

Extended-Spectrum β -Lactamase-Producing Enterobacteriaceae: In vitro Susceptibility to Fosfomycin, Nitrofurantoin and Tigecycline

M. Al-Zarouni^a A. Senok^c N. Al-Zarooni^a F. Al-Nassay^a D. Panigrahi^b

^aAlQassimi Hospital Laboratory Sharjah, Ministry of Health, and ^bDepartment of Clinical Sciences, College of Medicine, University of Sharjah, Sharjah, United Arab Emirates; ^cCollege of Medicine, Alfaisal University, Riyadh, Saudi Arabia

Key Words

Extended-spectrum β -lactamase · Fosfomycin · Tigecycline · Hyplex · Uropathogens

Abstract

Objective: To assess the susceptibility trends of community-acquired extended-spectrum β -lactamase (ESBL)-producing urinary isolates with particular reference to fosfomycin, nitrofurantoin and tigecycline. **Materials and Methods:** Seven hospitals across the United Arab Emirates participated in this study from June 2008 to March 2010. The antibiotic sensitivity of ESBL-producing uropathogens to a panel of antibiotics including tigecycline, fosfomycin and nitrofurantoin was assessed. The Hyplex ESBL identification system (h-ES-ID) was used for genotypic identification. **Results:** Two hundred and ninety-two ESBL-producing Enterobacteriaceae isolates were identified during the study period. Of these, 182 (62%) were urinary isolates and comprised of *Escherichia coli*: 149 (81.9%), *Klebsiella pneumoniae*: 30 (16.5%) and *Proteus mirabilis*: 3 (1.6%). Of the 182 urinary isolates, 179 (98.3%) were from patients with community onset urinary tract infections. The h-ES-ID system identified 172 (94.5%) of the urinary isolates as CTX-M positive. All isolates were susceptible to imipenem and meropenem. Over half of the isolates showed resistance to gentamicin (98; 53.8%), trimethoprim-

sulfamethoxazole (139; 76.4%) and ciprofloxacin (143; 78.6%). Sensitivity to nitrofurantoin and fosfomycin was 90 and 100%, respectively. Two CTX-M-positive *K. pneumoniae* isolates with tigecycline resistance (MIC >4 μ g/ml) were identified. **Conclusion:** There is dissemination of CTX-M ESBL-producing urinary pathogens into the community. Fosfomycin and nitrofurantoin were active against ESBL-positive uropathogens, and emergence of tigecycline resistance needs close monitoring.

Copyright © 2012 S. Karger AG, Basel

Introduction

Pathogens producing extended-spectrum β -lactamases (ESBL) cause infections which are often associated with high mortality, morbidity and health care costs. The ESBL enzymes are commonly found in the Enterobacteriaceae family and over 120 ESBL types, including the CTX-M and OXA enzymes, have been described and they hydrolyze penicillins, broad-spectrum cephalosporins

This paper was presented in part as a poster at the 20th European Conference on Clinical Microbiology and Infectious Diseases (ECCMID), 10–13 April, 2010, Vienna, Austria.

Table 1. Distribution of the total 292 isolates according to hospital

	Total number of isolates	Number of urinary isolates
Al Qassimi Hospital, Sharjah	79	29
Kuwaiti Hospital, Sharjah	29	21
Um Al Quwain Hospital	7	3
Sheikh Khalifa Hospital, Ajman	11	10
Al Wasel Hospital, Dubai	8	4
Dubai Hospital	127	99
Al Rashid Hospital, Dubai	31	16
Total	292	182

and monobactams [1]. However, they do not affect cephalosporins and carbapenems, and they are inhibited by clavulanic acid [1].

