Journal of Antimicrobial Chemotherapy (2000) **45**, 605–609

JAC

High prevalence of erythromycin-resistant, clindamycin/miocamycin-susceptible (M phenotype) *Streptococcus pyogenes*: results of a Spanish multicentre study in 1998

J. I. Alós[a]*, B. Aracil[a], J. Oteo[a], C. Torres[b], J. L. Gómez-Garcés[a] and the Spanish Group for the Study of Infection in the Primary Health Care Setting†

[a]*Servicio de Microbiología, Hospital de Móstoles, 28935 Móstoles, Madrid;* [b]*Area de Bioquímica y Biología Molecular, Universidad de la Rioja, Logroño, Spain*

Using the standard agar dilution method we studied the prevalence of susceptibility to 14-, 15- and 16-membered ring macrolides and clindamycin in *Streptococcus pyogenes* isolated in 1998 from 21 laboratories in Spain. The number of strains admitted to the study was proportional to the numbers of inhabitants in each geographical area. We also determined the susceptibility phenotypes and the genetic basis for the antibiotic resistance. A total of 486 unduplicated isolates of *S. pyogenes* were used. Throat swab samples provided 359 (73.9%) isolates, and the remaining 127 isolates were from other sources. One hundred and fourteen (23.5%) isolates were resistant to erythromycin, a 14-membered ring macrolide, and azithromycin, a 15-membered macrolide, whereas only 1% of isolates were resistant to miocamycin, a 16-membered macrolide and 0.8% were resistant to clindamycin. Of the 114 erythromycin-resistant strains, 109 (95.6%) were susceptible to clindamycin and miocamycin. Since induction with erythromycin did not modify susceptibility to the latter antibiotics, these 109 strains were considered to have the M phenotype. Twenty strains with the M phenotype, one per laboratory, were assayed by PCR and showed the presence of the *mef* gene, which is responsible for antibiotic resistance by an efflux system. Among comparable studies covering entire countries, ours demonstrates one of the highest rates of *S. pyogenes* erythromycin resistance and clindamycin and miocamycin susceptibility in the world. Strains with the M phenotype account for the great majority of these isolates.

Introduction

Streptococcus pyogenes is the most frequent bacterial cause of pharyngitis; this bacterium also causes impetigo, erysipelas and, less frequently, severe invasive diseases.[1] Penicillin has long been regarded as the treatment of choice for streptococcal tonsillitis.[2] In patients with known or suspected allergy to penicillin, clinicians avoid penicillin and β-lactam antibiotics in general. Erythromycin and other macrolides are considered alternative treatment for streptococcal pharyngitis and other non-serious infections caused by *S. pyogenes*, for which they have been demonstrated to be as effective and safe as penicillins.[3–5] In a recent study throughout Spain it was shown that >15% of all pharyngitis cases—not only streptococcal pharyngitis—were treated with macrolides.[6]

Therefore, the emergence and spread of resistance to macrolides in *S. pyogenes* constitute an important problem in the management of streptococcal infections. In recent years a variety of studies in different areas and countries have been published, showing great diversity in rates of resistance to macrolides.[7–11] Recent data from various parts of Spain indicate increasing prevalence of resistance to macrolides over the years.[12–15] The mechanisms of macrolide resistance have been elucidated and involve target modification mediated by a methylase (encoded by *erm* genes), which modifies an adenine in 23S rRNA,[16] and an efflux mechanism encoded by the *mef* gene.[17,18] The gene is

*Corresponding author. Tel: +34-91-6648695; Fax: +34-91-6471917.

