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High prevalence of erythromycin-resistant and clindamycin-susceptible (M phenotype) viridans group streptococci from pharyngeal samples: a reservoir of *mef* genes in commensal bacteria

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Sir,

An increase in the isolation of *Streptococcus pyogenes* resistant to macrolides, often mediated by the recently described M phenotype, has been reported in several countries.[1,2] Strains of the M phenotype carry the *mef*(A) gene, which confers resistance to 14- and 15-, but not 16-membered macrolides or clindamycin.[3] Pneumococcal resistance to macrolides mediated by the *mef*(A) gene has also been described.[4]

Some strains of viridans group streptococci (VGS) carry the *mef*(A) gene.[5] This ubiquitous group of bacteria frequently colonize the oropharynx in healthy individuals, and may thus be a reservoir for the dissemination of the *mef*(A) gene to *S. pyogenes* and *Streptococcus pneumoniae*. To ascertain the prevalence of VGS with the M phenotype, we studied strains of VGS isolated from pharyngeal exudates from patients with symptoms of pharyngitis and from healthy people. The prevalence of macrolide resistance and the different phenotypes were determined and, in some strains, the genes encoding resistance were characterized.

During 1999, 198 pharyngeal exudates obtained from outpatients in our Health Authority Area of Madrid, Spain, were studied. Most samples were from patients with symptoms of pharyngitis while 17 were from post-treatment controls of group A β-haemolytic streptococcal infection. All the samples were from children aged <14 years. In the same period, pharyngeal exudates from 50 healthy people in the same Health Authority Area (27 children and 23 adults), who had not received antibiotic treatment in the previous 3 months, were also collected. Samples were plated on Columbia sheep blood agar and α-haemolytic Gram-positive cocci that gave a negative catalase test were studied further. All colonies of each sample with different morphotypes (determined by lens examination) were studied. The optochin test was performed to differentiate VGS from *S. pneumoniae*.

Antimicrobial susceptibility testing was performed by the agar dilution method according to the guidelines of the NCCLS.[6,7] Antibiotics were obtained as 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). Plates were incubated overnight at 35°C in an atmosphere containing 5% CO_2. The range of interpretative categories for each antibiotic were those recommended by the NCCLS in the 2000 supplement.[6,7] The MIC breakpoint for miocamycin resistance was >4 mg/L, as defined by the Comité de l'Antibiogramme de la Société Française de Microbiologie.

Different macrolide resistance phenotypes were identified using discs containing erythromycin (15 μg) or clindamycin (2 μg) as described by Seppälä *et al.*[8] The resistance genes of 50 erythromycin-resistant strains (40 with the M phenotype and 10 with the MLS_B phenotype) from both healthy and symptomatic people were characterized by PCR amplification of *erm* genes, using degenerate *erm* primers and by PCR amplification of *mef*(A)/(E) genes using specific primers. VGS strains with a positive PCR with *mef*(A)/(E) primers were considered to have the *mef*(A) gene, following recent recommendations.[3] The χ^2 test was used for statistical analysis.

The 198 pharyngeal exudates from patients with pharyngitis yielded 296 VGS strains (1.49 strains per exudate) while the pharyngeal exudates from healthy people yielded 71 VGS strains (1.42 strains per exudate).

The Table shows the susceptibility of VGS obtained in the three groups studied (healthy children, healthy adults, and patients with symptoms of pharyngoamigdalitis or post-treatment or post-infection controls). Among the isolates from symptomatic patients, 23.6% (70 isolates) were susceptible to erythromycin, 60.8% (180 isolates from 115 patients) had the M phenotype and 15.6% (44 isolates, constitutive; two isolates, inducible) showed the MLS_B phenotype. Among the isolates from healthy children and adults, 36.6% (26 isolates from 17 patients) were susceptible to erythromycin, 50.7% (39 isolates from 30 patients) had the M phenotype and 12.7% (six isolates from five patients) had the MLS_B constitutive phenotype.

