Journal of Antimicrobial Chemotherapy (1994) **34**, 65–71

In-vitro antimicrobial susceptibility and β-lactamases of ampicillin-resistant *Escherichia coli* in Hong Kong

T. K. W. Ling, D. J. Lyon, A. F. B. Cheng and G. L. French*

Department of Microbiology, The Chinese University of Hong Kong, The Prince of Wales Hospital, Shatin, New Territories, Hong Kong

Three hundred and forty isolates of ampicillin-resistant *Escherichia coli* isolated from blood, bile and urine were collected during 1984 and 1988 in the Prince of Wales Hospital, Hong Kong and MICs of 21 β-lactam antibiotics were determined. β-Lactamases were identified by isoelectric focusing, and TEM-1 was found in 89·1% of isolates, PSE-1 in 3·2%, and OXA-1 in 2·9% of strains. Ten isolates (2·9%) possessed both TEM-1 and PSE-1. Five isolates (1·5%) produced only chromosomal enzymes and one produced an unidentified enzyme which focussed at pI 6·0. All but seven of 274 TEM-1 producers and all nine OXA-1 producers reacted with oligonucleotide probes in a colony blot technique. All isolates contained plasmids and 229 of 318 (72·0%) isolates transferred the ampicillin resistance factor. Of 42 transconjugants of TEM-1 producers selected for study, 39(92·9%) had only a single transferable plasmid bearing the TEM-1 gene.

Introduction

Sepsis caused by ampicillin-resistant *Escherichia coli* is common in Hong Kong (French & Ling, 1988; French *et al.*, 1990) but little is known of the β-lactamases and their genetics. Reports from other parts of the world have shown that TEM-1 is the most common β-lactamase found in these organisms (Cooksey *et al.*, 1990; Sousa *et al.*, 1991; Roy *et al.*, 1992; Liu *et al.*, 1992; Wu, Shannon & Phillips, 1992). We therefore undertook an extensive survey of the types of β-lactamases and their distribution and genetics that had been in ampicillin-resistant *E. coli* isolated from patients admitted to our hospital from 1984 to 1988.

Materials and methods

Bacterial strains

A total of 340 unique isolates of ampicillin-resistant *E. coli* were collected from blood, urine and bile. Their identity was established using the API 20E (API SA, Vercieu, France) and their susceptibility to ampicillin was determined by disc diffusion (Garrod, Lambert & O'Grady, 1981). Isolates were preserved by lyophilization and stored at ambient temperature.

*Present address: Department of Microbiology, United Medical and Dental Schools, Guy's Hospital, London SE1 7RT, UK.

0305–7453/94/070065+07 $08.00/0

Antibiotic susceptibility testing

Minimum inhibitory concentrations (MICs) of 21 β-lactam antibiotics were determined by a dilution method in microtitration trays, using Iso-Sensitest broth (Oxoid, Basingstoke). Antibiotics were either supplied by the manufacturers or were purchased from Sigma Chemical Co., U.S.A. The following strains were used as controls: *E. coli* ATCC 25922, ATCC 35218 and NCTC 10418, *Pseudomonas aeruginosa* ATCC 27853 and NCTC 10662, and *Staphylococcus aureus* ATCC 25923 and NCTC 6571. An inoculum of approximately 10^4 cfu was delivered to each well using an MIC-2000 inoculator (Dynatech Instrument, Inc., California, USA). The trays were incubated for 18 h, and the MIC was recorded as the lowest concentration that inhibited visible growth. Ampicillin was combined with sulbactam in a 1 : 1 ratio and amoxycillin with clavulanate in a 2 : 1 ratio results being expressed in terms of either the ampicillin or amoxycillin concentration.

Characterization of β-lactamases

Logarithmic phase bacterial cells were incubated in Iso-Sensitest broth at 37°C for 4 h, harvested and disrupted by sonication (MSE, Soniprep 150). The extracts containing β-lactamase were stored at -20°C and were identified by isoelectric focusing on polyacrylamide gel (Matthew *et al.*, 1975). β-Lactamase samples were applied to the gel which was focused by the Ultrophor Electrofocusing Unit (LKB 2217) with a power supply system set to 10 W constant power for 3 h at 10°C. The gel was developed by overlaying with 0·05% nitrocefin solution. The β-lactamase band patterns were photographed with a Polaroid MP-3 camera through a dark green filter (Hoya) with Polaroid type 667 positive instant film. Known β-lactamases (TEM-1, TEM-2, OXA-1 and PSE-1) were run as standards (strains were kindly supplied by Dr D. Livermore, Royal London Hospital).