†See Acknowledgements.

a novel one with sequence homology to membrane-associated pump proteins.[18] Target site modification due to methylase activity confers resistance to macrolides, lincosamides and streptogramin B (MLS_B) antibiotics, and is expressed constitutively or inducibly. The efflux mechanism selectively pumps 14- and 15-membered macrolides out of the cell but not 16-membered macrolides or lincosamides. The efflux determinant in streptococci seems to be distinct from the multicomponent macrolide efflux system in coagulase-negative staphylococci.[17]

Previous studies have used different methods (agar diffusion, agar dilution, broth microdilution), different interpretations of the results and different populations. Moreover, in several of these studies the genes responsible for antibiotic resistance were not determined.

The aim of the present study was to investigate, by the standard agar dilution method, the prevalence of susceptibility to 14-, 15- and 16-membered macrolides and clindamycin in *S. pyogenes* isolated in Spain in 1998. The numbers of strains collected were proportional to the numbers of inhabitants in each geographical area. The different susceptibility phenotypes and the genetic basis for the antibiotic resistance were also determined.

Materials and methods

Bacterial strains

A total of 486 unique isolates of *S. pyogenes* collected in 21 laboratories in Spain from February 1998 to September 1998 were used. The country was arbitrarily divided into 21 geographical areas. The sample size was stratified in proportion to the number of inhabitants of each area, with a ratio of approximately one strain per 80,000 inhabitants. Throat swab samples provided 359 isolates (73.9%), and the remaining 127 isolates were from other sources, including pus from cutaneous lesions ($n = 42$), otorrhoea ($n = 32$), vaginal swabs ($n = 19$) and others ($n = 34$). Three hundred and eighty-five (79.2%) were isolated from children and 101 (20.8%) from adults. Identification of strains was by standard criteria.[19] Strains were kept frozen in skim milk at –30°C.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards.[20] Antibiotics were obtained as standard reference powders of known potency from Sigma Chemical Co. (St Louis, MO, USA; penicillin G, erythromycin and clindamycin), Pfizer Inc. (New York, NY, USA; azithromycin) and Menarini (Barcelona, Spain; diacetil-midekamycin = miocamycin). The antimicrobials were incorporated into the medium in a log2 dilution series from 0.008 to 2 mg/L for penicillin G, from 0.06 to 64 mg/L for erythromycin, azithromycin and clindamycin and from 0.12 to 64 mg/L for miocamycin. Mueller–Hinton agar medium with 5% sheep blood was used. Inocula were prepared by diluting bacterial suspensions equivalent in turbidity to a McFarland 0.5 standard, resulting in *c.* 10^4 cfu/spot when applied by a Steer's replicator (Craft Machine Inc., Chester, PA, USA). The plates were incubated overnight at 35°C in an atmosphere containing 5% carbon dioxide. The interpretative categories for each antibiotic were those recommended by the NCCLS.[21] The MIC breakpoint for miocamycin resistance was 4 mg/L, as defined by the Comité de l'Antibiogramme de la Societé Française de Microbiologie.[22] *Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619 were used as quality control strains. All susceptibility tests were performed in the same laboratory to avoid interlaboratory variation in the results.

Discs containing erythromycin (15 μg) or clindamycin (2 μg) were used to identify antibiotic resistance phenotypes; different phenotypes of MLS resistance were identified according to the description by Seppälä *et al.*[23] After 24 h incubation at 35°C, blunting of the clindamycin zone of inhibition proximal to the erythromycin disc was taken to indicate inducible resistance. Resistance to clindamycin (confirmed by the agar dilution method) with no blunting of the clindamycin zone of inhibition indicated constitutive resistance. The novel resistance phenotype, designated the M phenotype, was characterized by susceptibility to clindamycin with no blunting of the inhibition zone around the clindamycin disc.

