Comparative in-vitro activities of amoxycillin and penicillin against Streptococcus pneumoniae isolates

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Sir,

In a recent issue of this Journal, Verhaegen & Verbist reported on the in-vitro activities of 21 \(\beta\)-lactams against penicillin-susceptible and -resistant isolates of Streptococcus pneumoniae. The results of this investigation are surprising because they suggest that amoxycillin is less active than penicillin against these strains. We have recently determined the in-vitro susceptibilities of 212 clinical isolates of S. pneumoniae by the microbroth dilution method according to recommendations of the National Committee for Clinical Laboratory Standards; the results are shown in the Table. The MICs of amoxycillin, in common with those of cefotaxime, were almost invariably one two-fold dilution lower than those of penicillin for strains with MICs of penicillin >0.06 mg/L. These observations in respect of pneumococci with reduced susceptibilities to penicillin are in accord with those of other investigators.

In addition, Verhaegen & Verbist claimed that “Apart from penicillin, there are currently no NCCLS-approved breakpoints for oral \(\beta\)-lactams for pneumococci.” This is incorrect. In 1995, the NCCLS recommended MIC breakpoints for both oral amoxycillin (susceptible, \(\leq 0.5\) mg/L; intermediate susceptibility, 1 mg/L; and resistant, \(\geq 2\) mg/L) and co-amoxiclav (susceptible, \(\leq 0.5\leq 0.25\) mg/L; intermediate susceptibility, 1/0.5 mg/L; and resistant, \(\geq 2/\geq 1\) mg/L). The fact that the susceptible and intermediate susceptibility breakpoints for amoxycillin are higher than those for penicillin (susceptible, \(\leq 0.06\) mg/L and intermediate susceptibility, 0.12–1 mg/L) reflect the superior pharmacokinetic profile of the former drug. This and its greater in-vitro activity explain why most pneumococci with reduced susceptibilities to penicillin remain fully susceptible or of intermediate susceptibility to amoxycillin, with or without clavulanic acid.

Table. MICs of penicillin, amoxycillin and cefotaxime for 212 S. pneumoniae isolates

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<th>0.008</th>
<th>0.015</th>
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<th>0.06</th>
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<td>Cefotaxime</td>
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References

Fluoroquinolones as treatment for patients with lower respiratory tract infections caused by Streptococcus pneumoniae

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Sir,

There has been considerable debate about the role of currently available fluoroquinolones as treatment of patients with lower respiratory tract infections caused by Streptococcus pneumoniae and this is set to continue as the emergence of strains with reduced susceptibilities to penicillin and other antibiotics increases the need for alternative therapies. The development of novel fluoroquinolones with enhanced activities against S. pneumoniae represents one approach to treating patients with such infections. However, if we are going to make rational decisions about the use of these compounds, we must have accurate information about levels of resistance, how these levels change with time and the relationship between in-vitro susceptibility and the outcome of therapy. Furthermore, it is apparent that not all quinolones are equivalent in terms of their potential to select resistant strains amongst particular species.

If we are to maximize the impact of the quinolones as therapy of patients with pneumococcal infections, it is imperative that they be treated with those compounds which are most active in vitro and which exhibit the best pharmacokinetic profiles in vivo. The situation is more complex, however, because we also need some idea of the magnitude of the selective pressures that exposure to older quinolones may have exerted on resistance. At present, data on the susceptibilities of S. pneumoniae isolates to quinolones are inadequate. In the USA, because the National Committee for Clinical Laboratory Standards has not yet issued guidelines for susceptibility testing of these drugs, their use is effectively avoided. In Europe, on the other hand, susceptibility testing of fluoroquinolones is undertaken, although reports concerning levels of resistance have varied from centre to centre. Difficulty in interpretation arises from the fact that most MIC breakpoints are chosen on the basis that they avoid peaks in the MIC distribution profiles; for most susceptible species, the breakpoint, of course, exceeds this peak. Determining a breakpoint for S. pneumoniae has proved particularly difficult because the MIC peak is often just below or around 1 mg/L. This means that quite subtle variations in testing procedures can markedly alter the apparent levels of susceptibility and resistance. We previously identified this as a problem in relation to determining the susceptibilities of enterococci by the disc diffusion method and concluded that it was necessary to increase the disc content from 1 μg to 5 μg in order to obtain reproducible results.

We have read with great interest the results of recent studies in which susceptibility to ciprofloxacin was determined with a fixed breakpoint of 1 mg/L. In the study of Felmingham et al., there was considerable variation in the percentages of isolates of S. pneumoniae susceptible to ciprofloxacin in different centres in the UK and, overall, an increase in the percentage of susceptible isolates compared with an earlier study. Although these observations are of interest, the authors themselves acknowledged that their clinical significance is unclear, given “the poor discrimination afforded by a breakpoint which is set at the mode of MIC distribution for this antimicrobial.” This problem might not have arisen if susceptibility testing had been undertaken with a higher breakpoint and it would be particularly interesting to know what the percentages of susceptible isolates would have been had such a breakpoint been used. ‘True’ fluoroquinolone resistance amongst pneumococci is, we suggest, relatively rare in the UK. On the other hand, the development of resistance has enormous implications for the efficacies of drugs such as moxifloxacin and trovafloxacin which are not yet commercially available, regardless of continued confidence in the current fluoroquinolones, and it is important that we have reliable information regarding the extent of the ‘true’ emergence of resistance to these antibiotics. We suggest, therefore, that studies involving ciprofloxacin or similar fluoroquinolones should adopt either a higher breakpoint for susceptibility or two breakpoints (high and low), one of which exceeds the mode of MIC distribution. With this information, we would be in a better position to predict not only potential clinical efficacy, but also if there had been a significant increase in the percentages of resistant isolates in a given population.

