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Extended-spectrum *β*-lactamase-producing strain of *Acinetobacter baumannii* isolated from a patient in France

J Antimicrob Chemother 1999; **43**: 157–158

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Sir,
Acinetobacter spp., especially *Acinetobacter baumannii*, are common opportunistic pathogens in immunocompromised patients and currently cause 10% of nosocomial infections in intensive care unit patients.[1] They tend to be resistant to multiple antibiotics and to produce cephalosporinases.[2] Indeed, hyperproduction of cephalosporinases, together with decreased outer membrane permeability, are the predominant mechanisms of resistance to ceftazidime amongst *A. baumannii* isolates.

Most extended-spectrum *β*-lactamases (ESBLs) are the result of mutations that alter the hydrolytic properties of the restricted spectrum penicillinases, TEM-1 and -2 and SHV-1.[3] These enzymes are principally mediated by plasmids which spread readily amongst Enterobacteriaceae and their presence is detected by the demonstration of synergy between clavulanic acid, which inhibits the *β*-lactamase, and a third-generation cephalosporin (most effectively ceftazidime) with the double-disc diffusion test.

In the course of routinely assessing all ceftazidime-resistant *Acinetobacter* spp. isolates by the double-disc diffusion test, we identified a strain of *A. baumannii* (Ama-1) exhibiting only slight synergy that was most evident when the ceftazidime disc was placed 2 cm from the clavulanic acid disc. The MICs of amoxycillin, ticarcillin, piperacillin, ceftazidime and imipenem for the isolate were determined by the agar dilution method according to recommendations of a working party of the British Society for Antimicrobial Chemotherapy.[4] Ama-1 was resistant to all of the *β*-lactams tested, with the exception of imipenem. In the presence of clavulanic acid at a fixed concentration of 2 mg/L, the MIC of ticarcillin was reduced from 512 mg/L to 256 mg/L and that of ceftazidime from 512 mg/L to 128 mg/L. Similarly, in the presence of sulbactam at a concentration of 4 mg/L, the MIC of ceftazidime fell from 512 mg/L to 256 mg/L. The activity of any *β*-lactamase(s) produced by this isolate is therefore inhibited only minimally by either *β*-lactamase inhibitor. In an attempt to determine the molecular basis of this resistance phenotype, we assayed for a putative ESBL gene by PCR analysis with TEM-specific primers (5′-GTATGGATCCTCAACATTTCCGTGTCG-3′ and 5′-ACCAAAGCTTAATCAGTGAGGCA-3′) and SHV-specific primers (5′-TTATCTCCCTGTTAGCCACC-3′ and 5′-GATTTGCTGATTTCGCCG-3′). Neither gene was demonstrated. However, primers used to detect the gene for the ESBL, PER-1 (5′-ATGAATGTCATTATAAAAGC-3′ and 5′-AATTTGGGCTTAGGGCAGAA-3′), yielded a 925 bp PCR product.[5] The sequence analysis of this product revealed total identity with $bla_{\text{PER-1}}$ which was originally detected in a strain of *Pseudomonas aeruginosa*.[5]

A survey recently carried out in Turkey showed that 46% of *Acinetobacter* spp. hospital isolates possessed PER-1-type *β*-lactamases.[6] In this study, attempts to demonstrate plasmid carriage of $bla_{\text{PER-1}}$ in Ama-1 and to transfer the ESBL resistance phenotype from Ama-1 to *Escherichia coli* DH10B by mating-out assays and by electroporation (electrotransformation) were unsuccessful.

Ama-1 was isolated following culture of a rectal swab obtained from a 90 year old female patient on the intensive care unit. She had previously been admitted to two other hospitals in Paris but, to the best of our knowledge, had not travelled to Turkey, nor had she been in contact with either Turkish patients or travellers to Turkey. PER-1-positive *A. baumannii* strains were not isolated from the rectal swabs of 16 other patients on the intensive care unit at the same time as the patient from whom Ama-1 was recovered. Moreover, comparison of *Apa*I-digested genomic DNA[7] extracted from Ama-1 with DNA from four randomly selected PER-1-type *β*-lactamase-positive and one PER-1-type *β*-lactamase-negative *A. baumannii* strains from Turkey by pulsed-field gel electrophoresis (PFGE) revealed different restriction patterns (Figure). Thus, the French isolate was not clonally related to the Turkish strains.

