antioxidants on the antibacterial activity of commonly used and therapeutically relevant β-lactam antibiotics.

The effect of glutathione (GSH) and ascorbic acid on the susceptibility of *E. coli* K-12 strain MG1655 to ampicillin and penicillin was investigated. MICs of these antibiotics in the presence and absence of 10 mM of these antioxidants were determined by agar dilution method as outlined by the CLSI (formerly the NCCLS). An inoculum of approximately $10^5 – 10^7$ cfu (simultaneously determined by plating) per spot (in a volume of 10 μL) was applied to the agar plates with increasing antibiotic concentration. MIC was the lowest concentration of antimicrobial agent that prevented visible growth after 20 h of incubation at 37°C. The experiments were carried out at least twice and the representative results are mentioned here. We observed that the presence of 10 mM GSH made MG1655 cells more susceptible to these β-lactams, because addition of GSH resulted in reduced MICs of ampicillin and penicillin from 8 and 64 mg/L to 4 and 48 mg/L, respectively. However, we found that this effect was specific to GSH, as the presence of ascorbic acid did not make any difference to the antibiotic susceptibility of MG1655.

Total intracellular glutathione (GSHin) is a sum of reduced glutathione (GSH) + oxidized glutathione (GSSG) amounts and bacterial cells can readily take up both GSH and GSSG, resulting in enhanced GSHin. As GSHin along with GSH to GSSG ratio are key regulators of various intracellular redox reactions, we investigated whether this glutathione-mediated phenotype is dependent on its redox status outside the cell. For this purpose the MICs of the above-mentioned β-lactams were determined in the presence of 5 mM GSSG. The results showed that GSSG also decreased the MICs of the antibiotics to the same extent as observed with GSH, implying that glutathione-mediated augmentation of β-lactam susceptibility is independent of its redox status outside the cell.

Gamma glutamyl-transeptidase (*ggt*, EC 2.3.2.2)-mediated cleavage of glutathione is required for the uptake of extracellular glutathione by bacterial cells. In addition, as *ggt* catalyses the transfer of the gamma glutamyl group of glutathione and related gamma-glutamyl amides to other amino acids and peptides (transpeptidation), it is likely to be involved in the transpeptidation step of bacterial cell wall synthesis. Considering that β-lactams act at the transpeptidation step of cell wall synthesis, glutathione-mediated augmentation of β-lactam antibacterial activity could be due to the competition between *ggt*-mediated cell wall synthesis and the glutathione uptake process. We investigated whether this possibility holds true by determining the MICs of ampicillin and penicillin for SH639, SH664 and SH673 strains (different *ggt* knockout strains of *E. coli*, a gift from Dr Hideyuki Suzuki) (see Suzuki et al. for details). However, we found that *ggt* knockout mutants did not differ from their parent strain in terms of ampicillin and penicillin susceptibility (data not shown), indicating that glutathione-mediated increased antibacterial activity of each β-lactam is not arbitrated by the status of *ggt* in *E. coli*. Another possible mechanism for this enhanced antibacterial effect could be altered expression of *cysB* in the presence of glutathione (*cysB* is an ORF encoding a regulator for GSH transport) as *cysB* is known to be involved in determination of bacterial susceptibility to β-lactams.

Irrespective of which mechanisms operate behind the above-mentioned phenotype, our observations are of significance, revealing that glutathione cannot only reduce the antibacterial effect of certain antibiotics (as previously demonstrated by us), but also enhance the antibacterial activity of β-lactam antibiotics. This study along with previous reports, reveal that glutathione differentially modulates the antibacterial activity of various groups of antibiotics. Additionally, since β-lactams are important antibiotics having immense therapeutic value, further investigations surrounding the effect of glutathione on antibacterial activity of β-lactams would be of interest in the future for development of improved treatment regimens for various infections.

**Acknowledgements**

We are grateful to Hideyuki Suzuki (Kyoto University, Japan) for providing the strains related to this study. We thank all the members of MMGES group for the valuable discussions related to the study. We also thank Dr S. K. Apte for his constant encouragement and support.

**Transparency declarations**

None to declare.