References

Correspondence


Ciprofloxacin prophylaxis and therapy of Yersinia pestis infection

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Sir,
In a recent report in the Journal of Antimicrobial Chemotherapy, Russell et al.1 demonstrated, with an animal model of Yersinia pestis pneumonia, that ciprofloxacin prophylaxis and therapy were effective up to 24 h after challenge, but not after 48 h. To the best of my knowledge, there has been only one report in the literature concerning ciprofloxacin and plague.2 That report described a 31-year-old male who had been treated with lincomycin and ciprofloxacin (dosage not stated) by his general practitioner, but was admitted to hospital with septic shock on the following day. The patient, who was diagnosed as suffering from pneumonic plague, died within 24 h of admission. Although one cannot draw meaningful conclusions from a single case of a patient whose death may not have been due to a lack of efficacy of ciprofloxacin, a note of caution is none the less warranted.

References


Amino acid substitutions at positions 69, 165, 244 and 275 of the PE R-1 β-lactamase do not impair enzyme inactivation by clavulanate


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Sir,
Inhibitor-resistant TEM β-lactamases (IRTs) are plasmid-mediated enzymes that have recently been identified in Gram-negative bacteria.1 They are derived from the two parental class A β-lactamases, TEM-1 and TEM-2, by various amino acid substitutions which result in a loss of susceptibility to inhibition by β-lactam/β-lactamase inhibitor combinations such as co-amoxiclav and ticarcillin/clavulanic acid. Most of the amino acid substitutions previously characterized in IRT enzymes, alone or in combination, have been at positions 69, 165, 244, 275 and 276 according to the Ambler numbering system.2 More recently, a mutant of TEM-1, TEM-50, which confers resistance to third-generation cephalosporins and is clavulanic acid-resistant, has been identified in a clinical isolate of Escherichia coli.3 This mutant was shown to harbour mutations which are present in both the inhibitor-resistant variant, IRT-4, and the extended-spectrum β-lactamase (ESBL), TEM-15, indicating that ESBLs can become less susceptible to inhibition by β-lactamase inhibitors (or vice versa).

The class A β-lactamase, PE R-1, is a plasmid-mediated ESBL which is not closely related to either TEM or to SHV; for example, it possesses only 26% amino acid identity with TEM-3 and 27% with SHV-2.4 Initially detected in a strain of Pseudomonas aeruginosa isolated in 1993, PE R-1 has recently been found in large numbers of strains of P. aeruginosa, Salmonella typhimurium and Acinetobacter spp. isolated in Turkey5—evidence of the high capacity of the blaPER-1 gene to spread from species to species. In E. coli, PER-1 confers resistance to penicillins, first-, second- and third-generation cephalosporins and monobactams, but not to cephamycins or carbapenems. Furthermore, as susceptibility to β-lactams is restored in the presence of clavulanic acid, the resistance phenotype conferred by PER-1 is indistinguishable from those conferred by other ESBLs.
In order to determine if PER-1 can become less susceptible, or even resistant, to inhibition by clavulanic acid, we used site-directed mutagenesis to substitute the amino acid residues at positions 69, 165, 244 and 275 in PER-1 with those found at the same positions in various IRT enzymes (Table); position 276 was not included because PER-1 and many IRT β-lactamases share an acidic residue at this position. Thus, the amino acid residues Leu69, Arg165, Cys244 and Leu275, which are found in IRT-5, IRT-10, IRT-1 and IRT-9 respectively, were substituted for the residues at the corresponding positions in PER-1. Two single mutants (Q69L and T244C) and two double mutants (Q69L N165R and Q69L N275L) (Table) were constructed and expressed in E. coli JM109 according to a method described previously. The resistance phenotypes of the E. coli strains producing the various mutants were determined by the disc diffusion method and compared with that conferred by the wild-type PER-1 enzyme expressed under the same conditions (data not shown). The bacteria harbouring the Q69L, T244C and Q69L N165R mutants remained susceptible to co-amoxiclav and exhibited a resistance profile indistinguishable from that of the strain harbouring PER-1. In contrast, the strain producing the enzyme with the Q69L N275L double mutation was susceptible to all of the β-lactams tested, suggesting that the mutant β-lactamase had lost its activity. The 50% inhibitory concentrations (IC₅₀) of clavulanic acid were determined with crude extracts prepared from the E. coli strains producing the wild-type PER-1 enzyme and the three active mutants, Q69L, T244C and Q69L N165R, with 100 μM cephalothin as the substrate. The IC₅₀s for the mutants ranged from 0.19 μM to 0.33 μM and that for PER-1 was 0.25 μM, thus confirming that the mutations previously identified in IRT β-lactamases do not markedly affect the inhibition of PER-1 by clavulanic acid.