Detection of macrolide resistance genes

Twenty-five erythromycin-resistant strains were selected, 20 with the M phenotype, one per laboratory, and all those with the MLS_B phenotype. The MLS resistance mechanism was determined by PCR with amplification of *erm* genes, using degenerate *erm* primers (E_1 5′-GARATIGGIIIIGGIAAGAGGICA-3′; E_2 5′-AAYTGRTTITTIGTRAA-3′),[24] and specific primers for *ermA* (A_1 5′-TCTAAAAAGCATGTAAAAGAA-3′; A_2 5′-CTTCGATAGTTTATTAATATTAGT-3′), *ermB* (B_1 5′-GAAAAGRTACTCAACCAAATA-3′; B_2 5′-AGTAACGGTACTTAAATTGTTTAC-3′), *ermC* (C_1 5′-TCAAAACATAATATAGATAAA-3′; C_2 5′-GCTAATATTGTTTAAATCGTCAAT-3′)[25] and *ermTR* genes (TR_1 5′-ATAGAAATTGGGTCAGGAAAAGG-3′; TR_2 5′-TTGATTTTTAGTAAAAAG-3′).[26] The conditions used in each case were as previously recommended.[18,24,26] The efflux pump mechanism was determined by PCR using primers and specific conditions for amplification of *mefA/E* genes (A/E_1 5′-AGTATCATTAATCACTAGTGC-3′; A/E_2 5′-TTCTTCTGGTACTAAAAGTGG-3′)[18,25] and the *msrA* gene (msrA1 5′-GCAAATGGTGTAGGTAAGACAACT-3′; msrA2 5′-ATCATGTGATGTAAACAAAAT-3′).[27] Positive and negative controls of our collection were used in all cases. Genomic DNA for PCR

reactions was obtained with the Instagene matrix system (BioRad, Hecules, CA, USA) according to the manufacturer's instructions.

Statistical analysis

The chi-squared test was used.

Results

The MIC ranges, MIC_{50} and MIC_{90} of the isolates, and the percentages of susceptible strains are given in Table I.

Penicillin remained highly active with an MIC_{90} of 0.015 mg/L.

One hundred and fourteen (23.5%) of the isolates were resistant to erythromycin (MIC breakpoint 1 mg/L). The resistance to both the 14- and 15-membered ring macrolides tested was 23.5%, whereas the resistance to miocamycin, a 16-membered ring macrolide, was 1.0% and the resistance to clindamycin was 0.8%.

The phenotypes of susceptibility to macrolides and lincosamides are shown in Table II. One hundred and nine (95.6%) of the 114 erythromycin-resistant strains were susceptible to clindamycin (MICs $\leqslant$ 0.06–0.25 mg/L) and miocamycin (MICs $\leqslant$ 0.12–1 mg/L), and induction with erythromycin did not modify susceptibility to the latter antibiotics; these strains were designated as having the M phenotype. Four isolates (3.5%) were resistant to erythromycin (MICs $>$ 64 mg/L), azithromycin (MICs $>$ 64 mg/L), miocamycin (MICs $>$ 64 mg/L) and clindamycin (MICs $>$ 64 mg/L), which indicates a constitutive type of resistance. The remaining erythromycin-resistant strain (MIC 4 mg/L) was susceptible to clindamycin (MIC 0.12 mg/L) and had intermediate resistance to miocamycin (MIC 2 mg/L), but showed an inducible type of resistance.

All 20 strains with the M phenotype assayed by PCR showed the presence of the *mef* gene, responsible for the efflux system. Two of the five strains with the MLS_B phenotype had the *ermB* gene, two had the recently described *ermTR* gene, and in the remaining strain, with a constitutive MLS_B phenotype, none of the genes searched for was found.

There were marked variations according to geographical area. As an example, 31/74 strains (41.9%) isolated in the three laboratories in the south of Spain were erythromycin resistant, versus 83/412 strains (20.1%) isolated in the rest of the country ($P = 0.00005$). There were no significant differences in the number of erythromycin-resistant strains recovered from children (93/385 or 24.1%) compared with adults (21/101 or 20.8%).

