


Extended high-level cross-resistance to antipseudomonal antibiotics amongst Pseudomonas aeruginosa isolates in a university hospital

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Sir,

Pseudomonas aeruginosa remains an important cause of hospital-acquired infections, particularly among immunocompromised patients, in whom such infections progress rapidly and are associated with high rates of mortality. The administration of appropriate antimicrobial therapy to these patients is therefore essential. However, because an innate characteristic of P. aeruginosa is the high frequency with which antibiotic-resistant variants emerge, knowledge of resistance rates among clinical isolates of this bacterium is necessary in order to ensure that effective antipseudomonal drugs are prescribed in different clinical settings throughout the hospital. In our region, high levels of antibiotic resistance among most bacterial species, especially Pseudomonas spp., have been reported. Furthermore, preliminary observations of the susceptibility patterns of P. aeruginosa strains isolated in our hospital confirm that a high percentage are resistant to commonly used antipseudomonal agents. The purpose of this study was to determine the in-vitro activities of and extent of cross-resistance among ten antipseudomonal agents currently used as therapy for patients with infections caused by P. aeruginosa.

Two hundred and forty-seven non-replicate strains of P. aeruginosa were recovered from consecutive hospitalized patients with active infections during 1996/7 in AHEPA University Hospital, Thessaloniki, Greece, a 700-bed general hospital with medical, paediatric, and surgical services, specialist intensive care units, and a dialysis ward. Identification of the isolates to species level was performed with the PASCO system (Difco Laboratories, Detroit, MI, USA) according to the manufacturer’s instructions. MICs were determined by a microbroth dilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The medium used was cation-supplemented Mueller–Hinton broth (Difco Laboratories) and the antimicrobial agents tested were amikacin, aztreonam, cefepime, ceftazidime, ciprofloxacin, imipenem, meropenem, piperacillin, piperacillin/tazobactam, and tobramycin. Stock solutions of each drug were prepared in concentrations of 2 g/L and stored at −20°C for a maximum of 1 month; imipenem solutions were prepared freshly on the day of testing. Fresh broth cultures of each bacterium were diluted to provide suspensions containing 5 × 10⁸ cfu/L in microtitre plates containing the antibiotics in appropriate ranges of dilution. P. aeruginosa ATCC 27853 was used as a control. The MIC was defined as the lowest concentration of each antibiotic that allowed no visible growth, and susceptibility categories were assigned according to NCCLS interpretative criteria.

The MIC₉₀, MIC₉₀₉₀₉₀, and MIC₉₀₉₀₉₀₉₀ of resistant strains are shown in the Table. On a weight-for-weight basis, meropenem was the most active agent, inhibiting 90% of strains at concentrations ≥ 16 mg/L, followed by imipenem and aztreonam, the MIC₉₀ of which were 32 mg/L; the two aminoglycosides, tobramycin and amikacin, and ceftazidime and piperacillin were the least active agents (MIC₉₀₉₀ ≥ 128 mg/L). However, when the results were compared according to the percentages of resistant isolates, the activities of all the antimicrobials tested were broadly similar (range 15.8–27.5%). Meropenem was still the most active agent, even when compared with imipenem (to which 22.3% of strains were resistant). It has been proposed that meropenem deactivates the class C β-lactamases of P. aeruginosa more effectively than does imipenem and is therefore less affected by the mutational loss of the D₂ outer membrane protein. This could explain the observation that a considerable number (19 of 55) of imipenem-resistant strains remained either susceptible or intermediately susceptible to meropenem. On the other hand, there were no strains...
that were susceptible to imipenem but resistant or intermediately susceptible to meropenem. Thus, the previously described β-lactamase-independent ‘intrinsic’ resistance, which mediates the reduced susceptibility to penicillins, cephalosporins, meropenem and unrelated drugs, but not to imipenem, was not observed in our bacterial population. It is also of interest that, of the β-lactam antibiotics tested, imipenem exhibited a higher rate of resistance, not only compared with meropenem, but compared with piperacillin and piperacillin/tazobactam as well. In particular, when piperacillin was combined with tazobactam, which effectively reduces the activities of class A penicillinases and to some extent those of partially elevated chromosomal class C β-lactamases, a larger number of strains remained susceptible. It is possible that the extensive use of imipenem for treatment of patients with infections caused by P. aeruginosa in our hospital has led to the selection of strains that either have lost the porin channel or that express carbapenemases that are more active against imipenem than meropenem. Conversely, the limited use, to date, of meropenem and piperacillin/tazobactam, which have only recently become available in Greece, may account for the relatively high percentages of strains susceptible to these agents. Post-marketing surveillance studies of the in-vitro activities of meropenem and piperacillin/tazobactam will be necessary in order to detect changes in susceptibility rates. A further interesting observation is that the percentage of isolates resistant to amikacin was higher than that to tobramycin, possibly reflecting the selective use of the former in our hospital.

