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Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility

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Sir,

We describe a clinical strain of methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to vancomycin (MIC = 8 mg/L). The strain was isolated from a surgical wound infection which was refractory to vancomycin therapy.

In May 1996, a 4 month-old male infant underwent heart surgery for pulmonary atresia. Two weeks following surgery, the infant became febrile and developed a purulent discharge from the sternal surgical incision site; culture of the purulent material yielded MRSA. The patient was treated with vancomycin (45 mg/kg daily) for 29 days, but fever and discharge of pus continued, and the C-reactive protein (CRP) remained elevated (40 mg/L). The treatment was changed to a combination of vancomycin and arbekacin (an aminoglycoside approved for MRSA infection in Japan). After 12 days of this regimen, the purulent discharge subsided, the wound began to heal, and the CRP declined from 40 to 9 mg/L. The antimicrobial therapy was discontinued. However, 12 days later the surgical site appeared inflamed with the development of a subcutaneous abscess accompanied by a sudden onset of fever and a raised CRP level of 35 mg/L. Therapy was resumed with the combination of arbekacin and ampicillin/sulbactam which has been shown to have synergic activity against MRSA.¹ After 6 days of therapy, the patient's fever had subsided and CRP declined below detectable levels (< 3 mg/L). During the next few days, however, the CRP level fluctuated between < 3 and 10 mg/L, suggesting persistence of infection. Debridement of the subcutaneous abscess was performed and the patient was discharged from the hospital after a further 17 days of therapy with arbekacin and ampicillin/sulbactam. CRP remained below detectable levels.

The MRSA strain (Mu50), which was isolated from the purulent discharge at the sternal incision site and from the debridement sample, had a vancomycin MIC of 8 mg/L by the broth microdilution method.² Vancomycin has the most reliable antimicrobial activity against MRSA. The emergence of resistance to vancomycin in *S. aureus* has been predicted^{3,4} based on the high levels of resistance to vancomycin in enterococci and because transfer of the *vanA*-containing plasmid from enterococci into *S. aureus* has been demonstrated.⁵ *S. aureus* strain Mu50 did not carry *vanA* or *vanB* genes as judged by PCR amplification of DNA. The exact mechanism of the organism's reduced susceptibility to vancomycin remains to be determined but it may be due to an intrinsic mechanism of augmented cell-wall synthesis. This is inferred from three findings (data not shown): the cell wall appeared twice as thick as the wall of control strains on electron microscopy; there was a three-fold increase in the production of both penicillin-binding protein (PBP) 2 and PBP2' as measured by Western blotting; and a three-fold increase, as judged by HPLC analysis, in production of cell wall murein precursors compared with vancomycin-susceptible MRSA strains (MIC \leq 2 mg/L). Low-level vancomycin resistance (MICs 8–16 mg/L) has been reported in clinical isolates of coagulase-negative staphylococci,^{6–8} but Mu50 is the first clinical strain of *S. aureus* to demonstrate this level of vancomycin resistance. In Juntendo University Hospital, strains with reduced vancomycin susceptibility showing pulsed-field gel electrophoresis patterns identical or similar to Mu50, have now been found in 2% of all MRSA isolates. Prolonged vancomycin therapy or, as in this case, combination therapy with other antimicrobial agents is necessary for infections caused by such strains. The situation reinforces the recommendation of the Centers for Disease Control and others to test all strains of staphylococci for resistance to vancomycin, and to use vancomycin prudently.³ Further work on the population analysis and mechanisms of resistance in these strains is proceeding.

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lysogenization can affect tolerance to vancomycin. The phage-free standard *S. aureus* NCTC 8325-4 strain was used. The bacteriophages used for lysogenization of the NCTC 8325-4 strain originated from five hospital isolates and two standard strains with or without tolerance to vancomycin (Table). The bacteriophages obtained were designated by the addition of ϕ to the parent strain number. All induced bacteriophages had been previously characterized in this laboratory.^{2,3} All these phages caused positive lysogenic conversion of staphylokinase. Three bacteriophages belonged to serological group A, two to serological group B and two to serological group F.

After lysogenization, seven different derivatives of *S. aureus* NCTC 8325-4 were obtained, each possessing one other phage integrated into the chromosome (Table).

The MIC and MBC of vancomycin as well as the MBC:MIC ratio were evaluated for all lysogenic derivatives, to determine the presence of tolerance. The MIC and MBC were determined by a macro-dilution method.⁴ The results were compared with those for the parent strains (Table).

