Epidem. Inf. (1989), **102**, 85–91
Printed in Great Britain

A community outbreak of group A beta haemolytic streptococci with transferable resistance to erythromycin

R. J. D. SCOTT,[1] J. NAIDOO,[2*] N. F. LIGHTFOOT[1]† AND R. C. GEORGE[2]

[1] *Public Health Laboratory, Musgrove Park Hospital, Taunton, Somerset TA1 5DB*
[2] *Antibiotic Reference Laboratory, Division of Hospital Infection, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT*

(*Accepted 16 August 1988*)

SUMMARY

Erythromycin resistance amongst group A streptococci (GAS) in Great Britain is a relatively rare occurrence and outbreaks have been sporadically reported. Over an 8-month period in 1986 ten associated cases occurred in the town of Bridgwater in Somerset. Isolates were group A, type M4 and resistant to erythromycin (MIC 8 mg/l) but sensitive to lincomycin and clindamycin. Erythromycin resistance was transferable from all isolates to a group A recipient strain. No plasmid DNA could be detected in the original isolates or transconjugants.

INTRODUCTION

Erythromycin-resistant group A streptococci (ERGAS) were first reported in Great Britain from an outbreak in a burns unit in Birmingham in 1959 (Lowbury & Hurst, 1959); a second outbreak was reported in 1968 (Kohn, Hewitt & Fraser, 1968). In 1984 outbreaks associated with schools in Liverpool (Youngs, 1984), Ayrshire (Hardie, 1984) and a hospital and school in Cambridge (Walker, Whetstone & Whipp, 1984) were reported. A number of sporadic isolates and a family outbreak were reported from North Yorkshire in 1986 (Barnham & Cole, 1986), the latter study reported 14/536 (2·6%) isolates of GAS resistant to erythromycin in an 18-month period during 1984–5. Reports from other countries have shown higher rates of erythromycin resistance in GAS; 5% in the United States (Istre *et al.* 1981), 0·5–2% in France (Horodniceanu, Bougueleret & Delbos, 1979), 4·4% in Taiwan (Tadano *et al.* 1978), 2·9% in Canada (Dixon & Lipinski, 1982) and 60% in Japan (Maruyama *et al.* 1979).

Erythromycin resistance in GAS is usually linked with resistance to other macrolide and lincosamide antibiotics though the phenotypic expression of these linked resistances may vary considerably (Horodniceanu, Bougueleret & Delbos, 1979). The resistance may be plasmid mediated (Clewell & Franke, 1974; Behnke *et al.* 1979) and has been shown to be transferable by transduction (Hyder &

* Present address: Department of Medical Microbiology, University of Liverpool, Royal Liverpool Hospital, Liverpool L69 3BX.
† To whom correspondence should be addressed.

Streitfield, 1978) and conjugation (Le Bouguenec *et al.* 1984), although in the latter case plasmid DNA could not always be demonstrated.

We describe an outbreak of ERGAS (Type M4) in Somerset in which the erythromycin resistance was transferable from the outbreak strains to a susceptible recipient organism. Ten cases occurred over a period of 8 months. The Public Health Laboratory at Taunton provides a microbiology service for a population of 400000; there had been no previous isolates of ERGAS in this area prior to 1986. The cases that occurred during 1986 were all in the Bridgwater area, a town with a population of 31000.

MATERIALS AND METHODS

Isolation of GAS

Beta-haemolytic colonies isolated from pus and throat swabs were subcultured onto blood agar with a 0·05 unit bacitracin disk as a preliminary screening test. In addition all erythromycin-resistant streptococci were grouped. Initial grouping was by latex agglutination (Phadebact); this was later confirmed by the Streptococcus Reference Laboratory (Division of Hospital Infection, Central Public Health Laboratory, Colindale), where M & T typing was also carried out.

Epidemiological investigations

Details of age, sex and general practitioner (GP) of affected cases were ascertained from laboratory request forms. GPs were asked for details of school attended, possible contacts and any recent antibiotic treatment. They were also asked about their antibiotic prescribing habits with special reference to erythromycin.