In conclusion, if the PER-1 β-lactamase was to become less susceptible to inhibition by β-lactamase inhibitors, this development would probably not be the result of mutations previously described in IRT enzymes. These results, taken together with those reported in a recent study in which PER mutants were characterized, suggest that the structure/activity relationships of PER-1 differ from those of other class A ESBLs.

**Correspondence**

We are indebted to Professor P. Nordmann and Dr T. Naas for the recombinant plasmid, pRAZ1, which encodes blaPER-1. We also thank N. Dagoneau-Blanchard for technical assistance. This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (INSERM, grant CR1 950601) and from Smithkline-Beecham Pharmaceuticals (Nanterre, France). A. T. Bouthors was a fellow of the Ministère de l’Enseignement Supérieur et de la Recherche.

### Table. Amino acid residues at various positions in TEM-1, the IRTs TEM-30 to TEM-39, PER-1 and constructed PER mutants

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<td>PER-Q69L N275L</td>
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<td>Leu</td>
<td></td>
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<td>Leu</td>
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</table>

**References**

of these enzymes, the amplification product was digested with
in most clinical isolates. Antibacterial Agents and Chemotherapy 41, 1322–5.


Characterization of the extended-spectrum β-lactamases and determination of the antibiotic susceptibilities of Klebsiella pneumoniae isolates in Hungary


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Sir,

Transferable β-lactamases which confer resistance to extended-spectrum cephalosporins are a common mechanism of resistance in aerobic Gram-negative bacilli, particularly Klebsiella pneumoniae. Of these enzymes, SHV-type extended-spectrum β-lactamases (ESBLs) confer a greater level of resistance than TEM-type β-lactamases, even to β-lactam/β-lactamase inhibitor combinations, thereby confounding efforts to effectively treat patients with infections caused by these bacteria. In most European countries and other parts of the world, there are published data for the types and prevalences of ESBLs in K. pneumoniae isolates. However, to date, no such data have been available in Hungary. The aims of the present study were to collect these data and to evaluate the activities of a wide range of antibiotics against K. pneumoniae isolates.

A total of 170 non-replicate K. pneumoniae clinical isolates were collected during 1996 by laboratories in five regions of the country (eight strains from western Hungary, ten from the north-west, 27 from northern Hungary, 11 from Budapest and 114 from Szeged). All of the strains isolated locally were identified according to standard laboratory techniques and those referred from other laboratories were identified by the ATB system (bioMérieux, Marcy l’Etoile, France). The antibiotics tested were as follows: amoxicillin, cefoxitin, cefuroxime, ceftazidime, cefotaxime, cepfime, cefpirome, aztreonam, imipenem, ampicillin/sulbactam (in a ratio of 2:1), co-amoxiclav (amoxycillin:clavulanic acid, 2:1), pipercillin/tazobactam (tazobactam at a fixed concentration of 4 mg/L), ceftazidime/clavulanic acid (clavulanic acid at a fixed concentration of 4 mg/L), ciprofloxacin, gentamicin, and amikacin. A double-disc diffusion test (DDDT), with co-amoxiclav, ceftazidime and cefotaxime, was used to detect ESBL production and the Etest ESBL strip (AB Biodisk, Solna, Sweden) was used to confirm the results of the DDDT. MICs were determined by the Etest on Mueller–Hinton agar according to the manufacturer’s instructions; susceptibility categories were assigned in accordance with recommendations of the National Committee for Clinical Laboratory Standards.

For the DNA analyses, plasmid DNA was extracted with a DNA purification kit (Promega, Madison, WI, USA) and used as a template for the amplification of the SHV gene with SHV-specific primers as described previously. The amplification product was digested with PstI (Pharmacia, Uppsala, Sweden) and separated on a polyacrylamide gel with known SHV standards; single-stranded DNA was stained with silver nitrate as described previously. E. coli strain containing the SHV-1 gene was used as a positive control. For the β-lactamase assays, the strains were grown in Mueller–Hinton broth, the cells disrupted by sonication and the crude enzyme in the supernatants quantified spectrophotometrically with nitrocefin as the substrate, as described previously. The protein contents of the samples were determined by the method of Lowry with bovine serum albumin as the standard.

Fifteen (9%) of the 170 K. pneumoniae isolates produced ESBLs, all of which were of the SHV type (12 SHV-2 β-lactamases and three SHV-5). Nine strains were isolated in Szeged, three in Budapest, two in northern Hungary and one in western Hungary. The activities of the 16 antibiotics against the 15 strains are shown in the Table. All of the isolates were resistant to amoxicillin and...
Table. In-vitro activities of various antibiotics for 15 ESBL-producing *K. pneumoniae* strains\(^a\) isolated in Hungary

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<tr>
<td>β-Lactamase activity</td>
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<td>328</td>
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<td>–</td>
<td>62</td>
<td>60</td>
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</table>

