In Vitro Resistance of Extended-Spectrum (ESBL) versus AmpC-Phenotype (AmpC) β-Lactamase-Producing *Escherichia coli* Urinary Isolates against Six First-line Oral Antibiotics Commonly Used for Community-Acquired Urinary Tract Infections

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ABSTRACT

Background: Serious infections due to organisms harboring broad-spectrum ß-lactamases, such as ESBL and/or AmpC ß-lactamases, have been reported worldwide with significant morbidity and mortality. We conducted a retrospective study to assess resistance profiles of ESBL-producing versus (vs) AmpC phenotype-expressing urinary strains of *E. coli* among nonhospitalized patients.

Methods: Over a 28 month period, all isolates of *E. coli*, *Klebsiella pneumoniae*, and *K. oxytoca* isolated from nonhospitalized patients were screened for ESBL production, followed by confirmatory testing if warranted, in accordance with CLSI (NCCLS) guidelines.The *in vitro* antimicrobial resistance profiles of ESBL-producing isolates and those expressing the AmpC phenotype were analyzed for six commonly used first-line oral antimicrobials, namely ampicillin (AM), cephalothin (CF), ciprofloxacin (CIP), nitrofurantoin (F/M), norfloxacin (NOR), and trimethoprim-sulfamethoxazole (TMP/SMX).

Results: A total of 107 urinary isolates were confirmed as AmpC phenotype or ESBL producers, all *E. coli.* All of the isolates were resistant to both AM and CF. Of the 59 AmpC phenotype vs the 48 ESBL producing strains, 11/59 (19%) vs 39/48 (81%) were resistant to both CIP and NOR; 16/59 (27%) vs 28/48 (58%) were resistant to TMP/SMX; and 1/59 (2%) vs 2/48 (4%) were resistant to F/M, respectively. Resistance of ESBL producers was significantly higher *in vitro* than that of AmpC phenotype expressing strains, tested against CIP ($p < 0.001$), NOR ($p < 0.001$), and TMP/SMX ($p = 0.002$). F/M was significantly more active against both AmpC phenotype and ESBL producing strains than the other antimicrobials tested ($p < 0.001$).

Conclusions: This study demonstrated that *E. coli* urinary isolates harboring ESBLs are significantly more likely than AmpC phenotype expressing isolates to be resistant to ciprofloxacin, norfloxacin, and trimethoprim-sulfamethoxazole. *In vitro* susceptibility to nitrofurantoin was retained in both AmpC phenotype and ESBL producing strains of *E. coli.*

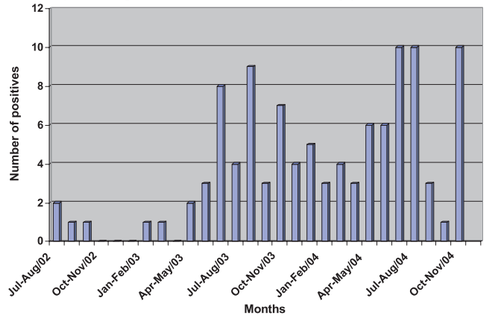
INTRODUCTION

Broad-spectrum ß-lactamases, such as extended-spectrum (ESBL) and AmpC type ß-lactamases are potent bacterial enzymes of increasing clinical significance. Serious infections due to organisms harboring these enzymes have been reported worldwide with significant morbidity and mortality. Their rapid increase is widely regarded as one of the major problems in medicine today.

Although ESBLs typically do not hydrolyze 7-α-methoxy-cephalosporins and are inactivated by ß-lactamase inhibitors (with the exception of certain newly discovered inhibitor-resistant enzymes of TEM derivatives), recent reports suggest that treatment of infections due to ESBL producing organisms with cephamycins or ß-lactam/ß-lactamase inhibitor combinations may result in clinical failure. As a result, both ESBL and AmpC producing organisms should be considered as potentially resistant to all ß-lactams and ß-lactam/ß-lactamase inhibitor combinations, except for carbapenems. Many of these strains are also resistant to other antimicrobial classes, such as fluoroquinolones and aminoglycosides.

A significant number of these strains had often been isolated from urine cultures of hospitalized patients. We conducted a retrospective study of urine cultures from nonhospitalized patients to assess resistance profiles of ESBL-producing versus AmpC phenotype-expressing urinary strains of *E. coli* against six first-line oral antibiotics commonly used in the treatment of community-acquired urinary tract infections (UTIs).

Figure 1. Number of urinary isolates of confirmed AmpC-phenotype/ ESBL-producing *E. coli* over the 28 month study period



METHODS

Bacterial Strains:
Over a 28 month period, from July 20, 2002 to November 19, 2004, urine specimens submitted from nonhospitalized patients for routine culture were processed by conventional procedures using commercially prepared media (Bio-Media Unlimited Ltd., Toronto, ON, Canada). All urinary isolates of *E. coli, Klebsiella pneumoniae*, and *K. oxytoca* were evaluated.

Antimicrobial Susceptibility Testing:
Suspensions of test organisms for susceptibility testing were prepared to a concentration equivalent to 0.5 McFarland standard and were inoculated onto Mueller-Hinton agar plates (PML Microbiologicals, Mississauga, ON, Canada), using antimicrobial disks (Becton Dickinson and Company, Sparks, MD, USA). Disk zone diameters were interpreted according to CLSI (NCCLS) interpretive criteria.

ESBL Screening:
All isolates were screened in accordance with CLSI (NCCLS) guidelines by cefpodoxime disk (10 µg) (Primary Screen), followed if screen-positive, by further testing (Secondary Screen) with aztreonam (ATM, 30 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), and ceftriaxone (CRO, 30 µg) disks. Secondary Screen-positive isolates were subsequently referred to the province's Public Health Laboratory for confirmatory testing.

