

A Study of Prevalence of Extended-spectrum β Lactamase Producing Enterobacteriaceae from Urinary Isolates in Community Setting

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ABSTRACT

Background: The development of drug resistance due to Extended Spectrum β -Lactamase (ESBL) makes it essential to have current knowledge on antimicrobial susceptibility pattern of uropathogens for appropriate therapy. ESBLs at present are regarded as a major problem across the world and are gradually spreading both in hospital as well as in community settings. Their occurrence varies from different countries and healthcare institutions. **Aim of this study:** Hence, we made an endeavor to study the prevalence of extended-spectrum β lactamase producing Enterobacteriaceae from urinary isolates in community setting at the Microbiology Laboratory of Premier diagnostic centre division of Prime healthcare group, Dubai, United Arab Emirates. **Methods:** A total of 2134 consecutive, nonreplicate fresh samples of urine were subjected to study from December 2011 to March 2012. Out of which, 200 gram-negative isolates belonging to the family of Enterobacteriaceae adjudged to be clinically relevant to the patient's infection were studied for ESBL production and associated resistance to a panel of antibiotics. The method used to detect ESBL production was BD Phoenix Automated ESBL detection system (Becton, Dickinson, Md., USA). **Results:** The tests were performed and interpreted to obtain the results, according to the manufacturer's instructions. Out of 200 isolates belonged to Enterobacteriaceae, 22(11%) were ESBL producers. Prevalent ESBL producers identified by BD Phoenix were *E. coli* 20(10%) and *K. pneumoniae* 2(1%). Additional finding was, 5(2.5%) and isolates were potential carbapenemase producers. Most active drugs against ESBL-positive isolates were Meropenem (100%) Imipenem (100%) and Piperacillin/tazobactam combination (100%). Susceptibility to ciprofloxacin (25%), gentamicin (65%) trimethoprim/sulphamethoxazole (7.7%) was fairly low, whereas 100% of ESBL-producing enterobacteriaceae were susceptible to amikacin and 86% of the isolates were susceptible to nitrofurantoin. **Conclusion:** The outcome in this study documents the emerging threat of ESBL pathogens with the occurrence of these strains as etiological agents of infection in the community. Hence, we support urgent need for regular screening and surveillance of these organisms. We also recommend to restrict the redundant use of antibiotics, this could have lead to the progression of drug resistance in the community in large.

Key words: Enterobacteriaceae, extended spectrum β lactamase (ESBL), drug resistance, *Escherichia coli*, *Klebsiella pneumoniae*

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INTRODUCTION

Urinary Tract Infection (UTI) is the most common infectious disease, both in the community and in hospitalized patients. Nearly 10% of individuals will suffer from UTI in their lifespan. Bacterial etiology is the most common and *E. coli* accounting to 80% of the urinary tract infections. Treatment of UTI is frequently empirical in the community setting. However, due to emerging drug resistance among urinary pathogens it becomes necessary to monitor the antibiotic susceptibility pattern among urinary isolates¹.

Drug resistance due to Extended-spectrum β lactamases (ESBLs) is the growing problem in recent days throughout the world. ESBLs are enzymes which

are capable of hydrolyzing β -lactam ring of newer penicillins, cephalosporin's and aztreonam but do not have activity against cephamycins or carbapenems. Their activity is inhibited in vitro by β -lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam².

It is commonly due to mutations in the genes encoding plasmid mediated TEM-1 and SHV-1 enzymes³. Most common isolates producing ESBLs are *Escherichia coli* and *Klebsiella* spp,⁴ however, spreading to other genera of the Enterobacteriaceae family, including *Citrobacter*, *Serratia*, *Proteus*, *Salmonella*, and *Enterobacter*⁵. Until recently, ESBLs were confined to hospitals as nosocomially acquired strains^{5,6}, but of late, community acquired strains have been defined and could be an emergent problem in most of the countries^{7,8,9}. Hence, the objective of this study is to know the prevalence and antibiotic susceptibility pattern of

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Extended-spectrum β -Lactamases (ESBL) producing Enterobacteriaceae among patients with urinary tract infection in Dubai, UAE using BD Phoenix Automated identification and susceptibility system.