Discussion

Antibiotic resistance is a public health problem. Better surveillance is needed to measure the impact of resistance on public health, the effect of intervention to prevent its emergence and spread, and to advise on empirical therapy.[28]

The design of our surveillance study, based on the collection of strains per unit population, avoids any bias resulting from some laboratories contributing a greater number of strains with respect to the size of population assigned to them. In addition, we tested all the strains in the same laboratory using standard agar dilution methodology, determined the susceptibility phenotype for each strain and identified the genes responsible for macrolide resistance in a selected sample.

Table II. Different phenotypes of susceptibility to macrolides and lincosamides in the 486 strains of *S. pyogenes* studied

Phenotype	Number of strains
Susceptible	372
M phenotype	109
Constitutive MLS_B phenotype	4
Inducible MLS_B phenotype	1

Table I. *In vitro* susceptibilities of 486 recent *S. pyogenes* strains to penicillin G, clindamycin and three macrolides

	MIC (mg/L)				MIC breakpoints (mg/L)[a]		
Antibiotic	range	MIC_{50}	MIC_{90}	Susceptibility rate (%)	S	I	R
Penicillin G	$\leqslant$0.008–0.03	0.008	0.015	100	$\leqslant$0.12		
Erythromycin	$\leqslant$0.06–$>$64	$\leqslant$0.06	8	76.5	$\leqslant$0.25	0.5	$\geqslant$1
Azithromycin	$\leqslant$0.06–$>$64	0.25	32	76.5	$\leqslant$0.5	1	$\geqslant$2
Miocamycin	$\leqslant$0.12–$>$64	0.5	1	99.0	$\leqslant$1		$>$4
Clindamycin	$\leqslant$0.06–$>$64	$\leqslant$0.06	0.012	99.2	$\leqslant$0.25	0.5	$\geqslant$1

[a]S, susceptible; I, intermediate; R, resistant.

In spite of the extensive use of penicillins and other β-lactam antibiotics, all our strains were susceptible to penicillin G, as in other parts of the world.[29]

Until the early 1990s there was a low rate of erythromycin resistance in *S. pyogenes* isolated in some parts of Spain.[30,31] However, the frequency of erythromycin resistance has increased in recent years.[12–15] In this national study we observed a high prevalence of resistance to both 14- and 15-membered macrolides, although regional variations were found. If we compare data obtained by the same methods in 1996[13] and 1998 from the same five laboratories, a dramatic increase in resistance is observed in the two-year period. In 1996, 15/103 (14.6%) isolates were erythromycin resistant, but in 1998 resistance had increased to 29.4% (32/109 isolates) ($P = 0.009$).

The prevalence of resistance to erythromycin but susceptibility to clindamycin and miocamycin in *S. pyogenes* is one of the highest reported for any country. The great majority of resistant strains (95.6%) have the M phenotype, conferred by the *mef* gene, as reported in some studies,[13–15,18,32–34] although not in other studies where greater variety in the percentages of various phenotypes was found.[7–9] Miocamycin, the 16-membered ring macrolide tested, and the lincosamides retained full activity against strains with the M phenotype, and could be an alternative for treatment, although more studies are needed to confirm its clinical efficacy. In our study, MICs of erythromycin and azithromycin for strains with the M phenotype are homogeneous. There appears to be a homogeneous population without subphenotypes as in the strains studied recently in Sweden.[32]

In Spain, with approximately 40 000 000 inhabitants, outpatient consumption of macrolides was 9.3×10^6 units in 1987 and 14.9×10^6 units in 1996, an increase of >60% (International Marketing Service, Madrid, Spain). In Finland an increase in erythromycin resistance was linked to increased use of erythromycin.[11] Therefore in Spain selection pressure by macrolides has probably played a significant role in the spread of resistance. In Finland, after a significant reduction of the use of macrolides in outpatients, there was a significant decrease in the rate of resistance to erythromycin in *S. pyogenes* isolates.[35] Judicious use of macrolides would probably help contain the spread of resistance, as has been seen in other countries.[35]

Among studies covering entire countries, our study demonstrates one of the highest rates of *S. pyogenes* erythromycin resistance with clindamycin and miocamycin susceptibility in the world. Strains with the M phenotype account for the great majority of erythromycin-resistant isolates. The high prevalence observed and the geographical variations suggest a need for all laboratories to test for at least a 14-membered macrolide and a 16-membered macrolide or clindamycin in their *S. pyogenes* isolates as routine practice, informing physicians of the results. Long-term monitoring of the antibiotic resistance to observe its evolution is also advisable, as is study of the prevalence of macrolide resistance in *S. pyogenes* in other countries, since bacteria do not recognize national boundaries.

