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Macrolide resistance determinants of group A streptococci in Ankara, Turkey

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***Objectives*: Macrolide–lincosamide–streptogramin B (MLS_B) resistance determinants of group A streptococci (GAS) in Ankara, Turkey, were defined for the first time.**

***Isolates and methods*: A total of 1355 GAS isolates, collected from three different regions of Ankara, were screened for erythromycin resistance. Resistance phenotypes were determined by a triple-disc test, and the gene determinants responsible were determined by PCR. MICs of erythromycin, clindamycin and spiramycin were measured for the resistant isolates, and susceptibility rates to some further antibiotics were determined.**

***Results*: Thirty-six isolates (2.6%) were resistant to erythromycin. Of these, 17 (47.2%) expressed macrolide-restricted resistance (M phenotype), while the remainder expressed inducible (16 isolates, 44.4%) or constitutive (three isolates, 8.3%) MLS_B resistance. All isolates of the M phenotype harboured the *mef*(A) gene. Of non-M isolates, 14 harboured *erm*(A) subclass *erm*(TR) and five had *erm*(B) genes. There was a significant relationship between tetracycline resistance and the inducible phenotype ($P < 0.05$). Macrolide resistance was significantly higher in adults ($P < 0.05$), and increased more than two-fold in 2002 compared with 2001 ($P < 0.05$).**

***Conclusion*: The prevalence of macrolide resistance in GAS is low in Ankara; therefore, routine antimicrobial susceptibility testing against these agents seems unwarranted.**

Keywords: *Streptococcus pyogenes*, macrolide–lincosamide–streptogramin B resistance, MLS_B

Introduction

Penicillin remains the treatment of choice for *Streptococcus pyogenes* pharyngitis, because of its proven efficacy and safety, its narrow spectrum and low cost. However, in certain circumstances such as penicillin allergy or presence of intracellular group A streptococci (GAS) protected from antimicrobial effects of extracellular penicillin, macrolides are often the recommended substitute.[1] Unfortunately, the worldwide development of macrolide resistance, with wide variations according to country, may occasionally limit the use of these antibiotics in the indications outlined above.

Macrolide, lincosamide and streptogramin B antibiotics are chemically distinct but share a similar mode of action. Bacteria resist these antibiotics by three mechanisms: drug inactivation, efflux of the drug and ribosomal target modification. The efflux in streptococci is conferred by the *mef* genes and results in a macrolide-restricted resistance (M phenotype). Target modification is controlled by *erm*-encoded methylases and manifests as either constitutive or inducible macrolide–lincosamide–streptogramin B (MLS_B) cross-resistance phenotypes.[2]

There are numerous reports concerning MLS_B resistance in GAS from different regions of the world.[3–10] The present study is the first report of MLS_B resistance phenotypes and genotypes of GAS from Turkey.

Materials and methods

GAS isolates

Between October 2000 and October 2002, a total of 1355 consecutive *S. pyogenes* isolates were obtained from throat swabs collected from three different regions of Ankara. Of these, 1277 isolates (94.2%) were obtained from children (1–16 years old; median age 7 years) and 78 (5.8%) from adults. Isolation and identification of the isolates were

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performed by standard laboratory techniques including latex agglutination test (Avipath-Strep A; Omega Diagnostics, UK).

Drug susceptibility testing

By using the individual colonies picked from the subcultures, all isolates were initially screened for their erythromycin resistance by the standard disc diffusion method, and then the resistant isolates were investigated for phenotypes of the resistance by a triple-disc test, with an erythromycin disc (15 μg) being placed between clindamycin (2 μg) and spiramycin (100 μg; a 16-membered macrolide used to represent streptogramin B) discs (Oxoid) 15–20 mm apart.[3] The MICs of erythromycin, clindamycin and spiramycin for the resistant isolates were determined by NCCLS agar dilution method. Since there is no international standard breakpoint for spiramycin, we used the breakpoints recommended by French Society for Microbiology (≤1 mg/L, susceptible; 2 mg/L, intermediate; ≥4 mg/L, resistant).[3] Susceptibilities of all the resistant isolates to azithromycin, clarithromycin, vancomycin, tetracycline, levofloxacin and chloramphenicol were determined using the standard NCCLS agar diffusion test. In all susceptibility tests, *S. pyogenes* ATCC 19615 was used for quality control.