This is the first report from outside Turkey of the detection of an ESBL in an *Acinetobacter* spp. clinical isolate. We believe that it is also the first description of the sequencing-based identification of an ESBL gene in an

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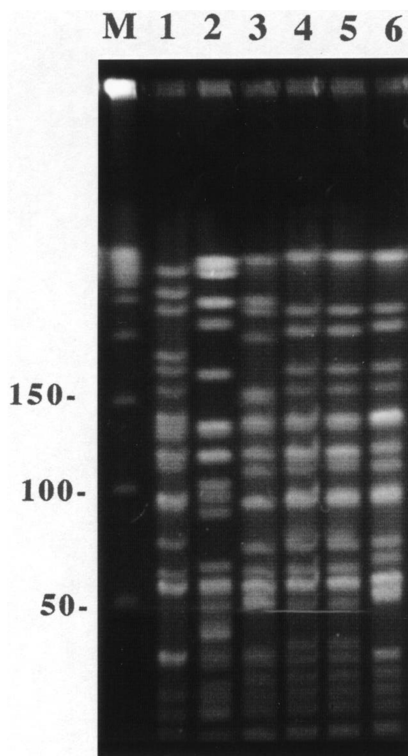

Figure. PFGE of *Apa*I-digested genomic DNA extracted from *A. baumannii* isolates. M, size markers (kb); lane 1, Ama-1; lanes 2, 4, 5 and 6: PER-1-type β-lactamase-positive Turkish strains; lane 3, PER-1-type β-lactamase-negative Turkish strain.

Acinetobacter spp. strain. ESBLs such as bla_{PER-1} may increase the level of naturally occurring multidrug resistance amongst isolates belonging to these species. Strains of *Acinetobacter* spp. should therefore be included in screening programmes designed to detect ESBL-producing aerobic Gram-negative bacilli as hospital outbreaks caused by these bacteria are common.[7] However, routine detection of such strains may be difficult because the synergy between third-generation cephalosporins and clavulanic acid, typically observed with ESBL-producing Enterobacteriaceae, tends to be minimal with *Acinetobacter* spp.

Acknowledgement

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Adherent bacteria and activity of antibiotics

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Sir,

We read with interest the recent correspondence in the journal from Gilbert & Brown,[1] in which they comment on the activity of antibiotics in biofilms.

In general, the dominant mechanisms of biofilm resistance may be related to the suppression of bacterial growth within the biofilm, to physicochemical interaction of the glycocalyx with certain antibiotics (via dipole–dipole-, H-, and ionic-bonds, and complexes) and to changes in the cell envelope following adhesion to hard and soft tissues. Suppressed growth rate and cell wall alterations subsequent to cell density transcriptional activation may be ultimately responsible for phenotypic resistance of adherent bacteria to certain antibiotics.[2] In addition to the cited Sorbarod technique, other models and methods for understanding specific aspects of antimicrobial resistance of sessile bacteria are useful to elucidate specific resistance mechanisms. Thus, for testing the effects of antibiotics on slow-growing

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bacteria we use phosphate-buffered saline (PBS) as a non-proliferating medium at 37°C.

We agree with Gilbert & Brown,[1] that antibiotics other than β-lactams would have markedly different results when the Sorbarod technique is employed. Relatively thick, hydrated, polyanionic-gelled polysaccharides and glycoproteins may act like ion exchange resins adsorbing cationic aminoglycosides until all binding sites are saturated.[3] Uptake of more hydrophilic antibiotics into bacteria varies with the drug's charge, size, and hydrophilicity and with the number of porin proteins, which changes with varying metabolic activity.[4] Ciprofloxacin, though highly effective against growing and non-growing Gram-negative bacilli,[5] had no detectable efficacy in an animal model with infected foreign bodies.[6]