Currently, the emergence and rapid dissemination of CTX-M-positive ESBL-producing bacteria have caused a change in ESBL epidemiology [1–3]. As these ESBL-producing strains often exhibit a multidrug-resistance phenotype, the increase in community-acquired urinary tract infections (UTIs) caused by CTX-M ESBL producers poses significant challenges for the empiric treatment of these infections in the outpatient setting. Furthermore, the recent identification of ESBL-producing isolates that have acquired carbapenemases has further limited the therapeutic options available for treatment of these multidrug-resistant microorganisms [4]. This scenario has resulted in the need for reevaluating the efficacy of old antimicrobials such as nitrofurantoin and fosfomycin for their activity against these community-acquired ESBL-producing uropathogens. In addition, there is growing interest in the evaluation of new-generation antibiotics such as tigecycline for treating nosocomial infections caused by ESBL-producing isolates. In this report, the antibiotic susceptibility trends of community-acquired ESBL-producing urinary isolates in the United Arab Emirates are presented – particularly, the *in vitro* activity of tigecycline, fosfomycin and nitrofurantoin against these organisms.

Materials and Methods

Two hundred and ninety-two ESBL-producing Enterobacteriaceae comprised of *Escherichia coli* (n = 225), *Klebsiella pneumoniae* (n = 60), *Proteus mirabilis* (n = 4), *Enterobacter cloacae* (n = 2) and *Acinetobacter baumannii* (n = 1) were obtained from participating hospitals as shown in table 1. Of the 292 isolates, 182

were urinary isolates and these were included for further study of antibiotic susceptibility testing and genotyping using the Hyplex ESBL identification system (h-ES-ID). Seven hospitals across the United Arab Emirates (UAE) participated in this study, which was carried out from June 2008 to March 2010. Nonduplicated ESBL-positive Enterobacteriaceae obtained from the participating hospitals were stored in 20% glycerol at –20°C and sent to the Microbiology Laboratory, Al-Qassimi Hospital, Sharjah, UAE, and reconfirmation of the ESBL phenotype was carried out.

Identification of ESBL Phenotype

ESBL screening was carried out using the VITEK II (bioMérieux, Marcy l’Etoile, France) and MicroScan Walk-Away (Siemens Healthcare Diagnostics, Sacramento, Calif., USA) automated systems. Manual screening of isolates was also done by disk diffusion using ceftazidime (30 µg) and cefpodoxime (10 µg) according to the Clinical and Laboratory Standards Institute’s (CLSI) recommended guidelines [5]. Confirmation of ESBL phenotype was carried out by using the ESBL E-test according to the manufacturer’s guidelines. *E. coli* ATCC 51446 and *K. pneumoniae* ATCC 700603 were used as positive controls and *E. coli* ATCC 25922 as negative control.

Determination of ESBL Genotype Using the h-ES-ID Multiplex PCR-ELISA System

The multiplex PCR and reverse-hybridization steps were carried out using the reagents and instructions provided by the manufacturer. Control reactions for evaluating the PCR and hybridization steps were performed in each run. For the identification of isolates with the *bla*_{CTX-M} gene, a CTX-M-specific probe was used. The multiplex PCR amplification was performed using 2 µl of the primer mix, 1 µl of nucleotide mix of the h-ES-ID PCR module and 5 µl of the sample supernatant, 5 µl of 10× PCR buffer, 1 µl UltraStart DNA polymerase and 50 µl of double-distilled water. The cycling conditions were as follows: initial denaturation step at 94°C for 5 min, 35 cycles of denaturation at 94°C for 25 s, annealing at 52°C for 25 s, elongation at 72°C for 20 s with increasing 1 s per cycle and a final extension of 3 min at 72°C. The amplification products were stored at –20°C. For reverse hybridization, amplification products were heated at 95°C for 10 min and then mixed with the hybridization buffer. Aliquots of this mixture were added to color-coded micro-well plate cavities adsorbed with the consensus probe for TEM, SHV, CTX-M and OXA (to confirm ESBL positivity) as well as a CTX-M-specific probe for identification of CTX-M genotype. Plates were incubated at 50°C for 30 min for hybridization and then incubated with 100 µl peroxidase conjugate per well for 30 min at room temperature and washed. Incubation with 100 µl of substrate solution was carried out and the reaction was terminated by addition of stop solution. Extinction measurement was done within 60 min of termination using a photometer at 450 nm and a reference length of 620–650 nm. Zero comparison was made against air and samples with extinction values >400 were considered positive, 200–400 borderline and <200 negative.