Antibiotic sensitivity tests

Initial sensitivity tests were carried out by the Stokes method on Isosensitest agar containing 5% lysed horse blood. Disks used contained the following concentrations of antibiotics: erythromycin 5 μg, oleandomycin 15 μg lincomycin 2 μg, clindamycin 2 μg, penicillin 1 unit, trimethoprim 2·5 μg tetracycline 10 μg, chloramphenicol 10 μg and rifampicin 2 μg. Agar dilution minimal inhibitory concentration values were estimated using a multi-point inoculator (Denley) to spot inoculate strains onto Diagnostic Sensitivity Test Agar (D.S.T. Oxoid) supplemented with 5% lysed horse blood and doubling dilutions of erythromycin, clindamycin, penicillin, ampicillin, tetracycline, chloramphenicol, vancomycin, trimethoprim and rifampicin. An inoculum of 10^4–10^5 colony forming units per ml was used.

Genetic analysis

All the outbreak isolates were studied for transferability of erythromycin resistance and presence of plasmid DNA.

Mating conditions

Brain Heart Infusion broth (BHI, Oxoid) was inoculated with overnight cultures of the streptococcal isolates and incubated at 37 °C with shaking for 6 h

The group A streptococcus recipient BM137 (resistant to rifampicin and fusidic acid) was kindly supplied by Dr T. Horaud (Horodniceanu *et al.* 1982). Matings were carried out by inoculating 1 ml of each donor and recipient into 3 ml of fresh BHI broth and passing the mixture through a sterile membrane filter (0·45 μm, 25 mm, Millipore Corp.). The filter with retained cells was placed on BHI agar and incubated for 18 h at 37 °C. In addition 1 ml of each donor and recipient was inoculated into 3 ml BHI broth and incubated for the same period of time. In the case of filter matings, cells were then resuspended in 5 ml BHI broth and, with the mixed broth cultures, dilutions were made and spread onto selective antibiotic agar (BHI agar containing 5% horse serum with 5 mg/l erythromycin and 80 mg/l rifampicin).

Isolation of plasmid DNA

Plasmid DNA was isolated by a modification of the method of Le Bouguenec *et al.* (1984). Cultures were grown for 18h at 37 °C in 30 ml BHI broth containing 1% glycine. Cells were then spun down and resuspended in 1 ml 25% sucrose (pH8) containing lysozyme (5 mg/ml). The cell suspension was incubated at 37 °C for 1 h. The resulting protoplasts were lysed, by the addition of 4% sodium dodecyl sulphate (SDS). For complete lysis samples were placed in a 56 °C water bath for 5 min.

5 M-NaCl was added to the crude lysate to a final concentration of 1 M. This was then gently mixed and stored at 4 °C overnight. The chromosomal NaCl/SDS complex was spun down at 15000 g at 4 °C. The cleared lysate was treated with ribonuclease (100 μg/ml) for 30 min at 37 °C, then extracted with an equal volume of phenol/chloroform (1 : 1). The DNA was recovered from the aqueous phase by precipitation with an equal volume of isopropanol. Electrophoresis was carried out on 0·8% agarose gels at 100 V for 4 h. *Streptococcus faecalis* strain 39-5 (Yagi *et al.* 1983) was used for plasmid molecular weight markers.

RESULTS

Epidemiology

Within the period of 8 months from May 1986 to January 1987 ten isolates of ERGAS were obtained from patients in the Bridgwater area (Table 1). Eight of the isolates were from children under 9 years old. Epidemiological studies revealed that three of the cases went to the same primary school (cases 1, 4 and 5) whilst four more had either children or siblings at the school (cases 2, 3, 9 and 10). No contact with the school could be found in the remaining three cases. The survey of antibiotic prescribing habits amongst local GPs revealed that erythromycin was only prescribed occasionally. It was not regarded as first-line therapy by any of the GPs. None of the patients had received erythromycin or lincomycin therapy within the preceding month of their infection.

Typing

All isolates were shown to be type M4 group A streptococci.

