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Principal β-Lactamases Responsible for Resistance to β-Lactam Antibiotics in Urinary Tract Infections

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Two independent surveys have been conducted to determine the prevalent bacterial species and β-lactamase types present in clinical populations of gram-negative, ampicillin-resistant isolates. A total of 208 isolates (112 from Nottingham Hospital and 96 from Charing Cross Hospital), all of which had been collected from out-patients suffering from urinary tract infections, were investigated. The incidence of ampicillin-resistant isolates (minimum inhibitory concentration, 8 μg/ml) was 24.1% and 18.8% within the Nottingham and Charing Cross samples, respectively. The surveys gave similar results within the ampicillin-resistant samples. *Escherichia coli* was the prevalent bacterial species (52.9%), followed by *Klebsiella pneumoniae* (30.3%). The majority of isolates, at least 54.8% and possibly as high as 74.5%, owed their principal β-lactamase activity to enzymes mediated by R-plasmids. The most prevalent β-lactamases were TEM-1 (53.3%), SHV-1 (30.9%), and OXA-1 (11.5%). Positive associations were found between *E. coli* and TEM-1 or OXA-1 and between *K. pneumoniae* and SHV-1.

There is indisputable evidence that antimicrobial agents become progressively less effective with continued clinical use due to the evolution of resistant strains. The principal mechanisms by which resistance to β-lactam antibiotics is achieved are changes in cell permeability (2, 12, 24) and enhanced production of β-lactamases (2, 24, 27). It is important to identify the principal β-lactamases responsible for resistance in clinical populations in order to assess the clinical potential of any novel β-lactam.

A series of surveys has been planned to determine the prevalent β-lactamases produced by gram-negative isolates in clinical populations from different geographical locations. The present communication reports studies carried out in London and Nottingham, England.

All isolates were taken from patients with urinary tract infections. The studies were restricted to out-patients to avoid the possibility of the repeated selection of indigenous "hospital" strains. Ampicillin resistance (minimum inhibitory concentration, 8 μg/ml) was taken as a marker of significant β-lactamase production (17). The principal β-lactamases of 112 such strains from London and 96 strains from Nottingham were identified using isoelectric focusing (16). Initially the enzymes were classified as being either chromosomally or R-plasmid mediated or of indeterminate genetic origin (14, 15, 18, 19, 27).

R-plasmid-mediated and potentially R-plasmid-mediated β-lactamases were considered to be more important epidemiologically, due to the ability of plasmids to transfer rapidly structural genes for antibiotic resistance (3, 28). Effort was therefore concentrated upon the typing of such β-lactamases and upon determination of their distribution throughout the bacterial population. Chromosomally mediated β-lactamases are species and subspecies specific (15), and hence further typing of such enzymes was by bacterial species alone.

MATERIALS AND METHODS

Strains. A total of 208 aerobic, gram-negative organisms (excluding pseudomonads) resistant to ampicillin (minimum inhibitory concentration, 8 μg/ml) were isolated from out-patients suffering from urinary tract infections. A total of 112 of the isolates were collected at Nottingham Hospital during the period from January to March 1977. The other 96 isolates were accumulated over a 4-year period (6 September 1973 to 8 December 1977) by A. Roberts at Charing Cross Hospital, London. The bacterial species of each organism was determined by using the API 20E biochemical test system (API System, SA, 38390, Montalieu-Verciell, France).

Media. Oxoid no. 1 nutrient agar (Oxoid Limited, London) was used for routine maintenance and storage of cultures. Overnight cultures for enzyme preparations were grown in Oxoid no. 1 nutrient broth.

Preparation of crude intracellular β-lactamase extracts. A loop of cells of each isolate was taken from fresh plate cultures and used to inoculate 250-ml Erlenmeyer flasks containing 100 ml of nutrient broth. After overnight incubation at 37°C on an orbital shaker (model INK-200, Gallenkamp) at 200 rpm, the bacterial cells were harvested by centrifugation at 5,000 × *g* for 45 min at 4° C (Mistral 6L; MSE Crawley,

Sussex) and discarding the supernatant. The pellet was suspended in 10 ml of 0.02 M neutral phosphate buffer, and the cells were disrupted by ultrasonic treatment (100 W Ultrasonic Disintegrator, MSE) for 3 min in an ice-water bath. Cell debris was removed by centrifugation at 17,000 × *g* for 60 min at 4°C (Hi speed 18, MSE). The crude enzymes were stored at −20°C.