**References**


**Journal of Antimicrobial Chemotherapy**

doi:10.1093/jac/dkm179
Advance Access publication 5 June 2007

**Temocillin susceptibility by BSAC methodology**

Jennifer M. Andrews*, Gail Jevons, Rebecca Walker, Janet Ashby and Adam P. Fraise

**Department of Microbiology, City Hospital, Dudley Road, Birmingham B18 7QH, UK**

Keywords: β-lactams, MICs, zone diameters
Sir,

Temocillin is a semi-synthetic β-lactam antibiotic (a derivative of ticarcillin) for parenteral administration. It has a mean elimination half-life of 4.5 h, considerably longer than that of other penicillins.1 Temocillin is highly stable to the bacterial of β-lactamases, including extended-spectrum types (ESBLs) and AmpC,2 but has low activity against Pseudomonas spp., Bacteroides spp., Campylobacter spp., Acinetobacter spp. and Staphylococcus aureus.1,3

In this study, temocillin MICs and zone diameters for a 30 μg temocillin disc (Oxoid, Basingstoke, UK) were obtained by BSAC methodology4,5 for 460 clinical isolates comprising 100 each of Escherichia coli (including ESBL, SHV, OXA-2 and AmpC producers) and Klebsiella spp. (including ESBL, CTX-M and K1 producers); 50 each of Citrobacter spp., Proteus mirabilis, Enterobacter spp. and Serratia spp.; 20 each of Proteus vulgaris, Morganella morganii and Providencia spp. Control strains E. coli NCTC 10418 and E. coli ATCC 25922 were also included and acceptable limits for the controls were determined by disc testing 100 times on pre-poured plates from Oxoid and bioMérieux (Basingstoke, UK). Zone diameters on the two types of media were combined and 95 percentiles calculated.6 Zone diameters for the clinical isolates were analysed using the BSAC MIC breakpoints5 (Figure 1).

Arithmetic means for the species tested were for E. coli 8.4 mg/L (range 2–32 mg/L), Klebsiella spp. 6.5 mg/L (range 1–64 mg/L), Enterobacter spp. 6.7 mg/L (range 1–128 mg/L), Citrobacter spp. 3.8 mg/L (range 1–16 mg/L), M. morganii 2.4 mg/L (range 2–4 mg/L), P. mirabilis 0.9 mg/L (range 0.25–1 mg/L), P. vulgaris 1 mg/L (range 0.5–2 mg/L), Serratia spp. 14.8 mg/L (range 4–64 mg/L) and Providencia spp. 2.8 mg/L (0.5–16 mg/L). The BSAC temocillin MIC breakpoints for systemic and urinary tract infections are 8 and 32 mg/L, respectively, with corresponding zone diameter breakpoints of 20 and 12 mm.6 Applying these criteria to the data obtained in this study for the systemic breakpoints, a false resistance rate of 2.2% (10/460) and a false susceptible rate of 2.6% (12/460) were observed and for the urinary breakpoints, a false resistance rate of 1.1% (5/460) and a false susceptible rate of 0.4% (2/460) were observed. Using the systemic criteria, the organisms that were interpreted as falsely susceptible had temocillin MICs of 16 mg/L and comprised one Klebsiella sp., one Citrobacter sp. and 10 Serratia spp. The organisms interpreted as falsely resistant were two Klebsiella spp. (not ESBL or AmpC β-lactamase producers), the control E. coli with plasmid-mediated β-lactamase AmpC β-lactamase, one E. coli with CTX-M-15, one E. coli AmpC and ESBL negative, one Serratia sp. and two Enterobacter spp. Using the urinary tract breakpoints, two Serratia spp. with temocillin MICs of 64 mg/L were interpreted as falsely susceptible. The organisms falsely interpreted as resistant comprised four E. coli with temocillin MICs of 32 mg/L (three reduced susceptibility to cefoxitin but not AmpC producers and one plasmid-mediated AmpC β-lactamase producer) and one Klebsiella sp. with a temocillin MIC of 16 mg/L (not an ESBL or AmpC β-lactamase producer).

