiella pneumoniae*24/63 (38.1%) and *Proteus mirabilis* 8/27 (29.6%) (Table 2). Regarding AmpC β lactamase and out 162 isolates only2 (1.2%) strains (*Escherichia coli*) and (*Klebsiella pneumoniae)* were found to be AmpC β lactamase producers (Table 2).7(04.3%) strains were found to be produce both ESBL and AmpC β lactamase (Co-Producer) (Table 2).
Antibiotic susceptibility of ESBL and AmpC β lactamase *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolates was evaluated for 10 antimicrobial agents. However, these strains are 100% resistant to the ceftazidime, ceftriaxone, cefotaxime, cefpodoxime, and aztreonam,While 39% were resistant to ciprofloxacin and 28% to imipenem (Table 3).

Table (1) Bacterial Isolates

Isolates/ Specimens	Urine No. (%)	Wound No. (%)	Blood No. (%)	Ear No. (%)	Total No. (%)
Escherichia coli	**52** (32.1)	**15** (09.2)	**04** (02.5)	**01** (00.6)	**72** (44.4)
Klebsiella pneumoniae	**23** (14.2)	**19** (11.7)	**21** (13.0)	**00** (00.0)	**63** (38.9)
Proteus mirabilis	**08** (04.9)	**16** (09.9)	**00** (00.0)	**03** (01.8)	**27** (16.7)
Total	**83** (51.2)	**50** (30.8)	**25** (15.5)	**04** (02.4)	**162** (100)

Table (2) ESBL and AmpC β lactamase production among isolates

Isolates	No.	ESBL Producer No.(%)	AmpC Producer No. (%)	Co-Producer No. (%)
Escherichia coli	**72**	**32** (44.4)	**01**(0.6)	**03**(01.9)
Klebsiella pneumonia	**63**	**24** (38.1)	**01**(0.6)	**04**(02.4)
Proteus mirabilis	**27**	**08** (29.6)	**00** (00.0)	**00** (00.0)
Total	**162**	**64** (39.5)	**02**(1.2)	**07**(04.3)

Table (3) The antibiotic resistance pattern among ESBL producing and none ESBL producing Isolates

No.	Antibiotic	ESBLS +ve (No. 64)		ESBLS –ve (No. 98)	
		No.	%	No.	%
1	Ceftazidime	**64**	100	**70**	71
2	Ceftriaxone	**64**	100	**70**	71
3	Cefotaxime	**64**	100	**70**	71
4	Cefpodoxime	**64**	100	**70**	71
5	Cefepime	**57**	89%	**68**	69
6	Aztreonam	**64**	100	**70**	71
7	Cefoxitin	**07**	11	**70**	71
8	Augmentin	**07**	11	**70**	71
9	Ciprofloxacin	**25**	39	**70**	71
10	Imipenem	**18**	28	**06**	06

Discussion

During the past decade, ESBL producing Gram-negative bacilli especially *Escherichia coli* and *Klebsiella pneumonia* have emerged as serious pathogens both in hospital and

community acquired infections worldwide. β-Lactam antibiotics such as long spectrum cephalosporins and carbapenems are the preferred treatment of Enterobacterial infections[14]. It is important to know the prevalence of ESBL and/or AmpC producing organisms so that judicious use of antibiotics could be done [15]. The spread of ESBL-producing bacteria has been strikingly rapid worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required. Based on the results of this study, the overall prevalence of ESBL among *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* was 39.5%; (44.4%, 38.1%, and 29.6% respectively). Therefore, we concluded that the prevalence of ESBL producing organisms is high in Khartoum, Sudan. Similarly, the prevalence of ESBL in different Enterobacteriaceae was reported 46.5% in *Escherichia coli* and 44.4% in *Klebsiella pneumoniae* isolates in India [16], 60% *Escherichia coli*, and 40% *Klebsiella pneumonia* in Tehran, Iran [17], 41% *Escherichia coli*, and 36% *Klebsiella pneumonia* in Pakistan [18], 41.5% *Escherichia coli*, and 54.5% *Klebsiella pneumonia* in Egypt [19], and 65% *Escherichia coli*, 68.8% *Klebsiella pneumonia* and 33.3% *Proteus mirabilis* in Khartoum-Sudan[20].The AmpC ß lactamase enzyme was detected only in 2 (1.2%) strains (*Escherichia coli* and *Klebsiella pneumonia*). 7 (4.3%) strains were found to be producing both ESBL and AmpC β lactamase (co-producer). Similarly, the prevalence of AmpC β lactamase enzyme in different Enterobacteriaceae was reported 4.4%, co-producer of ESBL and AmpC β lactamase was reported 2.2% [19].The ESBL and AmpC β lactamase producing *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* isolates exhibited co-resistance against most of the antibiotics tested. This is consistent with most of the recent findings [11,17,19,20,21,22]. About 28% of ESBL producing isolates were resistant to imipenem. This is in harmony with the findings of Yusuf, I., et. al. 2013 [22], and Reza et. al 2015 [23].The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant bacteria. Due to their broad spectrum of activity and stability to hydrolysis by most beta lactamases, carbapenems have been the drug of choice for treatment of infections caused by penicillin or cephalosporin-resistant Gram-negative bacilli especially, ESBL producing Gram-negative infections [24]. The carbapenems (imipenem and meropenem) are still the first choice of treatment for serious infections with ESBL-producing Enterobacteriaceae. In this study 18 of our ESBL producing isolates were carbapenems resistant. The emergence of the carbapenem-resistant Enterobacteriaceae, the ‘‘magic bullet’’ is actually difficult to find. Colistin is a choice which we can consider for the treatment of these organisms [25].

Conclusion

The present study determines the prevalence of ESBL and AmpC β lactamase producing *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis* with limited susceptibility to antimicrobials in Khartoum-Sudan. In order to combat these problems proper antibiotic policies should be formulated. Further, it was observed that the majority of ESBL and AmpC β lactamase producing isolates were susceptible to imipenem. This brings due relief as these are the drugs of choice in the treatment of infections caused by these organisms.

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