\(a\) Strains 1–12 produced SHV-2/β-lactamases and strains 13–15 produced SHV-5 β-lactamases.
The MICs of cefotaxime, ceftazidime and aztreonam were variable, but the MICs of ceftazidime and aztreonam for strains producing SHV-5 β-lactamases were, in general, higher than those for strains producing SHV-2 enzymes, whereas the converse applied to cefotaxime. Cefepime and cefpirome were more active than the third-generation cephalosporins, reflecting their low affinities for plasmid-mediated β-lactamases and their ability to resist hydrolysis by these enzymes. Cefpirome, but not cefepime, was less active against strain no. 9 which exhibited greater β-lactamase activity than the other strains. Imipenem was highly active against all of the strains and piperacillin/tazobactam was more active against SHV-5-producing isolates than those producing SHV-2, an observation that has been made previously by Jacoby & Carreras. Most of the isolates were resistant to ampicillin/sulbactam. On the other hand, all but three were susceptible to co-amoxiclav; moreover, the β-lactamase activities of the three resistant strains, all of which expressed SHV-2 β-lactamases, were up to 13-fold greater than those of susceptible strains. In the presence of clavulanic acid, the MICs of ceftazidime for all 15 strains were markedly reduced and even the resistant strains became susceptible. Thirteen of the strains were resistant to gentamicin and one exhibited resistance and a second reduced susceptibility to amikacin. All but one strain were susceptible to ciprofloxacin.

In the present study, the ESBLs expressed by Hungarian isolates of K. pneumoniae were shown to be either SHV-2 or SHV-5—a pattern that is consistent with those reported by investigators in many other countries. Despite cefepime and cefpirome having been shown to be more active in vitro against the isolates than the other β-lactams tested, with the exception of imipenem, these agents should be used with caution as treatment of patients with infections caused by ESBL-producing strains, particularly when there are large numbers of bacteria (producing corresponding large amounts of β-lactamases) at the sites of infection. More than 50% of strains producing SHV-2 β-lactamases, but none of those producing SHV-5 enzymes, exhibited resistance to piperacillin/tazobactam, while only a minority of the SHV-2 producers were resistant to co-amoxiclav. Jacoby & Carreras observed that the β-lactamase activities were significantly lower in SHV-derivatives, particularly SHV-5, than in SHV-1-producing strains, presumably because the substitutions that broaden the spectra of SHV-1 β-lactamases at the same time lower the catalytic efficiency of extended-spectrum enzymes.

In conclusion, this study has shown that K. pneumoniae isolates in Hungary produce SHV-type ESBLs exclusively and are often resistant to multiple antibiotics, including non-β-lactams.

References

Comparison of the 5 μg disc and the Neo-Sensitab for determining the susceptibilities of Staphylococcus aureus isolates to mupirocin


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Sir,

Mupirocin is a topical antibiotic that has excellent in-vitro activity against staphylococci (both methicillin-susceptible and -resistant) and streptococci. It is currently available in three formulations: a polyethylene glycol-based ointment for treating skin infections; a soft paraffin base for the eradication of Staphylococcus aureus nasal colonization; and a cream formulation that has recently been licensed in the USA for the treatment of patients with infected traumatic skin lesions.

The susceptibilities of clinical isolates to mupirocin can be determined by a variety of methods, including disc
Correspondence

diffusion, agar or broth dilution and the Etest,\(^1\) and interpretative criteria for defining the susceptibility categories of S. aureus strains causing infections, as determined by the National Committee for Clinical Laboratory Standards (NCCLS)-recommended disc diffusion method with a 5 \(\mu\)g disc, have been published.\(^2\) In addition, studies are currently under way to determine breakpoints for defining the susceptibility categories of isolates colonizing the nares; staphylococci with MICs up to 256 mg/L can be eliminated from these sites following the application of standard dosages of mupirocin.\(^3\)

Neo-Sensitabs are susceptibility test discs that are produced from compressed compound. The purpose of this study was to identify interpretative criteria for the mupirocin Neo-Sensitab and to compare the results of susceptibility testing with this disc with those obtained with the standard 5 \(\mu\)g mupirocin disc.

Ninety-seven S. aureus isolates with MICs ranging from \(\leq 0.06\) to \(>1024\) mg/L were included in the study, although an effort was made to select strains with MICs close to the breakpoint for susceptibility to mupirocin (\(\leq 4\) mg/L). Included amongst these were methicillin-resistant and -susceptible and \(\beta\)-lactamase-positive and -negative strains. Susceptibility was determined by the disc diffusion method with the 5 \(\mu\)g mupirocin disc (BBL, Cockeysville, MD, USA) and by an agar dilution method, in both cases according to recommendations of the NCCLS,\(^4,5\) and with Neo-Sensitabs containing 10 \(\mu\)g of mupirocin (Rosco, Taastrup, Denmark) which were used according to the manufacturer’s instructions. S. aureus ATCC 25923 was used as a control. Susceptibility was defined in terms of the following interpretative criteria: for the 5 \(\mu\)g disc, a zone of inhibition \(\geq 14\) mm;\(^2\) and for the Neo-Sensitab, a zone of inhibition \(\geq 18\) mm (as recommended by the manufacturer). The NCCLS guidelines for determining breakpoints with a scattergram\(^6\) specify that the proposed diameter of the zone of inhibition should be adjusted until results obtained with the disc diffusion method that are wrongly categorized as susceptible (very major errors) or resistant (major errors) are minimal. Very major errors should be detected with a frequency of <1.5% and major errors with a frequency of <3%.

The scattergram depicting the MICs of mupirocin as determined by the agar dilution method versus the zone diameters obtained with the Neo-Sensitabs is shown in the Figure. For the analysis of correlation, isolates with MICs \(\leq 0.06\) mg/L and those for which there were no zones of inhibition were eliminated from the calculations. For the remaining 49 isolates, the Pearson correlation coefficient was 0.927 for the Neo-Sensitab versus MICs.

