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Use of antibiotic prophylaxis in clean non-implant wounds

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Sir,

I enjoyed reading the leading article by David Leaper entitled 'Use of antibiotic prophylaxis in clean non-implant wounds'.[1] One message in particular needs to be written across the hearts and minds of all surgical specialist registrars desperate for research papers, as well as their consultant chiefs. It is that two-armed controlled trials which predict reductions in wound infection rates from 7.5% to 2.5% with a power of 80% require a total of approximately 1600 patients. Trials enrolling smaller numbers are at risk from a type II error, which means that the *P* value can falsely indicate non-significance at the conventional 5% level when there is actually a genuine underlying benefit from prophylaxis. We have previously expounded this point in detail in the surgical literature.[2]

Put simply, the harsh message is that a trial of 200 elective inguinal hernia cases, however meticulously carried out, is unlikely to demonstrate a genuine difference in any conceivable range of infection rates. Consequently, such trials should not be undertaken in the first place and, in the event that they are, the reports on which they are based should not be accepted for publication.

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The effect of the inoculum size on bactericidal activity

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Sir,

In a recent issue, König *et al*.[1] described the effect of the inoculum size on the activities of various antibiotics against *Escherichia coli* and *Staphylococcus aureus*. They showed that the activity of ciprofloxacin, expressed in terms of the MIC, was not markedly affected by this parameter. We wish, firstly, to point out that Chin & Neu[2] demonstrated 15 years ago that the inoculum size does not affect the MICs of the fluoroquinolones.

König *et al.*[1] also concluded that the bactericidal activity of ciprofloxacin, in terms of the MBC, was equally unaffected by the inoculum size. However, these investigators neglected to compare their results with other published data. For example, it had been shown previously that the bactericidal activities of ofloxacin and ciprofloxacin against *E. coli* and *S. aureus* are reduced when the initial inoculum size is increased from 10^9 cfu/L to 10^{11} cfu/Land are totally eliminated when inocula of *c.*10^{13} cfu/L are used.[3] This inoculum effect has been attributed to a greatly reduced oxygen tension at high bacterial densities.[4] Therefore, in contrast to the results of König *et al.*,there are data showing that the bactericidal activities of the fluoroquinolones are indeed influenced by the inoculum size. In further support of this contention, we provide here new information about the inoculum effect exhibited by fluoroquinolones and cefotaxime in relation to *Streptococcus pneumoniae*.

The bactericidal activities of levofloxacin, ofloxacin, cefotaxime (Hoechst Marion Roussel, Romainville, France), sparfloxacin (Rhône-Poulenc Rorer, Vitry sur Seine, France), and ciprofloxacin (Bayer, Newbury, UK) against *S. pneumoniae* C3LN4 were determined by a broth dilution method. The bacterium was inoculated into nutrient broth No. 2 (Unipath, Basingstoke, UK) supplemented with 7% (v/v) laked horse blood (Unipath) containing each drug at a concentration that reflected its potential maxi-

Table. Effect of the inoculum size on the percentage survival of *S. pneumoniae* C3LN4 following incubation for 3 h in the presence of various quinolones and cefotaxime

Antibiotic (concentration tested)	Percentage survival with the following inoculum:		
	10^9 (cfu/L)	10^{11} (cfu/L)	10^{13} (cfu/L)
Levofloxacin (5 mg/L)	0.1	3.9	33.0
Ofloxacin (5 mg/L)	0.1	10.0	38.0
Ciprofloxacin (3 mg/L)	0.5	19.9	64.0
Sparfloxacin (3 mg/L)	2.2	19.9	60.0
Cefotaxime (86 mg/L)	0.1	0.1	0.3

mum bactericidal activity (see the Table for concentrations); the fluoroquinolones were therefore tested at their respective optimum bactericidal concentrations (OBCs)[5] and cefotaxime was tested at a concentration equivalent to the mean peak serum concentration following a single 1 g iv dose.[6] The inocula, which were prepared as described previously,[3] ranged from 10^8 to 10^{13} cfu/L. The suspensions were incubated at 37°C for 3 h, after which 100 μL aliquots were withdrawn and inoculated on to nutrient agar No. 2 (Unipath) supplemented with 7% laked horse blood. After overnight incubation, the colonies were counted and the numbers of viable bacteria in the suspensions were calculated.

The percentages of *S. pneumoniae* C3LN4, at initial inoculum sizes of 10^9, 10^{11} and 10^{13} cfu/L, that survived after incubation for 3 h in the presence of the various antibiotics are shown in the Table. The bactericidal activities of levofloxacin and ofloxacin at inocula of 10^9 cfu/L were greater than those of ciprofloxacin and sparfloxacin, an observation in accord with our previous findings.[5] When the initial inocula were increased to 10^{11} cfu/L, the bactericidal activities of all the fluoroquinolones tested were reduced markedly, and at 10^{13} cfu/L the activities were effectively bacteriostatic. These results agree with those already reported for ciprofloxacin and ofloxacin against *E. coli* and *S. aureus*.[3] In contrast, increasing the inoculum size had no effect on the bactericidal activity of cefotaxime. It might be argued that, had the fluoroquinolones been tested at concentrations as high as that of cefotaxime, an inoculum effect with the former group of drugs would not have been observed. However, earlier data demonstrated an inoculum effect with the quinolones when they were used at concentrations as high as 500 mg/L.[3]

The results of the present study are in accord with those of König *et al*. in demonstrating that an inoculum effect varies from drug class to drug class.[1] However, in contrast to their findings, we have shown that the bactericidal activities of fluoroquinolones such as levofloxacin, ciprofloxacin, ofloxacin, and sparfloxacin are markedly affected by the inoculum size. This difference can probably be accounted for by variations in methodology, König *et al*. having used the MBC as a measure of the bactericidal activity of the quinolones, whereas we used the OBC. Further studies designed to determine whether novel fluoroquinolones also exhibit an inoculum effect are warranted, particularly if these drugs are to be used in clinical settings in which the numbers of bacteria at the sites of infections are very high.