The lysogenic derivatives of *S. aureus* NCTC 8325-4 as well as the maternal strains had similar MIC values (0.5–1.0 mg/L). This indicated that the lysogenization process did not influence the strain susceptibility to the bacteriostatic action of this antibiotic. Much more diverse results were

Lysogenic conversion as a factor influencing the vancomycin tolerance phenomenon in *Staphylococcus aureus*

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Sir,

Vancomycin is the drug of first choice in the treatment of MRSA and no naturally occurring strains of *Staphylococcus aureus* resistant to the bacteriostatic action of vancomycin have been detected although naturally occurring strains resistant to the bactericidal action of vancomycin have been described.¹ However, strains of vancomycin-resistant staphylococci have been obtained in the laboratory by serial passage in increasing vancomycin concentrations or conjugal membrane transfer of vancomycin resistance genes from *Enterococcus faecalis*. The objective of this study was to check whether the genetic modification of *S. aureus* strains related to bacteriophage

Table. MICs and MBCs of vancomycin for *S. aureus* NCTC 8325-4 and its derivatives lysogenized with staphylokinase-converting prophages

Strain	MIC (mg/L)	MBC (mg/L)	MBC/MIC
Standard strains			
NCTC 8325	1.0	16.0	16
NCTC 8325-4	1.0	8.0	8
PS 81	0.5	16.0	32
209P	1.0	4.0	4
Hospital strains			
M18	1.0	32.0	32
M21	1.0	32.0	32
M421-1	0.5	32.0	64
M507	1.0	16.0	16
M658-2	0.5	16.0	32
Lysogenized derivatives			
NCTC 8325-4 ϕ MPS81	1.0	32.0	32
NCTC 8325-4 ϕ M209P	1.0	4.0	4
NCTC 8325-4 ϕ M18	1.0	32.0	32
NCTC 8325-4 ϕ M21	1.0	32.0	32
NCTC 8325-4 ϕ M421-1	0.5	32.0	64
NCTC 8325-4 ϕ M507	1.0	16.0	16
NCTC 8325-4 ϕ M658-2	1.0	64.0	64

obtained for MBC values: these ranged from 4.0 to 64.0 mg/L of vancomycin. In the case of five derivatives, the MBC:MIC ratio ≥ 32 ; these were classified as tolerant strains. Such high increases in MBC values were observed in all derivative lysogenized with group A phages and in all lysogenized with group F phages. Among group A phages two were double-converting (SAK⁺, HLB⁻; ϕ M21, ϕ PS81) and one was triple-converting (SAK⁺, HLB⁻, SEA⁺; ϕ M421-1) (Table). The group F phages were double-converting (SAK⁺, HLB⁻; ϕ M18, ϕ M658-2), similar to those described by Coleman *et al.*⁵ The maternal strains from which the group A and F phages were induced were also found to be vancomycin-tolerant (Table). As a control, two derivatives lysogenized with group B phages induced from non-tolerant strains were examined. Those derivatives were also non-tolerant to vancomycin.

These results suggest that lysogenization status with some double-converting phages of serological group F and some double- or triple-converting phages of serological group A can influence tolerance of vancomycin phenomenon in *S. aureus*.

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Safety and efficacy of Intralipid emulsions of amphotericin B

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Sir,

Sievers *et al.*¹ recently reviewed the English literature in order to evaluate the efficacy and toxicity of Intralipid amphotericin B (IL-AmB) in the treatment of patients with systemic fungal infections. They concluded that the efficacy of IL-AmB in this clinical setting had not been convincingly demonstrated owing to the paucity of patients who had received treatment for documented fungal infections—a conclusion with which we concur. On the other hand, we cannot agree with their conclusion that the case for Intralipid improving the tolerability and safety of amphotericin B (AmB) has not been made. These authors have challenged the validity of those studies which compared IL-AmB with dextrose amphotericin B (D-AmB) on the grounds that the concentrations of AmB in the dextrose infusions were greater than those recommended. However, in contrast with the infusion duration, the concentration of AmB has never been shown to influence either the incidence of infusion-related adverse events or renal tolerance. Moreover, although a moderate degree of nephrotoxicity appears possible with IL-AmB, studies conducted by Caillot *et al.*,² Chavanet *et al.*³ and Moreau *et al.*⁴ have demonstrated convincingly that renal tolerability is better with IL-AmB than with D-AmB.

Sorkine *et al.*⁵ recently confirmed the reduced risk of nephrotoxicity with IL-AmB in a study in which AmB in a daily dosage of 1 mg/kg was administered in either 5% dextrose in water (prepared according to the manufacturer's instructions and giving a final concentration in the infusate of 100 mg/L) (group A) or in 250 mL 20% Intralipid (group B) to patients with infections caused by *Candida albicans*. There were 30 patients in each group. The mean cumulative dosages for the groups were similar (535 mg in group A and 642 mg in group B). Group A patients experienced a significantly higher incidence of infusion-related adverse events, including fever, rigors and a decrease in mean arterial pressure. The incidence of nephrotoxicity was also higher in patients receiving D-AmB, the serum creatinine concentration increasing from a mean baseline of 79.6 μ mol/L to a mean peak of 221 μ mol/L in group A, compared with an increase from a

mean baseline of 61.9 $\mu\text{mol/L}$ to a mean peak of 123.8 $\mu\text{mol/L}$ in group B. In addition, patients who received IL-AmB required less sodium and potassium supplementation than those treated with D-AmB. The efficacies of the two formulations were comparable.