Extracts of exopolysaccharide from slime-positive strains of *Staphylococcus epidermidis*[7] antagonized the antimicrobial efficacy of vancomycin but not that of rifampicin in a dose-dependent fashion. This could explain the increased level of resistance to vancomycin of organisms embedded in a biofilm. Obst *et al.*[8] also reported incomplete sterilization of *S. epidermidis* biofilms when vancomycin was suspended in peptone water or buffered peritoneal dialysis fluid, demonstrating the influence of the microenvironment. When rifampicin was tested in combination with nafcillin, vancomycin, clindamycin, pefloxacin, ciprofloxacin, trimethoprim, teicoplanin or erythromycin *in vitro*, the bactericidal activity of nafcillin, vancomycin and teicoplanin was significantly reduced and rifampicin-resistant strains emerged in combination with trimethoprim.[9]

It is difficult to deduce a general structure–activity relationship but some pharmacodynamical features may be important for the choice of biofilm-active drugs: intracellular accumulation, direct membrane damage, penetration of infected tissues, leukocytes, biofilms and lipophilicity may be advantageous for killing adherent, metabolically-inactive bacteria.

Lipophilic rifampicin with a high antistaphylococcal activity may be a drug that shows most of these properties.[10] Recently, some unpublished investigations on lipophilic drug combinations indicated that the addition of rifampicin to bacteriostatic agents such as erythromycin and fusidic acid resulted in antimicrobial activities more effective than the individual agents towards stationary growth phase bacteria in PBS (Figure). Addition of the bactericidal antibiotic mupirocin to bacteria in the stationary state was also effective. The molecular level of additive and synergic antimicrobial effects of rifampicin with erythromycin, mupirocin and fusidic acid was assumed to be a sequential activity of these substances on the RNA-polymerase dependent protein-synthesis level. Due to the highly lipophilic nature of the drugs, intracellular accumulation may enhance the post-antibiotic effect and therefore the killing efficacy. Lipophilic drug combinations containing rifampicin tested in this study may be beneficial for the outcome of implant infections by microorganisms in a stationary growth phase.

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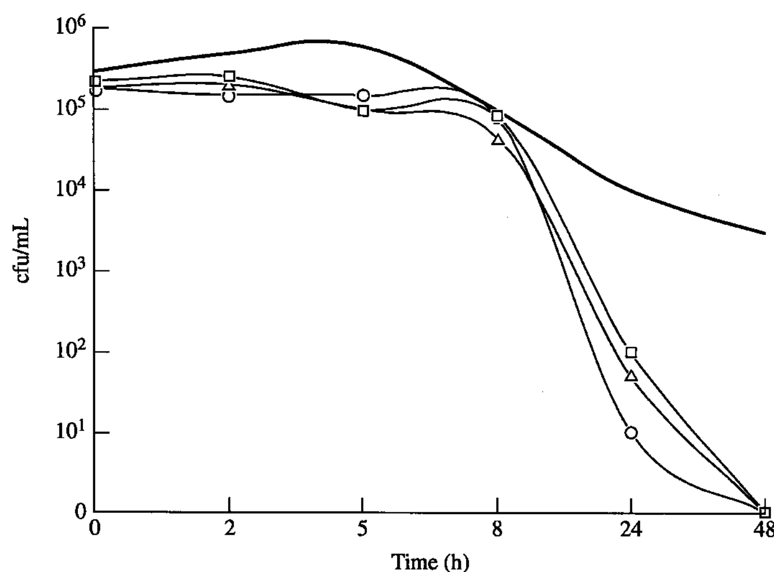

Figure. Killing kinetics of rifampicin (R) with mupirocin (M), erythromycin (E), and fusidic acid (F) in combination (0.5× MIC of each antibiotic) against *S. epidermidis* RP62 (10^5 cfu) in PBS buffer. In contrast to MICs of single agents (not shown), staphylococci in a stationary growth state were significantly more susceptible to antimicrobial combinations. △, R–F; □, R–E; ○, R–M; —, control.

