resistant strain would be even larger for both bacteriological (96.8% versus 33.3%; \( P < 0.0001; \) OR 60.5; 95% CI 10.19–382.72) and clinical (95.4% versus 76.9%; \( P = 0.032; \) OR 6.26; 95% CI 1.08–32.6) efficacy; whereas in the patients treated with penicillin V, no differences would be found with respect to bacteriological (\( P = 1.0 \)) and clinical efficacy (\( P = 1.0 \)) between patients with a clarithromycin-susceptible strain and those with a clarithromycin-resistant strain.

In the PP analysis of the group treated with clarithromycin, clinical cure at visit three was obtained in 95.2% (120 of 126) of patients with susceptible strains, and in 71.4% (10 of 14) of patients with resistant strains (\( P < 0.01 \)), with an OR of 8 (95% CI 1.38–39.76). As in the ITT analysis, the principal criterion for efficacy analysis (bacteriological eradication rate) was achieved at a much higher rate in the group with susceptible strains than in the group with resistant strains (89.6% (146 of 163) versus 28.6% (four of 14); \( P < 0.001 \)) with an OR of 42.5 (95% CI 8.90–221.3). In the penicillin V group, significant differences were also found for bacteriological efficacy (92% versus 60%; \( P = 0.002; \) OR 7.70; 95% CI 1.78–30.79) but not for clinical efficacy (\( P = 0.1 \)) between patients with clarithromycin-susceptible and those with clarithromycin-resistant strains. As with the ITT analysis, when patients classified as indeterminate are excluded, in the clarithromycin group for bacteriological (94.4% versus 33.3%; \( P < 0.0001; \) OR 34; 95% CI 6.72–183.75) and clinical (95.2% versus 76.9%; \( P < 0.038; \) OR 6; 95% CI 1.33–30.03) efficacy the differences between patients with clarithromycin-susceptible and -resistant strains persist. Nevertheless, in the penicillin V group, no differences are observed in either bacteriological (\( P = 0.5 \)) or clinical efficacy (\( P = 1.0 \)).

These data support the effect of macrolide resistance on bacteriological and clinical failure in GABHS pharyngitis, and indicate that failure in bacteriological eradication during clarithromycin therapy is more likely to occur in patients infected with clarithromycin-resistant GABHS. This finding may be of utmost importance in areas with a high prevalence of macrolide resistance, such as Spain.3 The difference observed in bacteriological efficacy in the penicillin V group between patients with clarithromycin-susceptible and patients with clarithromycin-resistant strains may have two explanations: (i) biologically, there is an association between macrolide resistance and cell invasiveness in GABHS strains combining macrolide resistance and ability to enter human respiratory tract cells may be able to escape cell invasiveness in GABHS (strains combining macrolide resistance and cell invasiveness in GABHS strains combining macrolide resistance and ability to enter human respiratory tract cells may be able to escape)

The major contribution of this study is the demonstration that macrolide resistance is clinically relevant in GABHS pharyngitis. Further studies with a larger number of resistant cases are needed to confirm these findings.

References


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Induction of telithromycin resistance in Streptococcus pneumoniae

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Sir,

Ketolides are a new class of semisynthetic macrolide derivatives that show excellent activity against Streptococcus pneumoniae, even against erythromycin-resistant isolates. Previous investigators have not found any telithromycin-resistant isolates among S. pneumoniae with the constitutive macrolide–lincosamide–streptogramin B (cMLSb) phenotype, even though staphylococci or Streptococcus pyogenes with the cMLSb phenotype can develop resistance to this drug.1,2 The lack of induction of methylase production by this drug is one reason for such a difference.3

Recently, Hamilton-Miller & Shah1 reported that resistance to the ketolide cethromycin (formerly ABT-773) could be induced in S. pneumoniae with the cMLSb phenotype by erythromycin or other related antibiotics. In this study, we examined whether 55 isolates of erythromycin-resistant S. pneumoniae (MIC ≥ 1 mg/L) carrying the mef(A) and/or erm(B) genes could develop resistance to telithromycin, a ketolide with different substitutions from cethromycin (ABT-773), when exposed to erythromycin.