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Reply

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Sir,

As there are only limited data on the number of bacteria present at sites of infection before antibiotic therapy is initiated, the primary aim of our study was to quantify the viable bacteria in clinical specimens obtained from infected patients. We found that the average number of bacteria in culture-positive samples was 2×10^{11} cfu/L, which is markedly greater than the inocula used in in-vitro susceptibility testing methods. We therefore extended the study and evaluated the effect of high inocula on the outcome of susceptibility testing by comparing the bactericidal activities determined with a standard inoculum and those determined with higher inocula in both a standardized medium (Mueller–Hinton broth) and, in an attempt to simulate in-vivo conditions, in peritoneal fluid as well. We analysed our results in the light of those reported by other investigators.

Contrary to what has been suggested by Morrissey & George,[1] we did not conclude that the bactericidal activity of ciprofloxacin was not affected by the size of the inoculum. Quite the opposite, we clearly stated in the abstract that 'The inhibitory and bactericidal activities of amikacin and ciprofloxacin determined with high inocula were two to four times lower than with standard inocula'. A similar statement about the reduced bactericidal activity of ciprofloxacin observed with high inocula appears in the Results section and the point is illustrated in Tables I and II.

Morrissey & George[1] have drawn attention to the methodological differences between the two experiments. We believe that these variations may explain the different observations. Among the methodological differences were the use of different bacterial species and different inocula. Most importantly, however, in the investigation of the effect of the inoculum size, different criteria were used as measures of bactericidal activity. We defined bactericidal activity in terms of the MBC, which was determined according to a well-recognized and validated protocol,[2] whereas Morrissey & George[1] used the optimum bactericidal concentration (OBC). The MBC and the OBC clearly represent different endpoints.

Bactericidal activity is usually expressed in terms of the MBC, which is defined as the lowest antibiotic concentration that causes ⩾99.9% reduction in the initial inoculum following incubation for 24 h. The MBC may or may not correspond to the OBC, since concentrations that exceed the MBC may be even more bactericidal, depending on the antibiotic and the bacterium being tested. Furthermore, an antibiotic concentration that results in optimum killing may not necessarily be bactericidal as defined in terms of the MBC if the initial inoculum is reduced by <99.9%. Morrissey & George[1] investigated the effect of high inocula on the bactericidal activities of various antibiotics against a strain of *Streptococcus pneumoniae* after incubation for 3 h and used the OBC as a measure of bactericidal activity. However, we do not believe that it is possible to predict from these data whether the OBCs of ciprofloxacin and sparfloxacin will lead to ⩾99.9% killing of the initial inoculum after a 24 h period of incubation.

Owing to major differences in the techniques used in the two studies, only a limited comparison of the results is possible. Depending on the focus of the study, either the MBC or the OBC can be used as a measure of bactericidal activity. Additional comparative studies to determine which criterion is of greater clinical relevance are warranted.

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Post-antibiotic effect of quinolones on *Pseudomonas aeruginosa*

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Sir,

Clinical experience has shown that many infections can be treated by intermittent dosage regimens rather than by continuous infusions. This has been attributed to the fact

Table. PAE of ciprofloxacin and norfloxacin on *P. aeruginosa*

Antibiotic	Concentration of antibiotic in multiples of MIC	PAE (h)[a] obtained with: inoculum size of 10^7 cfu/mL after exposure period of:		inoculum size of 10^8 cfu/mL after exposure period of:	
		30 min	1 h	30 min	1 h
Ciprofloxacin	0.5 × MIC	0.3	0.5	0.2	0.4
	MIC	1.6	1.8	1.5	1.6
	2 × MIC	2.0	2.2	1.8	2.0
Norfloxacin	0.5 × MIC	0.3	0.4	0.1	0.2
	MIC	1.4	1.6	1.0	1.3
	2 × MIC	1.6	1.7	1.3	1.5

[a]Average of five clinical isolates and standard strain.

that some bacteria exhibit a delay in regrowth after a short exposure to antibiotics—the post-antibiotic effect (PAE).[1] In this study we measured the PAE of ciprofloxacin and norfloxacin on five clinical isolates and one standard strain (ATCC 27853) of *Pseudomonas aeruginosa* and the factors affecting this PAE. MICs and PAE were determined by the macrobroth dilution technique[2] and broth dilution technique,[1] respectively, using Mueller–Hinton broth and agar (Hi Media, Bombay, India) supplemented with calcium (50 mg/L) and magnesium (25 mg/L).

To an inoculum of 10^7 cfu/mL, the antibiotic was added at varying concentrations: MIC, <MIC and 2 × MIC, separately. This point was defined as time zero of antimicrobial exposure. The antimicrobial bacterial mixture was incubated for 1 h. The antibiotic was then removed by washing.[1] The bacteria were counted (cfu/mL) at time zero, before and after washing and every 30 min until the appearance of visible turbidity. The PAE was calculated by the standard formula.[1]

The same procedure was repeated using a 30 min exposure period. The effect of increasing the inoculum size to 10^8 cfu/mL was studied after 30 min and 1 h exposure. The same procedures were repeated with all six strains and with both the quinolones. Data were analysed using the analysis of variance test and Tukey's test.[3]

MICs of ciprofloxacin for the tested strains were 0.25–0.5 mg/L and those of norfloxacin were 2–4 mg/L. Mean PAE determinations for ciprofloxacin and norfloxacin are given in the Table. There was no significant difference in the PAE seen with wild type and reference strains.