Nine major errors were detected with the Neo-Sensitabs when the \(\geq 18\) mm breakpoint was used; this represents a major error rate of 9.3%. From the Figure, it can be seen that, if a breakpoint of \(\geq 15\) mm is used, the number of major errors would be reduced to two (2%) which is within the suggested limit.

The modified zone diameter breakpoints were used to compare the results obtained with the Neo-Sensitab and the 5 \(\mu\)g disc. There were four discrepancies: two isolates were susceptible according to both the Neo-Sensitab and the MIC, but resistant according to the 5 \(\mu\)g disc; one was susceptible according to both the 5 \(\mu\)g disc and the MIC, but resistant according to the Neo-Sensitab; and one was resistant according to both the Neo-Sensitab and the 5 \(\mu\)g disc, but susceptible according to the MIC. The MICs for all four of these isolates were 4 mg/L which places them in the susceptible range.

The two diffusion methods appear to be reliable screening tools for the detection of isolates resistant to mupirocin. However, for strains with inhibition zone

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**Figure.** Scattergram of mupirocin MICs versus inhibitory zone diameters obtained with the Neo-Sensitab (n = 97). The double horizontal line represents the MIC breakpoint for susceptibility, the double vertical line represents the susceptibility breakpoint recommended by the manufacturer (\(\geq 18\) mm) for the Neo-Sensitab and the single vertical line represents the proposed breakpoint (\(\geq 15\) mm).
The high activity of co-amoxiclav suggests an isolate as resistant by the disc diffusion method and because this method does not differentiate between high- and low-level mupirocin-resistant isolates.

In conclusion, the mupirocin Neo-Sensitab is a reliable method of determining the susceptibilities of S. aureus isolates to this agent. The proposed breakpoints for strains causing infections are as follows: for the agar dilution test, susceptible \( \leq 4 \) mg/L and resistant \( \geq 8 \) mg/L; and for the Neo-Sensitab, susceptible \( \geq 15 \) mm and resistant \( \leq 14 \) mm.

**References**


The in-vitro activities of co-amoxiclav and other oral antibiotics against *Streptococcus pneumoniae* isolates exhibiting intermediate susceptibility to penicillin

**J** Antimicrob Chemother 1998; **42**: 405–406

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**a**The Cleveland Clinic Foundation, Cleveland, O H, USA; **b**GR MICRO Ltd, London, UK

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Sir,

The results of an ongoing, prospective, international, multicentre antibiotic susceptibility study (the A lexander Project), published in this Journal and elsewhere, have demonstrated that both amoxycillin and co-amoxiclav are more active in vitro than benzylpenicillin against strains of *Streptococcus pneumoniae* isolated from patients with community-acquired respiratory tract infections. The study also found that the activity of amoxycillin was greater than that of cephalosporins (cefaclor, cefuroxime and cefixime), macrolides, co-trimoxazole, ciprofloxacin and ofloxacin against *S. pneumoniae* strains exhibiting intermediate susceptibility to penicillin (Pen-I). A series of investigators participating in the Alexander Project, we report here recently compiled project data on the in-vitro activities of co-amoxiclav and other antibiotics used as oral therapy of patients with community-acquired respiratory tract infections against Pen-I strains isolated in the USA.

Between 1992 and 1996, 66 Pen-I strains recovered from either blood cultures or sputa were collected in five centres in the USA. On the basis of MIC susceptibility breakpoints recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (or, in the case of ciprofloxacin, a breakpoint of \( \leq 1 \) mg/L recommended by the Food and Drug Administration), the susceptibilities of the isolates to various antibiotics were as follows: co-amoxiclav, 92%; amoxycillin, 89%; erythromycin, azithromycin and clarithromycin, 85%; ciprofloxacin, 85%; cefuroxime, 68%; and co-trimoxazole, 52%. The corresponding data for cefprozil, cefixime and cefaclor could not be determined as NCCLS breakpoints for these agents have not yet been published.

The high in-vitro activity of co-amoxiclav against Pen-I strains demonstrated here is in accord with the results of an earlier study undertaken by Korgenski et al. who found that, overall, the MICs of this agent were lower than those of benzylpenicillin against Pen-I strains isolated from patients with respiratory tract infections at a centre in the USA. Recent surveillance studies in the USA have shown that 25% of strains of *S. pneumoniae* causing lower respiratory tract infections in outpatients and 39–42% of those causing upper respiratory tract infections are resistant to penicillin; many of these strains, especially those causing infections that are usually associated with children, such as acute otitis media, exhibited intermediate susceptibility. The high activity of co-amoxiclav suggests that this agent may be the preferred oral antibiotic for use as empirical therapy of patients with acute otitis media or other community-acquired respiratory tract infections frequently caused by Pen-I strains and/or \( \beta \)-lactamase-producing strains of *Haemophilus influenzae* or *Moraxella catarrhalis.*

**References**

38. Supplementary A. 71–84.

An LY 333328-dependent strain of Enterococcus faecalis isolated from a blood culture


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Newham District Microbiology Laboratories, St Andrews Hospital, Devon Road, Bow, London E 3 3NT, UK

