and desktop calculator/computer model No. 9825T Hewlett-Packard, Palo Alto, CA). Here, the friction coefficients for either 10 or 15 g sample sizes were treated as the dependent (response) variables and the physicochemical properties in Table 1 as the predictor variables (though, strictly speaking, not all are independent variables).

The results with the 10 g lubricant sample size suggested that 98% ($R^2 = 0.982$, Overall $F = 53.54$, degrees of freedom of denominator and numerator = 4, significant 99%) of the variation in friction coefficients could be accounted for by four variables. These variables were: projected surface area for particles, Martin's diameter, bulk density of powder, and projected area diameter.

Similarly, with the 15 g sample size, 80% ($R^2 = 0.800$, overall $F = 10.02$, degrees of freedom of numerator = 2, degrees of freedom of denominator = 5, significant 95%) of the variation in friction coefficients was explained by two variables, namely, projected surface area for particles and Martin's diameter.

Based on the above results it can be generalized that physical properties, particularly the project surface area of particles and Martin's diameter account for most of the variation in friction coefficients of the lubricants rather than the moisture content or melting point. Hence, to ensure reproducible functionality of tablet lubricants it could be suggested that particle size and/or surface area parameters be incorporated in product specifications, and in-house quality control tests be devised on such parameters.

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Cephaloridine resistance in Gram-negative bacteria isolated in Scotland

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Abstract—The incidence of cephaloridine resistance (minimum inhibitory concentration, MIC > 8mg L^{-1}) in isolates from urinary tract infections was 45·1% in Glasgow, 22·6% in Dundee and 25·9% in Edinburgh. The incidence of ampicillin resistance (MIC > 8 mg L^{-1}) was even higher:- being 45·2% in Dundee and 48·5% in Edinburgh. In Glasgow, the incidence was 71·9% which is the highest proportion of ampicillin resistance reported in the United Kingdom. The cephaloridine resistant strains were examined for β-lactamase production. Amongst these strains 50·8% produced only a chromosomal β-lactamase, whereas 47·9% produced β-lactamases which were potentially plasmid-mediated on the basis of biochemical tests. Only 1% of the resistant strains produced no detectable β-lactamase.

The recent increase in the number of available β-lactam antibiotics highlights the clinical importance of antibacterials. In the Royal Infirmary, Edinburgh, prescriptions for β-lactams accounted for 59% of all antibiotic prescriptions in 1986. Newer β-lactam antibiotics have been chemically manipulated to increase β-lactamase stability. However, a high proportion of older, less stable, compounds such as ampicillin or the 'first generation cephalosporins' are still in widespread clinical use for uncomplicated infections.

Several surveys have been conducted outside Scotland which examined the types of β-lactamase produced by ampicillin-resistant populations of Gram-negative enterobacteria (Simpson et al 1980, 1986; Roy et al 1983; Stobberingh et al 1985). However despite the increase in the use of cephalosporins, no surveys have been undertaken to investigate β-lactamase-mediated resistance to this class of compound.

The objectives of this study were to compare the relative incidences of cephalosporin and penicillin resistance, employing cephaloridine and ampicillin as class representatives, and to investigate the distribution of bacterial species and β-lactamase types within the cephaloridine-resistant strains.

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Materials and methods

Bacterial strains. A random population of 549 Gram-negative strains were collected from hospitals in Edinburgh, Dundee and Glasgow from January to April 1984. Only one isolate per patient was included in the study. Most of the strains (441) were isolated from significant urinary tract infections (UTI). From this population of 549, 164 cephaloridine-resistant (minimum inhibitory concentration, MIC > 8mg L^{-1}) strains were obtained.

A further 70 cephaloridine-resistant, Gram-negative strains selected on the same criteria, were collected from the three centres in April 1984. Thus the total number of cephaloridine-resistant strains studied was 234. The bacterial species of each resistant strain was determined by the API 20E biochemical test system (API system, SA38390, Montalieu-Vercieli, France).

