Susceptibility testing of extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae against oral antimicrobials, including fosfomycin and mecillinam

Julie A Creighton

Abstract

Background

During the last decade in New Zealand there has been a dramatic increase in the isolation rate of extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, particularly causing urinary tract infections (UTI) from patients residing in the community. These isolates are frequently co-resistant to many oral antimicrobials. In order to treat the patient in an ambulatory care setting, avoid costly and inconvenient IV administered antibiotics and limit the use of carbapenems, both the patient and the health system could benefit from more oral treatment options.

Aim

The aim of this study was to gather local data on the in-vitro susceptibilities of ESBL-producing Enterobacteriaceae from our institution, against two potentially useful oral antibiotics: fosfomycin and mecillinam.

Methods

Routine susceptibilities were performed by BD Phoenix™, with fosfomycin and mecillinam performed by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion methods. A total of 109 non-duplicate ESBL-producing Enterobacteriaceae isolates, from 102 patients were included in this study.

Results

Fosfomycin was active against 77/78 (98.7%) Escherichia coli and 19/21 (90.5%) Klebsiella pneumoniae, with an overall susceptibility of 96.3% against all isolates tested. Mecillinam was also highly active, with 90.8% of all isolates classified as susceptible, being particularly effective against E.coli 74/78 (94.9%) and K.pneumoniae 20/21 (95.2%). Nitrofurantoin was active against 72/78 E.coli (92.3%) but had diminished susceptibility against the other isolates tested. The remaining oral agents: augmentin, ciprofloxacin, trimethoprim, and trimethoprim/sulphamethoxazole, performed poorly against the isolates tested. Of the parenterally administered antimicrobials, gentamicin was only active in 50.5% of the isolates studied, but all isolates were susceptible to meropenem.

Conclusions

Fosfomycin and mecillinam emerged as highly effective oral antimicrobials in-vitro against these multidrug resistant pathogens and may be considered as useful oral treatment options, particularly for high risk patients with community acquired UTIs.

Key words

Enterobacteriaceae, ESBL, urinary tract infection, fosfomycin, mecillinam


Introduction

New Zealand is a country with relatively low rates of resistant clinical pathogens (1,2). However over the last decade there has been a dramatic increase in the isolation rate of extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae (3,4). At Canterbury Health Laboratories (CHL) in 2004 there were only 5 patients with ESBL-producing Enterobacteriaceae isolated, and all strains were E.coli. By 2012 the number of patients with ESBL-producing Enterobacteriaceae had climbed to nearly 200 patients, with eleven different species identified. This number had already been eclipsed in the first 8 months of 2013. Of most concern is the increasing prevalence of ESBL-producing Escherichia coli, causing urinary tract infections (UTIs) in community based patients: a trend which has been recognised globally (5-7).

ESBL-producing Enterobacteriaceae, particularly E.coli with CTX-M-15 type ESBLs, often associated with sequence type 131 (ST131), tend to be co-resistant to several antibiotic classes in addition to the expanded spectrum β-lactam antibiotics (8,9). The most recent study by the Institute of Environmental Science and Research showed a predominance of CTX-M-15 in New Zealand (10). Limited treatment options for these multi-resistant isolates have forced the use of carbapenems, particularly for serious infections, often resulting in the emergence of carbapenemase producing isolates – at an alarming rate in some countries (11). Patients in primary care, especially those with uncomplicated infections such as simple cystitis, could benefit from the availability of an effective oral agent, rather than costly and inconvenient IV administered antibiotics. In turn, the health system would benefit from reduced hospital admissions and other associated health costs.

Fosfomycin and mecillinam have been used extensively for the treatment of UTIs, mainly in Scandinavia and Europe, for decades while resistance rates remain low (12). Neither drug is currently licensed or funded in New Zealand. Fosfomycin tromethamine is the soluble salt form of fosfomycin, suitable for oral administration. High concentrations of fosfomycin are achieved in the urine as the drug is excreted unchanged. Bactericidal action is achieved by interference of peptidoglycan biosynthesis: an early stage of bacterial cell wall development (13). Fosfomycin has broad spectrum activity against a range of Gram positive and Gram negative organisms, although it not as useful against Pseudomonas aeruginosa and Acinetobacter baumanii (14). Although fosfomycin is only approved for the treatment of uncomplicated acute cystitis against susceptible strains of E.coli and Enterococcus faecalis, recent reviews have found it to be a viable option for the treatment of infections from a variety of body sites (15,16). Fosfomycin di-sodium salt is the parenteral version.