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Sir,

Further to the report by van Tiel & van den Bogaard¹ that LY 333328 (Eli Lilly Co., Indianapolis, IN, USA), a novel N-alkyl glycopeptide derivative, is more active in vitro than either vancomycin or teicoplanin for growth. It was also observed that the density of growth was progressively greater in proximity to increasing concentrations of vancomycin or teicoplanin. In contrast to previous reports,²⁻⁵ our strain did not grow in the presence of precursors of peptidoglycan biosynthesis. Growth was, however, supported by LY 333328 at concentrations ≥4 mg/L. The bacterium exhibited a high instability rate, with spontaneous glycopeptide-independent revertants being isolated at a frequency of 2.03 × 10⁷/h. It is not possible to determine the MIC of an antibiotic on which an organism is dependent for growth, but the MICs of vancomycin and teicoplanin for the revertant (1024 mg/L and 256 mg/L, respectively) match the criteria of Woodford et al.⁷ and are consistent with the VanA phenotype. The presence of the vanA gene in our isolate was confirmed by PCR at the Antibiotic Reference Unit, Central Public Health Laboratory (Colindale, UK).

Glycopeptides act by inhibiting cell wall (peptidoglycan) synthesis by binding non-specifically to saturate the outermost layer of the bacterial peptidoglycan structure. They then bind to the terminal D-Ala-L-Ala residues on the pentapeptide side chains of the peptidoglycan precursors. This interferes with elongation of the peptidoglycan backbone of the cell wall, resulting in inhibition of transglycosylation. Resistance has been attributed to the substitution of D-Ala-L-Ala with D-Ala-D-Lac for which glycopeptides have reduced affinities. Previous investigators⁸⁻¹⁰ have confirmed that VDE lack the VanA phenotype (i.e. dependence on or resistance to vancomycin and susceptibility to teicoplanin).³⁴ Our report questions the suggestion of van Tiel & van den Bogaard¹ that LY 333328 can be used as treatment for patients with infections caused by vancomycin-resistant enterococci (VRE).

A 36-year-old man with longstanding renal failure secondary to insulin-dependent diabetes mellitus attended our Accident and Emergency Department complaining of vomiting and abdominal pain. He had been admitted to the hospital on many occasions in the past and had received multiple courses of either vancomycin or teicoplanin. An isolate from a blood culture obtained from the patient grew exclusively around the vancomycin and teicoplanin discs; it was identified as a strain of E. faecalis by the Rapid ID 32A Strep system (bioMérieux, Basingstoke, UK), which does not depend on growth of the bacterium for identification. (A vancomycin-dependent enterococcus (VDE) had previously been isolated from blood cultures from this patient when he presented with a line-related infection following haemodialysis at another centre.⁹) Determination of vancomycin and teicoplanin MICs by the Etest method (Cambridge Diagnostics Ltd, Cambridge, UK) confirmed that the strain was a GDE requiring a minimum of 16 mg/L of vancomycin or 0.125 mg/L of teicoplanin for growth. It was also observed that the density of growth was progressively greater in proximity to increasing concentrations of vancomycin or teicoplanin. In contrast to previous reports,²⁻⁵ our strain did not grow in the presence of precursors of peptidoglycan biosynthesis. Growth was, however, supported by LY 333328 at concentrations ≥4 mg/L. The bacterium exhibited a high instability rate, with spontaneous glycopeptide-independent revertants being isolated at a frequency of 2.03 × 10⁷/h. It is not possible to determine the MIC of an antibiotic on which an organism is dependent for growth, but the MICs of vancomycin and teicoplanin for the revertant (1024 mg/L and 256 mg/L, respectively) match the criteria of Woodford et al.⁷ and are consistent with the VanA phenotype. The presence of the vanA gene in our isolate was confirmed by PCR at the Antibiotic Reference Unit, Central Public Health Laboratory (Colindale, UK).

Glycopeptides act by inhibiting cell wall (peptidoglycan) synthesis by binding non-specifically to saturate the outermost layer of the bacterial peptidoglycan structure. They then bind to the terminal D-Ala-L-Ala residues on the pentapeptide side chains of the peptidoglycan precursors. This interferes with elongation of the peptidoglycan backbone of the cell wall, resulting in inhibition of transglycosylation. Resistance has been attributed to the substitution of D-Ala-L-Ala with D-Ala-D-Lac for which glycopeptides have reduced affinities. Previous investigators⁸⁻¹⁰ have confirmed that VDE lack...
the functional native enzyme d-Ala-d-Ala ligase. Vancomycin induction of VanA or VanB ligase is thought to compensate for the absence of the native ligase by producing UDP-MurNAc-tetrapeptide-d-Lac, thereby allowing cell wall synthesis to proceed. This conclusion was reached because of the growth of VDE when supplemented with the dipeptide d-Ala-d-Ala in the absence of vancomycin.\(^3\)\(^5\)

The strain of GDE described here is unique, not only because of its resistance to LY 333328, but also because of its dependence on it for growth. Furthermore, the bacterium grew in the presence of glycopeptide antibiotics, but not in the presence of peptidoglycan precursors. The genetic mechanisms that are the basis of glycopeptide dependence in these mutants remain to be elucidated.

**Acknowledgement**

We are grateful to Dr N. Woodford of the Antibiotic Reference Unit, Central Public Health Laboratory, who kindly carried out the PCR work.