Assessment of β-lactamase activity of crude enzyme preparations. Samples of 0.1 ml of crude β-lactamase extract were added to 0.3 ml of the chromogenic cephalosporin nitrocefin (20) at a concentration of 50 μg/ml in a microtiter tray (Cooke Microtitre System, Sterilin). The time required for the color change was taken as an indication of the β-lactamase activity of the crude enzyme preparation. All β-lactamase preparations that failed to give a positive response within 2 min were concentrated by freeze-drying.

Concentration of β-lactamase preparations with low activity. Samples of 10 ml of crude β-lactamase extract were placed in sealed lengths of 8/32-in. (ca. 0.64-cm) Visking Tubing (The Scientific Instrument Centre, London) and dialyzed overnight at 4°C against distilled water to remove excess salts. Each dialyzed β-lactamase preparation was distributed equally between three 12-ml conical-based centrifuge tubes and rapidly frozen in an acetone-dry ice freezing mixture. The frozen extracts were placed overnight in a freeze-drier (model SB6; Chem-lab Instruments Limited, Ilford, Essex) operating at −30°C and 10^{-3} Torr. Freeze-dried preparations were stored at 4°C and reactivated by the addition of 0.1 to 0.5 ml of 0.02 M neutral phosphate buffer.

Identification of β-lactamases using analytical isoelectric focusing. The isoelectric focusing method was as outlined (15). The crude β-lactamase extracts were focused on plates supporting thin layers of polyacrylamide gel containing carrier ampholines (pH 3.5 to 10.0; LKB Instruments Limited, South Croydon, Surrey). The β-lactamases focused as sharp bands at their isoelectric point (pI) and were visualized by overlaying with nitrocefin (20). On primary identification runs, all 10 available tracks on the plates were occupied by the test extracts. On subsequent confirmatory runs, β-lactamases with similar pI's and patterns of satellite bands were checked for identity or nonidentity with each other or with well-documented "marker" enzymes, by being run in adjacent tracks and looking for confluent bands. Marker enzymes relevant to the present work are given in Table 1.

Classification of genetic origin of β-lactamases. It is probable that all bacterial isolates produce a β-lactamase of chromosomal origin (18, 27). The structural genes for such enzymes are bacterial species and subspecies specific (15). In addition, the structural genes for a small number of individually recognizable β-lactamases can also be located on extrachromosomal deoxyribonucleic acid (i.e., R-plasmids), and hence it is possible for an isolate to produce more than one β-lactamase.

It was not practicable to perform the necessary genetic or biochemical studies to determine beyond doubt whether each β-lactamase encountered was chromosomally or R-plasmid mediated. Classification of the genetic origin of each β-lactamase was therefore predicted by interpretation of the isoelectric focusing results. Thus, wherever an isolate produced a single β-lactamase with an isoelectric point typical of the bacterial species involved (15) and which did not correspond to any of the β-lactamases generally known to be R-plasmid mediated (14), then such a β-lactamase was classified as chromosomally mediated. Conversely, wherever an isolate produced an enzyme that showed identity with a known R-plasmid-mediated β-lactamase and was produced in addition to a recognizable chromosomally mediated β-lactamase, then such an enzyme was classified as R-plasmid mediated. In view of the lack of precise genetic evidence concerning the origin of each β-lactamase, a third "indeterminate" class was created. This class took account of the instances in which a strain produced a typically R-plasmid β-lactamase but failed to produce a detectable chromosomal type enzyme and hence could not be reliably classified.

β-Lactamase stability of commercial cephalosporins. The spectrophotometric method of O'Callaghan et al. (21) using crude β-lactamase preparations was used.