For the control strains E. coli ATCC 25922 and E. coli NCTC 10418, the temocillin MICs (zone ranges) were 8 mg/L (16–21 mm) and 2 mg/L (27–31 mm), respectively. The MIC for control ATCC 25922 straddles the BSAC MIC breakpoint and it is anticipated, as seen in this study, that zone diameters may often fall into the resistant population.

This study confirms the disc testing recommendations given by the BSAC;5 however, the high false susceptibility rate seen with Serratia spp. would suggest that an MIC determination may be more appropriate for determining the susceptibility of these genera.

Figure 1. Scattergram for a 30 μg temocillin disc for 460 clinical isolates of Enterobacteriaceae by BSAC methodology.

186
Acknowledgements

This study was supported by funding from Eumedica Pharmaceuticals, Brussels.

Transparency declarations

None to declare.

References


Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkm131
Advance Access publication 9 May 2007

In vitro efficacy of ceftriaxone/sulbactam against Escherichia coli isolates producing CTX-M-15 extended-spectrum β-lactamase

M. Shahid*, M. Singhai, A. Malik, I. Shukla, H. M. Khan, F. Shujatullah and F. Tahira

Correspondence

Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India

Keywords: E. coli, disc synergy, piperacillin/tazobactam, ESBLs

*Corresponding author. Tel: +91-571-2720382; Fax: +91-571-2721776; E-mail: shahidsahar@yahoo.co.in

Sir,

Resistance to third- and fourth-generation cephalosporins has become a major concern worldwide. Even more alarming is the emergence of carbapenem resistance; the carbapenems are often considered to be a ‘drug of choice’ and are increasingly used in empirical therapy. Against this rising resistance, the role of β-lactam/β-lactamase inhibitor combinations needs to be considered. Sulbactam has been approved recently in many countries to be combined with β-lactam antibiotics, including, recently, in India.

CTX-M enzymes are a recently emerged group of extended-spectrum β-lactamas (ESBLs) and CTX-M-15 has emerged as the most prevalent ESBL in many parts of the world, including the UK and India.2,3 We have evaluated the in vitro efficacy of ceftriaxone/sulbactam, a combination recently launched in India, against Escherichia coli harbouring blaCTX-M-15. Moreover, we describe the phenotypic detection of ESBL production by the synergy observed between cephalosporin and piperacillin/tazobactam discs and compare it with conventional disc synergy tests with co-amoxiclav.

Thirty-two urinary E. coli isolates previously shown to carry blaCTX-M-15, and 22 CTX-M-producing E. coli isolates from bronchoalveolar lavage and pus samples obtained during 2006, from Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh, India, were studied. The latter isolates were shown to carry blaCTX-M of genogroup-1 by PCR with primers 5'-AAA AAT CAC TGC GCC AGT-3' and 5'-AGC TTA TTC ATC GCC AGT-3'. Cycling conditions were initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 52°C for 45 s and 72°C for 1 min; and a final elongation at 72°C for 8 min. Precise CTX-M types were not identified in these 22 isolates because of the lack of sequencing facilities, but CTX-M-15 is the only variant that has been described to date in India.3,4 To ensure that multiple representatives of particular strains were eliminated, all isolates were

Table 1. Comparative susceptibilities of blaCTX-M-carrying E. coli isolates (n = 54) to individual β-lactams and β-lactam/β-lactamase inhibitor combinations

<table>
<thead>
<tr>
<th>Antibiotics discs (µg)</th>
<th>blaCTX-M-15 (n = 32)</th>
<th>blaCTX-M group-1 (n = 22)</th>
<th>total (n = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticarcillin (75)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ticarcillin/clavulanate (75/10)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Piperacillin (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Piperacillin/tazobactam (100/10)</td>
<td>9.4 (3)</td>
<td>18.1 (4)</td>
<td>12.9 (7)</td>
</tr>
<tr>
<td>Ceftriaxone (30)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ceftriaxone/sulbactam (30/15)</td>
<td>93.8 (30)</td>
<td>100 (22)</td>
<td>96.3 (52)</td>
</tr>
<tr>
<td>Imipenem (10)</td>
<td>93.8 (30)</td>
<td>100 (22)</td>
<td>96.3 (52)</td>
</tr>
</tbody>
</table>

187