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Molecular epidemiology of the plasmid-encoded TEM-1 β-lactamase in Scotland

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SUMMARY

A survey of the β-lactamases responsible for ampicillin resistance in urinary *Escherichia coli* isolated in central Scotland has been performed. The TEM-1 β-lactamase was found to be most prevalent occurring in 88·2% of ampicillin-resistant isolates tested. Forty-six percent of the TEM-1 producing strains were able to transfer this resistance to *E. coli* J62–2 at 37 °C. Analysis of the resulting transconjugants revealed that the degree of resistance to amoxycillin and amoxycillin in combination with clavulanic acid was related to the specific activity of the TEM-1 β-lactamase. The variation in specific activity was shown to be related to plasmid type as determined by restriction analysis. No obvious relationship between β-lactamase specific activity and resistance to amoxycillin and amoxycillin plus clavulanic acid could be demonstrated in the original plasmid donor strains.

INTRODUCTION

Bacterial resistance to β-lactam antibiotics is most commonly mediated by the action of β-lactamases [1, 2]. In order to counteract this the pharmaceutical industry has adopted two strategies. Firstly the development of successive generations of β-lactam antibiotics resistant to hydrolysis by the then known β-lactamases. Secondly the use of β-lactamase inhibitors which when administered in combination with the β-lactam antibiotic protect it from destruction by β-lactamases [1, 3].

However, bacteria have shown a remarkable capacity to adapt to overcome these advances in antibiotic development. The ability of the TEM-1 β-lactamase to mutate to confer resistance to third generation cephalosporins is well documented and over 30 of these extended spectrum β-lactamases have been identified [4, 5]. The use of β-lactamase inhibitors, of which the best known is clavulanic acid, appeared to have been more successful. However there have been several recent reports of decreased susceptibility to amoxycillin/clavulanate and ticarcillin/clavulanate combinations amongst *Escherichia coli* isolates attributed to hyperproduction of the TEM-1 β-lactamase [6–10].

Given the apparent role of the TEM-1 β-lactamase as a progenitor of resistance to third-generation cephalosporins [4, 5] and that changes in the level of production can mediate resistance to β-lactam/β-lactamase inhibitor combina-

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tions [6–10], a study was performed to investigate the molecular epidemiology of the TEM-1 β-lactamase. Isolates from urinary tract infections were studied as this represents one of the areas where β-lactam/β-lactamase inhibitor combinations are most often used.

The β-lactamase content of ampicillin resistant *E. coli* was examined and those strains found to be producing only a TEM-1 β-lactamase were tested for the transferability of this determinant to a standard strain. Once transferred to this isogenic background, the activity of the enzyme, the resistance it conferred and the genetic carrier for this were examined.

METHODS

Identification of strains

Nearly 1000 urinary isolates were obtained at the end of 1990; 491 from the Royal Infirmary Edinburgh and 500 from Glasgow Royal Infirmary. Strain identification by API20E (Biomerieux, Basingstoke, Hants) strips and breakpoint sensitivity testing with ampicillin at 32 mg/l according to BSAC guidelines [11] revealed a population of 240 ampicillin-resistant *E. coli* which were used in this survey.

Sensitivity testing and plasmid transfer

Minimum inhibitory concentrations and plasmid transfers were determined on Oxoid Isosensitest agar (Oxoid, Unipath, Basingstoke, Hants) as before [12]. The minimum inhibitory concentration of amoxycillin/clavulanate was performed at a ratio of 2:1 amoxycillin:clavulanic acid and expressed in terms of the amoxycillin concentration [13].

β-*lactamase identification*

β-lactamase identification was by means of analytical isoelectric focusing as described previously [12, 14] and β-lactamase bands were visualized by staining with the chromogenic cephalosporin nitrocephin. β-lactamase activity was measured by spectrophotometric assay of the hydrolysis of ampicillin [15] and the specific activity was obtained by measuring the protein concentration by the method of Waddell [16].

Plasmid and restriction analysis

The method of Takahashi and Nagano [17] was used to extract and visualize plasmid DNA. Restriction analysis was carried out with *Eco*R I (BRL, Paisley, Scotland, UK) according to manufacturer's instructions.