Acknowledgements

The Spanish Group for the Study of Infection in the Primary Health Care Setting: Hospital Juan Canalejo, La Coruña (A. Guerrero, A. Suárez); Hospital Montecelo, Pontevedra (F. Lueiro); Hospital Ntra Sra de Aránzazu, San Sebastián (E. Pérez-Trallero); Hospital de la Santa Creu i Sant Pau, Barcelona (G. Prats, B. Mirelis); C. A. P. 'Bon Pastor', Barcelona (M. Cervera); C. A. P. 'Manso', Barcelona (G. Roig); Hospital Clínico Universitario, Zaragoza (R. Gómez-Lus); Hospital Miguel Servet, Zaragoza (J. García-Moya); Hospital del Río Hortega, Valladolid (P. Pérez-Pascual); Hospital General de Segovia, Segovia (P. Carrero); Hospital General de Guadalajara, Guadalajara (J. Bixquert, T. Pérez-Pomata); Ambulatorio de Argüelles, Madrid (B. Orden); Hospital Universitario Clínico San Carlos, Madrid (F. de la Torre); Hospital Severo Ochoa, Leganés, Madrid (I. Wilhelmi); Hospital Universitario de Getafe, Getafe, Madrid (I. García-Bermejo); Instituto Valenciano de Oncología, Valencia (J. Maiquez); Hospital Morales Meseguer, Murcia (S. Moreno); Hospital Virgen de las Nieves, Granada (M. de la Rosa, A. Martínez-Brocal); Hospital Universitario de Valme, Sevilla (E. Martín); Hospital Puerta del Mar, Cádiz (P. Marín).

References

1. Bisno, A. L. (1995). *Streptococcus pyogenes*. In *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*, 4th edn, (Mandell, G. L., Bennett, J. E. & Dolin, R., Eds), pp. 1786–99. Churchill Livingstone, New York.

2. Bisno, A. L., Gerber, M. A., Gwaltney, J. M., Kaplan, E. L. & Schwartz R. H. (1997). Diagnosis and management of group A streptococcal pharyngitis: a practice guideline. *Clinical Infectious Diseases* **25**, 574–83.

3. Adam, D. & Scholz, H. (1996). Five days of erythromycin estolate versus ten days of penicillin V in the treatment of group A streptococcal tonsillopharyngitis in children: Pharyngitis Study Group. *European Journal of Clinical Microbiology and Infectious Diseases* **15**, 712–7.

4. Hooton, T. M. (1991). A comparison of azithromycin and penicillin V for the treatment of streptococcal pharyngitis. *American Journal of Medicine* **91**, S23–6.

5. Stein, G. E., Christensen, S. & Mummaw, N. (1991). Comparative study of clarithromycin and penicillin V in the treatment of streptococcal pharyngitis. *European Journal of Clinical Microbiology and Infectious Diseases* **10**, 949–53.

6. Romero Vivas, J., Rubio Alonso, M., Corral, O., Pacheco, S., Agudo, E. & Picazo, J. J. (1997). Estudio de las infecciones respiratorias extrahospitalarias. *Enfermedades Infecciosas y Microbiología Clínica* **15**, 289–98.

7. Cornaglia, G., Ligozzi, M., Mazzoriol, A., Masala, L., Lo Cascio, G., Orefici, G. *et al*. (1998). Resistance of *Streptococcus pyogenes* to erythromycin and related antibiotics in Italy. *Clinical Infectious Diseases* **27**, *Suppl. 1*, S87–92.