The rate at which IL-AmB is infused may influence tolerability. Schöffski *et al.*⁶ compared D-AmB, suspended and infused according to the manufacturer's recommendations, with AmB diluted in 250 mL of 20% Intralipid. In both groups the dosing regimen consisted of 0.25 mg/kg administered on day 1 and 0.75 mg/kg administered on days 2–8 and on alternate days thereafter; the formulations were infused over 1 h. Sudden dyspnoea was observed in one patient who received D-AmB and in eight given IL-AmB. No significant nephrotoxicity was reported in respect of either group, probably because of the low cumulative dosages of both formulations and the alternate daily administration after day 8. The unexpected pulmonary toxicity may have been related either to the rapid infusion of a large amount of lipid or to the aggregates created by combining AmB with the lipid. The results of this study suggest that IL-AmB should be infused over a period exceeding 1 h.

We believe that comparisons of therapeutic indices may be more appropriate than comparisons of the tolerabilities of the same daily or cumulative dosage. In a pilot study, Joly *et al.*⁷ demonstrated that IL-AmB in a dosage of 1 mg/kg/day was well tolerated and effective (mycological cure rate of 62%) in AIDS patients with cryptococcal meningitis. Increasing the daily dosage to 1.5 mg/kg was associated with nephrotoxicity in five of the six patients. The investigators then compared the tolerabilities and efficacies of D-AmB 0.7 mg/kg/day (infused over 6 h) and AmB 1 mg/kg/day in 125 mL Intralipid (infused over 2 h) in 44 and 46 AIDS patients with cryptococcal meningitis respectively. The efficacies of the two formulations were comparable, with clinical cure or improvement noted in 69.2% of patients who received D-AmB and 73.8% of those who were given IL-AmB; analysis of the time to the first negative cerebrospinal fluid culture showed a nearly significant difference that favoured IL-AmB ($P = 0.07$). The incidence of infusion-related adverse events was lower in patients treated with IL-AmB, and none of the patients given IL-AmB required the concomitant administration of hydrocortisone, compared with 40 of the 44 who received D-AmB. The percentage of patients with raised serum creatinine concentrations was significantly higher in the IL-AmB group than in the D-AmB group, but the median baseline creatinine concentration was also higher in the former than in the latter (113 $\mu\text{mol/L}$ versus 98 $\mu\text{mol/L}$). Irrespective of whether the baseline values were normal or elevated, the time to the onset of an increased serum creatinine concentration ($>150 \mu\text{mol/L}$) was significantly shorter in patients who received IL-AmB than in those who were given D-AmB. This suggests that combining

AmB with Intralipid does not improve the therapeutic index in patients with cryptococcal meningitis treated with this formulation.

None the less, we believe that IL-AmB warrants further evaluation as therapy of patients with other invasive fungal infections, as well as those with visceral leishmaniasis. Thakur⁸ compared AmB in Intralipid 10% (infused over 2 h) with D-AmB (infused over 4 h) in 22 patients with kala-azar. The dosage was progressively increased from 0.05 mg/kg/day to 1 mg/kg/day to give a total dosage of 20 mg/kg in 25 daily doses. All patients were cured parasitologically, irrespective of the formulation, but the incidence of infusion-related adverse events was significantly lower in the IL-AmB group than in the D-AmB group. Transient increases in the serum creatinine concentrations were observed in three patients who received D-AmB and in one given IL-AmB. The investigators concluded that while IL-AmB is associated with fewer infusion-related adverse events, the greater cost may discourage its use.

Although patients with kala-azar are rarely seen in the north-east of France, we have successfully treated a patient with this disease with IL-AmB.⁹ Unlike Thakur,⁸ we chose to give a higher daily dosage of AmB by diluting it in Intralipid. The patient, who received a dosage of 1 mg/kg on day 1 and 2 mg/kg on days 2–7, improved rapidly and was discharged one day after completing therapy. Parasitological cure was confirmed and no relapse occurred during the 14 month follow-up period. However, it was necessary to increase the infusion time from 4 h on days 1 and 2 to 8 h on the subsequent days because the patient experienced a grade 2 fever. The patient's serum creatinine concentration remained within the normal range throughout the course of treatment, but the creatinine clearance decreased from 118 mL/min before therapy was initiated to 81 mL/min on completion of the course. Although the renal function recovered within 5 days of discontinuing therapy, it appears that this short course of high-dosage IL-AmB was associated with mild nephrotoxicity. Transient, low-level hepatotoxicity (grade 2 increase in the serum transaminase concentration) was also observed. Whilst acknowledging that this is only a single case report, we believe that high-dosage IL-AmB as treatment of patients with kala-azar warrants further evaluation, if only because the marked decrease in the length of hospital stay is likely to be associated with a corresponding reduction in the overall management costs.