RESULTS

Incidence of ampicillin-resistant organisms. The incidence of ampicillin-resistant, gram-negative organisms among the original populations of Nottingham and Charing Cross

TABLE 1. *Marker β-lactamases used in the present work*

Enzyme	pI	Genetic origin	Bacterial species/ R-plasmid	Glaxo culture collection	References
K1	6.5	Chromosomal	*K. aerogenes*	1082E	(13, 27)
K14	5.3	Chromosomal	*K. pneumoniae*	1961E	Isolated by S. Goodwin, Northwich Park Hospital, Harrow, Middlesex
TEM-1	5.4	R-plasmid	RTEM	1193E	(7–9, 17, 27)
TEM-2	5.6	R-plasmid	RP1	1725E	(9, 17, 27)
SHV-1	7.6	R-plasmid	p543	2008E	(18, 23)
OXA-1	7.4	R-plasmid	RGN238	1527E	(5, 8, 9, 17, 27)
OXA-2	7.9, 8.0	R-plasmid	R1818	1573E	(4–6, 17, 27)
OXA-3	7.0	R-plasmid	R57b	1894E	(5, 9, 17, 27)

isolates was 24.1% and 18.8%, respectively (N. Pearson, Nottingham, and A. Roberts, Charing Cross, personal communications).

Distribution of bacterial species among ampicillin-resistant isolates. All 208 ampicillin-resistant isolates from the Nottingham and Charing Cross hospitals were characterized for bacterial species. The incidence of bacterial species from each hospital is given in Table 2.

A total of seven genera comprising 12 bacterial species were identified overall. Eleven of the species occurred in the Nottingham survey, four more than were found in the Charing Cross survey. Six species, namely, *Citrobacter freundii*, *Citrobacter koserii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Proteus morganii*, were common to both surveys.

E. coli was the prevalent species among the ampicillin-resistant isolates in both surveys (Nottingham, 42.9%; Charing Cross, 64.6%), followed in both instances by *K. pneumoniae* (Nottingham, 34.8%; Charing Cross, 25.0%). The next most common genus in the Nottingham survey, i.e., *Enterobacter* spp. (10.8%), was not encountered in the Charing Cross survey. *Proteus* spp. formed the next largest genus in both surveys (Nottingham, 7.2%; Charing Cross, 6.3%), followed by *Citrobacter* spp. (Nottingham, 2.7%; Charing Cross, 4.2%). *Serratia* and *Aeromonas*, which were present at low frequencies among the Nottingham organisms, were not found in the Charing Cross survey.

The number of bacterial species encountered in the Nottingham survey (i.e., 11) proved not to be significantly greater than the seven species found in the Charing Cross survey ($\chi^2_{(1)} = 0.143$). However, significant differences (Table 2) were found in the relative incidences of *Enterobacter* spp., and to a lesser extent *E. coli*, between the two surveys. No significant differences were found in the incidences of other genera.

Distribution of β-lactamases among ampicillin-resistant isolates. The β-lactamase(s) produced by each of the 208 isolates were characterized (Table 3). Fifty-three isolates produced a single chromosomally mediated β-lactamase, 99 isolates produced at least one R-plasmid-mediated β-lactamase in addition to a chromosomal enzyme, and 56 isolates produced β-lactamases of indeterminate origin (Table 3). In all 99 instances where an isolate produced both an R-plasmid and a chromosomally mediated β-lactamase, observation of the relative intensities of the β-lactamase bands on the isoelectric focusing gels, after staining with nitrocefin, indicated that the activity of the R-plasmid-mediated enzyme was at least equal to and generally greater than the activity exhibited by the chromosomal enzyme. Therefore, whenever an R-plasmid β-lactamase was identified, this was regarded as the principal enzyme responsible for β-lactam resistance.

Chromosomally mediated β-lactamases. Twenty-nine of the Nottingham isolates (25.9%) and 24 of the Charing Cross isolates (25.0%) owed their β-lactamase activity solely to typically chromosomally mediated enzymes (Tables 3 and 4). Although it was not practicable to further characterize these 53 enzymes experimentally, it was possible to predict the predominant substrate activity of each on the basis of published data for the bacterial species involved (27). The ratio of cephalosporinases to broad-spectrum β-lactamases thus obtained was 22:7 for the Nottingham survey and 20:4 for the Charing Cross survey (Table 4).

TABLE 2. *Distribution and statistical analysis of the bacterial species encountered in the Nottingham and Charing Cross surveys*

Bacterial species	No. of strains		$\chi^2_{(1)}$[a]	P
	Nottingham	Charing Cross		
Enterobacter agglomerans	7	0	12.5	<0.001
E. cloacae	5	0		
Escherichia coli	48	62	4.2	0.0.5–0.0
Klebsiella pneumoniae	39	24	1.3	NS[b]
Proteus inconstans	3	0	0	NS
P. mirabilis	3	4		
P. morganii	1	1		
P. vulgaris	0	1		
Aeromonas hydrophila	2	0	0	NS
Citrobacter freundii	2	1		
C. koserii	1	3		
Serratia liquefaciens	1	0		

[a] With Yates correction.
[b] NS, Not significant.