Antibiotic Susceptibility Testing

Susceptibility of the isolates to various antimicrobial agents was determined by the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar plates as previously described [6]. The following antibiotics were tested for all isolates: meropenem (10 µg),

imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), piperacillin/tazobactam (110/10 µg), ciprofloxacin (5 µg) and tigecycline (15 µg). The sensitivity of urinary isolates to nitrofurantoin (300 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg) was determined. Susceptibility to fosfomycin was assessed for 114 urinary isolates (92 *E. coli* and 22 *K. pneumoniae*). The results were recorded and interpreted according to CLSI criteria. Determination of MIC with the E-test was carried out for meropenem and tigecycline according to the manufacturer's guidelines as previously described [6].

Statistical analysis using the χ^2 test was performed using SigmaStatver 3.5 software (Systat Software Inc., San Jose, Calif, USA). $p < 0.05$ was considered statistically significant.

Results

The 182 urinary isolates studied comprised 149 (81.9%) *E. coli*, 30 (16.5%) *K. pneumoniae* and 3 (1.6%) *P. mirabilis*. Of these 182 urinary isolates, 179 (98.3%) were from patients with community onset UTIs, and the ESBL-positive isolates were correctly identified using automated methods, i.e. screening by disk diffusion, ESBL E-test and the h-ES-ID. Using the *bla*_{CTX-M} specific probe of the h-ES-ID system, 172 (94.5%) were found to be CTX-M positive while the remaining 10 (5.5%) were CTX-M-negative; these include *E. coli* (n =7); *K. pneumoniae* (n = 2) and *P. mirabilis* (n = 1). Of the 182 urinary isolates studied, 163 (90%) were susceptible to nitrofurantoin while 114 (62.6%) community onset urinary isolates were susceptible to fosfomycin; these include *E. coli* (n = 93), and *K. pneumoniae* (n = 21). As there are no accepted breakpoints for *K. pneumoniae*, those proposed by CLSI for *E. coli* were used. All 114 isolates were susceptible to fosfomycin with a mean inhibition zone diameter of 36.8 ± 7.0 mm (range 19–57). Sensitivity to nitrofurantoin and fosfomycin was significantly higher compared with gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole ($p < 0.001$).

Two (1.1%) CTX-M-positive *K. pneumoniae* isolates showed resistance to tigecycline with an MIC >4 µg/ml. These isolates were from adult female patients with community onset UTIs.

All isolates were sensitive to imipenem and meropenem. Of the 182 isolates, 175 (96%) were sensitive to amikacin and 172 (95%) were sensitive to piperacillin-tazobactam (fig. 1). In contrast, over half of the isolates showed resistance to gentamicin 98 (53.8%), trimethoprim-sulfamethoxazole 139 (76.4%) and ciprofloxacin 143 (78.6%) (fig. 1). Ciprofloxacin resistance was 80% among CTX-M-positive isolates compared to 37.5% in CTX-M-negative isolates.

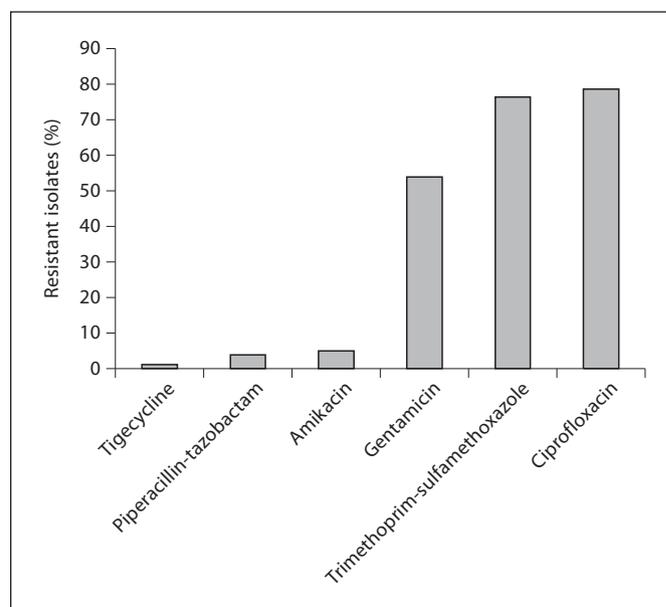


Fig. 1. Antibiotic resistance pattern of ESBL-positive urinary isolates.