8. Cornaglia, G., Ligozzi, M., Mazzariol, A., Valentini, M., Orefici, G. & Fontana, R. (1996). Rapid increase of resistance to erythromycin and clindamycin in *Streptococcus pyogenes* in Italy, 1993–1995. *Emerging Infectious Diseases* **2**, 339–42.

9. Hsueh, P., Chen, H., Huang, A. & Wu, J. (1995). Decreased activity of erythromycin against *Streptococcus pyogenes* in Taiwan. *Antimicrobial Agents and Chemotherapy* **39**, 2239–42.

10. Seppälä, H., Nissinen, A., Järvinen, H., Huovinen, P., Henriksson, T., Herva, E. *et al*. (1992). Resistance to erythromycin in group A streptococci. *New England Journal of Medicine* **326**, 292–7.

11. Seppälä, H., Klaukka, T., Lehtonen, R., Nenonen, E. & Huovinen, P. (1995). Outpatient use of erythromycin: link to increased erythromycin resistance in group A streptoccocci. The Finnish Study Group for Antimicrobial Resistance. *Clinical Infectious Diseases* **21**, 1378–85.

12. Baquero, F., García-Rodríguez, J. A., de Lomas, J. G. & Aguilar, L. (1999). Antimicrobial resistance of 914 beta-hemolytic streptococci isolated from pharyngeal swabs in Spain: results of a 1-year (1996–1997) multicenter surveillance study. The Spanish Surveillance Group for Respiratory Pathogens. *Antimicrobial Agents and Chemotherapy* **43**, 178–80.

13. García-Bermejo, I., Orden, B., Cacho, J., Alós, J. I. & Gómez-Garcés, J. L. (1998). Emergence of erythromycin-resistant, clindamycin-susceptible *Streptococcus pyogenes* in Madrid, Spain. *Antimicrobial Agents and Chemotherapy* **42**, 989–90.

14. Orden, B., Pérez-Trallero, E., Montes, M. & Martinez, R. (1998). Erythromycin resistance of *Streptococcus pyogenes* in Madrid. *Pediatric Infectious Disease Journal* **17**, 470–3.

15. Pérez-Trallero, E., Urbieta, M., Montes, M., Ayestaran, I. & Marimon, J. M. (1998). Emergence of *Streptococcus pyogenes* strains resistant to erythromycin in Gipuzkoa, Spain. *European Journal of Clinical Microbiology and Infectious Diseases* **17**, 25–31.

16. Rice, L. & Bonomo, R. A. (1996). Genetic and biochemical mechanisms of bacterial resistance to antimicrobial agents. In *Antibiotics in Laboratory Medicine*, 4th edn, (Lorian, V., Ed.), pp. 453–51. Williams & Wilkins, Baltimore.

17. Clancy, J., Petitpas, J., Dib-Hajj, F., Yuan, W., Cronan, M. A., Kamath, V. *et al*. (1996). Molecular cloning and functional analysis of a novel macrolide-resistance determinant, *mefA*, from *Streptococcus pyogenes*. *Molecular Microbiology* **22**, 867–79.

18. Sutcliffe, J., Tait-Kamradt, A. & Wondrack, L. (1996). *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. *Antimicrobial Agents and Chemotherapy* **40**, 1817–24.

19. Ruoff, K. L. (1995). *Streptococcus*. In *Manual of Clinical Microbiology*, 6th edn, (Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C. & Yolken, R. H., Eds), pp. 299–307. American Society for Microbiology, Washington, DC.

20. National Committee for Clinical Laboratory Standards. (1998). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard M7-A4*. NCCLS, Wayne, PA.

21. National Committee for Clinical Laboratory Standards. (1997). *Performance Standards for Antimicrobial Susceptibility Testing—Eighth Informational Supplement: M100-S8*. NCCLS, Wayne, PA.