MATERIALS AND METHODS

Sources of samples: The study was carried out at the Microbiology Laboratory of Premier diagnostic centre division of prime healthcare group, Dubai, United Arab Emirates. The base population of the study consisted of outpatients from our area from whom a clinical sample had been sent to our laboratory for culture and in whom a community-acquired urinary tract infection was suspected. Only one positive culture per patient was included in the study and repeated positive cultures from the same patient were excluded from the analysis. Isolates for which it was impossible to discriminate between contamination and infection were excluded from the analysis.

Collection of samples: From December 2011 to March 2012, up to 2134 consecutive, nonreplicate fresh samples of urine were collected, aseptically in sterile containers and inoculated onto media using sterile techniques. Of these 200 urinary isolates identified as enterobacteriaceae were included in the present study for ESBL detection and associated resistance to a panel of antibiotics.

Isolation and characterization of the organisms: All the urine samples were cultured directly on MacConkey agar and Blood agar using calibrated loop and incubated overnight at 37°C. Identification of isolates was done based on cultural characteristics and reactions in standard biochemical tests using BD Phoenix commercial identification system. Quality control strains *E. coli* ATCC 25922 was included in the study.

Detection of Extended-Spectrum-B-Lactamase (ESBL) production: Detection of ESBL phenotype were carried out using BD Phoenix ESBL Automated System (Becton, Dickinson, Md., USA) A total of 200 Enterobacteriaceae isolates were subjected for study. The isolates were subcultured on Mac-Conkey agar to obtain a pure culture from which a 0.5 McFarland suspension was obtained and tested according to the manufacturer provided protocol. We assessed the susceptibility patterns of the ESBL producing isolates to a panel of antibiotics including amoxicillin/clavulanate, ciprofloxacin, gentamicin, imipenem, meropenem and piperacillin/tazobactam, trimethoprim/sulphamethoxazole.

Phoenix analysis: “NMIC/ID-5 Phoenix panels (combined susceptibility and identification cassette) were inoculated and incubated according to the

manufacturer’s recommendations. The Phoenix ESBL test uses growth response to selected expanded-spectrum (cefpodoxime) and broad-spectrum (ceftazidime, ceftriaxone, cefotaxime) cephalosporins, with or without clavulanic acid, to detect the production of ESBL. The result of this test is integrated into the antibiogram through the interpretation of the BDxpert system. This system consists of a series of rules, based on CLSI guidelines which are triggered by various conditions, such as the ESBL test or specific bacterial identification and antibiotic susceptibility pattern. Each rule is identified by a numeric code. When a rule is triggered, a cautionary message is appended to the AST report and where appropriate the interpretations for individual antibiotics are modified from the interpretive result based on the MIC. The BDxpert rule associated with a positive ESBL test for *E. coli*, *K. pneumoniae* and *K. oxytoca* is rule no. 1505: “Enterobacteriaceae with ESBLs are resistant to all β -lactam drugs, except carbapenems.” If the BDxpert system detects resistance mechanisms related to ESBLs (e.g., AmpC in *E. coli* or K1 in *K. oxytoca*), the supplementary ESBL rule no. 1502 is noted: “Enterobacteriaceae that are susceptible to a carbapenem and resistant to ureidopenicillins and 3rd generation cepheems or cefpodoxime or aztreonam are also resistant to all β lactams, except carbapenems. If the ESBL test is negative, then no rule is supplied. A printed report of each test indicates the actual MIC, the raw categorizations, the categorizations after interpretation and the rule applied”¹⁰. BD phoenix ESBL identification system has the ability to detect ESBL types such as SHV-2, SHV-5 or SHV-12^{2, 10, 11, 12}.

RESULTS AND DISCUSSION

Out of 2134 urine samples subjected to study, 200 isolates belong to enterobacteriaceae family. The gram-negative bacteria isolated were *Escherichia coli* (166), followed by *K. pneumoniae* (21), *Citrobacter* species (6), *Enterobacter* (1), *Proteus mirabilis* (1), *Providentia rettgeri* (4), *Morganella morganii* (1). (Table 1). *E. coli* (83%) was a major uropathogen, similar to that has been reported previously^{13, 14}.