Detection of MLS_B resistance genes

All erythromycin resistant isolates were analysed by PCR for the presence of *mef*(A), *erm*(A), *erm*(B), *erm*(C) and *erm*(A) subclass *erm*(TR) determinants, as described elsewhere.[4,5] Three reference *Escherichia coli* strains carrying *erm*(A), *erm*(BP) and *erm*(C) genes, respectively, and one *S. pyogenes* strain positive for *mef*(A/E) (kindly provided by Dr Helena Seppälä, National Public Health Institute, Turku, Finland) were used as positive PCR controls. One of the *erm*(TR) PCR-positive bacterial DNA confirmed by sequence analysis was used for the following PCRs as subclass *erm*(TR) control. Reaction mixtures without DNA were used as negative controls.

Statistics

Statistical analysis of the data was done by χ^2 and Fisher's exact χ^2 tests in SPSS 10.0 software program, and P values <0.05 were considered significant.

Results and discussion

Of all isolates, only 36 (2.6%) were resistant to erythromycin. The resistance rates of isolates from adults and children were 7.7% (six of 78) and 2.3% (30/1277), respectively ($P < 0.05$). Resistance to macrolides in *S. pyogenes* were reported worldwide with an increasing incidence. In a study involving isolates from five countries, the Turkish *S. pyogenes* isolates have been reported to have 4.8% erythromycin resistance.[6] The overall erythromycin resistance rate of our isolates was lower (2.6%) compared with those in many countries, for example 63.2% in Taiwan, 23.5% in Spain, 26.8% in Italy and 32% in the USA.[2] The possible explanation of this situation is the low use of macrolide antibiotics in Turkey, especially for streptococcal pharyngitis. The low rate of erythromycin resistance in our isolates is quite similar to that in a recent report from Bulgaria (2.1%), a boundary neighbour of Turkey where erythromycin resistance has been found to be higher in isolates from children being opposite to the present study, but parallel to some other studies.[8,9]

In the triple-disc test, resistance to all tested drugs was assigned to a constitutive MLS_B (cMLS) resistance. Blunting of the clindamycin or spiramycin inhibition zones proximal to the erythromycin disc with or without a zone around it, indicated inducible MLS_B (iMLS) resistance. Susceptibility to spiramycin and clindamycin with no blunting indicated the M phenotype. Herein, iMLS subtypes determined by the triple-disc test are not discussed in details, because they are of no clinical utility.[2,3] The prevalence of M, iMLS and cMLS phenotypes detected was 47.2% (17/36), 44.4% (16/36) and 8.3% (three of 36), respectively. In a multicentre study involving 238 resistant isolates from eight different countries, the M phenotype was also the most common phenotype (73%).[7] iMLS, the most common phenotype in some geographical areas,[8,9] was the second most frequent phenotype among our isolates. cMLS prevalence was very low (8.3%), in contrast to some other reports.[9,10] Interestingly, cMLS prevalence in the multicentre study mentioned above was also quite low (4%).[7]

It is important to determine the macrolide resistance phenotype of a *S. pyogenes* isolated from a patient allergic or clinically resistant to penicillin: if the isolate has the M phenotype, it would be susceptible to lincosamides and streptogramin B/16-membered macrolides. On the contrary, presence of an inducible or constitutive MLS_B resistance would indicate resistance to all MLS_B antibiotics.[2]

The gene determinants of our *S. pyogenes* isolates responsible for the resistance to MLS_B drugs were *mef*(A) (47.2%), *erm*(A) subclass *erm*(TR) (39%) and *erm*(B) (13.9%), consistent with the earlier studies. All 19 MLS_B-resistant isolates carried either the *erm*(A) determinants (14 isolates) or the *erm*(B) determinants (five isolates). All constitutive MLS_B-resistant isolates possessed the *erm*(B), and all the isolates with M phenotype possessed the *mef*(A) gene determinants, in parallel with many prior reports.[3–5,7–10] All isolates carried only one of the resistance determinants screened in this study.