22. Societé Francaise de Microbiologie. (1996). Statement 1996 CA-SFM zone sizes and MIC breakpoints for non-fastidious organisms. *Clinical Microbiology and Infection* **2**, *Suppl. 1*, S46–9.

23. Seppälä, H., Nissinen, A., Yu, Q. & Huovinen, P. (1993). Three different phenotypes of erythromycin-resistant *Streptococcus pyogenes* in Finland. *Journal of Antimicrobial Chemotherapy* **32**, 885–91.

24. Arthur, M., Molinas, C. & Mabilat, C. (1993). PCR detection of *erm* erythromycin resistance genes by using degenerate oligonucleotide primers. In *Diagnostic Molecular Microbiology: Principles and Applications*, (Persing, D. H., Smith, T. F., Tenover, F. C. & White, T. J., Eds), pp. 534–8. American Society for Microbiology, Washington, DC.

25. Sutcliffe, J., Grebe, T., Tait-Kamradt, A. & Wondrack, L. (1996). Detection of erythromycin-resistant determinants by PCR. *Antimicrobial Agents and Chemotherapy* **40**, 2562–6.

26. Kataja, J., Seppälä, H., Skurnik, M., Sarkkinen, H. & Huovinen, P. (1998). Different erythromycin resistance mechanism in group C and group G streptococci. *Antimicrobial Agents and Chemotherapy* **42**, 1493–4.

27. Wondrack, L., Massa, M., Yang, B. V. & Sutcliffe, J. (1996). Clinical strain of *Staphylococcus aureus* inactivates and causes efflux of macrolides. *Antimicrobial Agents and Chemotherapy* **40**, 992–8.

28. Livermore, D. M., MacGowan, A. P. & Wale, M. C. J. (1998). Surveillance of antimicrobial resistance. *British Medical Journal* **317**, 614–5.

29. Macris, M. H., Hartman, N., Murray, B., Klein, R. F., Roberts, R. B., Kaplan, E. L. *et al*. (1998). Studies of the continuing susceptibility of group A streptococcal strains to penicillin during eight decades. *Pediatric Infectious Disease Journal* **17**, 377–81.

30. Betriu, C., Sánchez, A., Gómez, M., Cruceyra, A. & Picazo, J. J. (1993). Antibiotic susceptibility of group A streptococci: a 6-year follow-up study. *Antimicrobial Agents and Chemotherapy* **37**, 1717–9.

31. Pérez-Trallero, E., García-Arenzana, J. M. & Urbieta-Egaña, M. (1989). Erythromycin resistance in streptococci. *Lancet ii*, 444–5.

32. Jasir, A. & Schalén, C. (1998). Survey of macrolides resistance phenotypes in Swedish clinical isolates of *Streptococcus pyogenes*. *Journal of Antimicrobial Chemotherapy* **41**, 135–7.

33. Kataja, J., Huovinen, P., Skurnik, M. & Seppälä, H. (1999). Erythromycin resistance genes in group A streptococci in Finland. The Finnish Study Group for Antimicrobial Resistance. *Antimicrobial Agents and Chemotherapy* **43**, 48–52.

34. Klugman, K. P., Capper, T., Widdowson, C. A., Koornhof, H. J. & Moser, W. (1998). Increased activity of 16-membered lactone ring macrolides against erythromycin-resistant *Streptococcus pyogenes* and *Streptococcus pneumoniae*: characterization of South African isolates. *Journal of Antimicrobial Chemotherapy* **42**, 729–34.

35. Seppälä, H., Klaukka, T., Vuopio-Varkila, J., Muotiala, A., Helenius, H., Lager, K. *et al*. (1997). The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. *New England Journal of Medicine* **337**, 441–6.

Received 15 July 1999; returned 20 October 1999; revised 12 November 1999; accepted 26 November 1999