**References**


**In-vitro activity of N,N-dimethyl-2-propen-1-amin against Mycobacterium tuberculosis**


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Sir,

The magnitude of endemic tuberculosis is increasing and it is estimated that 1.7 billion people worldwide are infected with *Mycobacterium tuberculosis*.\(^1\) In addition to an increase in the number of cases, the increasing incidence of multidrug-resistant tuberculosis is one of the major public health challenges in the 1990s.\(^2\) The interaction between acquired immunodeficiency syndrome (AIDS) and tuberculosis has a major impact on the long-term survival of AIDS patients.\(^3\) Consequently, there is an urgent need to develop antimycobacterial drugs which may be more effective than the chemotherapy currently in use.

Recently, a series of N,N-dimethyl-2-propen-1-amine derivatives, 3-(4′-bromo[1,1′-biphenyl]-4-yl)-3-(4′-X-phenyl)-N,N-dimethyl-2-propen-1-amine, where X is H, Cl, Br, I, CH₃, OCH₃, SO₂CH₃ or NO₂, with excellent trypanocidal activities has been synthesized.\(^4\)\(^-\)\(^5\) These derivatives are substituted at the 4-position on the phenyl moiety and are obtained as a mixture of the E/Z isomers (nearly 1:1). In this study, we assayed the in-vitro activity of the 4-bromo derivative (X = 4-Br) and the unsubstituted derivative (X = 4-H), and their geometric E and Z isomers against *M. tuberculosis* (Figure).

The strains of *M. tuberculosis* (H37Rv, A TCC 25177) were grown in Middlebrook 7H9 broth (Difco, Detroit, MI, USA) at 37°C until the turbidity was equivalent to a McFarland no. 1 standard (3 × 10⁶ cfu/mL). In order to determine the MIC, stock solutions of the drugs were freshly prepared in dimethyl sulfoxide (Sigma) at 1 g/L and serial two-fold dilutions were prepared in 7H9 broth (Difco) to yield final concentrations of 2 to 256 mg/L.
These tubes and control tubes containing no drug were inoculated with approximately $1.5 \times 10^5$ cfu/mL of *M. tuberculosis* and were incubated at 37°C for 10 days. The microorganisms that grew in the presence and in the absence of the drugs were measured by the visual turbidity method and the MIC was taken as the lowest drug concentration that allowed no visible growth.

To determine the MBC we used the subculture in a Lowenstein–Jensen (Difco) medium with the dilutions of drugs that were equal to or higher than the MIC of each studied drug. The values of the MIC and MBC of *N,N*-dimethyl-2-propen-1-amine (X = H or Br) derivatives were 4 and 16 mg/L, respectively. The latter compound was twice as effective as the unsubstituted (X = 4-H) derivative, which had an MIC of 8 mg/L and an MBC of 32 mg/L. The MIC and MBC of the unsubstituted geometric Z isomer were 8 and 16 mg/L, respectively, while those of the E isomer were 16 and 128 mg/L, respectively.

Our results indicated that the 2-propen-1-amine derivatives have good bacteriostatic and bactericidal activity against *M. tuberculosis*. The bromo substitution (X = 4-Br) is also important for the antibacterial activity of these compounds in the same way as has occurred in Chagas' disease.5 The unsubstituted Z isomer showed great potential in this regard and should thus be evaluated as a chemotherapeutic agent against *M. tuberculosis*. In-vitro studies have been used to measure the activity of a drug against extracellular bacilli. Since mycobacteria infect and multiply in macrophages, it is necessary to improve the correlation between in-vitro MICs and MBCs of these drugs to their intracellular activity in experimentally infected macrophages. New 2-propen-1-amine derivatives are being synthesized in order to increase the antibacterial capacities, and in-vivo studies of these compounds in models of tuberculosis are in progress.

**Aknowledgement**

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**References**


**Pharmacokinetics of cefuroxime in healthy volunteers: an update**

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Sir,

After two decades, cefuroxime continues to be prescribed widely as treatment of patients with a broad range of community-acquired infections, particularly those affecting the respiratory tract. Pharmacokinetic studies of parenteral cefuroxime were first published between 1976 and 1978.1–3 The present study was undertaken to update the pharmacokinetics of this drug following a single dose given by the intramuscular (im) route and to evaluate the effects of the co-administration of lidocaine on these pharmacokinetic parameters. A secondary objective was to compare the plasma antibiotic concentrations of...
Correspondence