Pivmecillinam is the prodrug which releases the active agent mecillinam when absorbed. After oral administration mecillinam is rapidly absorbed, achieving peak plasma concentrations after 1 hour. In addition, much of the drug is excreted unchanged in the urine within the first 6 hours. Mecillinam is a β-lactam which uniquely binds to Gram negative penicillin binding protein 2 (BepP2), resulting in cell wall distortion, lysis and cell death (17). The main indication of use for mecillinam has also been for the treatment of acute uncomplicated cystitis.
The aim of this study was to gather local data on the in-vitro susceptibilities of ESBL-producing Enterobacteriaceae from our institution, against two potentially useful oral antibiotics: fosfomycin and mecillinam, and to compare the results with other routinely tested oral agents.

Methods and materials

Patient population
The Canterbury District Health Board (CDHB) oversees publicly funded health services to a wide geographical area, with a population in excess of 500,000. The largest of its fourteen hospitals is Christchurch Hospital, a 650 bed tertiary, teaching and research hospital. Canterbury Health Laboratories (CHL) provides laboratory testing services to the CDHB, private medical centres and national referring laboratories. During the study period the Bacteriology Department provided an integrated processing service for all community urine samples received by MedLab South Ltd (who were sharing CHL facilities following the destruction of their own laboratory in the 22 February 2011 earthquake).

Bacterial isolates
ESBL-producing Enterobacteriaceae isolates were collected at CHL from July to December 2011 and stored at -80°C in PROTECT cryopreservative fluid. Fosfomycin and mecillinam susceptibilities were retrospectively performed on non duplicate patient isolates. During the study period a total of 173 ESBL-producing isolates, from 112 patients, representing 3.45% of all patient isolates. During the study period the Bacteriology Department provided an integrated processing service for all community urine samples collected at CHL from July to December 2011 and stored at -80°C in PROTECT cryopreservative fluid. Fosfomycin and mecillinam, and to compare the results with other routinely tested oral agents.

Antimicrobial susceptibility testing
Routine susceptibility testing was performed with BD Phoenix, using either NMIC/ID-75 (448087) or NMIC/ID-95 (448783) combo panels. Interpretation criteria for susceptibility results were applied using the Clinical and Laboratory Standards Institute (CLSI) guidelines and Phoenix BDxpert rules (18).

Results
A total of 109 non duplicate isolates, from 102 patients were included in this study. The patients consisted of 73 (71.6%) female and 29 (28.4%) male patients, and ranged in age from 6 weeks to 100 years old, with the median being 65.5 years. The susceptibility of fosfomycin against all isolates tested was 98.7%, compared to other oral agents tested.

The percent susceptible results for all oral antimicrobials tested are summarised in Table 1. Parenteral antibiotics gentamicin and meropenem are also listed for comparison purposes. Fosfomycin and mecillinam were the most effective oral antimicrobials in-vitro, compared to other oral agents tested. The susceptibility of fosfomycin against all isolates tested was 96.3%, with E.coli showing the highest susceptibility rate of 98.7% and 19/21 (90.5%) K.pneumoniae isolates susceptible. Mecillinam also performed well, with 99/109 (90.8%) of all isolates classified as susceptible and it was particularly effective against 74/78 (94.9%) E.coli and 20/21 (95.2%) K.pneumoniae. Nitrofurantoin was active against 72/78 (92.3%) E.coli but had diminished susceptibility against the other isolates tested.

Table 1. Susceptibility results of ESBL-producing strains to fosfomycin, mecillinam and other oral agents

| Organism        | No. isolates | FOS | MEC | AUG | NIT | CIP | TRM | SXT | GN | MER |
|-----------------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| E.coli          | 78           | 98.7| 94.9| 38.5| 92.3| 29.5| 28.2| 32.1| 57.7| 100 |
| K.pneumoniae    | 21           | 90.5| 95.2| 19.1| 14.3| 28.6| 14.3| 14.3| 33.3| 100 |
| Others          | 10           | 90  | 50  | 10  | 50  | 80  | 40  | 40  | 30  | 100 |
| TOTAL           | 109          | 96.3| 90.8| 32.1| 73.4| 33.9| 22.9| 29.4| 50.5| 100 |