TABLE 3. *Summary of the distribution of bacterial species and β-lactamase types encountered in the Nottingham and Charing Cross surveys*[a]

Bacterial species	No. of isolates with:																							
	No. of isolates with chromosomal β-lactamase only		R-plasmid-mediated β-lactamase										β-Lactamases of indeterminate genetic origin											
			TEM-1		TEM-2		SHV-1		OXA-1		TEM-1 and SHV-1		TEM-1 and OXA-1		TEM-1		SHV-1		OXA-3		TEM-1 and SHV-1		TEM-2 and SHV-1	
	N	CX	N	CX	N	CX	N	CX	N	CX	N	CX	N	CX	N	CX	N	CX	N	CX	N	CX	N	CX
A. hydrophila	2																							
C. freundii	2	1																						
C. koserii	1	3																						
E. agglomerans	7																							
E. cloacae	3		1						1															
E. coli	4	14	33	40				1	11	6			1											
K. pneumoniae	7	4	1												1		28	13		1	2	1		5
P. inconstans	2				1																			
P. mirabilis		1													2	3								
P. morganii			1	1							1													
P. vulgaris		1																						
S. liquefaciens	1																							

[a] N, Nottingham; CX, Charing Cross.

R-plasmid-mediated β-lactamases. A large proportion of ampicillin-resistant isolates in both surveys produced β-lactamases that are typically R-plasmid mediated. The incidence of such strains was found to be remarkably similar for Nottingham (44.6%) and Charing Cross (51.0%).

Four distinct R-plasmid β-lactamases, namely, TEM-1, TEM-2, OXA-1, and SHV-1, were encountered in both surveys (Table 3). The majority of isolates produced a single R-plasmid-mediated β-lactamase. However, two isolates, one from each survey, were found to produce two different R-plasmid-mediated β-lactamases.

The predominant R-plasmid β-lactamase in both surveys was the TEM-1 enzyme (Tables 3 and 4). This enzyme was produced by a total of 37 isolates (33.0%) from Nottingham, including one isolate of *P. mirabilis* which also produced SHV-1 (Table 3). Similarly, 42 isolates (43.8%) from Charing Cross produced TEM-1, including an isolate of *E. coli* which also produced OXA-1.

The OXA-1 enzyme was produced by 7 of the Charing Cross isolates (7.3%) and 12 (10.7%) of the Nottingham isolates (Tables 3 and 4).

The R-plasmid-mediated SHV-1 enzyme was produced by a single isolate of *E. coli* from the Charing Cross survey, and was produced in addition to TEM-1 by a single isolate of *P. mirabilis* from the Nottingham survey (Table 3).

The TEM-2 enzyme was produced by a single isolate of *Proteus inconstans* from the Nottingham survey (Table 3).

β-Lactamases of indeterminate genetic origin. Fifty-six isolates, comprising 33 from Nottingham (29.5%) and 23 from Charing Cross (24.0%), produced β-lactamases of indeterminate genetic origin (Table 3). Only two bacterial species, *K. pneumoniae* (51 isolates) and *P. mirabilis* (5 isolates), were involved in the production of enzymes classified in this way.

In all instances, the β-lactamases involved were identified as TEM-1, TEM-2, SHV-1, or OXA-3. The structural genes for the TEM and OXA enzymes are normally located on R-plasmids. There are only two reported exceptions to this rule. First, the structural gene for TEM-2 has been laboratory manipulated onto the bacterial chromosome of *E. coli* (25). Second, a phage conferring ampicillin resistance in *E. coli* (26) was subsequently shown to mediate the TEM-1 enzyme (M. Matthew, personal communication). The gene mediating the SHV-1 enzyme can be located on the bacterial chromosome in *Klebsiella* and upon R-plasmids. Indeed, isolates have been recovered which carry the gene for SHV-1 on both elements of the

TABLE 4. *Incidences of specific β-lactamases and β-lactamase types found in the Nottingham and Charing Cross surveys*