Twelve healthy male volunteers (mean age 28.5 ± 5.4 years) were the subjects in this randomized, cross-over study. Each received cefuroxime 750 mg reconstituted with 3 mL of 1% lidocaine (regimen A), followed by a wash-out period of 4 days, and then a further dose reconstituted with 3 mL of water for injection (regimen B). Venous blood samples were obtained before the injection (t₀) and at 15, 30 and 45 min and 1, 1.15, 2, 3, 4, 6, 8, 10 and 12 h after the injection for HPLC and at t₀, 1, 2, 4, 6, 8, 10 and 12 h for the bioassay. The samples were collected in heparinized tubes which were centrifuged at 4°C; the plasma samples were immediately frozen and stored at -70°C until assayed. An agar diffusion method was used for the bioassay. The medium was Antibiotic medium 2 (Difco) and the test organism was Proteus mirabilis A TCC 21100. A standard curve was prepared with cefuroxime in normal human plasma at concentrations ranging from 0.125 to 8.0 mg/L and two controls of 3.2 mg/L and 0.4 mg/L were included with each assay. All tests were performed in triplicate and the results expressed as the means. The limit of sensitivity of the method was 0.2 mg/L and the coefficients of variation (CV) were 5.37% and 5% for the high and low controls respectively. For the HPLC method, extraction of samples by a chloroform-propanol mixture (60:20, v/v) at pH 1 was followed by back-extraction in buffer pH 7. Extracts were analysed by reverse-phase partition (Nucleosil C₁₈ column) and detection was by UV absorbance at 280 nm. CVs were not concentration-dependent and varied from 3.5% to 6.01%, while the detection limit of the assay was 0.05 mg/L. The pharmacokinetic parameters were calculated with a SİPHAR program (version 4.0, SIMED, Créteil, France). The parameters obtained by the two assay methods were compared by an analysis of variance and the level of significance was set at P = 0.05.

Peak plasma concentrations (Cmax) and other pharmacokinetic parameters (Table) were not significantly different for regimens A and B, indicating that lidocaine did not markedly affect the pharmacokinetics of cefuroxime. There was also no significant difference between the plasma concentrations determined by the two assay methods, thereby confirming the results of an earlier study in rabbits which also compared HPLC with a bioassay method. The CVs of the mean results for the bioassay were high in samples obtained soon after the injection, i.e., 83.2%, 62.5% and 41.7% for regimen A and 65.1%, 41.4% and 28.5% for regimen B at 15, 30 and 45 min respectively. They were also high with the HPLC method and this was probably related to inter-individual variation in the absorption phase. The CVs for later samples (after 8 h) were >60%, possibly because of low residual concentrations. In contrast, the CVs for samples obtained at intermediate times (between 1 and 6 h) were significantly lower for regimen B, suggesting a more homogeneous absorption and vascular distribution when cefuroxime was diluted in water compared with lidocaine. In previous studies, the elimination half-lives (t1/2) were 1.41 h and 1.37 ± 0.25 h after im injections of 750 mg, and 1.15 h following a 500 mg injection. These values did not differ significantly from those determined in the present study (t1/2 ranging from 1.4 ± 0.2 h to 1.6 ± 0.4 h). The values for the apparent total clearance (Cl/F), as determined by HPLC (14.6 L/h for regimen A and 14.0 L/h for regimen B), were consistent with previously published data (13.8 L/h and 12.6 L/h). In the present study, Cmax varied from 9.9 to 21.9 mg/L with the bioassay and from 9.9 to 33.5 mg/L with HPLC. The mean Cmax, which varied from 14.5 mg/L to 16.9 mg/L, differed from that reported by one previous group of investigators (34.9 mg/L), but was in accord with that reported by another (17.25 mg/L).

In conclusion, the updated pharmacokinetics of cefuroxime described here demonstrate that lidocaine does not significantly affect the pharmacokinetic para-

### Table

Mean ± sd (CV (%)) pharmacokinetic parameters following a single im injection of cefuroxime 750 mg diluted with lidocaine (regimen A) or water (regimen B), with the plasma concentration of cefuroxime determined by bioassay or HPLC

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>R regimen A</th>
<th>HPLC</th>
<th>R regimen B</th>
<th>Bioassay</th>
<th>HPLC</th>
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<tr>
<td>Cmax (mg/L)</td>
<td>14.5 ± 3.5 (24.4)</td>
<td>16.5 ± 7.2 (43.3)</td>
<td>15.3 ± 2.5 (15.2)</td>
<td>16.9 ± 4.4 (25.9)</td>
<td></td>
</tr>
<tr>
<td>AUC0–∞ (mg.h/L)</td>
<td>49.3 ± 5.3 (10.8)</td>
<td>52.4 ± 5.9 (11.3)</td>
<td>51.5 ± 7.9 (10.8)</td>
<td>54.3 ± 6.5 (12.1)</td>
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</tr>
<tr>
<td>CL/F (L/h)</td>
<td>1.6 ± 0.3 (18.8)</td>
<td>1.5 ± 0.2 (14.9)</td>
<td>1.6 ± 0.4 (23.9)</td>
<td>1.4 ± 0.2 (13.5)</td>
<td></td>
</tr>
<tr>
<td>Vd/F (L)</td>
<td>35.9 ± 8.5 (23.7)</td>
<td>31.2 ± 7.3 (23.4)</td>
<td>33.1 ± 9.1 (27.3)</td>
<td>29.1 ± 6.7 (23.1)</td>
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</tr>
<tr>
<td>MRT (h)</td>
<td>2.9 ± 0.7 (23.0)</td>
<td>2.8 ± 0.7 (26.0)</td>
<td>2.8 ± 0.5 (17.5)</td>
<td>2.6 ± 0.5 (17.5)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Cmax, peak plasma concentration; AUC0–∞, area under the concentration-time curve from zero to infinity; t1/2, elimination half-life; CL/F, total clearance on available fraction; Vd/F, volume of distribution; MRT, mean residence time.
meters of this antibiotic and that similar results are obtained with the bioassay and HPLC methods.

References