When patterns of cross-resistance were assessed, it was found that, of the 103 strains exhibiting resistance to at least one antimicrobial, the majority (59, 57.3%) were resistant to at least five drugs, and 25 (24.3%) were resistant to all of the antimicrobials tested. It appears that our multi-resistant strains of P. aeruginosa (i.e. those resistant to all antipseudomonal drugs) have acquired a variety of resistance mechanisms. The observation that nearly all of these particular isolates exhibited high-level resistance to the carbapenems (MICs >128 mg/L), as well as to other β-lactams, may be due to both reduced drug accumulation and totally derepressed synthesis of chromosomally encoded β-lactamases in these strains. However, neither reduced permeability nor a multi-drug efflux mechanism alone can account for the high-level cross-resistance to other unrelated antimicrobials that was also observed in the present study. These strains may possess supplementary resistance mechanisms, such as altered target DNA gyrases and enzymes that modify aminoglycosides, that would explain the high levels of resistance to ciprofloxacin and the aminoglycosides, respectively. The observation that multi-resistance was found predominantly among P. aeruginosa strains belonging to serotype O:12 (unpublished data) suggests that this characteristic has probably become established in clonally related organisms. Efforts to identify the source(s) of these multi-resistant strains and to minimize further their spread to other patients have been implemented.

References
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Comparison of three methods of determining the in-vitro susceptibilities of Acinetobacter baumannii isolates to imipenem

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Sir,

Acinetobacter baumannii is an important cause of nosocomial infections, especially in patients on intensive care units, and outbreaks caused by multiresistant strains of this pathogen have been reported widely. Although some of the outbreak-related strains have been susceptible only to carbapenems, sulbactam and colistin, sulbactam on its own is not currently available for clinical use in most countries and colistin is considered unsuitable because of its toxicity. These problems with alternative agents emphasize the importance of imipenem as therapy of patients with infections caused by multiresistant strains of A. baumannii. However, because of the instability of imipenem in solution, difficulties in determining the MICs of this agent have been reported when commercially prepared, dried and frozen microbroth dilution panels have been used.

This study was undertaken in order to compare the results of three different methods of determining the in-vitro susceptibilities of A. baumannii isolates to imipenem.

Fifty consecutive isolates of A. baumannii were collected in the Department of Microbiology of the University Hospital V. Macarena in Seville, Spain between 1995 and 1996. The identities of the isolates were confirmed by the API 20 NE system (bioMérieux, Marcy l’Etoile, France), supplemented with biochemical tests recommended by Bouvet & Grimont. The strains were maintained in 10% glycerol in tryptic soy broth at −70°C.

The methods of susceptibility testing included the microbroth dilution method (MBD) (reference method), the agar dilution method (AD) and the Etest. Imipenem was obtained as a powder of known potency from Merck Sharp and Dohme, Madrid, Spain and the media used were cation-adjusted Mueller–Hinton broth (Difco) for the MBD and AD methods and Mueller–Hinton agar (Difco) for the Etest. Etest strips were obtained from Biodisk, Solna, Sweden. The MBD and AD methods were performed according to guidelines issued by the National Committee for Clinical Laboratory Standards (NCCLS) and the Etest method was carried out according to the manufacturer’s instructions. All media were incubated at 35°C and the MICs read after 18–20 h; for the Etest, MICs that fell between two two-fold dilutional steps were rounded up to the higher concentration. Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and A. baumannii ATCC 19606 were used as controls. The MICs determined by the three methods were compared and discrepancies between the results obtained by these methods were expressed in terms of the number of two-fold dilutions. These data were then used to calculate the percentages of isolates for which there was agreement between the tests (i.e. MICs differing by no more than one two-fold dilution). From the results of each of the three test methods, the isolates were assigned to susceptibility categories (susceptible, intermediate susceptible and resistant) according to interpretative breakpoint criteria recommended by the NCCLS and the categorizations were compared and the numbers of minor, major and very major discrepancies noted. The three types of discrepancy were defined as follows: minor, intermediate susceptibility according to either the reference method or the comparative method, susceptible or resistant according to the other method; major, susceptible according to the reference method, resistant according to the comparative method; very major, resistant according to the reference method, susceptible according to the comparative method.

To assess the stability of the imipenem Etest strips, MICs for the three control strains were determined by the Etest method twice weekly over a period of 8 months; the same batch of strips was used throughout. On each day of testing, a ten-strip batch was thawed and then refrozen until all of the strips in the batch had been used.

The MICs of imipenem for the 50 strains, as determined by the MBD method, were as follows: ≤0.06 mg/L (four strains); 0.125 mg/L (six); 0.25 mg/L (three); 0.5 mg/L (ten); 1 mg/L (five); 4 mg/L (eight); 8 mg/L (eight); 16 mg/L (five); and 32 mg/L (one). In 24 of the 50 strains tested, colonies were observed within the elliptical zones of inhibition around the Etest strips. This confounded efforts to define the endpoints/MICs accurately. For each of the strains exhibiting this phenomenon, four colonies growing within the zones of inhibition were subcultured twice on