Confirmatory Testing:
An isolate was confirmed as an ESBL producer by the CLSI (NCCLS) Phenotypic Confirmatory Test (Disk Diffusion). An increase in zone diameter of ≥ 5 mm for either CTX or CAZ tested in combination with clavulanic acid versus its zone diameter when tested alone was considered confirmatory of ESBL production. If the isolate expressed no synergy with clavulanic acid, was resistant or intermediate to cefoxitin, and the CTX or CAZ zone diameters were ≤ 27 mm or ≤ 22 mm, respectively, this was considered indicative of the AmpC phenotype.

Table 1: Susceptibilities of study strains of AmpC phenotype and ESBL-producing *E. coli* ($n = 107$) against six first-line oral antimicrobial agents

Antimicrobial Agent	Susceptible AmpC *n* (%)[1]	Susceptible ESBL *n* (%)	Intermediate AmpC *n* (%)	Intermediate ESBL *n*(%)	Resistant AmpC *n*(%)	Resistant ESBL *n*(%)	Total *n*[2]
Ampicillin	0(0)	0(0)	0(0)	0(0)	59(100)	48(100)	107
Cephalothin	0(0)	0(0)	0(0)	0(0)	59(100)	48(100)	107
Ciprofloxacin	48(81)	9(19)	0(0)	0(0)	11(19)	39(81)	107
Nitrofurantoin	55(93)	40(83)	3(5)	6(12)	1(2)	2(4)	107
Norfloxacin	48(81)	9(19)	0(0)	0(0)	11(19)	39(81)	107
Trimethoprim/ sulfamethoxazole	41(69)	20(41)	2(3)	0(0)	16(27)	28(58)	107

[1] Percentages may not add up to 100, due to rounding of fractions.
[2] The total number of isolates comprises 59 AmpC phenotype-expressing and 48 ESBL-producing isolates.

Profile Analysis:
Antimicrobial susceptibility profiles were analyzed for six oral antimicrobials, ampicilin, cephalothin, ciprofloxacin, nitrofurantoin, norfloxacin, and trimethoprim-sulfamethoxazole.

RESULTS & DISCUSSION

A total of 14,270 *E. coli* and 1,455 *Klebsiella* urinary isolates were screened for ESBL production, in accordance with CLSI (NCCLS) guidelines. Of these 15,725 isolates, 131 were potential broad-spectrum ß-lactamase producers. Confirmatory testing detected 107 strains that were either AmpC phenotype (59 isolates) or ESBL (48 isolates)-producing isolates, all *E. coli.*

The mean age of patients with AmpC phenotype or ESBL-positive organisms was 49.9 years, with a range of <1 to 94 years. More isolates were recovered from females (0 - 21 years, $n = 11$; 22 - 65 years, $n = 61$; > 65 years, $n = 21$) than from males (0 - 21 years, $n = 1$; 22 - 65 years, $n = 10$; > 65 years, $n = 3$). This high female to male ratio is consistent with urinary isolation rates encountered in routine practice.

As can be seen in Figure 1, the number of AmpC-phenotype/ESBL-producing *E. coli* isolates increased significantly during the study period. At seven month intervals, the number of isolates were 5, 27, 29 and 46. The increase in the isolation rates of broad-spectrum ß-lactamase producing organisms is consistent with studies showing increasing prevalence of infections caused by these types of resistance.

Table 1 summarizes the antimicrobial test profiles of the 107 isolates. Since the source of isolation was urine, where drugs are physiologically concentrated, the intermediate category in the susceptibility profile may imply clinical applicability.

At the low resistance rates of 2% and 4%, nitrofurantoin was significantly more active *in vitro* than the other antimicrobials tested in this study against AmpC phenotype and ESBL producing isolates, respectively ($p < 0.001$). The generally low acquired resistance of nitrofurantoin has been attributed to the broad-based nature of its mode of action by inactivating or altering ribosomal proteins and other target macromolecules, thus inhibiting essential biochemical processes of nucleic acid synthesis and protein synthesis.

ESBL- and AmpC-producing *E. coli* strains are often multidrug resistant. The *in vitro* resistance of ESBL-producing *E. coli* isolates in this study was however significantly higher than that of the AmpC phenotype-expressing isolates against ciprofloxacin ($p < 0.001$), norfloxacin ($p < 0.001$), and trimethoprim-sulfamethoxazole ($p = 0.002$). To our knowledge, this is the first report describing a statistically significant diffrence between two types of broad-spectrum ß-lactamase resistance against these three oral antimicrobials. Further studies are needed to assess these findings using nosocomial isolates against these and other antimicrobial classes.

CONCLUSIONS

- There has been an increase in the isolation of community-acquired UTI-associated broad-spectrum ß-lactamase producing *E. coli* over the past two years.
- *E. coli* isolates harboring ESBLs are significantly more likely than AmpC phenotype-expressing isolates to be resistant to ciprofloxacin, norfloxacin, and trimethoprim-sulfamethoxazole.
- This study demonstrated the efficacy of nitrofurantoin as significantly more active *in vitro* than the other oral agents tested in this study against both AmpC-phenotype and ESBL-producing *E. coli* strains.

ACKNOWLEDGMENTS

This study was supported in part by Becton Dickinson Canada Inc., Bio-Media Unlimited Ltd. (Canada) and PML Microbiologicals (Canada). The excellent assistance of Ana Liza Guardian and Rufina Lee is gratefully acknowledged