Discussion

In this study, the majority of ESBL-producing Enterobacteriaceae identified were urinary isolates associated with community onset UTIs. These urinary isolates were highly resistant to ciprofloxacin and trimethoprim-sulfamethoxazole, the commonly used antibiotics in the outpatient setting for treatment of UTIs. Although they remained predominantly sensitive to carbapenems (imipenem and meropenem), amikacin and piperacillin-tazobactam, these are parenterally administered and reserved for inpatient use. Our findings demonstrated fosfomycin susceptibility in all 114 isolates tested. Fosfomycin is a cell wall inhibitor whose broad spectrum of activity, therapeutic range and oral administration make it pertinent for use in the treatment of UTIs in the outpatient setting [7]. Although *E. coli* rapidly develops resistance to fosfomycin, experimental in vitro studies have shown that resistant isolates have lowered fitness and poor survival [7]. Hence, fosfomycin remains active against many common uropathogens, including ESBL producers. A recent report has also described good activity of fosfomycin against carbapenem-resistant Enterobacteriaceae, including NDM-1-positive isolates [8]. In a survey of 428 ESBL-producing isolates in Spain, fosfomycin resistance was 0.3 and 7.2% in *E. coli* and *K. pneumoniae*, respectively [9]. In Brazil, where there is high us-

age of fosfomycin, up to 98.8% of *E. coli* isolates from community-acquired UTIs were fosfomycin sensitive [10]. Our findings of 100% susceptibility among the ESBL-producing isolates tested provide further supportive evidence for recommending fosfomycin for the management of community acquired ESBL-producing urinary isolates.

Nitrofurantoin is a synthetic nitrofurantoin antimicrobial agent which has been used for over 50 years in the outpatient setting for the treatment of uncomplicated UTIs. In this study, 90% of the isolates were susceptible to nitrofurantoin. Varying levels of nitrofurantoin sensitivity in urinary ESBL isolates have been reported in the literature. Whilst high sensitivity levels have been reported from surveys in the USA (>88%), India (89%) and Hong Kong (98%), reports of lower sensitivity levels have emerged from Italy, Latin America and France [7, 11, 12]. Compared with these surveys, the 90% susceptibility to nitrofurantoin reported in this study is relatively high and similar to that reported data from Kuwait [13]. In addition, it is also significantly higher compared to data from neighboring countries in the region, i.e. 73 and 61.8% of urinary isolates from Saudi Arabia and Bahrain, respectively, were reported to be sensitive to nitrofurantoin [14, 15]. These findings indicate that both nitrofurantoin and fosfomycin represent alternative therapeutic agents for the treatment of community onset UTIs caused by ESBL-producing uropathogens in our setting.

Tigecycline is a glycylcycline derivative of minocycline with broad-spectrum activity against Gram-positive and Gram-negative bacteria. Tigecycline is not affected by the presence of coresistance mechanisms in unrelated antimicrobials, thus representing an attractive alternative in ESBL isolates which often exhibit other re-

sistance phenotypes. Two *K. pneumoniae* isolates with elevated tigecycline MICs were identified, suggestive of the emergence of isolates resistant to this antibiotic. Similar resistance to tigecycline has recently been reported by Livermore et al. [8] among carbapenemase-producing *Klebsiella* and *Enterobacter* isolates in the United Kingdom. Although these findings indicate that tigecycline remains useful for isolates circulating in our setting, the implementation of good antibiotic stewardship and active surveillance is recommended particularly in the community and outpatient setting.

Compared with other reports from the Arabian Gulf region, the findings in this study demonstrate a significantly higher frequency of *bla*_{CTX-M} ESBL-positive isolates. In recent reports from the UAE and Kuwait, the proportions of Enterobacteriaceae isolates with the CTX-M genotype were 87 and 77.9%, respectively [16, 17]. Lower figures have emerged from Saudi Arabia, with two recent reports showing *bla*_{CTX-M} detection in 34 and 71% of Enterobacteriaceae isolates [14, 18]. In an earlier report, ESBL producers were identified mainly as agents of nosocomial infection in the UAE [6]. These new findings are of particular concern as they indicate that there is a trend towards increasing dissemination of CTX-M ESBL isolates into the community.