A PAE of 1.6 h was found following 30 min exposure of the clinical strains of *P. aeruginosa* to ciprofloxacin at the MIC. With an increase in MIC there was a significant increase in the duration of the PAE (1.6–2.0 h). However, at sub-MIC concentrations a very short PAE was observed (0.3–0.5 h). Increasing the exposure period of the clinical strains to 1 h for ciprofloxacin resulted in longer PAEs (1.7 h at MIC) while increasing the inoculum size resulted in a significant decrease in the PAEs (1.6–1.5 h).

With norfloxacin, on exposure of *P. aeruginosa* strains to the MIC and 2 × MIC for 30 min, an increasing PAE of 1.4–1.6 h was found, depending on the concentration. At sub-MIC concentrations a short PAE was obtained. With an increase in the inoculum size and exposure period, the results obtained were similar to those found with ciprofloxacin.

The results of this study show that both quinolones exhibit a PAE for *P. aeruginosa*. The PAE could be due to binding of quinolones to DNA gyrase and/or to DNA, which would affect those members of a population of bacteria that were not immediately killed by exposure to the quinolones.[4] The PAE could be the result of an increased post-treatment lag phase, which might be followed by a near normal multiplication or abnormal rapid cell division.[5]

The quinolones thus show a concentration-dependent killing action, as earlier demonstrated by Fuursted.[6] Significantly higher PAEs were noted on increasing the duration of exposure to the quinolones from 30 min to 1 h. This was seen at the MIC and 2 × MIC. Chin & Neu[4] also demonstrated an increase in the PAE with increasing duration of exposure to ciprofloxacin for *P. aeruginosa*. Increasing the inoculum size resulted in shorter PAEs for the quinolones at all concentrations tested.

We believe that the rapid killing obtained with quinolones and their ability to produce a significant PAE provide a rationale for the twice-daily dosage regimens that are used clinically.

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Susceptibility of methicillin-resistant *Staphylococcus aureus* to tea tree oil and mupirocin

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Sir,

The antimicrobial properties of essential oils of plant origin have been recognized for centuries.[1] Carson & Riley[2] demonstrated that the antimicrobial activities of one such compound, tea tree oil, which is obtained from *Melaleuca alternifolia*, are attributable to its hydrocarbon and terpine constituents, including terpinen-4-ol, α-terpineol and linalool.

With the increasing prevalence of methicillin-resistant strains of *Staphylococcus aureus* (MRSA) as pathogens in both hospitals and the community, the eradication of the carrier state has become an important control measure. Mupirocin has been used widely for this purpose[3] but, recently, there have been reports of clinical isolates of MRSA exhibiting either high- or low-level resistance to it.[4,5] This has prompted a search for alternative agents and this search has extended to natural oils. The present study was undertaken in order to evaluate the in-vitro activities of tea tree oil and mupirocin against 100 recent clinical isolates of MRSA.

Tea tree oil (batch no. 3691) was obtained from Thursday Plantation Laboratories Ltd (Ballina, NSW, Australia); the batch complied with the International Standard (ISO 4730) in that the 1,8-cineole content was 15% and the terpinen-4-ol content was 30%. Mupirocin was provided by SmithKline Beecham Pharmaceuticals Ltd (Harlow, UK). The strains of MRSA were 100 non-replicate clinical isolates collected from laboratories throughout England and Wales. *S. aureus* NCTC 7447 was used as a control.

The susceptibilities of the strains to tea tree oil and mupirocin were determined by a microbroth dilution method described previously by Carson *et al*.,[6] except that the medium used was nutrient broth (Oxoid, Basingstoke, UK); the medium was supplemented with 0.1% Tween 80 (Sigma Aldrich Ltd, Poole, UK) when susceptibility to tea tree oil was determined. Doubling dilutions were performed in 96-well microtitre plates (Greiner Laboratories Ltd, Dursley, UK), giving tea tree oil concentrations ranging from 0.039% to 2.5% (v/v) and mupirocin concentrations ranging from 0.25 mg/L to 2048 mg/L. An overnight culture of each isolate was adjusted and inoculated into the wells to give suspensions containing *c*. 2.5×10^9 cfu/L. The plates were incubated in air for 24 h at 30°C (chosen because of the volatility of the oils at higher temperatures). MICs were read with a programmable microtitre plate reader (Titertek Multiscan, Flow Laboratories, High Wycombe, UK) at 540 nm. MBCs were determined by withdrawing 5 μL aliquots from wells in which there was no visible growth and inoculating into 100 μL of nutrient broth supplemented with 0.1% Tween 80 in microtitre wells. The plates were incubated at 30°C for 24 h and scanned with the microtitre plate reader. This method of determining MICs and MBCs was chosen because it overcomes the inhibitory carryover effect of tea tree oil which can be a problem when methods involving determining viable counts are used.

The median MIC of tea tree oil for the MRSA isolates was 0.32% (range, 0.16–0.32%), while the median MBC was 0.64% (range, 0.32–1.25%); the median MIC fell within the range of previously published values.[6] The median MIC of mupirocin was 16 mg/L (range, 2–>2048 mg/L) and the median MBC was 32 mg/L (range, 4–>2048 mg/L). According to the definition of Poupard,[5] 23% of the MRSA strains were categorized as susceptible to mupirocin, 45% as exhibiting low-level resistance and 32% high-level resistance. The isolates exhibited remarkably uniform susceptibilities to tea tree oil, whereas the ranges of the MICs and MBCs of mupirocin were much broader. There was no difference between isolates that were susceptible or resistant to mupirocin in terms of their susceptibilities to tea tree oil—data which are in accord with those of Carson *et al*.[6]

As the proportion of MRSA isolates that are resistant to mupirocin increases, topical agents, such as tea tree oil, that might be used as alternatives to eradicate MRSA carriage, will assume greater importance.[4] However, as Nelson has already pointed out,[1] the widespread use of tea tree and other essential oils in sub-inhibitory concentrations in cosmetics and other topical formulations could undermine the potential efficacies of these compounds as antiseptic agents.