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Macrolide resistance among *Streptococcus pneumoniae* and *Streptococcus pyogenes* isolates from out-patients in the USA

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Sir,

Erythromycin, clarithromycin and azithromycin are similar in terms of their in-vitro spectra of activity against Gram-positive bacteria.^{1–3} Since they became available, the newer macrolide and azalide compounds have been used more commonly for the treatment of out-patients with respiratory tract infections, especially those who are

allergic to penicillins. At the same time, studies have documented the increasing prevalence of penicillin-resistant pneumococci,^{2,3} a situation that underscores the need for alternative therapeutic agents. The objective of this investigation was to evaluate the susceptibilities of clinical isolates of *Streptococcus pneumoniae* and *Streptococcus pyogenes* to erythromycin, clarithromycin and azithromycin.

During the winter of 1993–1994, consecutive, non-replicate, respiratory tract isolates from patients attending out-patient clinics in 12 different medical centres throughout continental USA were collected at this institute. This exercise yielded 333 strains of *S. pyogenes*, 197 from children of ≤ 12 years of age and 136 from adults, and 260 of *S. pneumoniae*, 100 from children and 160 from adults.

The susceptibilities of the isolates to erythromycin, clarithromycin and azithromycin were determined by a broth microdilution method according to a protocol of the National Committee for Clinical Laboratory Standards (NCCLS);⁴ susceptibility to benzylpenicillin was determined concurrently. All four drugs were serially diluted in cation-adjusted Mueller–Hinton broth containing 2–3% lysed horse blood. The microdilution trays were inoculated with approximately 5×10^8 cfu/L and then incubated at 35°C in an atmosphere without additional CO₂. After 20–24 h, the MICs were recorded as the lowest concentration permitting no visible growth; the interpretative breakpoints were those defined by the NCCLS⁴ i.e. for erythromycin and clarithromycin, susceptible ≤ 0.25 mg/L and resistant ≥ 1.0 mg/L, and for azithromycin, susceptible ≤ 0.5 mg/L and resistant ≥ 2 mg/L.

All of the *S. pyogenes* strains were shown to be highly susceptible to penicillin (MIC ≤ 0.06 mg/L). Of the pneumococci, on the other hand, only 62% of isolates from children and 82% of those from adults were susceptible to this antibiotic; 8% exhibited high-level penicillin resistance (MIC ≥ 2.0 mg/L), while the remaining strains (27% and 11% of those from children and adults respectively) were of intermediate susceptibility (MIC = 0.12–1.0 mg/L). These prevalence figures are consistent with those recently reported by Doern *et al.*² for the USA.

The susceptibilities of the respiratory tract pathogens to erythromycin, clarithromycin and azithromycin are shown in the Table. Of the *S. pyogenes* isolates, only 2% or fewer were resistant to all three macrolides. Similar prevalence figures have been reported from Spain.⁵ Of the penicillin-susceptible strains of *S. pneumoniae*, between 1% and 3% were resistant to each macrolide, compared with approximately 16% that exhibited intermediate susceptibility and approximately 57% that were penicillin-resistant. Soriano & Fernandez-Roblas⁶ have made similar observations in respect of isolates from Spain.

The increasing prevalence of penicillin-resistant pneumococci is causing serious therapeutic problems in

Table. In-vitro activities of two macrolides and an azalide against clinical isolates of *S. pyogenes* and *S. pneumoniae*

Bacterium (n)	Antibiotic (mg/L)	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	Range	% Resistant strains ^a
<i>S. pyogenes</i> (333)	erythromycin	0.03	0.06	0.016–>32	2.0
	clarithromycin	0.03	0.03	0.016–>32	1.8
	azithromycin	0.12	0.12	0.03–>32	2.0
<i>S. pneumoniae</i> (260)	penicillin-susceptible (190)	erythromycin	0.03	0.008–8.0	3.1
		clarithromycin	0.03	0.004–4.0	1.1
		azithromycin	0.06	0.008–8.0	2.6
	penicillin-intermediate (49)	erythromycin	0.03	0.016–>32	16.3
		clarithromycin	0.03	0.016–>32	16.3
		azithromycin	0.06	0.03–>32	16.3
	penicillin-resistant (21)	erythromycin	4.0	0.016–>32	57.1
		clarithromycin	2.0	0.016–>32	57.1
		azithromycin	8.0	0.03–>32	57.1

^a According to the following breakpoints: erythromycin and clarithromycin, MIC \geq 1.0 mg/L; and azithromycin, MIC \geq 2.0 mg/L.

many communities. Although the newer macrolides have been shown in this study to be highly active *in vitro* against *S. pyogenes* isolates and penicillin-susceptible pneumococci, macrolide resistance among the penicillin-resistant pneumococcal strains was common, and this is a major cause of concern.