a = K.oxytoca (2), E.cloaceae (3), C.farmeri (2), C. braakii (1), Raoultella ornithinolytica (1), and Serratia fonticola (1)
FOS=fosfomycin, MEC=mecillinam, AUG=augmentin, NIT=nitrofurantoin, CIP=ciprofloxacin, TRM=trimethoprim, SXT=sulphamethoxazole/trimethoprim, GN=gentamicin, MER=meropenem
The remaining oral agents: augmentin, ciprofloxacin, trimethoprim, and trimethoprim/sulphamethoxazole, performed poorly against the isolates tested, with overall susceptibilities ranging from as little as 22.9% for trimethoprim to 33.9% for ciprofloxacin. ESBL-carrying plasmids often contain other resistance mechanisms, making the isolates more likely to be multi-resistant, particularly to quinolones such as norfloxacin and ciprofloxacin. This co-resistance was demonstrated in our study by the low level of susceptibility to ciprofloxacin by both E.coli and K.pneumoniae (29.5% and 28.6% respectively). Furthermore, isolates that were resistant to ciprofloxacin were also more likely to be resistant to other antimicrobials including aminoglycosides. Results are summarised in Table 2.

**Table 2. Percent of resistance to other antimicrobials as determined by ciprofloxacin resistance**

<table>
<thead>
<tr>
<th></th>
<th>% resistant</th>
<th>No isolates</th>
<th>GN</th>
<th>TOB</th>
<th>TRM</th>
<th>SXT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin resistant</td>
<td></td>
<td>72</td>
<td>59.5</td>
<td>64.3</td>
<td>78.3</td>
<td>81.1</td>
</tr>
<tr>
<td>Ciprofloxacin susceptible</td>
<td></td>
<td>37</td>
<td>40.5</td>
<td>40.0</td>
<td>65.2</td>
<td>56.8</td>
</tr>
</tbody>
</table>

GN=gentamicin, TOB=tobramycin, TRM=trimethoprim, SXT=sulphamethoxazole/trimethoprim

Of the parenterally administered antimicrobials, gentamicin, a common empirical choice in a patient with suspected urosepsis, was only active in 50.5% of the isolates studied, but all isolates were susceptible to meropenem.

**Discussion**

Urinary tract infections are one of the most common bacterial infections in both hospital and community settings, with E.coli remaining the most predominant uropathogen. It is a concerning trend that ESBL-producing E.coli strains have gained such a foothold, especially in community acquired infections. Many UTIs can progress to serious illnesses including bacteraemia: in which there is a recognised higher mortality rate for patients who have multidrug resistant isolates coupled with a delay in receiving appropriate antibiotic therapy (20-22). In this study the predominant organism/source group was E.coli isolated from urine. While there were only four isolates recovered from blood culture during the study period, three of the four patients had either a concurrent UTI or urology comorbidity, and we have seen an increasing prevalence of patients infected with ESBL-producing E.coli septicemia over the last 12 months at our institution (data not shown).

Enterobacteriaceae are spread by hand carriage, contaminated food and water. Risk factors for acquisition of ESBL-producing Enterobacteriaceae include previous exposure to antibiotics, previous health care intervention or resident of a long term care facility, or urinary catheter use (22,23). In New Zealand, Freeman et al. found that travel to the Indian subcontinent was a risk factor for community-onset UTI (24). A recent study in Sweden showed a high rate of faecal flora colonisation with ESBL-producing Enterobacteriaceae following international travel (25). Destinations of highest risk included the Indian subcontinent and Asia, with travellers being unlucky enough to suffer a gastrointestinal illness and those aged >65 years being the most vulnerable.