Enzyme name	Principal substrate activity	Genetic origin	Observed occurrence of enzyme(s)		Overall occurrence	Incidence (%)
			Nottingham	Charing Cross		
	Cephalosporins	Chromosomal	22	20	42	19.3
	Broad spectrum	Chromosomal	7	4	11	5.1
TEM-1	Broad spectrum	R-plasmid	37	42	88	40.4
		Indeterminate	5	4		
TEM-2	Broad spectrum	R-plasmid	1	0	6	2.8
		Indeterminate	0	5		
SHV-1	Broad spectrum	R-plasmid	1	1	51	23.4
		Indeterminate	30	19		
OXA-1	Penicillins	R-plasmid	12	7	19	8.7
OXA-3	Penicillins	Indeterminate	0	1	1	0.5

genome (19). Thus, in the absence of a recognizable chromosomal β-lactamase, the genetic location of genes for the above enzymes and especially SHV-1 cannot be predicted on the basis of these isoelectric focusing results alone.

Generally the level of activity of chromosomal β-lactamases from *P. mirabilis* is low and difficult to detect using isoelectric focusing (unpublished data). Hence the production of TEM-1 by all five isolates is likely to be R-plasmid mediated, but cannot be legitimately classified as such due to the apparent absence of a chromosomal enzyme.

The situation is more complicated with the 51 isolates of *K. pneumoniae*. Since the production of TEM-1 and OXA-3 has always been shown to be associated with R-plasmids, it is highly likely that the two isolates of *K. pneumoniae* found to produce these enzymes do so by means of an R-plasmid-associated gene. None of the remaining 49 isolates produced a β-lactamase, other than SHV-1, which could be regarded as the chromosomal enzyme. This would suggest that for the majority of these isolates the SHV-1 enzyme was itself the indigenous chromosomal β-lactamase. Since chromosomal β-lactamases are subspecies specific (15), this would indicate a marked predominance of one particular subspecies of *K. pneumoniae* (i.e., 49 of 61 isolates) within the ampicillin-resistant samples. Since the isolates had been collected from two independent geographical locations, such an explanation would seem unlikely. There has been a marked increase in the incidence of SHV-1 over the last 10 years (see Discussion), which can only be satisfactorily accounted for by R-plasmid carriage of the gene for this enzyme. Furthermore, the observation that two isolates of *K. pneumoniae* failed to produce a recognizable chromosomal β-lactamase while producing TEM-1 or OXA-3 indicates that nondetection of a chromosomal β-lactamase is a possibility for each of the 49 isolates producing SHV-1. Irrespective of the genetic origin of SHV-1, circumstantial evidence strongly suggests R-plasmid mediation of the TEM enzymes that were produced in addition to SHV-1 by eight isolates of *K. pneumoniae*.

Overall incidences of β-lactamase types. Statistical analysis of the incidences of readily identifiable β-lactamases, i.e., TEM-1, TEM-2, SHV-1, OXA-1, and OXA-3, irrespective of their genetic origin, and the incidences of isolates owing their β-lactamase activity solely to typically chromosomally mediated enzymes revealed no significant differences between the Nottingham and Charing Cross surveys (Table 4). Hence the results were pooled.

The most prevalent individual β-lactamase encountered was the TEM-1 enzyme, which was produced by a total of 88 isolates (40.4%). The SHV-1 enzyme, which has a broad-spectrum activity similar to that of TEM-1 (14, 23), was produced by 51 isolates (23.4%). The third broad-spectrum β-lactamase, TEM-2, was much less common (2.8%). The OXA-1 enzyme, which has activity predominantly against penicillins and isoxazolyl β-lactams (9, 18, 27) was the third

most prevalent individual β-lactamase, being produced by 19 isolates (8.7%). The remaining individually identified β-lactamase, OXA-3, was produced by only one isolate.

A total of 53 isolates (24.4%) with readily classifiable β-lactamases owed their sole β-lactamase activity to chromosomally mediated cephalosporinases (19.3%) or chromosomally mediated broad-spectrum β-lactamases (5.1%).

Association between bacterial species and specific β-lactamases. Inspection of the distribution of the TEM-1, TEM-2, SHV-1, OXA-1, and OXA-3 β-lactamases in relation to the distribution of bacterial species revealed further marked similarities between the Nottingham and Charing Cross surveys (Table 3). Statistical analysis of the data with respect to each survey alone was not possible. However, in view of the obvious similarity between the surveys, pooled results were used in all subsequent analyses.

