concentrations closely similar to the range found in our present study. A conspicuous early ultrastructural feature of alcoholic liver damage is large, swollen mitochondria, suggesting that mitochondrial damage may be an important factor in the pathogenesis of alcoholic liver disease. Enzyme analysis of isolated hepatocyte mitochondrial fractions from patients with alcoholic steatosis, however, has failed to show evidence of mitochondrial damage.⁵ Concentrations of enzymes associated with mitochondrial membranes were unchanged but concentrations of enzymes in the mitochondrial matrix, including glutamate dehydrogenase, were significantly raised in alcoholic steatosis. This suggests that raised serum concentrations of glutamate dehydrogenase might be due to either hepatocyte damage or alcohol induction of liver mitochondrial enzyme activity.

Our study has therefore failed to confirm the usefulness of

serum concentrations of glutamate dehydrogenase as an indicator of liver damage in patients with chronic alcoholism.

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Dissemination of resistance plasmids among gentamicin-resistant enterobacteria from hospital patients

SIDONIE KNIGHT, MARK CASEWELL

Abstract

Out of 184 patients who were infected or colonised with gentamicin-resistant enterobacteria, 17 (9%) harboured more than one strain. Single antibiotic-resistance plasmids were common to more than one of the different organisms isolated from nine patients, strongly suggestive of in-vivo conjugation. An "epidemic" plasmid with a molecular weight of approximately 110 megadaltons was found in 11 distinct strains isolated from four patients. Seven of the organisms harbouring this plasmid were *Klebsiella aerogenes*.

Spread of multiple-resistance plasmids among endemic gentamicin-resistant enterobacteria is not uncommon, and these organisms provide a reservoir of plasmids that may ultimately spread to more pathogenic genera.

Introduction

Interbacterial transfer of antibiotic-resistance plasmids by conjugation is a theoretically potent mechanism for limiting the clinical efficacy of many antimicrobial agents. Although the spread of plasmids from epidemic strains of multiply-resistant coliforms to other Gram-negative bacilli is recognised¹⁻³ little is known about the frequency of plasmid spread among hospital bacteria during endemic colonisation and infection.

We therefore sought evidence for the spread of single plasmids among gentamicin-resistant enterobacteria isolated from patients in our hospital during a non-epidemic period.

Patients and methods

Between 20 February 1979 and 27 March 1980 we identified all inpatients at St Thomas's Hospital whose bacteriological specimens

received by the diagnostic microbiology laboratory yielded more than one strain of gentamicin-resistant enterobacteria. Three strains from one patient at another hospital (case 9) were provided by D G White. Bacteria were identified by standard methods⁴ and controlled disc-diffusion was used for sensitivity testing.⁵ *Klebsiella capsular* (K) typing was as described.^{6,7}

Gentamicin-resistant enterobacteria from patients with more than one such strain were tested for plasmids that were transferable to *Escherichia coli* K12.⁸ Transconjugants were tested for resistance to chloramphenicol (Cm), tetracycline (Tc), ampicillin (Ap), carbenicillin (Cb), cephaloridine (Cr), cephalothin (Kf), cephalexin (Cfx), cefuroxime, cefoxitin (Fox), sulphamethoxazole (Su), trimethoprim (Tp), streptomycin (Sm), kanamycin (Km), neomycin (Nm), gentamicin (Gm), tobramycin (Tm), amikacin, nitrofurantoin, and nalidixic acid. Plasmid molecular weights were determined by single-colony lysate agarose-gel electrophoresis.⁹

Results

During the 13 months 184 patients at St Thomas's Hospital were found to have gentamicin-resistant enterobacteria; 17 (9%) harboured more than one strain, and the isolates from 14 of these patients possessed plasmids that were transferable to *E. coli* K12. Eight of the St Thomas's Hospital patients with multiple strains of gentamicin-resistant enterobacteria and the patient from another hospital were found to harbour two or more strains with common plasmids which were recognisable by their transferable resistance pattern and indistinguishable electrophoretic mobility. The table shows the resistance patterns and approximate molecular weights of these plasmids, together with the identification of their host bacteria and the clinical sources.

Klebsiella aerogenes was among the clinical isolates from eight of these patients and was the first gentamicin-resistant species isolated from seven patients. All but two of the patients had at least two distinct strains in their urine.

K. aerogenes K21, bearing a transferable plasmid of approximately 110 megadaltons which conferred the resistance pattern ApCbCm-TcSuTpSmKmNmGmTm, was isolated from four patients; this plasmid was found in 11 epidemiologically distinct strains belonging to four different genera (see table).