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Increasing prevalence of methicillin resistance amongst *Staphylococcus aureus* blood culture isolates

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Sir,

Staphylococcus aureus is the second commonest bacterial species isolated from bacteraemic patients.[1] Based on reports of the results of susceptibility testing of blood culture isolates forwarded to the Public Health Laboratory Service, we observed that the prevalence of methicillin resistance amongst *S. aureus* isolates increased from *c.* 1.5% in 1989–91 to 21.1% in 1996.[2,3] On reviewing the data from 1997, we noted a further 50% increase compared with the previous year, with 31.7% of 7311 strains having been reported as resistant. This relentless increase in the number of methicillin-resistant *S. aureus* isolates will inevitably be associated with a corresponding increase in the use of glycopeptides as treatment of patients with infections caused by these organisms. This, in turn, will lead to increases in the selective pressure for glycopeptide-resistant enterococci, which are already being isolated in many hospitals, and for *S. aureus* isolates exhibiting intermediate resistance to vancomycin which, to date, have been detected in Japan, America and France.[4–6]

Acknowledgement

These data were included in the recent report by the standing Medical Advisory Committee.[7]

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Correlation between serotype and in-vitro antibiotic susceptibility of pneumococci isolated in Saudi Arabia

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Sir,

Reduced susceptibility to penicillin amongst specific serotypic clusters of *Streptococcus pneumoniae* isolates has been described previously.[1] Regional differences in susceptibility patterns have also been recognized.[2] In the present study, we investigated the relationship between serotype and antibiotic susceptibility for pneumococci isolated between 1994 and 1996 in the central and western regions of Saudi Arabia.

One hundred and thirty-one randomly selected, non-replicate clinical isolates of *S. pneumoniae* were identified by standard laboratory procedures. MICs of penicillin, cefotaxime, imipenem, trimethoprim/sulphamethoxazole, erythromycin, ciprofloxacin, vancomycin and chloramphenicol for the strains were determined by the Etest method (AB Biodisk, Solna, Sweden). The medium used was thymidine-free Mueller–Hinton agar supplemented with 5% lysed sheep blood and the turbidity of the inoculum was equivalent to that of a 0.5 McFarland standard. Susceptibility categories were assigned according to MIC breakpoints recommended by the National Committee for Clinical Laboratory Standards.[3] Serotyping was performed by the Quellung reaction with pneumococcal antisera by Marguerite Lovgren at the National Centre for Streptococcus, Edmonton, Alberta, Canada; the Danish nomenclatural system was used.[2]

Of the 131 isolates, 111 were typable and 48 of these exhibited reduced susceptibility to penicillin (37 intermediate susceptibility and 11 resistance). As the purpose of the study was to attempt to correlate the serotype and the antibiotic profile of strains exhibiting reduced susceptibility to penicillin, only data relating to these 48 strains were analysed. Of the 37 (33.3%) strains exhibiting intermediate susceptibility, the predominant serotypes were 14 (eight (21.6%) strains) and 23F (seven (18.9%) strains), whereas seven of the 11 (63.6%) that were resistant to penicillin belonged to serotype 9V. The antibiotic susceptibilities of the pneumococci exhibiting reduced susceptibility to penicillin are summarized in the Table. According to criteria proposed by Butler *et al.*,[4] 38 (34.2%) of the 111 typable isolates were categorized as multiresistant, i.e. resistant to two or more of the six major antibiotic groups tested (data not shown); the predominant serotypes were 9V, 14 and 23F. No correlation between penicillin-resistant strains belonging to serotype 9V and either temporal and/or geographic clustering was detected.

Periodic surveys conducted over the past 15 years have traced the development of antibiotic resistance, particularly penicillin resistance, among pneumococci isolated from various parts of Saudi Arabia, together with regional similarities and variations in serotype.[5] Thirty-six per cent of our pooled strains, isolated in two regions of the country separated by a considerable distance, currently exhibit reduced susceptibility to penicillin (MICs > 0.1 mg/L) and many of these are resistant to multiple other antibiotics. These observations are consistent with the increases in the incidences of multidrug-resistant pneumococci reported in other countries and the observation that most penicillin-resistant isolates also carry genetic determinants encoding resistance to multiple antibiotics.[6]

The emergence of 9V as the predominant serotype amongst our penicillin-resistant strains is a recent observation and parallels the increase in the incidence of this

Table. Antibiotic susceptibilities of 48 pneumococcal isolates exhibiting intermediate susceptibility (P-IS) or resistance (P-R) to penicillin