Transfer of drug resistance plasmids

The transfer of R-plasmids was demonstrated by conjugation experiments (Meynell, Meynell & Datta, 1968). *E. coli* K12 14R525 which is free of plasmids and resistant to nalidixic acid was the recipient. The ampicillin-resistant, nalidixic acid-sensitive isolates were cultured separately from the recipients in 10 mL brain heart infusion (BHI) broth (Oxoid) until each had reached the late exponential phase. A loopful of approximately 0·01 mL of each isolate was placed in 10 mL of fresh BHI broth. The conjugation mixture was then incubated for 24 h without aeration. Transconjugants were selected by plating on to MacConkey agar containing nalidixic acid 50 mg/L and ampicillin 32 mg/L. The transfer frequency was defined as the number of transconjugant colonies per recipient cell.

Plasmid analysis

Plasmids were extracted from ampicillin-resistant strains and their transconjugants by the method of Kado & Liu (1981) and electrophoresed in a horizontal gel apparatus (GNA-200, Pharmacia Fine Chemicals, Uppsala, Sweden) using 0·7% agarose gels and tris-borate-EDTA buffer pH 8·3 (Meyers *et al.*, 1976). The gel was stained with 0·5 mg/mL ethidium bromide for 30 min before exposure to ultraviolet (UV) irradiation. DNA bands were viewed under UV light of wavelength 260 nm (Transilluminator, Ultra Violet Product, Inc., USA) and photographed through a red filter (Hoya, Tokyo, Japan) using Polaroid T667 (Polaroid, USA) positive/negative black and white film.

DNA hybridization

Oligonucleotide probes to detect nucleotide sequences homologous to TEM-1 (5′-CCCAACTGATCTTCA-3′), TEM-2(5′-CCCAACTTATCTTCA-3′) and OXA-1 (5′-CCAAAGACGTGGATG-3′) (Ouellette *et al.*, 1987, 1988) were purchased from OCS Laboratories (Texas, USA). The probes were labelled with ^{32}P using a 3′ end labelling kit (Amersham N4020). The membranes were pre-hybridized for 1 h at 37°C as described previously by Maniatis, Fritsch & Sambrook (1982). Hybridization was then carried out at 37°C for 16 h by the method of Ouellette *et al.* (1987, 1988). For colony blotting, the bacterial strains were grown for 6 h on Hybond-N membrane and the DNA then prepared as described by Maniatis *et al.* (1982). For the Southern blotting (Southern, 1975), plasmid DNA first separated on agarose gel was transferred to a Hybond-N membrane using a vacuum blotting system (Vacugene, LKB, Bromma, Sweden).

Results

Antibiotic susceptibility testing

All isolates were resistant to ampicillin and amoxycillin (MIC > 8 mg/L) (Table I) and using a breakpoint concentration of 8 mg/L (Working Party Report, 1991), 50·3% of isolates were resistant to ampicillin/sulbactam and 41·2% to amoxycillin/clavulanate. One isolate had an MIC of 2 mg/L of cefotaxime and an MIC of 8 mg/L of ceftazidime. This strain produced only a chromosomal β-lactamase with a pI of > 8.

Ampicillin-resistance factor

All organisms contained between two and eight plasmids, with molecular sizes ranging from 0·87 to 110 Megadaltons (MDa). No common plasmid pattern was established although plasmids of similar molecular size were noted in different strains. Of 318 ampicillin-resistant, nalidixic acid sensitive strains selected for conjugation experiments, 229 (72·0%) strains transferred the ampicillin-resistance factor, the frequency of transfer ranging from $7{\cdot}1 \times 10^{-2}$ to $4{\cdot}5 \times 10^{-12}$. Not all of the plasmids present in the parent strains transferred to the recipient strains. Of 42 TEM-1 producers chosen at random for further study, 26 strains (61·9%) transferred one plasmid and 10 strains (23·8%) two plasmids to the recipient. The distribution of plasmids in the 42 TEM-1 producing *E. coli* and their transconjugants is shown in Table II.