RESULTS

Resistance to amoxycillin and amoxycillin in combination with clavulanic acid of ampicillin resistant urinary E. coli

The 240 ampicillin-resistant *E. coli* isolated from urinary tract infections were tested for their minimum inhibitory concentrations to amoxycillin and amoxycillin plus clavulanic acid in a 2:1 ratio. The results (Table 1) indicted a number

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Table 1. *Phenotypes in amoxycillin-resistant* Escherichia coli

Minimum inhibitory concentration (mg/l)			
Amoxycillin	Amoxycillin + clavulanic acid	Number	Percentage
1024	64	3	1·2
1024	32	8	3·3
1024	16	86	35·8
1024	8	74	30·8
512	256	1	0·4
512	64	1	0·4
512	32	4	1·6
512	16	2	0·8
512	8	44	18·3
512	4	7	2·9
256	128	1	0·4
256	32	1	0·4
256	16	1	0·4
256	8	1	0·4
256	4	4	1·6
128	32	2	0·8

Table 2. *The proportion of each β-lactamase type in the amoxycillin/clavulanic-acid resistant strains of* E. coli

β-lactamase	No. of isolates	Percentage of isolates
TEM-1 alone	80	72·7
TEM-1 plus chromosomal	11	10·0
TEM-1 plus SHV-1	3	2·7
TEM-1 plus TRC-1	3	2·7
SHV-1 alone	3	2·7
Chromosomal alone	4	3·6
TRC-1 alone	2	1·8
OXA-1 alone	1	0·9
OXA-3 alone	1	0·9
No enzyme detected	2	1·8

of resistance phenotypes. Those bacteria exhibiting a raised MIC to the combination of amoxycillin and clavulanic acid (16 mg/l or above) were selected for further study.

β-lactamase profile of isolates with an MIC of amoxycillin/clavulanate of 16 mg/l or above

One hundred and ten isolates, with raised MICs to amoxycillin/clavulanate were examined for their *β*-lactamase content. The proportion of isolates within each *β*-lactamase type is shown in Table 2. In common with other studies, the TEM-1 *β*-lactamase was the most prevalent and was present in 88·2% of isolates either alone or with other plasmid or chromosomal enzymes. The SHV-1 *β*-

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Table 3. *Plasmid analysis of TEM-1 producing transconjugants from Edinburgh and Glasgow*

No. of transconjugants	City	No. of plasmids	Size (kb)	Restriction pattern	Antibiogram*
3	Edinburgh	1	75	1a	Ap Sx
2	Edinburgh	1	82	1b	Ap Sx
3	Edinburgh	1	78	1c	Ap Sx
2	Edinburgh	1	84	2	Ap
1	Edinburgh	1	61	3	Ap Sx Tp
1	Edinburgh	3	53. 24. 5	17	Ap
2	Edinburgh	1	116	4	Ap
1	Edinburgh	1	95	5	Ap
1	Edinburgh	1	54	6	Ap Gm
1	Edinburgh	3	58. 13. 5	16	Ap
4	Glasgow	1	57	7	Ap
2	Glasgow	1	87	8	Ap
3	Glasgow	1	74	9a	Ap Sx
1	Glasgow	1	59	9b	Ap Sx
1	Glasgow	1	106	10	Ap
1	Glasgow	1	81	11	Ap
1	Glasgow	1	73	12	Ap Sx Tp
1	Glasgow	1	51	13	Ap
3	Glasgow	1	42	14	Ap
1	Glasgow	1	62	15	Ap
2	Glasgow	3	68. 14. 5	18	Ap

* Ap. Ampicillin; Gm. gentamicin; Sx. sulphamethoxazole; Tp. trimethoprim.

lactamase was the next most prevalent occurring in 5·4% of isolates. The novel β-lactamase, TRC-1, was found in 4·5% of the isolates. Oxacillin hydrolysing β-lactamases were present in 1·8% of isolates and no β-lactamase activity was detected in 1·8% of strains. A further 3·6% of isolates produced only a chromosomal β-lactamase.

Transferability of the TEM-1 β-lactamase and plasmid and restriction analysis of the resulting transconjugants

All strains exhibiting only a TEM-1 β-lactamase were tested for their ability to transfer this resistance determinant by conjugation to the standard bacterial strain *E. coli* J62–2. Thirty-seven isolates (46%) transferred ampicillin resistance at 37 °C. Those which did not transfer resistance at 37 °C were retested at 30 °C but no further transfer was observed.