Our data indicates that majority of the UTIs occurred in adult patients (93.5%), mainly women (91.5%), thereby confirming the previous report that adult women have a higher prevalence of UTI than men¹⁵.

While many international studies have addressed the emergence of ESBL producing Enterobacteriaceae in

Table 1: ESBL pattern of Enterobacteriaceae

Enterobacteriaceae	ESBL	(%)
<i>E. coli</i>	20	10
<i>Klebsiella pneumonia</i>	2	1
Total	22	11

ESBL: Extended-spectrum β lactamase

community acquired UTIs^{16,17}; locally very few reports on this subject. In the Gulf region much of the study is conducted on hospitalized patients and particular sites of infection (e.g., urine, blood)³. The percentage of ESBL producers in hospital setting ranges from 41% in United Arab Emirates, 52.2% in Bahrain and 31.7% in Kuwait^{18,19,20}. Hence, in this report, we present the direct relative data on the prevalence and antibiotic susceptibility pattern of ESBL-producing Enterobacteriaceae causing community-acquired urinary tract infections.

In our study 22(11%) of the isolates were ESBL producers, prevalent strains were *E. coli* 20(10%) and *K. pneumoniae* 2(1%) (Table 1 and 2). Our finding of 11% ESBL producers is analogous to the finding of previous study who reported 12% in community acquired UTIs¹⁴. On the contrary, our prevalence is on the higher end of the range compared to the Khanfar *et al.*³ (4.5%) and Luzzaro *et al.*²¹ (3.5%). Thus, based on different studies, it is apparent that the problem of ESBL producers is much more worrisome in hospitalized settings and not too far that the resistance is spreading in the community²². Thus, it becomes essential to advocate increased surveillance as well comprehensive multicenter/multinational studies to address this emerging problem of ESBL-associated infections even in the nonhospitalised patients.

In our study all the ESBL positive strains were resistant to ceftazidime and Aztreonam by the testing method. Drugs potentially active against ESBL-positive Enterobacteriaceae include β -lactam/ β -lactamase inhibitor combinations, cephamycins, carbapenems, aminoglycosides and fluoroquinolones. As shown in Table 3, Meropenem (100%) Imipenem (100%) and piperacillin/tazobactam combination (100%) were the most active drugs against ESBL producers, which were consistent with other studies¹⁹. Cefoxitin which is not hydrolyzed by ESBLs, was active against 95.5% of isolates. High level cefoxitin resistance is demonstrated among ESBL producing *Klebsiella* spp. (50%), likely due to the expression of the chromosomal AmpC β -lactamase⁵. Susceptibility to ciprofloxacin (25%), gentamicin (65%) trimethoprim/sulphamethoxazole (7.7%) was fairly low, whereas 100% of ESBL-producing enterobacteria were susceptible to amikacin. Nitrofurantoin was susceptible among (86%) of the ESBL producers. Concerning the community, *E. coli* and *Klebsiella pneumoniae* appeared to be the most common ESBL-producing strains. This was not unexpected, since isolates were obtained from urinary tract infections. Although a predominance of either *K. pneumoniae* or *E. coli* ESBL isolates has been identified in different geographical regions, *E. coli* has emerged as the major source of ESBLs producers in our study (91%) which is comparable to previous reports^{21,23,24}. Therapy for infections caused by ESBL-producing Enterobacteria is

Table 2: Enterobacteriaceae species

Species	No of isolates (%)	ESBL isolates (%)	Non-ESBL (%)
<i>Escherichia coli</i>	166(83)	20(10)	146(73)
<i>Klebsiella pneumoniae</i>	21(10.5)	2(1)	19(9.5)
<i>Citrobacter</i>	6(3)	-	6(3)
<i>Enterobacter</i>	1(0.5)	-	1(0.5)
<i>Proteus mirabilis</i>	1(0.5)	-	1(0.5)
<i>Providentia rettgeri</i>	4(2)	-	4(2)
<i>Morganella morganii</i>	1(0.5)	-	1(0.5)

Table 3: BL-positive strains: percent susceptibilities to potentially active drugs for different species % resistance