Conclusion

Our findings have demonstrated a high level of CTX-M ESBLs with dissemination into the community. Further, both nitrofurantoin and fosfomycin were effective therapeutic agents in the treatment of community onset UTIs caused by ESBL-positive pathogens.

References

- 1 Paterson DL, Bonomo RA: Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* 2005;18:657–686.
- 2 Livermore DM, Hawkey PM: CTX-M: changing the face of ESBLs in the UK. *J Antimicrob Chemother* 2005;56:451–454.
- 3 Pitout JD, Laupland KB: Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 2008;8:159–166.
- 4 Nordmann P, Poirel L, Toleman MA, Walsh TR: Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? *J Antimicrob Chemother* 2011;66:689–692.
- 5 National Committee for Clinical Laboratory Standards: Performance Standards for Antimicrobial Susceptibility Testing. Wayne, National Committee for Clinical Laboratory Standards (CLSI), 2005, 15th Informational Supplement (m100-s15).
- 6 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 Princ Pract* 2008;17:32–36.
- 7 Garau J: Other antimicrobials of interest in the era of extended-spectrum beta-lactamases: fosfomycin, nitrofurantoin and tigecycline. *Clin Microbiol Infect Dis* 2008; 14(suppl 1):198–202.

- 8 Livermore DM, Warner M, Mushtaq S, Doumith M, Zhang J, Woodford N: What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. *Int J Antimicrob Agents* 2011;37:415–419.
- 9 de Cueto M, Hernandez JR, Lopez-Cerero L, Morillo C, Pascual A: Activity of fosfomycin against extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*. *Enferm Infecc Microbiol Clin* 2006;24:613–616.
- 10 Biondo CM, Rocha JL, Tuon FF: Fosfomycin in vitro resistance of *Escherichia coli* from the community. *Braz J Infect Dis* 2011;15:96.
- 11 Zhanel GG, Hisanaga TL, Laing NM, DeCorby MR, Nichol KA, Weshnoweski B, Johnson J, Noreddin A, Low DE, Karlowsky JA, Hoban DJ: Antibiotic resistance in *Escherichia coli* outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). *Int J Antimicrob Agents* 2006;27:468–475.
- 12 Fadda G, Nicoletti G, Schito GC, Tempera G: Antimicrobial susceptibility patterns of contemporary pathogens from uncomplicated urinary tract infections isolated in a multi-center Italian survey: possible impact on guidelines. *J Chemother* 2005;17:251–257.
- 13 Al Benwan K, Al Sweih N, Rotimi VO: Etiology and antibiotic susceptibility patterns of community- and hospital-acquired urinary tract infections in a general hospital in Kuwait. *Med Princ Pract* 2010;19:440–446.
- 14 Bindayna K, Senok A, Jamsheer A: Extended Spectrum Beta-lactamase-producing Enterobacteriaceae in Bahrain: Prevalence and antibiotic sensitivity pattern. *J Infect Public Health* 2009;2:129–135.
- 15 Khanfar HS, Bindayna KM, Senok AC, Botta GA: Extended spectrum beta-lactamases (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*: trends in the hospital and community settings. *J Infect Dev Ctries* 2009;3:295–299.
- 16 Alfaresi MS, Elkoush AA, Alshehhi HM, Abdulsalam AI: Molecular characterization and epidemiology of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in the United Arab Emirates. *Med Princ Pract* 2011;20:177–180.
- 17 Al Hashem G, Al Sweih N, Jamal W, Rotimi VO: Sequence analysis of *bla*_{CTX-M} genes carried by clinically significant *Escherichia coli* isolates in Kuwait hospitals. *Med Princ Pract* 2011;20:213–219.
- 18 Al-Agamy MH, Shibl AM, Tawfik AF: Prevalence and molecular characterization of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in Riyadh, Saudi Arabia. *Ann Saudi Med* 2009;29:253–257.