Each transconjugant had its plasmid content examined and the relatedness of these plasmids investigated by restriction analysis with *Eco*R I. Those plasmids with closely related restriction digest patterns were assigned to the same category number. If all the bands matched exactly no suffix was added whereas if they differed by up to two bands, the plasmids were assigned different suffixes. The results (Table 3) revealed that a number of different plasmid types were present. Most of them were completely distinct from each other but in categories 1 and 9, alphabet suffixes were used to distinguish closely related plasmids. The resistance profiles of the transconjugants were also determined by testing for resistance to ampicillin 8 mg/l, gentamicin 2 mg/l, chloramphenicol 8 mg/l, sulphamethox-

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Table 4. *Relationship between plasmid type, specific activity and resistance to amoxycillin and amoxycillin/clavulanate for the TEM-1 producing transconjugants. The results are presented with increasing specific activity of the β-lactamase*

Source	Minimum inhibitory concentration (mg/l) Amoxycillin	Amoxycillin/ clavulanate	Plasmid Type	Plasmid Size (kb)	Specific activity*
Glasgow	512	4	8	87	0·042
Glasgow	512	4	8	87	0·051
Glasgow	512	4	9b	59	0·057
Glasgow	512	4	7	57	0·066
Edinburgh	512	4	1a	75	0·069
Glasgow	512	4	12	73	0·099
Edinburgh	512	4	1c	78	0·102
Edinburgh	512	4	1b	82	0·105
Edinburgh	512	4	16	58, 13, 5	0·114
Edinburgh	512	4	1b	82	0·132
Glasgow	512	4	7	57	0·150
Glasgow	512	4	14	42	0·153
Edinburgh	1024	8	3	61	0·156
Edinburgh	512	8	5	95	0·156
Edinburgh	512	4	1a	75	0·159
Glasgow	1024	8	15	62	0·162
Edinburgh	512	4	1c	78	0·162
Edinburgh	512	4	2	84	0·168
Edinburgh	512	4	2	84	0·174
Glasgow	512	4	7	57	0·177
Edinburgh	512	4	1c	78	0·180
Edinburgh	512	4	4	116	0·183
Glasgow	512	4	14	42	0·183
Edinburgh	512	4	1a	75	0·186
Edinburgh	512	4	4	116	0·192
Glasgow	512	4	7	57	0·213
Glasgow	512	4	14	42	0·231
					Cut off
Edinburgh	1024	8	6	54	0·285
Glasgow	2048	8	9a	74	0·363
Glasgow	2048	16	11	81	0·387
Glasgow	2048	8	18	68, 14, 5	0·396
Glasgow	2048	8	18	68, 14, 5	0·423
Glasgow	2048	8	9a	74	0·495
Edinburgh	2048	8	17	53, 24, 5	0·507
Glasgow	2048	16	10	106	0·612
Glasgow	2048	8	9a	74	0·627
Glasgow	2048	16	13	51	1·032

* μmol ampicillin hydrolysed/min per mg protein.

azole 32 mg/l and trimethoprim 8 mg/l. In transconjugants which had been obtained from isolates in Edinburgh, eight plasmid types were identified. One type however (conferring resistance to ampicillin and sulphamethoxazole) predominated and was found in 47% of the strains. The plasmids originating in

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Table 5. *Relationship between specific activity and resistance to amoxycillin and amoxycillin/clavulanate for the original TEM-1 producing isolates. The results are presented with increasing specific activity of the β-lactamase*

Source	Minimum inhibitory concentration (mg/l) Amoxycillin	Amoxycillin/ clavulanate	Specific activity*
Edinburgh	2048	16	< 0·001†
Glasgow	128	32	< 0·001†
Edinburgh	2048	16	0·021
Glasgow	2048	16	0·051
Edinburgh	1024	16	0·060
Glasgow	512	16	0·065
Glasgow	1024	64	0·066
Edinburgh	2048	16	0·075
Edinburgh	2048	8	0·090
Edinburgh	1024	16	0·093
Glasgow	1024	32	0·108
Edinburgh	2048	16	0·120
Glasgow	2048	16	0·123
Edinburgh	2048	16	0·126
Edinburgh	1024	16	0·132
Glasgow	2048	32	0·132
Edinburgh	2048	16	0·135
Edinburgh	2048	16	0·138
Glasgow	2048	16	0·147
Edinburgh	1024	16	0·147
Glasgow	2048	16	0·180
Glasgow	2048	16	0·183
Glasgow	2048	16	0·204
Edinburgh	2048	16	0·225
Glasgow	2048	16	0·243
Glasgow	2048	16	0·300
Edinburgh	1024	16	0·312
Glasgow	2048	16	0·384
Glasgow	2048	16	0·444
Glasgow	2048	16	0·477
Glasgow	2048	16	0·504
Edinburgh	2048	16	0·552
Glasgow	2048	16	0·621
Glasgow	2048	16	0·657
Edinburgh	2048	16	0·687
Glasgow	2048	16	0·921
Edinburgh	2048	32	1·464

* μmol ampicillin hydrolysed/min per mg protein.

† The enzymes have very slow activity but could easily be identified as TEM-1 after prolonged incubation of an isoelectric focusing gel with nitrocephin.