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Fluoroquinolone resistance among recent clinical isolates of *Streptococcus pneumoniae*

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Sir,

Recent studies of *Streptococcus pneumoniae* have defined the appearance of ciprofloxacin-resistant pneumococci *in vitro* and *in vivo*.[1,2] Two types of mutation have been identified: mutations of *parC* typically display low-level resistance (MIC 4.0–8.0 mg/L), while further mutations in the gene(s) encoding DNA gyrase, usually *gyrA*, result in high-level resistance (MIC 16–64 mg/L). These two types of mutational event must occur in sequence to result in high-level resistance to ciprofloxacin and to other fluoroquinolones.[1] The clinical relevance of low-level resistance is not entirely clear, but continued exposure to fluoroquinolones could select mutants with high-level resistance.

The purpose of this study was to define the normal distribution of fluoroquinolone MICs against 600 contemporary isolates of *S. pneumoniae* gathered from 11 medical centres in North America during the 1996–97 winter season. Each facility contributed 30–81 separate isolates of pneumococci that were thought to be clinically relevant; duplicate isolates from the same patient episode were excluded. Only 68% of the strains were penicillin-susceptible, 15% were intermediate in susceptibility and 17% were penicillin-resistant; 17% were also macrolide-resistant.

We performed broth microdilution susceptibility tests as described by the National Committee for Clinical Laboratory Standards.[3] Ciprofloxacin, ofloxacin, levofloxacin and sparfloxacin were studied. The population statistics for MICs of each fluoroquinolone (Table) describe normal distributions that might be expected if one strain were retested 600 times or 600 strains were tested once. For each fluoroquinolone, >99% of all MICs were within the range defined by the mode ± one doubling concentration and <1% of all MICs were two doubling concentrations from the mode.

In the case of levofloxacin and sparfloxacin, the MIC mode is two doubling concentrations below the susceptible breakpoint and, consequently, only one or two strains gave MICs in the intermediate range (mode + 2 doubling concentrations) and none were in the resistant category.

The modal MICs of ciprofloxacin and of ofloxacin are located at their susceptible breakpoints of 1.0 and 2.0 mg/L, respectively. Consequently, repeated testing should shift a substantial number of strains from the susceptible to the intermediate category or *vice versa*, but rarely from susceptible to resistant, or *vice versa*. For <1% of all pneumococci, ciprofloxacin MICs were in the upper portion of the normal distribution curve (resistant). If those ciprofloxacin-resistant pneumococci represent first-step (*parC*) mutants, we would expect them to show cross-resistance to other fluoroquinolones and that was not observed consistently.

We are unaware of any evidence that supports or refutes the assumption that pneumococci with ciprofloxacin MICs of 4.0 mg/L are likely to fail to respond to therapy. We do not yet know whether any of those strains are progeny from parents that have undergone the first-step *parC* mutation and are now ready to undergo a second-step mutation that can lead to high-level resistance. Among the pneumococci that we collected from 11 USA medical centres, there were no strains with ciprofloxacin MICs >8.0 mg/L; thus the prevalence of high-level resistance is <0.2% (<1 in 600). Pneumococci with a ciprofloxacin MIC of 4.0 mg/L (low-level resistance?) occurred in about 1% of our pneumococci. That is consistent with the findings of Simor *et al*.[4] who evaluated 1089 clinical isolates from Canadian medical centres. Of the 600 pneumococci, 11.8% had ciprofloxacin-intermediate MICs of 2.0 mg/L: the clinical significance of strains in this category remains unclear.

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Table. Distribution of fluoroquinolone MICs against 600 recent isolates of *S. pneumoniae* recovered from patients in 11 different medical centres throughout North America during the 1996–97 winter months

	Number of *S. pneumoniae* isolates with each MIC[a]			
MIC (mg/L)	ciprofloxacin	ofloxacin	levofloxacin	sparfloxacin
8.0		2		
4.0	5	**52**	**2**	
2.0	**71**	521	21	
1.0	420	25	531	**1**
0.5	101		46	101
0.25	3			447
0.12				48
0.06				3
0.03				
% Susceptible	87.3	91.0	99.7	99.8
% Intermediate	11.8	8.7	0.3	0.2
% Resistant	0.8	0.3	0	0

[a]The data in bold type indicate strains in intermediate categories. Isolates with MICs above that level are assumed to be resistant; others are susceptible. The percentage of strains in each category is noted at the bottom of this table.

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Prevalence of *Acinetobacter* spp. isolates with reduced susceptibility to imipenem, as determined by a USA-wide electronic surveillance network

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Sir,

Resistance to multiple antibiotics amongst aerobic Gram-negative bacilli has led to increased use of the carbapenems. While acquired resistance to these agents is rare, several authors have recently expressed concern about car-

bapenem resistance amongst *Acinetobacter* spp.[1–6] Reports of carbapenem-resistant isolates belonging to this genus have originated in South America,[1,4,5] Europe[1–3,6] and the Far East and Middle East,[1] but little is known of their prevalence. The present study used routine laboratory data, collected via The Surveillance Network (TSN) Database–USA, to investigate the pattern of isolation of *Acinetobacter* spp. with reduced susceptibility to carbapenems in the USA.

TSN Database–USA electronically collects all routine susceptibility data daily from, currently, 158 laboratory databases throughout the USA. These data are selected in such a way that ensures that they are geographically, demographically and methodologically representative and they are pooled and filtered through expert rules so that repeat isolates and isolates with unusual antibiograms that cannot be confirmed, are identified and excluded from analysis. The database currently comprises >13,565,000 results, collected between 1 January 1994 and 24 June 1998, for 976,927 strains from 652,454 patients. All of this information is available for on-line analysis by participating institutions via the TSN website (www.thetsn.com).