Isoelectric focusing

The β-lactamases identified by isoelectric focusing in the 340 strains were TEM-1 (89·1%), PSE-1 (3·2%), OXA-1 (2·9%), and TEM-1 plus PSE-1 (2·9%). Five organisms (1·5%) produced only chromosomal (CHR) enzymes and one organism possessed an unidentified enzyme with pI = 6·0.

Table I. Susceptibility of 340 ampicillin-resistant *E. coli* to 21 β-lactam antibiotics

Antimicrobial agent	MICs (mg/L) range	MIC_{50}	MIC_{90}
Ampicillin	32–$\geqslant$ 4096	512	$\geqslant$ 4096
Amoxycillin	128–$\geqslant$ 4096	4096	$\geqslant$ 4096
Ampicillin/sulbactam	4–$\geqslant$ 64	16	$\geqslant$ 64
Amoxycillin/clavulanate	4–64	8	32
Carbenicillin	4–$\geqslant$ 256	$\geqslant$ 256	$\geqslant$ 256
Piperacillin	2–$\geqslant$ 256	32	$\geqslant$ 256
Cephalexin	2–64	8	16
Cephradine	2–64	8	64
Cephalothin	0·5–64	8	16
Cephaloridine	1–64	8	32
Cefaclor	0·5–64	2	8
Cefamandole	0·25–64	2	16
Cefoxitin	0·12–64	4	8
Cefuroxime	0·25–128	4	8
Cefoperazone	$\leqslant$ 0·03–64	1	8
Ceftazidime	$\leqslant$ 0·03–8	0·12	0·25
Cefotaxime	$\leqslant$ 0·03–4	$\leqslant$ 0·03	0·12
Ceftriaxone	$\leqslant$ 0·03–1	$\leqslant$ 0·03	0·12
Latamoxef	$\leqslant$ 0·03–16	0·12	0·25
Aztreonam	$\leqslant$ 0·03–8	0·12	0·5
Imipenem	0·06–0·5	0·12	0·25

Table II. Distribution of plasmids in 42 TEM-1 producing *E. coli* and their transconjugants and location of the TEM-1 gene in transconjugant plasmids

Plasmid size (MDa)	Number (%) of isolates with plasmid	Number (%) of transconjugants with plasmid	Transferable plasmids hybridizing with TEM probe
< 11	34 (27·2)	5 (7·7)	2 (4·4)
11–20	1 (0·8)	0 (0·0)	0 (0·0)
21–30	5 (4·0)	1 (1·5)	1 (2·2)
31–40	7 (5·6)	5 (7·7)	3 (6·7)
41–50	10 (8·0)	6 (9·2)	3 (6·7)
51–60	24 (19·2)	18 (27·7)	16 (35·6)
61–70	18 (14·4)	13 (20·0)	8 (17·8)
71–80	15 (12·0)	9 (13·9)	4 (8·9)
81–90	9 (7·2)	6 (9·2)	6 (13·3)
> 90	2 (1·6)	2 (3·1)	2 (4·4)
Total	125	65	45

[a]Number of isolates with greater than or equal to one plasmid in the size range < 11 to > 90 MDa.

DNA hybridization

Three hundred and ten of the ampicillin-resistant *E. coli* that produced TEM-1 (274 isolates), TEM-1 + PSE-1 (10 isolates), PSE-1 (11 isolates), OXA-1 (9 isolates), CHR (5 isolates) and the isolate that produced the unidentified β-lactamase were examined by a colony blot technique using TEM-1, TEM-2 and OXA-1 oligonucleotide probes. The

TEM-1 probe hybridized with 267 (97·5%) TEM-1 producers and all 10 of TEM-1 + PSE-1 producers and none of the non-TEM-1 producers. The TEM-2 probe did not hybridize with any of the isolates. The OXA-1 probe hybridized with all 9 of the OXA-1 producing *E. coli* and none of the non-OXA-1 producers.

Forty-two TEM-1 producers were selected at random and their transconjugants were further studied for the location of TEM-1 genes using Southern blot hybridization. Among the 42 transconjugants, 39 strains (92·9%) were shown to have only a single transferable plasmid bearing the TEM-1 gene with a molecular size ranging between 3·3 to 96·0 MDa. The distribution of plasmids bearing the TEM-1 gene is shown in Table II and more than half (53·4%) of the transferable plasmids bearing this gene were in the 51–70 MDa range.

