Aminoglycoside resistance among isolates of nosocomial Enterobacteriaceae

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Summary
Fifty-seven gentamicin-resistant isolates of Enterobacteriaceae, obtained from patients attending hospital, were examined for the production of aminoglycoside-modifying enzymes. Of the 51 strains producing such enzymes, 34 were presumptively plasmid-mediated as indicated by conjugation experiments.

As long ago as 1972, Woods et al. were able to demonstrate plasmid-mediated aminoglycoside resistance among coliform bacilli occurring in East London (urban) and in Transkei (rural). Resistant plasmids have a pool of genetic elements available, including transposons. The expression of resistance can be determined by the plasmid with respect to plasmid copy number or gene amplification. In different genera, interaction between plasmid-coded and cellular components produces varying degrees of expression of antibiotic resistance. Previously reviewed work indicates that resistance to aminoglycosides is frequently due to the presence of aminoglycoside-modifying enzymes. This study concerns gentamicin-resistant strains isolated from clinical material in Cape Town.

Materials and methods

Bacterial strains
The pool of organisms, isolated from various types of specimens, was made up of Escherichia coli (8 strains), Enterobacter spp. (9), Klebsiella spp. (27), Proteus spp. (4), Salmonella spp. (2) and Serratia spp. (7). These organisms were selected on the basis of resistance to gentamicin and were often resistant to tobramycin and amikacin as well. Additional resistance to other antibiotics was common.

Initially, criteria for resistance were arbitrarily laid down as resistance to minimal inhibitory concentrations of gentamicin 6 mg/l, tobramycin 6 mg/l and amikacin 12.5 mg/l, as these were clinically realistic levels, obtainable in serum on the local dosage regimen. The inoculum in each case was made up of 10<sup>3</sup> - 10<sup>4</sup> colony-forming units plated onto antibiotic-containing isosensitivity testing agar (Oxoid). The cation content of the batch had previously been confirmed as being within physiological limits.

Demonstration of enzyme activity
Individual strains were cultured overnight at 37°C in Difco nutrient broth No. 2 and ultrasonicated. Each lysate was tested for enzyme-mediated transfer of radioactivity from acetyl coenzyme A and adenosine-5'-triphosphate to aminoglycosides, using the phosphocellulose binding system. This method makes use of the fact that aminoglycoside antibiotics are strongly basic compounds with a high affinity for negatively charged phosphocellulose. Using radioactive co-substrates, a labelled group may be transferred by means of an enzyme to an aminoglycoside and then detected by binding to phosphocellulose, since the labelled donors, being negatively charged, do not bind.

Plasmid transfer
A method for mating on solid media was used. Experiments were performed by mixing 4 volumes of the presumptive plasmid-carrying strain and 5 volumes of the plasmid-negative recipient Esch. coli J62 nal<sup>+</sup> at 20°C, followed by incubation at 37°C for 2 hours. Selection was for the antibiotic marker on the presumptive plasmid.

Results
In this series of clinical isolates 51 out of 57 strains produced demonstrable aminoglycoside-modifying enzymes (Table I); some strains produced two or more such enzymes (Table II). Enzyme-mediated resistance was successfully transferred from producer donors to recipients in all but 17 instances. In 10 of these, transfer was not attempted because of difficulty with cross-selection associated with multiple antibiotic resistance of the donors.

Discussion
The aim of this study was to explore aminoglycoside-resistant Enterobacteriaceae for the presence of antibiotic-modifying enzymes and the capacity to transfer this property to sensitive strains. This mechanism of antibiotic resistance is the predominant mode in Cape Town at present and similar findings have been reported elsewhere. One difference we found was that a single resistant strain could produce more than one gentamicin-modifying enzyme (see Table II). Whether this is due to a single resistance factor specifying several aminoglycoside-modifying enzymes or the existence of several different plasmids in each bacterial cell, is not clear from the information available at present. Failure to transfer resistance may imply the absence of resistance transfer factor on the plasmid and not necessarily a resistance mechanism otherwise mediated.

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The transfer and expression of resistance to antibiotics poses an ever-growing problem in hospital practice and the prudent use of antibiotics and enforcement of hygiene are essential in limiting the occurrence of plasmid-mediated aminoglycoside resistance.

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REFERENCES


Nuus en Kommentaar/News and Comment

Epidural opiates

The use of epidural opiates to relieve pain is one of the modern techniques that requires no statistical evaluation, according to Zenz (Disch. med. Wschr., 1981, 106, 483). He points out that the identification of opiate receptors in the central nervous system is one of the significant findings in pain research. Both intrathecal and epidural injection of opiates can ensure prolonged analgesia without impairment of consciousness, mobility, sensation other than pain, or sympathetic function. The dosage required is much lower than that needed intra muscularly or intravenously, which implies a local effect on the spinal cord (and nerve roots). Zenz uses the following doses:

- morphine 2-5 mg, buprenorphin 0,15-0,3 mg, fentanyl 0,1 - 0,4 mg.

Side-effects include respiratory depression some hours after an epidural injection (reversible by naloxone without loss of analgesia), itching, probably due to preservatives in the solution, nausea and vomiting (less than with systemic opiates), and occasional retention of urine in the male.

Indications include pain in terminal cancer, acute pain after major abdominal operations, and multiple rib fractures. The technique should, however, not be used if satisfactory alternatives for pain relief are available.

Prophylactic heparin in intermediate coronary syndrome

During the days or weeks before a myocardial infarction 27 - 67% of patients have one or more brief episodes of ischaemic chest pain with ECG changes. Various proposals have been made for preventing infarction in this 'intermediate coronary syndrome' group, and Telford and Wilson (Lancet, 1981, 1, 1225) report a randomized double-blind trial of heparin and/or atenolol for this purpose in 214 patients.

Patients were monitored for 7 days unless circumstances dictated a change, and after this all patients under 65 were given maintenance anticoagulant treatment with warfarin for 8 weeks. Patients given 1,5 - 2 ml of heparin in solution (5000 IU/ml) intravenously 6-hourly for 1 week fared significantly better than those given atenolol (100 mg daily) or placebo. Transmural myocardial infarction developed in 9 out of 54 on placebo and 8 out of 60 on atenolol, but only in 1 out of 51 on heparin and 2 out of 49 on heparin plus atenolol. They recommend that intravenous heparin be included in the medical management of the intermediate coronary syndrome.