During the >4-year study period, imipenem susceptibility data for 10,578 isolates of *Acinetobacter* spp. were collected. They were identified as *Acinetobacter calcoaceticus* or *Acinetobacter baumannii* (9229 isolates), *Acinetobacter lwoffii* (938) and other *Acinetobacter* spp. (411); for the purpose of the present analysis, *A. calcoaceticus* and *A. baumannii* isolates are grouped together as the 'ACB complex' because these species cannot be reliably distinguished by routine laboratory tests. Susceptibility to meropenem was determined for only a few strains. All of the reporting laboratories used methods and interpretative criteria recommended by the National Committee for Clinical Laboratory Standards, with resistance to imipenem being defined as a MIC of >8 mg/L or an inhibition zone diameter of ⩽13 mm with a 10 μg disc and intermediate susceptibility as a MIC of 8 mg/L or a zone diameter of >13 mm but <16 mm.[7]

One hundred and forty-eight (1.4%) isolates were categorized as being of intermediate susceptibility to imipenem and a further 292 (2.76%) as resistant. The percentages of ACB complex, *A. lwoffii* and *Acinetobacter* spp. strains that were resistant were similar, while most of the isolates that exhibited intermediate susceptibility belonged to the ACB complex (Table). There was no significant difference between strains that had been tested by the microbroth dilution and disc diffusion methods in terms of rates of resistance, the implication being that resistance was not a methodological artefact.

Following trends in the patterns of susceptibility to imipenem amongst ACB complex isolates for the last 3 years of the collection period revealed year-to-year increases in the incidences of strains exhibiting intermediate susceptibility, i.e. from 3.1% (79 of 2565 strains) to 4.6% (174 of 3773) to 5.8% (113 of 1961) in 1996, 1997 and the first half of 1998 respectively; these differences did not reach statistical significance ($0.1 > P > 0.05$). Similar trends were observed when the susceptibility patterns of the resistant isolates were considered. However, marked changes in the susceptibility patterns of *A. lwoffii* and *Acinetobacter* spp. isolates were not observed.

ACB complex strains isolated from the lower respiratory tract were more often of intermediate susceptibility or resistant to imipenem than those from the upper respiratory tract, cerebrospinal fluid, skin and soft tissues or urinary tract, but these differences were not statistically significant ($P > 0.1$). Similarly, there were no differences in terms of susceptibility to imipenem between ACB complex strains isolated from intensive care unit patients (five of 988 (0.5%) strains exhibiting intermediate susceptibility and 24 of 988 (2.5%) that were resistant) and those isolated from patients on other wards (16 of 4247 (0.4%) strains exhibiting intermediate susceptibility and 78 of 4247 (1.8%) that were resistant). On the other hand, the rates of reduced susceptibility amongst ACB complex isolates recovered from patients in hospitals with >500 beds (113 of 5693 (2%) strains exhibiting intermediate susceptibility and 161 of 5693 (2.8%) that were resistant) were higher than those amongst isolates recovered from patients in smaller institutions (24 of 3296 (0.7%) strains exhibiting intermediate susceptibility and 55 of 3296 (1.7%) that were resistant), although the differences only approached statistical significance ($0.1 > P > 0.05$).

Table. Susceptibilities of *Acinetobacter* spp. isolates to imipenem as determined by the disc diffusion (DD) and microbroth dilution (MBD) methods

Species	No. of isolates	No. tested by MBD/DD methods	No. (%) susceptible		No. (%) intermediate susceptibility		No. (%) resistant	
			MBD	DD	MBD	DD	MBD	DD
ACB complex	9229	8002/1156[a]	7657 (95.7)	1122 (97.1)	136 (1.7)	5 (0.5)	209 (2.6)	29 (2.5)
A. lwoffii	938	807/122[a]	777 (96.3)	118 (96.7)	4 (0.5)	0	26 (3.2)	4 (3.3)
Acinetobacter spp.	411	324/81[a]	312 (96.3)	79 (97.5)	0	0	12 (3.7)	2 (2.5)

[a]The remaining isolates (not shown) were tested by the Etest or agar dilution method.

MICs for 741 of the 9229 ACB complex isolates were determined with a full range of two-fold dilutions and values of 1, 2, 4, 8 and >8 mg/L were recorded in respect of 69 (9.3%), 25 (3.4%), seven (1%), five (0.7%) and 16 (2.1%) strains respectively, compared with MICs of 0.12–0.5 mg/L for susceptible *Acinetobacter* spp. isolates.[1] Borderline MICs (i.e. those between 4 and 8 mg/L) are difficult to interpret, low-level β-lactamase-mediated resistance in other species having been associated with clinical failures, although no such correlation has been observed with *Acinetobacter* spp. and carbapenems. Extrapolating the percentages of isolates with MICs of 2 and 4 mg/L, which closely approximate the MIC susceptibility breakpoint, to the entire population of ACB complex strains studied suggests that the number of isolates for which potentially misleading in-vitro susceptibility test results are obtained is large.

We conclude that the incidence of reduced susceptibility to imipenem amongst ACB complex strains in the USA is increasing. This increase may reflect clonal spread or plasmid dissemination and resistance may or may not be attributable to carbapenemases. Molecular studies will be necessary to resolve these issues, but the vast collection of phenotypic data available through electronic surveillance programmes, such as TSN, can be used to rapidly assess the prevalence of emerging resistance profiles of public health importance reported by research and reference laboratories. Only with such systems can we monitor and respond to the problem of emerging drug resistance.

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Geographical distribution of quinolone resistance among *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp. isolates from 20 European university hospitals

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Sir,

In recent years there have been increasing numbers of reports of the emergence of resistance to ciprofloxacin.[1] This development is regarded as the inevitable consequence of the exposure of bacterial populations to progressively greater quantities of this antibiotic. There is, therefore, a need for new quinolones with reduced propensities for promoting resistance and improved activities against Gram-positive bacteria, whilst retaining broad-spectrum activity against aerobic Gram-negative bacilli.