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In-vitro susceptibilities of multiresistant strains of *Acinetobacter baumannii* to eight quinolones

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Sir,
Acinetobacter baumannii is an important nosocomial pathogen which may cause severe infections, particularly in intensive care unit patients.¹ In recent years, several

outbreaks of nosocomial infections caused by multiresistant strains of *A. baumannii* have been described. Until the late 1980s, fluoroquinolones exhibited good in-vitro activities against clinical isolates of these organisms.² Since then, however, the proportion of susceptible strains has declined rapidly to the extent that most of the isolates in our hospital are currently resistant to commercially available fluoroquinolones.

In the past few years, fluoroquinolones with both broader spectra of activity and increased activities against certain bacterial species have been developed. The purpose of this study was to compare the activities of the original fluoroquinolones with those of novel fluoroquinolones against multiresistant strains of *A. baumannii*.

Thirty clinical isolates recovered from different patients in the University Hospital of Seville between 1991 and 1995 were collected. The sources of the isolates were as follows: bronchoalveolar lavage fluid (16 strains), wound exudate (five), urine (three), pleural fluid (three), blood (two) and a catheter tip (one). Preliminary identification was made using the PASCO system (panel 6P; Difco, Detroit, MI, USA) and identification to species level was performed by biotyping according to the method of Bouvet & Grimont.³ On the basis of breakpoints recommended by the National Committee for Clinical Laboratory Standards (NCCLS),⁴ the isolates were shown to be resistant to amikacin, cefotaxime and ceftazidime (except for two strains for each of which the MIC was 8 mg/L); seven strains were also resistant to imipenem (MIC \geq 16 mg/L).

MICs were determined by a broth microdilution method that conformed with NCCLS guidelines. The antibiotics tested were nalidixic acid (Sigma, Madrid, Spain), norfloxacin (Sigma), ciprofloxacin (Bayer, Leverkusen, Germany), ofloxacin (Hoechst, Barcelona, Spain),

pefloxacin (Rhône-Poulenc, Paris, France), sparfloxacin (Rhône-Poulenc), clinafloxacin (Parke-Davis, Ann Arbor, USA) and trovafloxacin (Pfizer, Groton, USA). The medium used was cation-adjusted Mueller-Hinton broth and the inoculum was $1-5 \times 10^8$ cfu/L. Following incubation for 20 h at 35°C, the MIC was taken as the lowest antibiotic concentration that allowed no visible growth. The resistance breakpoints were ≥ 2 mg/L for ciprofloxacin, pefloxacin, clinafloxacin, sparfloxacin and trovafloxacin, ≥ 4 mg/L for ofloxacin, ≥ 8 mg/L for norfloxacin and ≥ 32 mg/L for nalidixic acid. The breakpoints for ciprofloxacin, ofloxacin, norfloxacin and nalidixic acid were those recommended by the NCCLS,⁴ while the breakpoint chosen for the novel fluoroquinolones was that recommended for ciprofloxacin.

The susceptibilities of the *A. baumannii* isolates to the quinolones tested are shown in the Table. More than 93% were resistant to all of the commercially available agents (nalidixic acid, norfloxacin, ciprofloxacin, ofloxacin and pefloxacin). The percentages of strains resistant to the newer agents were markedly lower, i.e. sparfloxacin (66.7%), trovafloxacin (56.7%) and clinafloxacin (43.3%). Clinafloxacin, for which the MIC₉₀ was 2 mg/L, was the most active agent.

Resistance of *A. baumannii* to the fluoroquinolones has been attributed to changes in the structure of topoisomerase II or topoisomerase IV which are usually mediated by mutations in the *gyrA* or *parC* genes.⁵ Because *Acinetobacter* spp. are, in general, less permeable to antimicrobial agents than other aerobic Gram-negative bacilli,⁶ resistance to the quinolones can also result from alterations to the outer membrane that lead to decreased uptake. The basis of the increased activities of the novel fluoroquinolones has not yet been determined, but could, at least in part, be related to greater intrabacterial penetration, owing to the lower hydrophobicity of these drugs, or to an increased affinity for bacterial topoisomerases. Further studies are currently in progress to clarify this issue.

In conclusion, clinafloxacin and, to lesser extents, trovafloxacin and sparfloxacin, showed greater in-vitro activities against multiresistant strains of *A. baumannii* than currently available quinolones. In the light of the limited therapeutic options for the treatment of infections caused by such organisms, additional studies to evaluate the potential clinical uses of these drugs are warranted.