	Susceptibilities of isolates exhibiting P-IS (n = 37) or P-R (n = 11)		
Antibiotic	susceptible	intermediate	resistant
Cefotaxime	36/2 (79.2%)	1/9 (20.8%)	0
Imipenem	36/9 (93.8%)	1/2 (6.2%)	0
Trimethroprim/ sulphamethoxazole	15/1 (33.3%)	15/0 (31.3%)	7/10 (35.4%)
Erythromycin	29/9 (79.2%)	0	8/2 (20.8%)
Ciprofloxacin	22/4 (54.2%)	15/7 (45.8%)	0
Vancomycin	37/11 (100%)	0	0
Chloramphenicol	37/11 (100%)	0	0

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serotype noted in other surveys.[4] Periodic surveillance of pneumococcal antibiotic/serotype relationships serves a useful purpose in terms of facilitating local therapeutic strategies and formulating vaccines that are relevant to the local population.

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Classification of *Streptococcus pneumoniae* based on in-vitro susceptibility to oxyiminocephalosporins

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Sir,

Penicillin-resistant *Streptococcus pneumoniae* was first isolated in Australia,[1] and highly penicillin-resistant and multiply antibiotic-resistant strains of *S. pneumoniae* in South Africa.[2] In Japan, some cases of clinical failures with treatment by β-lactam antibiotics due to penicillin resistance have also been reported.[3,4] In a previous report,[5] we investigated the in-vitro susceptibility of 40 clinical strains of *S. pneumoniae* which were isolated in Japan between 1982 and 1990. Some strains were susceptible to penicillins, but resistant to cephalosporins that had oxyimino groups at the 7-position on the cephem skeleton (i.e. oxyiminocephalosporins). This suggested that there was a structure-specific resistance in *S. pneumoniae* for β-lactam agents.

In this study, 324 distinct clinical strains of *S. pneumoniae* isolated in Japan between 1993 and 1996 were investigated for their in-vitro susceptibilities to determine the presence of oxyiminocephalosporin resistance in penicillin-susceptible strains.

The antibiotics used in this study were as follows; amoxycillin, cefaclor and cefuroxime (Sigma Aldrich Japan, Tokyo, Japan), benzylpenicillin (Banyu Pharmaceutical Co. Ltd, Tokyo, Japan), and cefotiam (Takeda Pharmaceutical Co. Ltd, Osaka, Japan). Cefteram, cefpodoxime and cefixime were synthesized in our laboratory. MICs were determined by an agar dilution method using brain heart infusion agar (BHIA; Eiken Co. Ltd, Tokyo, Japan) supplemented with 5% defibrinated sheep blood as culture medium.

S. pneumoniae has been conventionally classified into three groups, (susceptible (PSSP), intermediate (PISP) and resistant (PRSP) to penicillin) based on their MICs of benzylpenicillin. Among the strains tested, these were 227, 77 and 20 strains of these groups, respectively. The distribution of MICs of benzylpenicillin, amoxycillin, cefaclor and cefotiam for PSSP were narrow and peaked at one-point. However those of oxyiminocephalosporins (cefteram, cefpodoxime, cefixime and cefuroxime) for PSSP were broad and peaked at two points. For example, the MICs of cefteram were distributed from $\leqslant$0.00625 to 1.56 mg/L, and peaked at both 0.0125 (susceptible peak) and 0.1 mg/L (resistant peak).

These results confirmed our previous report[5] and indicated that PSSP could be classified into two groups based on the MICs of oxyiminocephalosporins. Therefore, we defined the strains for which the MICs of cefteram were $\leqslant$0.025 mg/L and $\geqslant$0.05 mg/L as OS-PSSP (oxyiminocephalosporin-susceptible PSSP) and OR-PSSP (oxyiminocephalosporin-resistant PSSP), respectively.

The MICs of eight β-lactam agents for OS-PSSP, OR-PSSP, PISP and PRSP are shown in the Table. There were 121 OS-PSSP strains and 106 OR-PSSP. Although the MIC ranges, MIC_{50}s and MIC_{90}s of benzylpenicillin, amoxycillin, cefaclor and cefotiam for OR-PSSP were similar to those for OS-PSSP; those of oxyiminocephalosporin for OR-PSSP were higher than those for OS-PSSP. The MIC_{50} and MIC_{90} of oxyiminocephalosporins for OR-PSSP were four-fold higher than those for OS-PSSP. The distribution of MICs of cefteram, cefpodoxime, cefuroxime and cefixime for OR-PSSP peaked at 0.1, 0.2, 0.39 and 1.56 mg/L, respectively.