Discussion

The antibiotic susceptibility of our isolates of ampicillin-resistant *E. coli* is broadly comparable to that published elsewhere although there was a high proportion of strains with elevated MICs (> 8 mg/L) of ampicillin/sulbactam (50·3%) and amoxycillin/clavulanate (41·2%). This high level of resistance is unlikely to be an artifact since we have previously shown that these strains give similar results after repeated testing in agar dilution and broth dilution and the isolates also have reduced zones of inhibition in disc diffusion tests (French & Ling, 1988). This resistance may be explained by hyperproduction of TEM β-lactamase as suggested by Martinez *et al.* (1987) and Williams *et al.* (1988), or to other factors, such as variation in permeability. Further study of resistance to ampicillin/sulbactam and amoxycillin/clavulanate in our strains is warranted.

We detected β-lactamases in all of our 340 ampicillin resistant isolates and found TEM-1 to predominate, being the only enzyme in 89·1% of strains. These results are similar to those reported from the USA (Cooksey *et al.*, 1990), Spain (Roy *et al.*, 1992) UK (Wu *et al.*, 1992), (Liu *et al.*, 1992) and Portugal (Sousa *et al.*, 1991) but differed from many other reports by the absence of TEM-2 and SHV-1 and the presence of PSE-1. TEM-2 is closely related to TEM-1 (Medeiros *et al.*, 1980) and is often found in *E. coli* and other members of the Enterobacteriaceae. We have noted TEM-2 to be present in 21·0% of ampicillin-resistant strains of *Proteus mirabilis* in our hospital (unpublished results) and we therefore have difficulty explaining the absence of this enzyme in our *E. coli* isolates. PSE-1 is reported to be found mainly in *P. aeruginosa* and rarely in the Enterobacteriaceae (Wiedemann, Kliebe & Kresken, 1989). Yet we found PSE-1 alone in 3·2% of strains and TEM-1 plus PSE-1 in 2·9%. This latter combination of β-lactamases has been previously reported by Simpson *et al.* (1986). Despite the predominance of TEM-1, there was a wide variety of resistance patterns (124 in all) and plasmid profiles. The absence of any extended spectrum β-lactamases is to be expected, since our strains were collected during the years 1984–1988. Five of our strains produced only β-lactamases with a pI of > 8·0 and were resistant to cephalothin but sensitive to carbenicillin. These strains failed to transfer the resistance factor to the recipient suggesting that the β-lactamase gene was located on the chromosome. Similar strains have been reported by Medeiros, Kent & O'Brien (1974).

A further objective in this study was to evaluate oligonucleotide probes for the detection of TEM and OXA producing organisms. As has been previously reported (Huovinen, Huovinen & Jacoby, 1988), these proved to be both sensitive and specific.

The plasmids carrying the ampicillin resistance factor varied widely in size, although most were in the range of 40–70 MDa. Plasmids of size < 11 MDa were more commonly seen in the parent strains but rarely were able to transfer the ampicillin-resistance factor.

Acknowledgement

We are grateful to Mr Tony Woo for his technical support.

References

Cooksey, R., Swenson, J., Clark, N., Gay, E. & Thornsberry, C. (1990). Patterns and mechanisms of β-lactam resistance among isolates of *Escherichia coli* from hospitals in the United States. *Antimicrobial Agents and Chemotherapy* **34,** 739–45.

French, G. L., Cheng, A. F. B., Duthie, R. & Cockram, C. S. (1990). Septicaemia in Hong Kong. *Journal of Antimicrobial Chemotherapy* **25,** *Suppl. C,* 115–25.

French, G. & Ling, T. (1988). Amoxycillin/clavulanate resistant *Escherichia coli. Lancet i,* 704.

Garrod, L. P., Lambert, H. P. & O'Grady, F. (1981). *Antibiotics and Chemotherapy,* 5th edn. Churchill Livingstone, Edinburgh.

Huovinen, S., Huovinen, P. & Jacoby, G. A. (1988). Detection of plasmid-mediated beta-lactamases with DNA probes. *Antimicrobial Agents and Chemotherapy* **32,** 175–9.