In this study we have found both fosfomycin and mecillinam to have a high *in-vitro* activity against a range of ESBL-producing Enterobacteriaceae, especially against E.coli. These results are similar to those found in other studies (13,26-28). A recent study in Turkey of 52 patients with UTI showed a successful clinical response at follow up in 49 patients, after 3 doses of fosfomycin (29). Notably many of these patients had complicated infections. Of some concern is the study by Oteo et al. that showed increasing fosfomycin resistance, possibly due to a resurgence of fosfomycin use in the community setting (30). It is also possible, however, that the spike in fosfomycin resistance was aligned with a local clonal spread of CTX-M-15-producing E.coli ST131. A multicenter evaluation in Japan of 192 CTX-M-producing E.coli found some transferable fosfomycin resistance enzymes co-existing on the CTX-M enzyme plasmid, indicating that dual transmission could occur. However, fosfomycin has been used for many years in Japan, and the overall activity of fosfomycin was 96.4% (26). Resistance to fosfomycin was recently extensively reviewed by Karageorgopoulos et al. (31). They concluded that fosfomycin appeared to be a reliable agent for the treatment of UTIs, due to high levels of drug excreted into urine, together with an acidic urine environment and low level of biological fitness of fosfomycin-resistant mutants. They cautioned against its use as a sole agent in serious infections and against organisms other than E.coli.

Although mecillinam has performed well in *in-vitro* studies against ESBL-producing Enterobacteriaceae, authors have warned against its use as mono therapy due to the apparent effect of high bacterial inoculum, and hence high concentrations of β-lactamase production, which increases the MIC level of mecillinam (32,33). Thomas et al. and Brenwald et al. both suggest that mecillinam would be more effective if given in combination with a β-lactam inhibitor such as clavulanate. Similarly, in a more recent study from Greece, mecillinam was found to be highly active *in-vitro* against 47 of 48 (97.9%) ESBL-producing E.coli uropathogens, with the authors also suggesting the administration of a β-lactam inhibitor in combination with mecillinam for more effective treatment (34).

On a side note, we have also found fosfomycin to be a useful agent against Enterococcus faecalis and some E.faecium. In a separate in-house study, an evaluation of fosfomycin against 100 consecutive Enterococci isolated from urine (consisting of 91 E.faecalis and 9 E.faecium), showed excellent activity of fosfomycin against E.faecalis (97.8% susceptible), but less activity against E.faecium (63.6% susceptible). These organisms can cause UTI in elderly patients, many of whom can have limited treatment options due comorbidities such as renal insufficiency or penicillin allergy, perhaps ruling out the use of nitrofurantoin or ampicillin respectively.

The poor results of other antimicrobials in this study highlighted the limited oral treatment choices against ESBLs. Nitrofurantoin has been used for the treatment of simple cystitis and for prophylaxis for more than 50 years, and it did show high rates of susceptibility against E.coli. However, it was not as effective against *K.pneumoniae* and is not suitable for the treatment of members of the Proteus/Providencia/Morganella genera. Nitrofurantoin is contraindicated in patients with renal failure – which can be common in elderly patients; and its longer dosing regimen may have implications for patient noncompliance. The poor performance of ciprofloxacin, trimethoprim and trimethoprim/sulphamethoxazole do not make these antibiotics a preferred choice, especially not for empirical treatment in patients with high risk factors of ESBL infections.

Although susceptibility against parenteral meropenem was 100%, it would seem prudent to conserve the use of carbapenems to selective cases only, or to limit their use to an initial 24 hour empirical treatment, changing to an oral agent once the results of laboratory susceptibility testing are known.

Further investigation on our study isolates could include the analysis of ESBL enzyme type, phylogenetic group or sequence type, to determine any clonal relationship or association with multidrug resistance in our population.

**Conclusions**

With the prevalence rate of ESBL-producing Enterobacteriaceae continuing to climb, there is a need to seek suitable alternative treatment options to carbapenems and other IV administered drugs.
This need is especially acute for the treatment of community-based patients and goes hand-in-hand with the desire to preserve the use of carbapenems where ever possible. The results of this study has shown that fosfomycin and mecillinam were highly active agents in-vitro against ESBL-producing Enterobacteriaceae in our population and may be considered as useful oral options, either for treatment or empirical use, particularly in high risk patients with community acquired UTIs. Laboratories need to be vigilant in their screening for ESBL-producing pathogens and could consider the addition of fosfomycin and mecillinam testing to their extended antibiotic panel.

Acknowledgments
Grateful thanks to Sandra Hainsworth and Mirjam Horsburgh (formerly MedLab South employees) for performing much of the disk diffusion testing on the study isolates.

Author information
Julie Creighton, DipMLT, Senior Medical Laboratory Scientist1 and Clinical Lecturer2
1Canterbury Health Laboratories, PO Box 151, Christchurch and
2University of Otago, Christchurch, New Zealand
Email: julie.creighton@cdhb.health.nz

References


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