The incidence of OR-PSSP did not change between 1993 and 1996, remaining at >20%. Continued surveillance for such strains remains essential.

It has been proposed that penicillin-binding protein (PBP) 2X is altered in cefotaxime-resistant mutants and that this is an essential PBP in determining susceptibility to

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Table. MICs of β-lactams for 227 clinically isolates of *Streptococcus pneumoniae* collected in Japan between 1993 and 1996

β-Lactams	Type of structure[a]	R_1	R_2	Strain	MIC range (mg/L)	MIC (mg/L)	
						MIC_{50}	MIC_{90}
Benzylpenicillin	A		–	OS-PSSP[c]	⩽0.00625–0.05	0.025	0.025
				OR-PSSP[d]	⩽0.00625–0.05	0.025	0.05
				PISP[e]	0.1–0.78	0.39	0.78
				PRSP[f]	1.56	1.56	1.56
Amoxycillin	A		–	OS-PSSP	⩽0.00625–0.1	0.025	0.05
				OR-PSSP	⩽0.00625–0.2	0.025	0.1
				PISP	0.125–1.56	0.39	0.78
				PRSP	⩽0.00625–3.13	1.56	1.56
Cefaclor	B		—Cl	OS-PSSP	0.1–3.13	0.78	1.56
				OR-PSSP	0.39–3.13	0.78	1.56
				PISP	1.56–>100	12.5	100
				PRSP	50–>100	100	>100
Cefotiam	B			OS-PSSP	0.025–0.78	0.1	0.2
				OR–PSSP	0.05–0.78	0.1	0.2
				PISP	0.1–12.5	1.56	3.13
				PRSP	1.56–12.5	3.13	12.5
Cefteram	B			OS-PSSP	⩽0.00625–0.025	0.0125	0.025
				OR-PSSP	0.05–1.56	0.1	0.2
				PISP	0.05–1.56	0.39	1.56
				PRSP	0.78–3.13	0.78	1.56
Cefpodoxime	B		$—CH_2OCH_3$	OS-PSSP	⩽0.00625–0.2	0.025	0.05
				OR-PSSP	0.1–1.56	0.2	0.39
				PISP	0.1–3.13	0.78	3.13
				PRSP	1.56–6.25	3.13	3.13
Cefuroxime	B		$—CH_2OCONH_3$	OS-PSSP	⩽0.00625–0.39	0.025	0.1
				OR-PSSP	0.05–6.25	0.2	0.78
				PISP	0.2–3.13	0.78	3.13
				PRSP	0.39–6.25	3.13	3.13
Cefixime	B		$—CH{=}CH_2$	OS-PSSP	>0.1–3.13	0.2	0.39
				OR-PSSP	0.2–12.5	1.56	6.25
				PISP	1.56–25	6.25	12.5
				PRSP	12.5–50	12.5	25

[a]Type A, penicillins: Type B, cephalosporins:

[b]The boxed part of the structure is the oxyimino group.
[c]Oxyiminocephalosporin-susceptible, penicillin-susceptible *S. pneumoniae* (benzylpenicillin and cefteram MICs ⩽0.05 and⩽0.025 mg/L) (n = 121).
[d]Oxyiminocephalosporin-resistant, penicillin-susceptible *S. pneumoniae* (benzylpenicillin and cefteram MICs ⩽ 0.05 and ⩾ 0.05 mg/L) (n = 106).
[e]Penicillin intermediate resistant *S. pneumoniae* (benzylpenicillin MICs 0.1–0.78 mg/L) (n = 77).
[f]Penicillin-resistant *S. pneumoniae* (benzylpenicillin MICs ⩾ 1.56 mg/L) (n = 20).

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cefotaxime.[6] From our results it would appear that the resistant mutants were OR-PSSP. More detailed studies of mutations in PBP2X are necessary in order to determine the oxyiminocephalosporin-resistance mechanism. In the meantime, we propose that PSSP should be classified into two groups, OS-PSSP and OR-PSSP.