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Table. Susceptibilities of 30 clinical isolates of *A. baumannii* to eight quinolones

Antibiotic	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	Range (mg/L)	% Resistant strains ^a
Ciprofloxacin	32	128	0.03–128	93.3
Clinafloxacin	1	2	0.015–2	43.3
Trovafloxacin	2	16	0.015–16	56.7
Nalidixic acid	>32	>32	4–>32	93.3
Norfloxacin	>32	>32	4–>32	93.3
Ofloxacin	16	32	0.06–32	93.3
Pefloxacin	32	>32	0.125–>32	93.3
Sparfloxacin	4	8	0.015–16	66.7

^aAccording to the following breakpoints: ≥ 2 mg/L for ciprofloxacin, pefloxacin, clinafloxacin, sparfloxacin and trovafloxacin; ≥ 4 mg/L for ofloxacin; ≥ 8 mg/L for norfloxacin; ≥ 32 mg/L for nalidixic acid.

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Intracellular and extracellular killing of a penicillin-resistant, serotype-9 strain of *Streptococcus pneumoniae* by polymorphonuclear leucocytes in the presence of sub-inhibitory concentrations of clavulanic acid

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Sir,

In addition to its β -lactamase inhibitory activity, clavulanic acid possesses weak antibacterial activity, decreases the growth rate of *Staphylococcus aureus*¹ and causes alterations to non-penicillinase-producing strains of *S. aureus* that influence their susceptibilities to phagocytosis.² Several studies have failed to demonstrate any effect of β -lactam antibiotics on the bactericidal mechanisms of polymorphonuclear leucocytes (PMNLs),

although, at sub-inhibitory concentrations, these agents have been shown to cause alterations in bacterial morphology (by a poorly understood mechanism) that affect their susceptibilities to host defences.² We have investigated the bactericidal activity of sub-inhibitory concentrations of clavulanic acid in combination with human PMNLs against a penicillin-resistant, serotype-9 strain of *Streptococcus pneumoniae* (penicillin, amoxycillin, amoxycillin/clavulanic acid (2:1): MIC = 1 mg/L, MBC = 2 mg/L; clavulanic acid: MIC \geq 256 mg/L, MBC \geq 256 mg/L) in an attempt to explain the enhanced immunomodulatory activity of amoxycillin that was previously observed when it was combined with this β -lactamase inhibitor.³

Human peripheral PMNLs were harvested from untreated healthy donors, pooled and adjusted to give suspensions containing 10^{10} cells/L in gelatin-Hanks' balanced salt solution (HBSS) supplemented with calcium (1.2 mM) and magnesium (0.8 mM). The total numbers of leucocytes were determined with a Neubauer counting chamber and the viability of the PMNLs was verified by Trypan Blue exclusion, both at the beginning and the end of each experiment; the suspensions initially contained >95% viable cells. Bacterial suspensions in the logarithmic growth phase were incubated on a shaking water-bath (Lab-line Environ-shaker) until an absorbance at 580 nm of 0.11, measured spectrophotometrically with a Hitachi U-100 spectrophotometer, was reached. Aliquots of 200 μ L were added to sterile tubes containing one of the following: HBSS (control, K); HBSS containing 20% human serum (K + S); HBSS containing 20% human serum and PMNLs (3×10^8 /L) (K + S + PMNLs); HBSS containing 20% human serum and clavulanic acid at one of three concentrations (1, 2 or 4 mg/L) (K + S + C); and HBSS containing 20% human serum, PMNLs (3×10^8 /L) and clavulanic acid at one of three concentrations (1, 2 or 4 mg/L) (K + S + PMNLs + C). The volume was adjusted to 2 mL. The suspensions initially contained 3×10^9 cfu/L and the final ratio of the number of bacteria to the number of PMNLs was 10:1.

The tubes were incubated at 37°C in a shaking water-bath at 110 oscillations/min for 3 h, thereby precluding antibiotic leakage into the PMNLs as a consequence of the cellular damage associated with prolonged incubation.⁴ Aliquots of 100 μ L were removed after 1 h and 3 h and added to 9.9 mL of sterile water in order to disrupt the leucocytes and release the intracellular bacteria; this allowed both intracellular and extracellular killing to be measured. Ten-fold serial dilutions of the suspensions were made and 20 μ L of each dilution were dispensed on to blood agar plates. Following incubation for 18–20 h at 37°C in an atmosphere containing 5% CO₂, the numbers of colonies were counted. Each experiment was carried out in duplicate and the results expressed as the mean. Bactericidal activity was defined in terms of a change in the initial bacterial count and expressed as a

Table. Percentage changes in the initial counts of a strain of *S. pneumoniae* following incubation in the presence of PMNLs and/or clavulanic acid