When we compared the strains resistant and intermediate

Table. Number of strains and percentage susceptibility in VGS isolated from healthy children, healthy adults, and symptomatic patients

Antibiotic	Healthy children (n = 27) 29 strains			Healthy adults (n = 23) 42 strains			Symptomatic patients (n = 198) 296 strains		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Erythromycin	13 (44.8)	2 (6.9)	14 (48.3)	25 (59.5)	2 (4.8)	15 (35.7)	70 (23.6)	19 (6.5)	207 (69.9)
Azithromycin	13 (44.8)	2 (6.9)	14 (48.3)	27 (64.3)	2 (4.8)	13 (30.9)	73 (24.7)	22 (7.4)	201 (67.9)
Clindamycin	25 (86.2)	0 (0)	4 (13.8)	36 (85.7)	0 (0)	6 (14.3)	251 (84.8)	0 (0)	45 (15.2)
Miocamycin	3 (10.4)	19 (65.5)	7 (24.1)	5 (11.9)	31 (73.8)	6 (14.3)	33 (11.1)	221 (74.7)	42 (14.2)

S, susceptible; I, intermediate; R, resistant.

to erythromycin (226/296) and azithromycin (223/296) in the patient group versus the healthy people group (33/71 and 32/71, respectively), a higher prevalence of macrolide resistance in the patient group was found, with a statistically significant difference ($P < 0.001$) for both antibiotics.

In the comparison of the two groups of healthy people, children and adults, the strains proved to be more resistant to macrolides in children than in the adult group, with 16/29 strains erythromycin resistant or intermediate in children versus 17/42 in adults, and 16/29 strains azithromycin resistant or intermediate in children versus 15/42 in adults. These differences are statistically significant for the two antibiotics ($P < 0.05$).

The MICs of erythromycin and azithromycin for strains of VGS with the M phenotype were between 2 and 8 mg/L. In each of 40 strains with the M phenotype studied, only the *mef*(A) gene was found. In the 10 strains with the MLS_B phenotype, *erm* genes were found.

This work shows the existence of a high percentage of individuals, both healthy and with pharyngitis, who carry VGS with the M phenotype of resistance due to the *mef*(A) gene in their pharyngeal flora. It is reasonable to suppose that the resistance gene could be transmitted to respiratory pathogens that are genetically similar to VGS, such as *S. pyogenes* and *S. pneumoniae*. Others have demonstrated the transfer of macrolide resistance from VGS to *S. pneumoniae*[9] and vice versa, and from strains of *S. pyogenes* to *S. pyogenes* BM137 and *Enterococcus faecalis* JH2-2.[2] The mechanism of transmission of the genetic material was in one case by conjugation[9] because the experiments were performed in the presence of DNases to confirm that the mechanism was not by transformation.

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References

1. Alós, J. I., Aracil, B., Oteo, J., Torres, C., Gómez-Garcés, J. L. & the Spanish Group for the Study of Infection in the Primary Health Care Setting. (2000). High prevalence of erythromycin-resistant, clindamycin/miocamycin-susceptible (M-phenotype) *Streptococcus pyogenes*: results of a Spanish multicentre study in 1998. *Journal of Antimicrobial Chemotherapy* **45**, 605–9.

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

3. Roberts, M. C., Sufcliffe, J., Courvalin, P., Jensen, L. B., Rood, J. & Seppälä, H. (1999). Nomenclature for macrolide and macrolide–lincosamide–streptogramin B resistance determinants. *Antimicrobial Agents and Chemotherapy* **43**, 2823–30.

4. Johnston, N. J., De Azavedo, J. C., Kellner, J. D. & Low, D. E. (1998). Prevalence and characterization of the mechanisms of macrolide, lincosamide, and streptogramin resistance in isolates of *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy* **42**, 2425–6.

5. Arpin, C., Canron, M. H., Maugein, J. & Quentin, C. (1999). Incidence of *mefA* and *mefE* genes in viridans group streptococci. *Antimicrobial Agents and Chemotherapy* **43**, 2335–6.

6. National Committee for Clinical Laboratory Standards. (2000). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Fifth Edition: Approved Standard M7-A5*. NCCLS, Wayne, PA.

7. National Committee for Clinical Laboratory Standards. (2000). *Performance Standards for Susceptibility Testing: Tenth Informational Supplement M100-S10 (M7)*. NCCLS, Wayne, PA.

8. 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.

9. Luna, V. A., Coates, P., Eady, E. A., Cove, J. H., Nguyen, T. T. H. & Roberts, M. C. (1999). A variety of Gram-positive bacteria carry mobile *mef* genes. *Journal of Antimicrobial Chemotherapy* **44**, 19–25.