Fifty-five clinical isolates of erythromycin-resistant S. pneumoniae (obtained from the sputum of patients with lower respiratory tract infections between 1998 and 2000) were used. These isolates were identified by their sensitivity to optochin and the bile solubility test, and by PCR amplification of the ftaa gene. S. pneumoniae ATCC 6305 was used as the quality control strain.

Reference samples of the following antimicrobial agents of known potency were kindly supplied in powder form by the indicated manufacturers: erythromycin (Shionogi Pharmaceutical Co., Osaka,
Japan), clarithromycin (Taisho Pharmaceutical Co., Tokyo, Japan), azithromycin (Pfizer Laboratories, Groton, CT, USA), rokitamycin (Asahi Kasei, Tokyo, Japan), telithromycin (Nippon Hoechst-Marion-Roussel, Tokyo, Japan) and clindamycin (Upjohn, Tokyo, Japan).

All isolates were tested for susceptibility to the six antibiotics listed above at concentrations between 0.015 and 128 mg/L. MICs were determined by the two-fold agar dilution method using susceptibility test agar (Mueller–Hinton agar medium; Eiken Chemicals, Tokyo, Japan) with 8% Strepto Haemo supplement (SHS; Eiken Chemicals).

DNA was obtained as previously reported, and the presence of macrolide resistance genes was investigated by PCR using primers and amplification conditions that have been described previously.\(^6\)

Induction of telithromycin resistance was examined by the following two methods.

(i) Agar dilution method: induction of telithromycin resistance was evaluated by comparing the MICs for telithromycin in the presence and absence of a subinhibitory concentration of erythromycin (0.1 mg/L). Two susceptibility agar plates were prepared for each isolate: one contained only telithromycin and the other contained telithromycin plus erythromycin. The MIC was determined in accordance with the susceptibility test.

(ii) Disc diffusion method: A bacterial suspension (2 mL of \(\sim 10^8\) cfu/mL) was inoculated onto 10 mL of susceptibility test agar containing 8% SHS and spread over the surface. After excess suspension was removed, paper discs (8 mm diameter high discs; Tokyo Roshi Kaisha, Tokyo, Japan) containing erythromycin or rokitamycin at 20 \(\mu\)g/disc or telithromycin at 5 \(\mu\)g/disc were placed on the surface of the agar plate. Then the plates were incubated overnight at 35°C and induction of telithromycin resistance was assessed from the shape of the zone of inhibition around the telithromycin disc nearest either the erythromycin or the rokitamycin disc.

Fifteen isolates carrying only the \(mef(A)\) gene had the M phenotype. Among the other 40 isolates, 25 carried the \(erm(B)\) gene, and 15 had both the \(mef(A)\) and \(erm(B)\) genes. All 40 isolates showed a high level of resistance to clindamycin (MIC ≥ 128 mg/L) and were also resistant to macrolides, lincosamides and streptogramin B (MLS\(_B\) phenotype). As shown in Figure 1, 26 isolates with this phenotype showed decreased susceptibility to telithromycin after exposure to erythromycin, although the MICs were still <1 mg/L. The remaining 14 isolates showed a minimal decrease in susceptibility to telithromycin (two-fold dilution). However, upon adding a telithromycin disc to an erythromycin–rokitamycin two-disc diffusion test plate, the zone of inhibition around the telithromycin disc was blunted near the erythromycin disc for all isolates with the MLS\(_B\) phenotype, including the 25 isolates considered to show a true cMLSB phenotype based on constitutive resistance to rokitamycin (MIC ≥ 4 mg/L). Furthermore, when this was done for the 16 isolates highly resistant to rokitamycin (MIC ≥ 64 mg/L), the zone of inhibition around the telithromycin disc was blunted near both the erythromycin and rokitamycin discs.

These results indicate that telithromycin resistance can be induced by erythromycin, even in \(S. pneumoniae\) isolates with the MLS\(_B\) phenotype as Hamilton-Miller & Shah reported.\(^3\) However, telithromycin still inhibited all 40 isolates at concentrations <1 mg/L, a result that may reflect the strong affinity of the drug for methylated 23S rRNA.\(^7\)

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