Gatifloxacin and trovafloxacin are novel quinolones that meet these criteria and are active against a broad range of Gram-positive and Gram-negative bacteria. Gatifloxacin is a fluoroquinolone with a 3-methylpiperazine group at position 7 of the quinolone ring and a methoxy group at position 8,[2] while trovafloxacin possesses a novel 3-aza-bicyclohexyl substituent at the C-7 position.[3]

The SENTRY Antimicrobial Surveillance Programme is a longitudinal surveillance programme that was created for the purpose of monitoring the antimicrobial resistance patterns of the principal causes of nosocomial and community-acquired infections both nationally and internationally; included among these infections are bacteraemias, outpatient respiratory tract infections caused by fastidious organisms, nosocomial pneumonias, wound infections and urinary tract infections. This initiative was employed in the present study, which was undertaken before the launch of gatifloxacin and trovafloxacin, to monitor the levels of resistance to quinolone antibiotics in 12 European countries and to investigate geographical differences in the emergence of resistant strains.

Table. Geographical distribution of resistance to ciprofloxacin (C), trovafloxacin (T) and gatifloxacin (G) among MSSA, *E. coli* and *Klebsiella* spp. isolates from 20 European university hospitals

	MSSA					*E. coli*					*Klebsiella* spp				
		MIC_{90} (mg/L)					MIC_{90} (mg/L)					MIC_{90} (mg/L)			
Country	no. strains	C	T	G	%S[a]	no. strains	C	T	G	%S[a]	no. strains	C	T	G	%S[a]
Austria	57	0.5	0.12	0.12	96.5	57	0.03	0.06	0.06	100	14	0.06	0.25	0.12	100
Belgium	15	0.5	0.12	0.25	93.3	57	0.03	0.12	0.06	94.7	12	1	1	1	91.7
France	65	>2	1	4	78.5	96	0.12	0.25	0.25	95.8	23	0.25	0.25	0.25	100
France	96	>2	2	2	83.3	127	0.25	0.25	0.25	95.3	29	0.5	0.25	0.25	100
France	93	>2	1	2	80.7	175	0.25	0.25	0.25	96.0	31	0.03	0.12	0.06	96.8
France	78	>2	1	2	80.7	93	0.25	0.5	0.25	94.6	22	0.25	0.25	0.25	100
Germany	65	0.25	0.06	0.12	98.5	101	≤0.015	0.06	0.06	98.0	26	0.25	1	0.5	96.2
Germany	110	0.5	0.06	0.12	94.6	110	0.25	0.5	0.25	91.8	36	0.25	0.5	0.25	100
Greece	57	1	0.12	0.25	91.2	108	0.12	0.25	0.25	93.5	46	2	2	2	84.8
Italy	47	>2	1	2	80.9	77	0.5	1	0.5	93.5	22	>2	4	4	77.3
Italy	26	>2	2	2	80.8	42	0.25	0.25	0.25	90.5	9	>2	>4	4	88.9
The Netherlands	42	0.25	0.06	0.12	100	79	0.25	0.5	0.25	94.9	24	0.06	0.25	0.25	100
Poland	21	0.25	0.06	0.06	95.2	27	0.06	0.25	0.12	100	15	2	2	1	86.7
Poland	44	0.25	0.12	0.12	97.7	41	>2	>4	>4	82.9	11	>2	>4	4	54.6
Portugal	68	>2	1	2	88.2	72	>2	>4	>4	77.8	37	0.03	0.25	0.12	100
Spain	52	>2	4	4	57.7	75	>2	>4	>4	88.0	25	0.25	0.5	0.5	96.0
Spain	55	0.25	0.06	0.12	92.7	168	>2	>4	4	85.1	28	0.03	0.25	0.06	100
Spain	49	2	0.25	0.25	89.8	142	>2	>4	>4	76.7	36	>2	>4	>4	88.9
Switzerland	54	0.5	0.12	0.12	90.7	160	≤0.015	0.06	0.06	100	47	0.12	0.25	0.25	100
UK	85	>2	1	2	84.7	87	0.12	0.25	0.25	92	27	0.5	1	0.5	92.6
Total	1179	>2	1	1	86.7	1894	0.5	1	0.5	91.6	520	0.5	1	0.5	93.4

[a]Percentage of isolates susceptible to ciprofloxacin according to an MIC breakpoint of ≤1 mg/L.

Three of the predominant causes of severe bacterial infections, namely *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp., were selected as the main foci of the study; only methicillin-susceptible *S. aureus* (MSSA) isolates were included, since, across Europe, all but 10% of the methicillin-resistant *S. aureus* isolates tested ($n = 369$) were resistant to ciprofloxacin. The strains studied therefore included 1179 MSSA, 1894 *E. coli* and 520 *Klebsiella* spp. clinical isolates collected between April 1997 (the start of the European SENTRY programme) and April 1998; these strains comprised nearly 35% of the organisms tested during the first year of the programme. The isolates were referred to the Eijkman–Winkler Institute for Medical Microbiology, Utrecht, The Netherlands, which served as the regional co-ordinating centre for 20 university hospitals in the 12 European countries. The susceptibilities of the isolates to ciprofloxacin, gatifloxacin and trovafloxacin were determined by a reference microbroth dilution method recommended by the National Committee for Clinical Laboratory Standards.[4]

The susceptibility data for the isolates are summarized in the Table. The activities of gatifloxacin and trovafloxacin against the MSSA strains were comparable (MIC_{90}s 1 mg/L) and superior to that of ciprofloxacin (MIC_{90} >2 mg/L); on the basis of an MIC breakpoint of ≤1 mg/L,[4] a mean of *c*. 87% of all MSSA isolates were categorized as susceptible to ciprofloxacin. The prevalence of quinolone resistance among the MSSA isolates was high in the participating hospitals in France, Italy, Portugal and the UK and in one of the three hospitals in Spain.