Antibiotic sensitivities

All the ERGAS were sensitive to penicillin (MIC < 0·06 mg/l), ampicillin (MIC < 0·06 mg/l), tetracycline (MIC < 0·5 mg/l), chloramphenicol (MIC < 2

Table 1. *Erythromycin-resistant group A streptococci from the Bridgwater area*

Case no.	Date isolated	Age (years)	Sex	Group and type	Source	Epidemiology
1	6 May	7	F	A M4	Rash, red throat	Attends primary school A
2	13 May	4	M	A M4	Recurrent impetigo	Sister attends primary school A
3	19 May	4	F	A M4	Ear discharge	Sister attends primary school A
4	1 July	5	F	A M4	Vaginal soreness	Attends primary school A
5	16 Sept.	8	F	A M4	Ingrowing toenail	Attends primary school A
6	16 Sept.	32	M	A M4	Sore throat	No details. Lives 5 miles from Bridgwater
7	29 Sept.	8	M	A M4	Rash, sore throat	No contact with primary school A
8	23 Oct.	1	M	A M4	Otitis externa	Pre-school age
9	30 Dec.	2	M	A M4	Discharging ear	Sister attends primary school A
10	6 Jan.	27	F	A M4	Pharyngitis with glands	Son attends primary school A

Table 2. *Transfer of erythromycin resistance from outbreak isolates of group A streptococci to recipient BM137*

Donor	Mating*	Frequency of Transfer†
32211	Broth	$< 1 \times 10^{-8}$
	Filter	1×10^{-6}
33686	Broth	$< 2 \times 10^{-8}$
	Filter	2×10^{-7}
35327	Broth	$< 1 \times 10^{-8}$
	Filter	1×10^{-5}
44952	Broth	$< 9 \times 10^{-8}$
	Filter	6×10^{-6}
62735	Broth	$< 3 \times 10^{-8}$
	Filter	1×10^{-6}
63110	Broth	$< 9 \times 10^{-8}$
	Filter	1×10^{-7}
66075	Broth	$< 9 \times 10^{-8}$
	Filter	1×10^{-7}
72443	Broth	$< 1 \times 10^{-8}$
	Filter	5×10^{-6}
88206	Broth	$< 6 \times 10^{-8}$
	Filter	2×10^{-6}
01056	Broth	$< 2 \times 10^{-8}$
	Filter	2×10^{-6}

* No transconjugants were ever obtained from mixed broth culture.
† Number of transconjugants per final number of donors.

mg/l), trimethoprim (MIC < 0·5 mg/l), rifampicin (MIC < 0·25 mg/l), lincomycin (2 μg disk), clindamycin (MIC < 0·25 mg/l) and vancomycin (MIC < 0·5 mg/l). They were resistant to erythromycin (MIC 8 mg/l) and oleandomycin (15 μg disk) only. There was no induction of resistance to lincomycin or clindamycin by growing strains on 5 mg/l erythromycin or when erythromycin, clindamycin and lincomycin disks were placed on agar plates at varying distances apart (Horodniceanu, Bougueleret & Delbos, 1979).

Transfer of erythromycin resistance (EM^r)

All donor wild strains were able to transfer EM^r at low frequency. Frequencies of transfer of Em^r to recipient BM137 are shown in Table 2. Transconjugants arising on selective media were tested for resistance to fusidic acid as counter selection. All transconjugants were resistant to rifampicin, fusidic acid, erythromycin and oleandomycin. They were sensitive to the lincosamides, lincomycin and clindamycin, like the original donors. No erythromycin-resistant transconjugants were obtained from the mixed broth cultures. Controls were always set up incubating the donor and recipient separately on filters. No erythromycin-resistant mutants were ever obtained from these.

Isolation of plasmid DNA

Attempts to detect extrachromosomal DNA in donor ERGAS strains and Em^r transconjugants were unsuccessful.

DISCUSSION

We report a protracted outbreak of infections caused by ERGAS (Type M4) in the South West of England. Seven of the ten cases either attended or had contact with the same primary school. There are no other diagnostic microbiology laboratories in the area served by Taunton Public Health Laboratory and thus these isolates represent the total of documented ERGAS infections in this population of 400000 over an 8-month period. The relatively small number of disclosed ERGAS infections may mask a wider dissemination of this particular organism. General practitioners in this area do not perform bacteriological sampling on all cases and it is possible that a number of episodes of empirically treated infection and colonization occurred. The survey of antibiotic prescribing habits among local GPs revealed that erythromycin was not used widely and rarely, if at all, as first-line treatment; therefore it is unlikely that erythromycin treatment failure associated with ERGAS infections would have been observed. When the epidemiological association with the primary school was first noted, mass swabbing was considered but not undertaken because of our desire not to cause alarm. The emergence of antibiotic resistance in organisms causing common community acquired infections provides an unfortunate but useful marker for the study of the circulation of these pathogens in the community.