Kado, C. I. & Liu, S. T. (1981). Rapid procedure for detection and isolation of large and small plasmids. *Journal of Bacteriology* **145,** 1365–73.

Liu, P. Y. F., Gur, D., Hall, L. M. C. & Livermore, D. M. (1992). Survey of the prevalence of β-lactamases amongst 1000 Gram-negative bacilli isolated consecutively at the Royal London Hospital. *Journal of Antimicrobial Chemotherapy* **30,** 429–47.

Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbour.

Martinez, J. L., Cercenado, E., Rodriguez-Creixems, M., Vincente-Perez, M. F., Delgado-Iribarren, A. & Baquero, F. (1987). Resistance to beta-lactam/clavulanate. *Lancet ii,* 1473.

Matthew, M., Harris, A. M., Marshall, M. J. & Ross, G. W. (1975). The use of analytical isoelectric focusing for detection and identification of β-lactamases. *Journal of General Microbiology* **88,** 169–78.

Medeiros, A. A., Kent, R. L. & O'Brien, T. F. (1974). Characterization and prevalence of the different mechanisms of resistance to beta-lactam antibiotics in clinical isolates of *Escherichia coli. Antimicrobial Agents and Chemotherapy* **6,** 791–801.

Medeiros, A. A., Ximenex, J., Blickstein-Goldworm, K., O'Brien, T. F. & Acar, J. (1980). Beta-lactamases of ampicillin-resistant *Escherichia coli* from Brazil, France and the United States. *Current Chemotherapy and Infectious Diseases* **1,** 761–2.

Meyers, J. A., Sanchez, D., Elwell, L. P. & Falkow, S. (1976). Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. *Journal of Bacteriology* **127,** 1529–37.

Meynell, E., Meynell, G. G. & Datta, N. (1968). Phylogenetic relationships of drug-resistance factors and other transmissible bacterial plasmids. *Bacteriology Review* **32,** 55–83.

Ouellette, M., Gerard, C. P., Philippon, A. M. & Roy, P. H. (1988). Oligonucleotide probes (TEM-1, OXA-1) versus isoelectric focusing in beta-lactamase characterization of 114 resistant strains. *Antimicrobial Agents and Chemotherapy* **32,** 397–9.

Ouellette, M., Rossi, J. J., Bazin, R. & Roy, P. H. (1987). Oligonucleotide probes for the detection of TEM-1 and TEM-2 β-lactamase genes and their transposons. *Canadian Journal of Microbiology* **33,** 205–11.

Roy, S., Teruel, D., Reig, R., Hermida, M. & Teixell, M. (1992). β-Lactamases and susceptibility phenotypes to β-lactam antibiotics in *Escherichia coli* strains. *Journal of Antimicrobial Chemotherapy* **29,** 593–4.

Simpson, I. N., Knothe, H., Plested, S. J. & Harper, P. B. (1986). Qualitative and quantitative aspects of β-lactamase production as mechanisms of β-lactam resistance in a survey of clinical isolates from faecal samples. *Journal of Antimicrobial Chemotherapy* **7,** 725–37.

Sousa, J. C., Carneiro, G., Peixe, M. L., Queirós, M. L. & Rebelo, I. (1991). Characterization of β-lactamases encoded by pathogenic strains of *Escherichia coli* from Portugal. *Journal of Antimicrobial Chemotherapy* **27,** 437–40.

Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98,** 503–17.

Wiedemann, B., Kliebe, C. & Kreksen, M. (1989). The epidemiology of β-lactamases. *Journal of Antimicrobial Chemotherapy* **24,** *Suppl. B*, 1–22.

Williams, H., King, A., Shannon, K. & Phillips, I. (1988). Amoxycillin/clavulanate resistant *Escherichia coli. Lancet i,* 304–5.

Working Party Report of the British Society for Antimicrobial Chemotherapy (1991). A guide to sensitivity testing. *Journal of Antimicrobial Chemotherapy* **27,** *Suppl. D.*

Wu, P. J., Shannon, K. & Phillips, I. (1992). β-Lactamases and susceptibility to β-lactam antibiotics in *Escherichia coli. Journal of Antimicrobial Chemotherapy* **30,** 868–71.

(*Received 2 June 1993; revised version accepted 15 February 1994*)