The *E. coli* and *Klebsiella* spp. isolates were highly susceptible to all three quinolones tested. Based on the MIC_{90}s, ciprofloxacin and gatifloxacin (MIC_{90}s 0.5 mg/L) were slightly more active than trovafloxacin (MIC_{90} 1 mg/L) and, according to the MIC breakpoint (≤1 mg/L), almost 92% of all *E. coli* isolates and 93% of *Klebsiella* spp. isolates were susceptible to ciprofloxacin. High prevalence of quinolone resistance among the *E. coli* strains was observed in the participating hospitals in Portugal and Spain and in one of the two hospitals in Poland, whereas high prevalence among the *Klebsiella* spp. isolates was observed in the participating hospitals in Greece, Italy and Poland and in one of three hospitals in Spain.

Although the percentages of susceptible strains belonging to some species varied markedly from one university hospital to another, the relative activities of the quinolones tested were similar in each institution; this reflects cross-resistance to the quinolones among different bacterial species. In general, ciprofloxacin resistance was associated with reduced susceptibility to the newer quinolones.

There are several explanations for the observed regional variations in susceptibility to ciprofloxacin, including differences in the sources of the isolates referred for susceptibility testing (i.e. different wards and patient populations) and differences in quinolone usage within the various hospitals. Case-control studies have demonstrated that the emergence of fluoroquinolone resistance correlates with extensive use of these drugs and that previous treatment with ciprofloxacin is a major risk factor for the isolation of ciprofloxacin-resistant bacteria.[5] Quinolone resistance is also frequently associated with specific centres or wards. Once a resistant clone emerges, the rate of quinolone resistance usually increases rapidly within that specific hospital setting as the result of clonal spread.[6] In addition to quinolone usage, this clonal spread might account for the differences in the prevalence of ciprofloxacin-resistant MSSA, *E. coli* and *Klebsiella* spp. in the 20 European hospitals. Further epidemiological typing studies are underway to analyse the clonal relatedness of these resistant bacteria and to clarify the role of antibiotic usage in promoting quinolone resistance in different hospitals.

In summary, compared with ciprofloxacin, gatifloxacin and trovafloxacin exhibited superior in-vitro activities against *S. aureus*, and ciprofloxacin and gatifloxacin were more active than trovafloxacin against *E. coli* and *Klebsiella* spp. Pre-existing ciprofloxacin resistance was associated with reduced susceptibility to the newer quinolones. Finally, geographical differences in the prevalence of quinolone-resistant bacteria might be explained by extensive quinolone usage and/or horizontal clonal spread of resistant bacteria in the various hospitals.

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A reverse-phase, isocratic high-performance liquid chromatography assay for levofloxacin

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Sir,

Levofloxacin, the L-isomer of ofloxacin, was licensed for use in the USA in 1996 and has recently become available in the UK. It has a broad spectrum of in-vitro activity and has been shown to be more active than ofloxacin.[1] The mean peak plasma concentrations of levofloxacin (t_{max} 1–2 h) after 250 mg and 500 mg oral doses have been reported to be 2.8 and 5.2 mg/L respectively.[2] As with ofloxacin, the oral and iv preparations have been formulated to allow for interchange and, with a half-life of approximately 8 h, once-daily dosing may be feasible when treating patients with mild to moderate infections.[3] We have previously developed a simple high-performance liquid chromatography (HPLC) assay for ofloxacin[4] and describe here a modification of that technique for the assay of levofloxacin.

The stationary phase was Spherisorb 5 ODS in a stainless steel column, 25 cm × 4.6 mm (HPLC Technology, Macclesfield, UK), heated to 50°C (column block heater, HPLC Technology, Macclesfield, UK). The mobile phase was 0.16% *ortho*-phosphoric acid, adjusted to pH 3 with tetrabutylammonium hydroxide; 50 mL of acetonitrile were added to the 1 L solution after the pH adjustment. Serum samples were mixed with equal volumes of methanol, allowed to stand for 5 min and centrifuged at 25,000**g** for 5 min; 20 μL of the supernatants were injected on to the column. The flow rate was 1.5 mL/min. Detection was by fluorescence (excitation wavelength, 310 nm; emission wavelength, 467 nm; model LC240, Perkin Elmer, Beaconsfield, UK).

A chromatogram of levofloxacin is shown in the Figure. The reproducibility of the assay, expressed as the percentage coefficient of variation (% CV), was <4% when the assay was repeated six times with aqueous and serum samples spiked with 0.9 or 4.6 mg/L of levofloxacin. The detection limit (in serum), defined as a levofloxacin concentration equivalent to a peak three-fold greater than that of the base-line noise, was 0.05 mg/L. Linearity and serum recovery were investigated by assaying aqueous and serum specimens containing levofloxacin at concentrations of 0, 0.4, 0.9, 1.9, 4.6 and 8.0 mg/L. When the peak height of levofloxacin was plotted against drug concentration and a regression analysis performed, the correlation between these two variables for both the aqueous and serum levofloxacin samples ($r = 0.996$ and 0.982 respectively) was good. The percentage serum recovery (serum peak height/aqueous peak height × 100) approached 100% at each drug concentration tested. The accuracy of the assay was investigated by assaying serum samples containing 1.5, 3.0 or 5.0 mg/L of levofloxacin with a single standard of 1.9 mg/L. Accuracy, expressed as the percentage error ((measured concentration – target concentration)/target concentration × 100) was 0.7%, 6.7% and 0.2% for the 1.5, 3.0 and 5.0 mg/L samples respectively. The assay is specific. Following the assay of 22 commonly used antibiotics (including other fluoroquinolones) and antifungal agents, as well as samples containing unknown drugs which were obtained from 24 patients, no chromatographic peaks that could potentially interfere with that of levofloxacin, with the exception of the peak for ofloxacin which, in any event, would not be