Glasgow showed more heterogeneity; 10 types were identified and no plasmid group accounted for more than 20% of the isolates. The majority of plasmids, 62% from both sources conferred resistance to no other antibiotic besides ampicillin. Most transconjugants contained only a single plasmid type with only four isolates containing more than one plasmid.

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Specific activity of the TEM-1 β-*lactamase*

The specific activity of the TEM-1 β-lactamase was determined for the original isolate and for the resultant transconjugant. The results are shown in Tables 4 and 5. The MIC of amoxycillin and amoxycillin in combination with clavulanic acid was also determined for the TEM-1 transconjugants. When arranged in order of increasing specific activity it is clear that for the transconjugants there is a relationship between the MIC to amoxycillin and amoxycillin plus clavulanic acid and the β-lactamase specific activity with only three strains not fitting the pattern (Table 4). In contrast with the original strains no correlation between specific activity and MIC was observed (Table 5). With the transconjugants the crucial point seemed to be at a specific activity of 0·28 μmol ampicillin hydrolysed/min per mg protein with the MIC rising at this point. Interestingly when the plasmid type, as defined by restriction pattern, was related to specific activity and a cut off of 0·28 μmol ampicillin hydrolysed/min per mg protein taken, this divided the plasmids into two groups suggesting that the differences in specific activity and hence resistance were a result of properties of the host plasmid that the TEM-1 gene was located on.

The specific activities of the β-lactamases in the transconjugants were compared to the levels found in the original isolates. No clear pattern emerged as the ratio ranged from 0·12 to $> 5{\cdot}43$. This did not correlate with the ratios of the MICs further suggesting that the resistance levels in the original strains do not simply derive from β-lactamase activity.

DISCUSSION

The results of this study confirm that the TEM-1 β-lactamase predominates as the cause of resistance to β-lactam agents in urinary isolates from Edinburgh and Glasgow, as it was found in 88·2% of strains tested. This finding is similar to many other surveys which have demonstrated the dominance of this enzyme [18–20]. Other plasmid-encoded β-lactamases were represented in small numbers; most notably the newly-identified TRC-1 β-lactamase which has been shown specifically to overcome the inhibition of clavulanic acid [21]. In the isolates from Edinburgh and Glasgow, clavulanic acid reduced the MIC of amoxycillin to below 32 mg/l in the majority of the amoxycillin resistant strains tested and this is similar to other studies [13]. The role of the TEM-1 enzyme as the progenitor for extended spectrum-β-lactamases and, recently, for clavulanic acid resistant β-lactamases has been demonstrated [5, 21, 22]. More contentious, however, is the role that TEM-1 may play in the development of resistance to β-lactam/β-lactamase inhibitor combinations by hyperproduction to overcome the inhibition by clavulanic acid. Hyperproduction of the TEM-1 β-lactamase as a mechanism of clinical resistance to amoxycillin/clavulanic acid combinations has been reported from a number of centres [6–10]. The exact mechanism for this is not clear but a number of mechanisms have been proposed including gene duplication and plasmid rearrangement to give small multi-copy plasmids [9, 23]. However, others argue that strains that hyperproduce TEM-1 do not arise as a response to amoxycillin/clavulanic acid treatment, rather that TEM-1 hyperproducing strains

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were present, in the clinical population, before the development of β-lactamase inhibitors and their incidence has not risen despite the greatly increased use of amoxycillin in combination with clavulanic acid [24, 25].

It has been speculated that the reason for these differing results is that those areas that report increasing incidences of hyperproducing strains are experiencing local outbreaks caused by a ubiquitous strain or plasmid [25]. This study would suggest that this may indeed be the case as the variation in TEM-1 specific activity in the transconjugants was related to the plasmid type. This was demonstrated by the difference observed between the two centres. Plasmid types producing high levels of TEM-1 and consequently a higher level of resistance to amoxycillin and amoxycillin/clavulanate were more common in Glasgow than in Edinburgh.

The fact that no obvious relationship between specific activity and MIC to amoxycillin and amoxycillin/clavulanic acid combinations was detected for the original donor strains may also prove to be an important point. Clearly other resistance factors are involved in these strains, an explanation also favoured by the observation that the original strains were all more resistant to amoxycillin and amoxycillin plus clavulanic acid than the transconjugants. The fact that the interplay of different resistance factors may mask any changes in hyperproduction should be borne in mind when considering this problem. Whilst it is easy to demonstrate with standard laboratory-derived strains a clear correlation between TEM-1 β-lactamase activity and the amount of clavulanic acid required to potentiate amoxycillin [24], the clinical importance of hyperproducing TEM-1 strains is as yet not clear cut.

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