Antimicrobial drug sensitivity testing. Sensitivity-testing and MIC determinations of the antimicrobials were determined as previously described (Amyes & Gould 1984). Resistance to a defined antimicrobial agent was described as an MIC of greater than 8 mg L^{-1}.

β-Lactamase preparation and isoelectric focusing. Cell-free extracts containing β-lactamase for isoelectric-focusing (IEF)

were prepared from bacteria grown on nutrient agar slopes (Livermore et al 1984). Cultures which failed to produce a β-lactamase by this method were induced as follows. An overnight nutrient broth culture was diluted 10^{-2} in prewarmed nutrient broth no. 2 (Oxoid CM67) and shaken for 2h at 37°C. Cefoxitin, a potent inducer of β-lactamase production (Minami et al 1980) was then added to the growing culture at one quarter the MIC value for that particular strain and incubation was continued for a further 16 h. The bacteria were then harvested and the β-lactamase extracted as previously described (Simpson et al 1980).

Isoelectric focusing. The isoelectric points of the β-lactamases were determined by the method of Matthew et al (1975). Polyacrylamide gels (25mL) were prepared which included pH 3·5–10 ampholines (Catalogue no. 1809-101) (LKB, Bromma, Sweden) to form the pH gradient. β-Lactamases samples, in volumes up to 100 μL according to their activity, were applied to the surface of the gel. The gels were run overnight at constant power (1 watt) at 4°C. The pH range across the surface of the gel was determined with a pH surface electrode and the β-lactamase bands were visualized by staining with the chromogenic cephalosporin, nitrocephin. The isoelectric point of each β-lactamase was determined and compared with the position of standard β-lactamases run on the same gel (Simpson et al 1980). On this basis, most of the β-lactamases could be identified and classified as plasmid- or chromosomally mediated. Those few that could not be identified were given a preliminary classification based on their isoelectric point (Simpson et al 1980).

Results

Incidence of cephaloridine resistance. One hundred and sixty four (29·9%) of the 549 strains surveyed were resistant to cephaloridine. Of these 549 strains, 441 were isolated from UTIs and the incidences of cephaloridine and/or ampicillin resistance within this group of strains for each of the three survey centres is shown in Table 1. Patients in Glasgow had the largest proportion (45·1%) of cephaloridine-resistant strains, significantly greater than the proportions in Dundee (22·6%) or Edinburgh (25·9%) ($P<0·001$; χ^2 (2) = 14·6). Similar proportions of ampicillin-resistant UTI isolates were found in strains from Edinburgh and Dundee, 48·5% and 45·2%, respectively, which again are significantly lower than the figure of 71·9% obtained in Glasgow ($P<0·001$; χ^2 (2) = 16·3). Most (120/127) of cephaloridine-resistant (CER^r) strains were also ampicillin-resistant (AMP^r). The remaining seven CER^rAMP^s strains included five species: *Escherichia coli* (1), *Hafnia alvei* (1), *Enterobacter cloacae* (1), *Proteus mirabilis* (3) and *Acinetobacter calcoaceticus* (1).

Table 1. Incidences of resistance to cephaloridine and/or ampicillin amongst 441 Gram-negative bacteria responsible for urinary tract infections.

Resistance profile*		Number of strains (%)			
		Dundee	Edinburgh	Glasgow	Total
CER^s	AMP^s	51 (54·8)	130 (48·9)	23 (28·0)	204 (46·2)
CER^r	AMP^s	0 (0)	7 (2·6)	0 (0)	7 (1·6)
CER^r	AMP^r	21 (22·6)	62 (23·3)	37 (45·1)	120 (27·2)
CER^s	AMP^r	21 (22·6)	67 (25·2)	22 (26·8)	110 (24·9)
Total		93	266	82	441

* CER^s, AMP^s MIC of cephaloridine (CER) or ampicillin (AMP) of $<8mg\ L^{-1}$. CER^r, AMP^r MIC of CER or AMP $>8mg\ L^{-1}$.