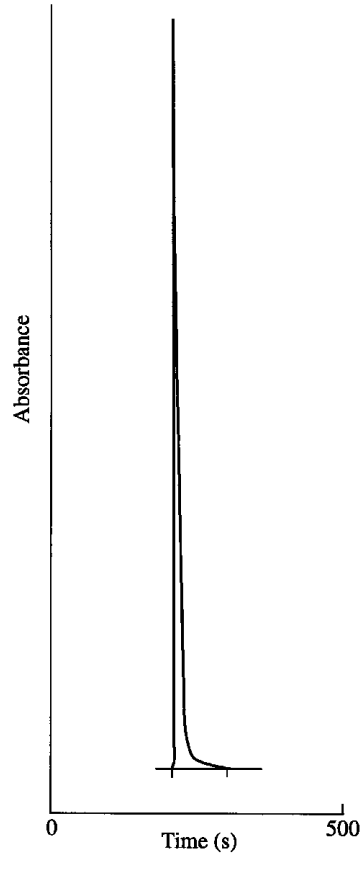

Figure. Chromatogram of levofloxacin (retention time, 300 s).

co-administered with levofloxacin, were observed. We conclude that the technique described here is a rapid assay for levofloxacin than can be routinely performed in clinical diagnostic laboratories.

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Haemolytic anaemia following treatment with piperacillin in a patient with cystic fibrosis

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Sir,

We describe a patient who developed severe haemolytic anaemia following treatment with piperacillin. Although haemolysis is a widely recognized complication of penicillin therapy,[1,2] we believe this to be the first published report in the UK of piperacillin-induced haemolysis.

A 34 year old female patient with cystic fibrosis was admitted complaining of increased shortness of breath and production of purulent sputum. She had no known allergies, although she had previously experienced pruritis while receiving ceftazidime, and tingling in her hands with azlocillin. She had also completed courses of amoxycillin and flucloxacillin and, 2 years previously, had been given piperacillin for 12 days, in all cases without adverse effects. Culture of a recent sputum specimen yielded a strain of *Pseudomonas aeruginosa* that was susceptible only to imipenem and colistin and the patient was, therefore, treated with meropenem and colistin, both by the iv route, for 16 days. However, as she was still producing large amounts of purulent sputum, the meropenem, to which the strain of *P. aeruginosa* had become resistant, was discontinued and treatment with iv piperacillin, to which it had become susceptible, in a dosage of 6 g tds, was initiated; the patient continued to receive colistin. After 12 days, piperacillin/tazobactam was substituted for the piperacillin because the patient had failed to improve further and because the pathogen had become resistant to the latter drug, although it was susceptible to the combination. On the following day, the patient complained of headache and nausea and noticed that she was passing pink urine. She was febrile (38.8°C) and clinically anaemic. Her haemoglobin concentration had fallen from 10.9 g/dL on admission to 5.5 g/dL, with spherocytes, polychromasia, anisocytosis and nucleated red cells. A positive direct Coombs' test, a serum bilirubin concentration of 28 mmol/L, a low serum haptoglobin concentration (0.3 g/L) and the presence of haemoglobin in the urine were consistent with an immune-mediated haemolytic crisis. The piperacillin/tazobactam was discontinued, prednisolone and folic acid were administered and the patient was transfused. Her haemoglobin concentration subsequently remained stable at 14 g/dL and she was well when reviewed in the outpatients' clinic 2 weeks later.

A panel of antibiotics that might be given to this patient in the future as treatment of infections caused by *P. aeruginosa* and/or other pathogens, including piperacillin, piperacillin/tazobactam, azlocillin, ticarcillin, temocillin, flucloxacillin, imipenem, meropenem, aztreonam, chloramphenicol, gentamicin, colistin, cefuroxime and ceftazidime, were incubated with her serum. Of these drugs, only piperacillin and piperacillin/tazobactam caused agglutination in an indirect agglutination test; sera from other patients (controls) did not react in this way. We concluded, therefore, that this patient's acute haemolytic anaemia had been induced by the piperacillin.

Piperacillin is frequently used to treat infections caused by *P. aeruginosa* in cystic fibrosis patients, in whom adverse reactions to this drug tend to be more common than in non-cystic fibrosis patients.[3–5] In one study, nine of 38 patients who were given piperacillin developed swinging pyrexias after treatment for a mean of 13.5 days,[3] while, in another, 11 of 31 patients developed fever and rash after a mean of 9.1 days.[4] In a third study, 14 of 46 patients developed serum sickness-like illnesses which appeared to be dosage-related.[5]

The patient described here developed brisk severe intravascular haemolysis after 12 days of treatment with piperacillin. The mechanism of this reaction is an antibody/hapten immune response. The hapten/protein complex is formed when piperacillin combines with protein on red cell membranes. Haemolysis, which is usually extravascular, classically occurs after approximately 10 days in patients with penicillin-induced haemolysis.[1,2] Our patient did not

experience haemolysis with either amoxycillin or flucloxacillin, presumably because they form different hapten complexes. Haemolysis in this case was highly specific to piperacillin, no agglutination having been observed *in vitro* with the closely related drugs azlocillin and ticarcillin which, therefore, remain therapeutic options for the future.

This is the first documented report from the UK of piperacillin-induced haemolysis (Wyeth, personal communication). The case is unusual because there was evidence of intravascular haemolysis. Patients with cystic fibrosis have a greater predisposition than non-cystic fibrosis patients to develop allergic reactions to piperacillin, which occur 9–15 days after treatment has been initiated. It is important, therefore, to monitor the full blood counts of these patients during the second week of treatment with piperacillin and to be aware of the potential for this serious complication to develop.

References

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