Discussion

Our finding that as many as 9% of patients with clinical isolates of gentamicin-resistant enterobacteria were infected or colonised by more than one strain, and that almost half of these

Department of Microbiology, St Thomas's Hospital Medical School, London SE1 7EH

SIDONIE KNIGHT, BSC, research assistant

MARK CASEWELL, MD, MRCPATH, senior lecturer in microbiology (now senior lecturer in medical microbiology, The London Hospital Medical College, London E1 2AD)

patients harboured several different strains or even different species bearing indistinguishable plasmids, indicates that considerable dissemination of plasmids does occur during periods of endemic colonisation and infection. Although plasmid transfer may have occurred between several enterobacteria before each patient acquired the strains, it seems more probable that our results reflect the transfer of plasmids from one strain to another after they were acquired by the patient—that is, by in-vivo conjugation.

In-vivo conjugation has been observed in the bowel of man, with¹⁰ and without¹¹ antibiotic selective pressure, and in the burns of mice.¹² The frequency with which we have noted urinary isolates and the relative difficulties of achieving in-vivo conjugation in the human bowel¹³ suggest that the urinary bladder is a

Patients harbouring several gentamicin-resistant enterobacteria with plasmids common to more than one strain

Case No	Clinical isolates			Plasmid	
	Date	Site	Organism	Resistances transferred†	Molecular weight (megadaltons)
1	20/2/79	Blood	<i>K aerogenes</i> K21	ApCbCmTcSuTpSmKmNmGmTm	110
	23/2/79	Urine	<i>K aerogenes</i> NT		
	23/2/79	Urine	<i>E coli</i>		
2	14/5/79	Urine	<i>K aerogenes</i> K21	ApCbCmTcSuTpSmKmNmGmTm	110
	16/5/79	Faeces	<i>E coli</i>		
3	21/6/79	Ulcer	<i>K aerogenes</i> K21	ApCbCmTcSuTpSmKmNmGmTm	110
	19/7/79	Faeces	<i>E coli</i>		
	20/7/79	Wound	<i>E cloacae</i>		
4	3/7/79	Wound	<i>K aerogenes</i> K21	ApCbCmTcSuTpSmKmNmGmTm	110
	3/7/79	Wound	<i>K aerogenes</i> K3		
	4/7/79	Urine	<i>C freundii</i>		
	4/7/79	Urine	<i>E coli</i>		
5	27/7/79	Wound	<i>K aerogenes</i> K64	ApCbCmTcTpGmTm	105
	5/9/79	Urine	<i>K aerogenes</i> K21		
	5/9/79	Urine	<i>K aerogenes</i> K60		
6	9/8/79	Urine	<i>E coli</i>	ApCbCrCmTcSuTpSmKmNmGm	57
	15/8/79	Urine	<i>K aerogenes</i> NT		
7	23/7/79	Urine	<i>E coli</i>	ApCmSuTpKmNmGmTm	80
	23/7/79	Urine	<i>S marcescens</i>		
8	27/3/80	Urine	<i>K aerogenes</i> K16	ApCbCrKfCmTcSuTpSmKmNmGm	62
	27/3/80	Urine	<i>E coli</i>		
9*	22/10/79	Urine	<i>K oxytoca</i> K43	ApCbCrKfCfxFoxCmTcSuTpGm	50
	6/11/79	Urine	<i>E coli</i>		
	7/12/79	Urine	<i>K aerogenes</i> K64		

*Not a patient at St Thomas's Hospital.

†Ap, ampicillin. Cb, carbenicillin. Cm, chloramphenicol. Tc, tetracycline. Su, sulphamethoxazole. Tp, trimethoprim. Sm, streptomycin. Km, kanamycin. Nm, neomycin. Gm, gentamicin. Tm, tobramycin. Cr, cephaloridine. Kf, cephalothin. Cfx, cephalexin. Fox, cefoxitin.

more likely anatomical site for conjugation between enterobacteria.

K aerogenes K21, which is one of the more prevalent strains in Britain,¹⁴ was isolated from four patients in two small clusters of cross-infection, and for these patients it was the first isolate to be found with the plasmid of 110 megadaltons which was ultimately found in nine other strains of enterobacteria. Thus this plasmid seems to be already "epidemic" at St Thomas's Hospital.

Resistance genes from coliform-derived plasmids have already been implicated in ampicillin-resistant¹⁵ and chloramphenicol-resistant¹⁶ *Haemophilus influenzae*, in penicillinase-producing *Neisseria gonorrhoeae*,¹⁷ and in carbenicillin-resistant *Pseudomonas aeruginosa*.¹⁸ The results of this study indicate that endemic gentamicin-resistant enterobacteria, especially *Klebsiella* spp.,¹⁹ provide an abundant reservoir of transferable plasmids. In the absence of controlled antibiotic usage and epidemiological containment of these strains, it seems probable that resistance genes derived from these plasmids will increasingly disseminate among more pathogenic genera.

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