Table 2. Distribution of bacterial species and β-lactamase types in Scottish survey.

Bacterial species and no. of strains	Distribution of β-lactamase types										
				R-plasmid mediated							
				TEM-		OXA-			PSE-	TEM-1 and SHV-1	TLE-2 and TEM-1 and SHV-1
	None		Chr. only	1	2	1	2	3	4		
A. calcoaceticus	2		1	1							
C. freundii	7		5	2							
Ent. aerogenes	2		2								
Ent. agglomerans	1		1								
Ent. cloacae	25		16	4	3		1		1		
Ent. sakazakii	1			1							
E. coli	104		25	76	2				1		
H. alvei	1					1					
K. oxytoca	12		9	2	1						
K. ozaenae	3		2							1	
K. pneumoniae	8		5	1					1		1
P. mirabilis	12	3	3	4	2						
P. morganii	9		7	2							
P. vulgaris	10		10								
Prov. stuartii	1		1								
Ps. aeruginosa	22		20	2							
Ps. fluorescens gp.	3		3								
Ps. maltophilia	1			1							
Ps. maltophilia	1			1							
S. marcescens	8		7					1			
S. odorifera	2		2								
TOTALS	234	3	119	96	8	1	1	2	2	1	1

To demonstrate the range of resistance mechanism, a further 70 cephaloridine-resistant strains from the three centres were obtained after the three month survey, giving a total of 234 cephaloridine-resistant strains for biochemical analysis.

Distribution of bacterial species. When the 234 cephaloridine-resistant strains were pooled, a total of ten genera comprising 20 species were identified (Table 2). *E. coli* (44·4%) was the most prevalent species, followed by Proteus (13·7%), Enterobacter (12·4%), Pseudomonas (11·1%) and Klebsiella (6·8%).

Distribution of β-lactamase among cephaloridine-resistant strains. The β-lactamases produced by the 234 cephaloridine-resistant strains were identified by IEF of the crude enzyme extracts. Ten resistant strains, which did not appear to produce a chromosomal species-specific β-lactamase, were induced with cefoxitin at one quarter of their MIC. Comparison with standard marker enzymes enabled preliminary classification of the β-lactamases according to whether they were of plasmid or chromosomal origin (Simpson et al 1980).

Only three Enterobacterial strains did not produce a detectable β-lactamase and all three were *Proteus mirabilis* from different sources. One hundred and nineteen strains (50·9%) comprising all the genera encountered, produced only β-lactamases which were typically chromosomally mediated. One hundred and twelve strains (47·9%) produced an enzyme which was typically plasmid-mediated in addition to the chromosomal β-lactamase (Table 2).

Typically plasmid-mediated β-lactamases. The 112 strains producing potentially plasmid-mediated β-lactamases, comprised 14 of the 20 bacterial species encountered. The PSE-4 β-lactamase was produced by two strains; an *Enterobacter cloacae* A113 and a *Klebsiella pneumoniae* 241. This enzyme has not been identified before in a genus other than Pseudomonas. Another

strain of *Klebsiella pneumoniae* produced three potentially plasmid mediated β-lactamases: TEM-1, SHV-1 and a novel enzyme with a pI of 6·5. Preliminary studies indicate that the novel enzyme may be related to the TEM β-lactamase and it has been designated TLE-2.

Amongst the remaining 109 strains, six well-characterized β-lactamases were identified (Table 2). These were the TEM-1, TEM-2, OXA-1, OXA-2, OXA-3 and SHV-1 β-lactamases. The TEM-1 β-lactamase was the most common and was present in 98 strains (41·9%) comprising 11 species. The next most common β-lactamase was the TEM-2 enzyme which was present in eight strains. The other β-lactamases in this category were produced by only one or two strains.