Species	No of isolates	% resistance						
		AMC	TZP	IPM	MEM	AMK	GEN	CIP
<i>Escherichia coli</i>	20	100	0.0	0.0	0.0	0.0	30	75
<i>Klebsiella pneumoniae</i>	2	100	0.0	0.0	0.0	0.0	50	50

Drugs are as follows: AMC: Amoxicillin-clavulanate, TZP: Piperacillin-tazobactam, IPM: Imipenem, MEM: Meropenem, AMK: Amikacin, GEN: Gentamicin, CIP: Ciprofloxacin

usually difficult, since these organisms not only are resistant to penicillin's, cephalosporins and monobactams but often is characterized by associated resistance to other classes of antimicrobials. Based on our findings a marked association between ESBL production and resistance to ciprofloxacin (75%) was observed, especially in *E. coli* which was analogous to that has been reported earlier^{14,25}. This resistance may be due to previous exposure to the drug fluoroquinolones which has been recently acknowledged as an independent risk factor for ciprofloxacin-resistant *E. coli* from community acquired UTI²⁶. Our data is consistent to earlier reports which showed a substantial reduction in susceptibility to antibiotics frequently used in the community such as Ampicillin, amoxicillin-clavulanic acid, trimethoprim/sulphamethoxazole and ciprofloxacin. Because of this, they are of little use as empirical therapeutic options in outpatient settings.

Our result shows nitrofurantoin is the only oral agent that remains relatively active against most ESBL producing uropathogens and can be used for empiric therapy in uncomplicated UTI, particularly in the community and this finding is in accordance with the previous study¹⁴. On contrary to our study, Bindayna *et al.*¹⁹ reported much lower percentage of susceptibility to nitrofurantoin in Bahrain, signifying the presence of resistant stains in some areas which can even might extend to other regions. Overall, data supports the choice of carbapenems as empirical therapy in the case of life-threatening infections UTIs. Use of fluoroquinolones may be justified only in selected cases of ESBL-related infections.

Excluding carbapenems, amikacin (100%) and piperacillin-tazobactam (100%), were the most effective drugs *in vitro*. Based on these data, piperacillin-tazobactam alone or together with amikacin would be a useful option

for urinary tract infections. This finding is consistent with the findings of previous studies²². Hence, we also concur to previously recommended, in the case of non-life-threatening infections and in no outbreak situations, it is not necessary to administer carbapenems. This approach is intended to preserve the therapeutic value of these precious drugs²⁷.

The increasing frequency of ESBL-producing Enterobacteriaceae among patients is an important problem for both microbiologists and clinicians, because of the difficulty in correctly detecting, reporting and treating infections caused by these organisms. The true community-acquired infections caused by ESBL-producing Enterobacteria have also been described formerly²⁸. Time again and again good number of studies is to be carried out in this region to know the occurrence of ESBL in clinical isolates. Additional finding was, 5(2.5%) isolates were potential carbapenemase producers, suggests possible KPC or Carbapenem-Resistance in Enterobacteriaceae²⁹. Notably, potential carbapenemase producers reported in this study indicating an emerging problem of possible KPC or Carbapenem-Resistance in Enterobacteriaceae which needs increased attention by clinical microbiologists.

CONCLUSION

In conclusion, the outcome in this study documents the emerging threat of ESBL pathogens in our surroundings with the occurrence of these strains as etiological agents of infection in the community. While the findings shed light on *E. coli* and *Klebsiella species* which are the predominant ESBL producers, we recommend further work on evaluating the ESBL types in these isolates as well as the prevalence of other ESBL-producing Gram negative bacteria which are emerging as pathogens of concern in the clinical setting. We do agree that there might be a relationship between antibiotic usage and resistance that play a major role in the development of resistance among uropathogens as there is a high level of antibiotic prescription and misuse in community setting practice. Hence we are in opinion that antibiotic policies should be in place to determine the choice of antimicrobial agents and infection control measures should be implemented and to be meticulously, as choice of the drugs in case of ESBL producing strains are limited. Finally, Education and Awareness of clinicians to clinical significance of these enzymes play a pivotal role in fight against drug resistance.

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