Further inspection of the results suggests nonrandom distribution of these enzymes throughout the bacterial species encountered. Statistical analyses were restricted to the distribution of TEM-1, OXA-1, and SHV-1 with respect to *E. coli*, *K. pneumoniae*, and the remaining species as "others" (Table 5). The TEM-1 enzyme, which was produced by a total of 88 isolates comprising five species (Table 3), showed highly significant association with *E. coli* and significantly low association with *K. pneumoniae*. The production of the OXA-1 enzyme was limited to only 19 isolates, of which 18 were *E. coli*. This association was also found to be highly significant. The SHV-1 enzyme exhibited highly significant association with *K. pneumoniae*. However, due to the ambivalent genetic origin of this enzyme in *K. pneumoniae*, it is not possible to determine the extent to which this result reflects the predominance of one particular subspecies or the host range of R-plasmids specifying SHV-1.

DISCUSSION

The prevalent β-lactamases responsible for bacterial resistance in urinary tract infections have been determined in two geographically independent samples of ampicillin-resistant, gram-negative organisms.

No comparable study has been undertaken previously to determine the incidences of clinically important β-lactamase types, irrespective of genetic origin. Estimates of the relative incidences of different R-plasmid β-lactamases have been obtained by collating published and unpublished results on known R-plasmids (14). Furthermore, the 363 isolates cited by Matthew (14), used in preparing these estimates, were recovered in many countries from a variety of infections and biological sources (Matthew, personal communication).

The difference in the observed incidences of ampicillin-resistant isolates in the Nottingham (24.1%) and Charing Cross (18.8%) surveys was found to be statistically significant only at the 5% level ($\chi^2_{(1)} = 3.71$; value for $P = 0.05 - 3.84$; see also reference 1; $d = 2.00$, $P = 0.05 - 0.02$). The Charing Cross value represents an average for the 4-year collection period, whereas the Nottingham value was obtained for a comparatively short (3 months) collection period which overlapped with the later stages of the Charing Cross survey. Thus, the Charing Cross value would be expected to be lower due to the significant increase in the incidence of ampicillin-resistant isolates in recent years (H. Knothe, Klinikum der Johann Wolfgang Von Goethe, Universität, Zentrum der Hygiene, Frankfurt, Germany, unpublished data). The incidence of *E. coli* within the ampicillin-resistant samples (i.e., 52.9% were similar to those observed in

TABLE 5. *Summary of statistical analyses for the distribution of TEM-1, OXA-1, and SHV-1 enzymes throughout bacterial species groups encountered in the Nottingham and Charing Cross surveys*

β-lactamase	Presence	Bacterial species groups				Distribution analyzed	df	χ^{2a}	P
		E. coli	Others	*K. pneumoniae*	Total				
TEM-1	+	74	9	5	88	All species groups	2	62.7	<0.001
	−	36	26	58	120	*E. coli* vs non-*E. coli*	1	57.5	<0.001
						K. pneumoniae vs Others	1	4.5	0.05–0.01
OXA-1	+	18	1	0	19	All species groups	2	15.1	<0.001
	−	92	34	63	189	*E. coli* vs non-*E. coli*	1	12.9	<0.001
SHV-1	+	1	49	1	51	All species groups	2	165.1	<0.001
	−	109	34	14	157	*K. pneumoniae* vs non-*K. pneumoniae*	1	134.4	<0.001

[a] Yates correction used.

comparable populations of clinical isolates not preselected with an antibiotic [11; Knothe, unpublished data]). In contrast, the incidences of *K. pneumoniae* and *Proteus* spp. were appreciably higher and lower, respectively, than in non-ampicillin-selected populations, reflecting the differing bacterial species susceptibility to this antibiotic.

The incidence of isolates in the present study (pooled results) owing their principal β-lactamase activity to enzymes typically mediated by R-plasmids was surprisingly high. A total of 91 isolates (47.6%) were readily classified as producing such enzymes. A further 15 isolates (7.2%) produced TEM-1, TEM-2, or OXA-3, and these were probably R-plasmid mediated. In addition, 49 isolates (23.6%) of *K. pneumoniae* produced the SHV-1 enzyme, but it was not possible to determine in how many instances this was R-plasmid mediated. Thus, at least 54.8% and possibly as high as 74.5% of isolates produced β-lactamases of R-plasmid origin. This incidence clearly demonstrates the importance of such enzymes in effecting bacterial resistance to β-lactam antibiotics.

