Mecillinam resistance in *Enterobacteriaceae* urinary tract isolates

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Introduction

The rapid and global spread of multi-drug resistant Gram-negative bacteria is a therapeutic challenge. *E.coli* and other *Enterobacteriaceae*, are important pathogens causing urinary tract infection and sepsis. Urinary tract infection (UTI) is amongst the most common infection seen both in the community and hospital settings. In England alone over 35,000 cases of *E.coli* bacteraemia were reported from April 2014 to March 2015[1]. The judicious antimicrobial management of Gram-negative bacterial infection is therefore essential.

Mecillinam (pro-drug pivmecillinam) is an attractive oral option in the treatment of uncomplicated urinary tract infection[2]. Mecillinam acts by inhibiting the transpeptidase activity of penicillin binding protein-2 (PBP2) responsible for cell elongation and division of Gram-negative bacilli[4-7]. Resistance is believed to be secondary to mutations in gene targets involved in the elongation process.

Recent studies show that mecillinam resistance is associated with highly diverse multi-resistance profiles and imposes a significant fitness cost to an organism suggesting a low propensity for clonal spread[7,8].

European surveillance data has indicated that mecillinam susceptibility of uropathogens is high at 95.9% with little variation between participating countries previously being noted[2,3]. However increasing resistance rates have been observed in Sweden in recent years[9]. UK specific resistance data remains largely unknown despite mecillinam being recommended as first-line empirical treatment of uncomplicated UTI by the European Society for Microbiology and Infectious Diseases[9].

Aims

The objective of this study was to determine whether resistance to mecillinam was associated with beta-lactamase production in *Enterobacteriaceae* and if co-resistance towards other antibiotic classes was displayed.

Method

Mecillinam resistant *Enterobacteriaceae* urinary tract isolates were analysed over a 10-month period (September 2014 to June 2015). Clinical isolates were collected from our microbiology laboratory based at the Glasgow Royal Infirmary, a large teaching hospital which serves a population of 560,00 patients.

Species identification was performed using the automated system, VITEK MS (Matrix Assisted Laser Desorption Ionization Time-of-Flight, or MALDI-TOF). Determination of mecillinam susceptibility was performed using the automated system, VITEK 2 or the EUCAST standardised disc diffusion method. A sensitive result for mecillinam is a minimum inhibitory concentration (MIC) ≤ 8µg/ml or zone diameter ≥ 15mm. Phenotypic detection of a beta-lactamase resistance mechanism was performed for each isolate as described by Schreckenberger *et al*[12]. Co-resistance towards 6 other antibiotic classes (ciprofloxacin, gentamicin, nitrofurantoin, fosfomycin, temocillin and trimethoprim) commonly used in the treatment of UTI was also analysed. Resistance was verified using EUCAST methodology; MIC determination was verified with E-test (bioMérieux, Solna, Sweden) according to the manufacturer's instructions.

Table 1 Mecillinam resistant *Enterbacteriacaea* analysed

Organism	No. Of isolates
E.coli	93
Enterobacter spp	2
Enterobacter amnigenus	1
Enterobacter cloacae	1
klebsiella spp	23
Klebsiella oxytoca	10
Klebsiella pneumoniae	13
Morganella morganii	7
Proteus mirabilis	12
Serratia marcescens	7
Total	**144**

Figure 1 Beta-lactamase production phenotypically detected in mecillinam resistant isolates

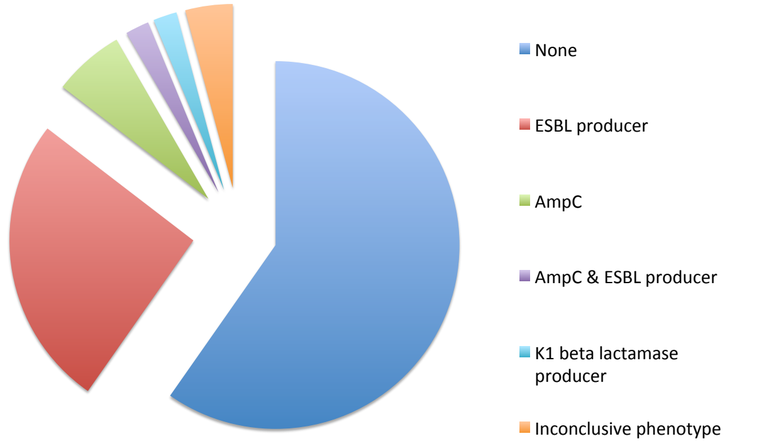

Table 2 Co-resistance to other antibiotic classes amongst mecillinam resistant isolates