The MICs of erythromycin, clindamycin and spiramycin detected here (Table 1) were also highly comparable to earlier reports. The isolates with M phenotypes were moderately resistant to erythromycin (MIC range 4–16 mg/L), while entirely susceptible to clindamycin and spiramycin. For M phenotype, the most commonly reported upper limit of erythromycin MIC is 8 mg/L, which was exceeded in some of our isolates. The three cMLS expressing isolates were inhibited by MICs > 128 mg/L for all three drugs, as has been detected in the majority of prior studies. With the exception of four that were resistant to all MLS_B drugs, all the iMLS-expressing isolates carried the iMLS-C subtype, and consequently expressed relatively low-level erythromycin resistance (MIC 8 mg/L), consistent with previous reports. These isolates were susceptible to clindamycin (MIC ≤ 0.25 mg/L) and, except three, to spiramycin. It should be remembered that spiramycin has neither an international standard breakpoint nor a regional one for Turkey. The resistance to spiramycin and/or clindamycin we observed in iMLS isolates can be explained by the own induction capabilities of these drugs in streptococci, whereas in staphylococci, only 14- to 15-membered macrolides can induce MLS_B resistance.[2]

Of the six resistant isolates obtained from adults, five (83.3%) had iMLS phenotypes with the *erm*(A) determinant. Among 30 resistant isolates from children, there were 16 (53.3%) with the *mef*(A) determinant, nine (30%) with the *erm*(A) determinant expressing iMLS and five with the *erm*(B) determinant expressing cMLS (three of five) or iMLS (two of five) phenotypes. The relative high rates of iMLS phenotype in adults and M phenotype in children were not significant ($P > 0.05$).

The resistance rates in 2000, 2001 and 2002 were 0% (zero of 96), 1.75% (13/740) and 4.4% (23/519), respectively. In 2002, there was a significant increase in the erythromycin-resistance rate compared with 2001 ($P < 0.05$), while the differences between 2000 and the others were not significant, probably owing to the low number of

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Table 1. MICs of erythromycin, clindamycin and spiramycin against the erythromycin-resistant isolates

Phenotype (n^a)	Antimicrobial agent	MIC (mg/L)		
		MIC_{50}	MIC_{90}	range
M (17)	erythromycin	8	16	4–16
	clindamycin	0.125	0.125	0.125–0.25
	spiramycin	0.5	1	0.25–1
cMLS (3)	erythromycin	>128	>128	>128[b]
	clindamycin	>128	>128	>128[b]
	spiramycin	>128	>128	>128[b]
iMLS (16)	erythromycin	8	>128	2–>128
	clindamycin	0.125	>128	0.125–>128
	spiramycin	2	32	0.25–>128

[a]Number of isolates.
[b]Concentrations >128 mg/L were not tested.

Table 2. Susceptibility of erythromycin-resistant isolates ($n = 36$) to some routinely used antibiotics

Drug	Susceptibility rates according to phenotypes (%)			
	M	cMLS	iMLS	Total
Azithromycin	0	0	0	0
Clarithromycin	0	0	0	0
Vancomycin	100	100	100	100
Tetracycline	94	66	19	61.1
Chloramphenicol	100	100	88	94.4
Levofloxacin	100	100	100	100

isolates obtained in only the last 3 months of 2000. Although the overall resistance rate seems very low, the significant increase in 2002 may be reflecting a general tendency previously observed in some geographical areas.

The susceptibility profiles of the resistant isolates to the antibiotics routinely used for treatment of streptococcal infections are shown in Table 2. Eleven tetracycline-resistant isolates (79%) were shown to express the iMLS phenotype ($P < 0.05$). There are studies indicating an association between tetracycline resistance and MLS_B resistance pattern. The meaning and the cause of this association is not yet clear.[7]

In conclusion, macrolide resistance of GAS was low (2.6%) in our isolates, and fortunately a large part of it (47.2%) was expressed as the M phenotype. Because of the current low prevalence of resistance, routine antimicrobial susceptibility testing of MLS_B antibiotics for *S. pyogenes* isolates seems unwarranted in Ankara. However, to obtain correct information about the epidemiology of macrolide resistance in Turkey and, when required, to take the proper measures, more comprehensive and multicentre surveillance studies should be performed with representative isolates from different regions of the country.

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