As anticipated from the widely reported geographical and clinical distribution of R-plasmids specifying TEM-1, this enzyme proved to be the most prevalent β-lactamase in the Nottingham and Charing Cross surveys. OXA-1 was found to be the next most prevalent of the enzymes readily classified as R-plasmid mediated and was also the third most prevalent individual enzyme overall. Both TEM-1 and OXA-1 exhibited significant association with *E. coli*.

The SHV-1 enzyme was the second most prevalent individual β-lactamase, being produced by 51 isolates of which 49 were *K. pneumoniae*. An R-plasmid specifying SHV-1 was first reported in 1972 (25). Since then other such R-plasmids have been found in several different countries (17, 20), but they have been regarded as a novelty due to the apparent low incidence of the enzyme. Matthew (14) estimated the incidence of such R-plasmids to be a significant 4.1% among all organisms investigated and 5.6% among bacterial species normally associated with urinary tract infections. It has been suggested (14, 21) that the rapid increase in the incidence of this enzyme is attributable to the translocation of its structural gene, probably originating from the *Klebsiella* chromosome, onto a plasmid. Subsequent recombination events between plasmids could dramatically increase the potential promiscuity of this gene. Such a process has already been suggested for the carriage of the gene for TEM-1 on many different R-plasmids (10). The fact that 49 of the 51 isolates producing the SHV-1 enzyme were *K. pneumoniae* agrees with previous findings (23, 27) and supports the hypothesis (14, 21) that the SHV-1 β-lactamase gene evolved as a chromosomal gene in *Klebsiella*. We know of no surveys undertaken to determine either the incidence of the various subspecies of *K. pneumoniae* or the incidence of the chromosomally mediated SHV-1 enzyme with respect to other chromosomally mediated β-lactamases from *K. pneumoniae*. However, in the present surveys, of the 11 isolates of *K. pneumoniae* with solely chromosomal β-lactamases (Table 3), eight different enzymes were involved, indicating considerable genetic variation. This observation would tend to suggest the nonchromosomal location of the structural gene for the SHV-1 enzyme in the majority of isolates. Location of the structural gene for SHV-1 on R-plasmids would explain the epidemic increase of this enzyme over the last 5 years.

Clearly, resistance to β-lactamases must be an important consideration, both in the clinical application of and in the search for new β-lactam antibiotics. On the basis of the present findings, the most important β-lactamases are TEM-1, SHV-1, and OXA-1. Cefoxitin and cefuroxime exhibit the greatest stability of currently available cephalosporins to hydrolysis by these three enzymes (Table 6). Screening procedures for new β-lactams should take account not only of the most prevalent β-lactamases but also the variety of β-lactamases responsible for bacterial resistance. In the present study, 25% of the β-

TABLE 6. *Comparison of relative rates of hydrolysis of eight commercial cephalosporins by each of the five R-plasmid β-lactamases found in the Nottingham and Charing Cross studies*

R-plasmid β-lactamase	Relative rates of hydrolysis							
	Cephaloridine	Cephalothin	Cephalexin	Cefazolin	Cephradine	Cefamandole	Cefuroxime	Cefoxitin
TEM-1	100	20	1	18	<1	76	<1	<1
TEM-2	100	21	6	19	<1	50	<1	0
SHV-1	100	24	2	<1	1	40	<1	0
OXA-1	100	484	65	13	62	22	15	0
OXA-2	100	147	7	800	7	167	0	0

lactamases characterized were grouped together as being of chromosomal origin (Table 3). Nevertheless these enzymes are different and may exhibit extreme variation in their abilities to hydrolyze new β-lactam antibiotics. This potential source of bacterial resistance will be bacterial species specific (15) and thus possible to screen for by using a modest but representative sample of bacterial species.

Further surveys of this nature are planned to establish the relevance of this U.K. finding on a broader geographical basis. Moreover, in view of the rapid transmission of the structural gene for SHV-1, it will be necessary to regularly monitor the situation in the U.K. and modify screening procedures, should such an outbreak occur.

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