All ten ERGAS isolated had demonstrable transferable erythromycin resistance providing a potential reservoir for spread of this type of resistance to other group A strains and other species of streptococci. The resistance could also be transferred to group B streptococci and *S. faecalis* (unpublished data). No transfer was

achieved in mixed broth culture indicating that the donor and recipient cells needed to be in close contact for a conjugative transfer to occur. However, no plasmid DNA could be detected in either donors or transconjugants. Conjugative transfer of resistance markers in group A streptococci in the absence of plasmid DNA has been reported before (Le Bouguenec *et al.* 1984; Horodniceanu, Bougueleret & Bieth, 1981) but there was no definitive explanation for the phenomenon. This sort of translocation of chromosomal resistance could be explained if the genes for erythromycin resistance were located on a conjugative transposon (Clewell, 1986). Alternatively, the resistance maybe plasmid mediated but we have been unable to detect any extrachromosomal DNA by the isolation procedure used. Previous reports of plasmid-mediated erythromycin resistance describe linked macrolide/lincosamide resistance which may be inducible or constitutive, erythromycin MICs usually being in excess of 100 mg/l (Horodniceanu, Bougueleret & Delbos, 1979). The strains reported here show low level but clinically significant transferable erythromycin resistance. Neither the original isolates or transconjugants could be induced to lincosamide resistance by the techniques used. Thus these strains of ERGAS do not show the typical characteristics of plasmid-mediated erythromycin resistance.

Erythromycin resistance has been reported in several different serotypes of GAS. A detailed study of antibiotic resistance in haemolytic streptococci in Canada (Dixon & Lipinski, 1982) showed erythromycin resistance to be most common among types T4 and T12, though resistance was noted in 12 other types. In the UK most reports of ERGAS have been types M4 and M12 (Youngs, 1984; Walker, Whetstone & Whipp, 1984; Hardie, 1984) and the isolates reported here were all type M4. The presence of transferable genes for erythromycin resistance in GAS documented in this report may be a harbinger for wider dissemination of erythromycin resistance among GAS. This is of particular concern if resistance to a safe and generally effective antibiotic becomes common in types of GAS associated with rheumatic fever. A recent editorial (Anonymous, 1987) has stressed the importance of culturing throat swabs in cases of pharyngitis. The therapeutic role of erythromycin was emphasized in view of its activity against GAS and the putative pathogen *Arcamobacterium* (*Corynebacterium*) *haemolyticum*. Our findings support the recommendations of performing cultures and susceptibility testing whenever infections with GAS are suspected, and especially if erythromycin is to be used as first-line therapy.

We gratefully acknowledge serotyping studies performed by the Streptococcus Reference Laboratory, Division of Hospital Infection, Central Public Health Laboratory and the co-operation given by local GPs in the investigation of this outbreak. We are grateful to our typists Mrs G. Broom and Miss Gill Phillips.

REFERENCES

Anonymous (1987). Bacterial pharyngitis. *Lancet* ii, 1241–1242.

Barnham, M. & Cole, G. (1986). Erythromycin resistant beta-haemolytic streptococci in North Yorkshire. *Journal of Infection* **13**, 200–202.

Behnke, D., Golubkov, V., Malke, H., Boitsov, A. & Totolian, A. (1979). Restriction

endonuclease analysis of group A streptococcal plasmids determining resistance to macrolides, lincosamides and streptogramin-B antibiotics. *FEMS Microbiological Letters* **6**, 5–9.

CLEWELL, D. B. (1986). Conjugative transposons and the dissemination of antibiotic resistance in streptococci. *Annual Review of Microbiology* **40**, 635–659.