Period of incubation	Percentage change in bacterial count							
	K	K + S	K + S + PMNL	K + S + C, 1 mg/L	K + S + C, 1 mg/L + PMNL	K + S + C, 2 mg/L	K + S + C, 4 mg/L	K + S + C, 4 mg/L + PMNL
1 h	-535.8	-535.8	-420.8	-56.7	66.5	57.8	-25.9	70.8
3 h	-5229.3	-10371.3	-8154.1	-3489.2	-205.5	2.28	-1396.3	25.0

A negative value indicates an increase in the viable count compared with the initial count.
K, control HBSS; S, 20% human serum; C, clavulanic acid.

percentage of the change in the initial bacterial count i.e. $\Delta\text{IBC} = 100 - (100 \times I_T/I_I)$ where I_T is the bacterial count after incubation at 37°C for 1 h or 3 h and I_I is the initial bacterial count.

The results are shown in the Table. In comparison with the control (broth only), the presence of serum caused a two-fold increase in the bacterial count after 3 h of incubation, while the presence of PMNLs exerted no additional effect over that of the serum. On the other hand, in the presence of clavulanic acid, the generation time of the pneumococcus increased, the growth rate declining in inverse relation to the concentration of the β -lactamase inhibitor. The presence of both PMNLs and clavulanic acid produced a reduction in the initial count of >50% after 1 h of exposure to each of the three concentrations tested. Regrowth was observed after incubation for 3 h, but the bacterial count following exposure to the highest concentration of clavulanic acid was still 25% of the initial count; regrowth was also lowest at this concentration.

By showing that sub-inhibitory concentrations of clavulanic acid (similar to those obtained *in vivo* following a 125 mg oral dose⁵) in the presence of human serum and PMNLs retard bacterial growth, we have confirmed that low concentrations of the β -lactamase inhibitor ($\leq 1/64 \times \text{MIC}$) possess immunomodulatory activity. This effect may account for the observation in a previous study, in which the highest achievable serum concentrations were tested, that the combination of amoxycillin, clavulanic acid and PMNLs caused a greater reduction in the initial count of the same strain of pneumococcus than amoxycillin and PMNLs in the absence of clavulanic acid.³ If these results can be confirmed with other penicillin-resistant pneumococcal strains of the same and different serotypes, they will suggest that co-amoxiclav might be of benefit in patients with infections requiring maximal bactericidal activity,⁶ such as those with bacteraemic pneumococcal pneumonia.

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Successful use of tetracycline as therapy of an immunocompromised patient with septicaemia caused by a vancomycin-resistant enterococcus

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Sir,

Vancomycin-resistant enterococci (VRE) were first detected in 1987 and have become increasingly common causes of nosocomial infections; by the end of 1995, 71 hospitals in the UK had reported isolating strains of VRE.¹ Effective therapy of patients with infections caused by these pathogens can be problematic as most isolates are resistant to multiple antibiotics. We report here the successful use of tetracycline to treat a bacteraemic episode caused by a vancomycin-resistant strain of *Enterococcus faecium* in a profoundly immunocompromised patient.

The patient, a 6-year-old male, had undergone allogeneic bone marrow transplantation for central nervous system relapse of acute lymphoblastic leukaemia. In the immediate post-transplantation period he received standard antimicrobial prophylaxis, comprising ciprofloxacin, acyclovir and itraconazole. Eight days after transplantation he became profoundly neutropenic and developed a pyrexia for which he was treated with piperacillin/tazobactam 90 mg/kg tds and gentamicin 4.5 mg/kg od. Therapy with teicoplanin 10 mg/kg bd was commenced on day 15 when he became febrile again. On

the following day, blood cultures taken from the Hickman line yielded a Gram-positive coccus which was subsequently identified as *E. faecium*. The Antibiotic Reference Unit, Central Public Health Laboratory, London, UK, confirmed that the isolate was resistant to benzylpenicillin, ampicillin, chloramphenicol, ciprofloxacin, erythromycin, rifampicin, teicoplanin and vancomycin, exhibited low-level resistance to gentamicin and was susceptible to tetracycline (MIC, 1 mg/L) and RP59500 (a combination of the two pristnamycin derivatives, dalfoprstin and quinupristin) (MIC, 1 mg/L). Despite removal of the central venous catheter the patient remained unwell and on the twenty-first day after transplantation, treatment with RP59500 160 mg tds (provided on compassionate grounds by Rhône-Poulenc Rorer) was administered in combination with gentamicin; blood and stool cultures obtained before this regimen was initiated yielded the strain of *E. faecium* isolated previously. The patient's temperature remained high and peripheral blood cultures taken 2 and 6 days into the course continued to yield VRE. In view of the in-vitro susceptibility of the pathogen to tetracycline, and after due consideration of the potential risks of this drug in children, he was commenced on iv tetracycline 12.5 mg/kg bd; all other antibiotic therapy was discontinued. Within 36 h the patient became afebrile and, over the next 48 h, the serum C-reactive protein concentration fell from 102 mg/L to 43 mg/L. Blood and stool cultures obtained 48 h after starting tetracycline did not yield VRE. The patient received iv tetracycline for 5 days and was maintained on oral doxycycline (with occasional periods of iv tetracycline when he was unable to tolerate oral therapy) for an additional 2 months. During his hospital stay, he experienced two further episodes of infection, but on neither occasion were VRE isolated from samples of blood or stool.