	Resistant isolates [n (%)]					
Organism	CIP	GENT	NIT	FOS	TEM	TMP
E.coli	59 (63)	31 (33)	22 (24)	3 (3*)	5 (5)	84 (90)
Enterobacter spp	0	2 (100)	-	1 (50)	0	2 (100)
klebsiella spp	6 (26)	11 (48)	10 (43)	9 (39)	0	16 (70)
Morganella morganii	0	4 (57)	7 (100)	7 (100)	1 (14)	5 (71)
Proteus mirabilis	3 (25)	9 (75)	12 (100)	1 (8)	0	12 (100)
Serratia marcescens	0	3 (43)	7 (100)	2 (33*)	3 (50*)	4 (57)

*Susceptibility result/MIC not available for 1 isolate. CIP, ciprofloxacin; GENT, gentamicin; NIT, nitrofurantoin; FOS, fosfomycin; TEM, temocillin; TMP, trimethoprim

Results

Over the 10-month period a total of 144 mecillinam resistant isolates were analysed, Table 1. Repeat samples from the same patient were removed and excluded.

Of the corresponding patients, 69% (100/144) were female. 49% (71/144) and 12% (17/144) of the isolates were taken from in-patients under the care of Medical and Surgical specialties respectively. 43% (62/144) of isolates were cultured from mid-stream urine (MSU), 29% (42/144) from clean-catch urine, and 17% (25/144) from catheter urine specimens (CSU).

Of the *Enterobacteriaceae* analysed, no beta-lactamase resistance was detected phenotypically in 60% (86/144) of isolates. Extended-spectrum beta-lactamase (ESBL) production was detected in 26% (37/144) of isolates. AmpC production was detected in 6% (9/144) of isolates and K1 beta-lactamase production was detected in 2% (3/144) of isolates (Figure 1). For 6 isolates, an inconclusive phenotype was detected that did not meet the interpretation criteria of Schreckenberger *et al.*

Interestingly 83% (120/144) of the isolates displayed resistance to more than 2 other classes of antibiotic, with 47% (67/144) displaying co-resistance to more than 3 classes.

Conclusions

With the emergence of multidrug-resistant Gram-negative bacteria mecillinam in recent years has shown a promising role for the treatment of UTI. In Scotland, the Scottish Antimicrobial Prescribing Group (SAPG) advocates mecillinam as directed therapy for uncomplicated UTI [13].

Previous analysis of *Enterobacteriaceae* urinary tract isolates by our laboratory in 2012 found no statistically significant association between mecillinam resistance and ESBL production (p=0.075, Chi squared test). Of the 499 clinical isolates tested, 336 (67%) were identified as ESBL producers. Of these 12.2% (41/336) were Mecillinam resistant[11]. Analysis of the collection of *Enterobacteriaceae* urinary tract isolates in this study detected no associated beta-lactamase production phenotypically amongst mecillinam resistance isolates. Interestingly, the isolates displayed a high level of resistance to the other antibiotic classes. However, the number of isolates analysed was relatively small as VITEK and mecillinam susceptibility testing are not routinely performed for all urinary tract isolates in this laboratory; nor were MIC values for mecillinam determined. We believe that further genetic analysis is warranted to investigate for possible association with specific resistance lineages.

Despite the universal recommendation and subsequent use of mecillinam as a treatment option in Scotland, local and UK specific resistance data remains largely unknown. This study highlights that a more robust surveillance strategy is required to assess antimicrobial resistance of uropathogens in our laboratory. Furthermore evaluation of clinical outcome of mecillinam therapy is required in order to gain insight into clinical failure versus laboratory-reported resistance.

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