CLEWELL, D. B. & FRANKE, A. E. (1974). Characterisation of a plasmid determining resistance to erythromycin, lincomycin and vernamycin B in a strain of *Streptococcus pyogenes*. *Antimicrobial Agents and Chemotherapy* **5**, 534–537.

DIXON, J. M. S. & LIPINSKI, A. E. (1982). Occurrence of antibiotic resistance in haemolytic streptococci. In *Microbiology 1982* (ed. D. Schlesinger), pp. 147–150. Washington, D. C.: American Society for Microbiology.

HARDIE, R. (1984). Erythromycin resistant *Streptococcus pyogenes* in Ayrshire. *Communicable Diseases of Scotland* **33**, xi–xii.

HORODNICEANU, T., BOUGUELERET, L. & BIETH, G. (1981). Conjugative transfer of multiple resistance markers in beta-haemolytic group A, B, F and G streptococci in the absence of extra-chromosomal deoxyribonuclease acid. *Plasmid* **5**, 127–137.

HORODNICEANU, T., BOUGUELERET, L. & DELBOS, F. (1979). Phenotypic aspects of resistance to macrolide and related antibiotics in B-haemolytic group A, B, C and G streptococci. In *Recent Developments in Laboratory Identification Techniques 1979*, (ed. R. Facklam, G. Laurell and I. Lind), pp. 122–131. Uppsala: Excerpta Medica.

HORODNICEANU, T., BUU-HOI, A., LE BOUGUENEC, C. & BIETH, G. (1982). Narrow host range of some streptococcal R plasmids. *Plasmid* **8**, 199–206.

HYDER, S. & STREITFIELD, M. (1978). Transfer of erythromycin resistance from clinically isolated strains of *Streptococcus pyogenes* via their endogenous phage. *Journal of Infectious Diseases* **188**, 281–286.

ISTRE, G. R., WELCH, D. F., MARKS, M. I. & MOYER, N. (1981). Susceptibility of group A beta-haemolytic streptococcus isolates to penicillin and erythromycin. *Antimicrobial Agents and Chemotherapy* **20**, 244–246.

KOHN, J., HEWITT , J. H. & FRASER, C. A. M. (1968). Group A streptococci resistant to lincomycin. *British Medical Journal* i, 703.

LE BOUGUENEC, C., HORAUD, T., BIETH, G., COLIMON, R. & DAUGUET, C. (1984). Translocation of antibiotic resistance markers from a plasmid-free *Streptococcus pyogenes* (group A) strain into different streptococcal haemolysin plasmids. *Molecular and General Genetics* **194**, 377–387.

LOWBURY, E. J. L. & HURST, L. (1959). The sensitivity of staphylococci and other wound bacteria to erythromycin, oleandomycin and spiramycin. *Journal of Clinical Pathology* **12**, 163–169.

MARUYAMA, S., YOSHIOKA, H., FUJITA, K., TAKIMOTO, M. & SATAKE, Y. (1979). Sensitivity of group A streptococci to antibiotics. *American Journal of Diseases in Childhood* **133**, 1143–1145.

TADANO, J., KOSAKAI, N., OGURI, T. & WANG, C. M. (1978). A study on beta haemolytic streptococci isolated from the throats of healthy school children in Taiwan: changing patterns of type distribution and drug susceptibility. In *Current Chemotherapy 1978* (ed. W. Siegenthaler and R. Luthy), pp. 547–550. Washington, D.C.: American Society for Microbiology.

WALKER, M., WHETSTONE, R. J. & WHIPP, J. (1984). Erythromycin-resistant *Streptococcus pyogenes* in Cambridge. *Journal of Infection* **8**, 88–89.

YAGI, Y., KESSLER, R. E., SHAW, J. H., LOPATIN, D. E., AN, F. & CLEWELL, D. B. (1983). *Plasmid content of Streptococcus faecalis* strain 39-5 and identification of a pheromone (CPDI)-induced surface antigen. *Journal of General Microbiology* **129**, 1207–1215.

YOUNGS, E. R. (1984). Erythromycin-resistant *Streptococcus pyogenes* in Merseyside. *Journal of Infection* **8**, 86–87.