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General stress response master regulator *rpoS* is expressed in human infection: a possible role in chronicity

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Sir,

Recently, there has been much interest in an aspect of the stationary phase of non-sporulating environmental bacteria termed the general stress response (GSR), which results in cells resistant to numerous physical and chemical stresses.[1] The final, non-replicating stages have been variously described as quiescent, resting, or dormant. We wished to test the hypothesis that this response to stress could be a critical event in chronic infection, resulting in at least a subpopulation of cells contributing to the characteristic antibiotic resistance of chronic infections. Using RT-PCR we have found strong mRNA expression of the master regulator of GSR in sputum from cystic fibrosis (CF) patients with chronic *Pseudomonas aeruginosa* lung infection.

A general tendency for nutrient depletion and slow or zero growth to be associated with antibiotic resistance[2] has long been established. In retrospect, it seems probable that, in addition to the particular consequences of adaptation to specific nutrient depletion or starvation and of reduced growth rate, the GSR played a significant role in resistance. There is also evidence that nutrient depletion has major effects on the susceptibility of *P. aeruginosa*[3] and of *Burkholderia cepacia*[4] to phagocytosis (and cationic proteins) and to serum killing. Nevertheless, there are as yet few papers on the effects of the general stress response on the susceptibility of microbes to host defences and antibiotics.

The *rpoS*-encoded sigma factor σ^s is a master regulator in a complex network of stationary-phase-responsive genes in *Escherichia coli*,[1] whereas in *P. aeruginosa* it seems likely that at least two sigma factors, RpoS and AlgU, and density-dependent *N*-acyl homoserine lactone (AHL) quorum sensing molecules act in concert to orchestrate the organism's remarkable persistence in the CF lung. There is a hierarchical link between AHLs and RpoS expression.[5] Not only do AHLs influence virulence factor production by *P. aeruginosa*, they also have immunomodulatory activity.[6] Furthermore, AHLs influence biofilm development.[7] Thus, the density-dependent regulation of virulence determinants (and secondary metabolites) in *P. aeruginosa* is integrated with biofilm formation and adaptation and survival in the stationary phase.

In the CF lung, colonization by *P. aeruginosa*, expressing an iron-restricted phenotype,[8] is due to cells present as biomasses or adherent biofilms[9] which are slow growing or non-growing, from which cells may break away to cause acute exacerbations of infection. For prescribing purposes it is these latter, growing cells that are typically characterized *in vitro* in terms of antibiotic susceptibility. Cell density will be relatively high in the biomass or adherent biofilm fraction of the population and such populations would be expected to demonstrate density-related effects at a stage when their free-growing planktonic counterparts of equivalent population numbers would not.

Sputum samples from CF patients undergoing routine physiotherapy were collected and immediately resuspended in a 0.05% solution of sodium azide and stored in dry ice before transport and long-term storage at −20°C. This azide step was included to disrupt the production of

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ATP and prevent the synthesis of mRNA resulting from any general stress response associated with sample preparation and processing. A multiplex PCR (M-PCR) test based on the simultaneous amplification of two *P. aeruginosa* outer membrane lipoproteins was performed on these samples. M-PCR detected five positives out of 19 sputum samples. RNA extracted from these samples using an RNeasy Extraction Kit (Qiagen, Crawley, UK) was used as the template for RT-PCR. *P. aeruginosa* specific primers designed to amplify a 528 bp *rpoS* product were used in conjunction with Titan One-Tube RT-PCR kits (Boehringer, Mannheim, Germany) to measure gene expression. In all cases *rpoS* was found to be strongly expressed *in vivo* by the clinical isolates. Its identity was confirmed by sequence analysis.

Biofilm cultures of bacteria, including *P. aeruginosa*, are much more resistant than equivalent cells grown in suspension. The reasons for the extreme resistance of biofilms are not clear. We propose that biofilm growth leads to an early accumulation of density-dependent signals and to an early general stress response and possibly a more complete expression of the response relative to that in conventional planktonic culture. This may partially explain both the exceptional antibiotic resistance of *P. aeruginosa* in CF lung infection in particular and of biofilms generally.

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