Discussion

In contrast to previous surveys, resistance to the cephalosporin, cephaloridine, was used to identify β-lactam-resistant Gram-negative strains. The incidences of cephaloridine and ampicillin resistance in strains isolated in Dundee and Edinburgh were similar to those reported in London by Grüneberg (1984). However, the Glasgow portion of our survey revealed that 71·9% of the strains examined were ampicillin-resistant and 45·1% were cephaloridine-resistant. Ampicillin resistance as frequent as this has not been reported before in the United Kingdom (Simpson et al 1980; Grüneberg 1984).

In this Scottish survey, the selection of resistant strains using cephaloridine has resulted in a higher proportion of resistant Enterobacter and Proteus species strains than had been found in the previous surveys where ampicillin resistance was the selection criterion (Simpson et al 1980, 1986; Roy et al 1983; Stobberingh et al 1985). On the other hand, the use of ampicillin as the selection agent in these latter surveys resulted in a higher incidence of resistant Klebsiella species than we found in our study. Our results suggest that a higher proportion of Enterobacter and Proteus species are inherently resistant to cephaloridine than to ampicillin. Similarly, cephaloridine screening may be responsible for a high proportion of strains (50·8%) producing only a chromosomally mediated β-lactamase and harbouring no plasmid-encoded enzyme. The chromosomal DNA of Enterobacter and Proteus species can produce a variety of β-lactamases which are well able to hydrolyse the cephalosporins. Indeed, in our survey there was a particularly high proportion of Enterobacter species. The presence of chromosomally determined β-lactamases in these strains may render the necessity, to import plasmids carrying β-lactamase genes, less immediate. This may be especially true in a selective environment of cephaloridine because it could be argued that the plasmid-encoded β-lactamases are less efficient at hydrolysing first-generation cephalosporins, such as cephaloridine, than the penicillins (Amyes 1988).

β-Lactamases normally associated with plasmids were detected in 112 strains (47·9%). In common with previous surveys, the most prevalent plasmid-encoded enzyme was TEM-1 (85·2% of all plasmid-encoded enzymes tested). This compares with 53% found by Simpson et al (1980). In later surveys the proportion of the TEM-1 β-lactamase increased to 77% (Simpson et al 1986) and 80% (Roy et al 1983), levels approaching those we found in our own study. In the previous studies, the most successful plasmid-encoded β-lactamase after TEM-1 was a similar enzyme, SHV-1, which accounted for 31% (Simpson et al 1980), 13% (Roy et al 1983) and 12% (Simpson et al 1986), respectively, of all plasmid β-lactamases. In our study, the SHV-1 β-lactamases was found on only two occasions (1·7%) and then only in the presence of another plasmid-encoded β-lactamase in the same bacterial cell. The only plasmid-encoded enzyme found more than twice in our study, other than TEM-1, was TEM-2 but this accounted for only 7% of these β-lactamases.

Except for the identification of PSE-4 β-lactamase in two strains other that Pseudomonas and the novel β-lactamase TLE-2, the distribution of typically plasmid-mediated enzymes was similar to surveys employing ampicillin screening in Europe (Simpson et al 1980, 1986; Roy et al 1983; Stobberingh et al 1985) However, the distribution of β-lactamases was different from a similar survey performed in India where a series of new plasmid-encoded β-lactamases have been found (Nandivada & Amyes 1987).

Nearly 30 plasmid-encoded β-lactamases have been found world-wide (Amyes 1988). Although many of them are not found frequently there are a few, such as TEM-1, which are found with considerable and increasing regularity. These β-lactamases have a particular affinity for penicillins and are easily selected by the use of common penicillins. The common oral penicillins, such as ampicillin and even amoxycillin, are not completely absorbed by the gut and the drug that remains provides an excellent selective environment for the emergence of β-lactamases in the commensal gut bacteria. Bearing this in mind, some care should be exercised in the continued use of some common penicillin antibiotics.

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