The treatment of patients with serious enterococcal infections has usually involved the administration of bactericidal combinations of antibiotics. Tetracyclines have been prescribed rarely because they have only bacteriostatic activities, although there has been one previous report of a patient with a presumed central line infection caused by a strain of VRE that responded to removal of the line and treatment with doxycycline.² Our patient was severely immunocompromised and, despite removal of the central line and the administration of appropriate antibiotics, blood cultures continued to yield VRE. The speed and magnitude of the clinical response to tetracycline were remarkable.

In 1995, the MICs of tetracycline for 131 of 463 (28.3%) strains of VRE submitted to the Antibiotic Reference Unit of the Central Public Health Laboratory were ≤ 1.0 mg/L (N. Woodford, personal communication). We suggest that tetracycline might be of benefit as treatment of some patients with serious infections caused by VRE.

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Prophylaxis of mycobacterial infection and acquired drug resistance

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Sir,

Disseminated infection caused by *Mycobacterium avium* is a hallmark of advanced disease in human immunodeficiency virus (HIV)-positive patients. In two recently published studies, the benefits of prophylaxis with one of two macrolides, clarithromycin or azithromycin, or the rifamycin derivative, rifabutin, were investigated.^{1,2} Although each regimen clearly led to a reduction in the risk of developing disseminated *M. avium* infection, a paradox, which unfortunately was not noted by the investigators at the time, has become apparent: all of the mycobacterial isolates subsequently recovered from patients in whom rifabutin prophylaxis had failed were susceptible to rifabutin,^{1,3} while varying percentages (11–58%) of the strains recovered from patients in whom macrolide prophylaxis had failed were resistant to these agents.^{1,2} What is the explanation for this discrepancy? Although some of the breakthrough infections in patients receiving either rifabutin or a macrolide can be attributed to non-compliance, it is likely that a fundamental difference between rifabutin and clarithromycin/azithromycin accounts for the remainder.

In attempting to resolve this issue, it is necessary to have an understanding of both the pharmacology of these antibiotics and their resistance mechanisms. Firstly, pre-treatment *M. avium* isolates are invariably susceptible to both clarithromycin and azithromycin,⁴ while a small proportion may exhibit innate, low-level resistance to rifabutin (or at least to the peak serum concentrations achieved *in vivo*).⁵ Secondly, acquired drug resistance is the result of single-step mutations; for rifamycins, mutations occur in the *rpoB* gene,⁶ while for clarithro-

mycin/azithromycin, they occur in the macrolide binding region of the 23S rRNA gene.⁷ This suggests that the chance of encountering a resistant strain is related to both the bacterial load and the mutational frequency; acquired drug resistance is therefore enhanced by monotherapy of mycobacterial infections. Thirdly, clarithromycin is readily absorbed from the gastrointestinal tract following oral administration, with only small amounts of the drug being excreted in the bile and faeces;⁸ 14-OH clarithromycin, the principal metabolite of clarithromycin, is much less active against *M. avium* than the parent compound. On the other hand, rifamycins, such as rifabutin, are poorly absorbed from the gastrointestinal tract,^{9,10} total faecal excretion of the parent compound and its derivatives being between 30% and 50%.

The gastrointestinal tract appears to be the main portal of entry of *M. avium*. In the light of the pharmacological properties of rifabutin, the mechanisms of resistance to this drug and the results of studies of prophylaxis, we hypothesize that, owing to its poor absorption and relatively high faecal excretion, rifabutin is present in sufficiently high concentrations to interfere with the multiplication of *M. avium* within the gastrointestinal tract, thereby reducing the bacterial load and, consequently, the probability of acquired drug resistance. Breakthrough bacteraemias caused by this organism might therefore be the result of either innate, low-level resistance or non-compliance. In contrast, the low concentrations of the macrolides in the gut are unlikely to affect the proliferation of *M. avium*, thereby permitting the organism to multiply locally and creating the appropriate conditions for mutations mediating macrolide resistance. Consistent with this hypothesis is the observation that macrolide-resistant strains of *M. avium* have been detected late (300–400 days) into prophylactic courses.^{1,2} If our hypothesis is subsequently confirmed, a non-absorbable macrolide acting locally in the gastrointestinal tract would be a suitable target for drug development.

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