

## RESEARCH NOTE

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### Circulating serotypes and antimicrobial sensitivity of *Streptococcus pyogenes* isolates from children in Cyprus

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#### ABSTRACT

The most common T-serotypes among group A streptococci ( $n = 88$ ) isolated from pharyngeal samples of children referred to a tertiary hospital in Cyprus for pharyngitis or scarlet fever during a 14-month period (2003–2004) were T28 (25%), T8/25/Imp19 (22.7%) and T12 (9.1%). All 88 isolates were sensitive to penicillin and clindamycin, but 1.1% and 18.2% of isolates were resistant to erythromycin and tetracycline, respectively. Macrolide consumption was estimated at 1.7 defined daily doses/1000 inhabitants/day. The low percentage of resistance to macrolides may have been related, at least in part, to the low consumption of macrolides.

**Keywords** Antibiotic resistance, Cyprus, erythromycin, group A streptococci, macrolide consumption, T-serotypes

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*Streptococcus pyogenes* (group A streptococcus; GAS) is a bacterial pathogen that causes diverse diseases, ranging from superficial to serious invasive infections [1,2]. Characterisation of GAS is classically based on the identification of T and M cell-wall protein antigens, and detection of the serum opacity factor [3]. Detection of the M protein, or the *emm* gene encoding the M protein, is an important part of GAS characterisation, as the M protein represents a major and very important virulence factor [4]. T-typing and opacity factor determination are useful in clinical situations where the number of GAS types is limited, and represent useful supplements to M/*emm*-typing [3,5]. The simplicity and cost-effectiveness of T-typing have established this method as the first step for screening GAS isolates.

Penicillin continues to be the drug of choice for superficial GAS infections, with erythromycin also being used as an alternative treatment [6]. Despite GAS exhibiting sensitivity to  $\beta$ -lactam antibiotics, resistance to macrolides has been increasing in many countries, ranging from 6.2% in North America to >20% in some European countries, e.g., Spain and Greece [7–9].

Data concerning the epidemiology of GAS infections in Cyprus are limited. The present study describes the characterisation of GAS isolates from pharyngeal infections in children, including their sensitivity to penicillin, erythromycin, clindamycin and tetracycline. The study was conducted at the Archbishop Makarios Hospital (AMH), Nicosia, Cyprus, which is a tertiary 250-bed healthcare facility. The catchment area of the hospital is estimated to include *c.* 300 000 individuals (Nicosia district); in addition, some patients are referred from other districts, since AMH is considered to be a main paediatric referral centre.

GAS strains were isolated from pharyngeal swabs of all paediatric patients who visited the outpatient department or were admitted to the hospital between 1 November 2003 and 31 December 2004 with throat infections. Isolates were identified as *S. pyogenes* by colony morphology,  $\beta$ -haemolysis and sensitivity to bacitracin 0.1 IU (Abtek Biologicals, Liverpool, UK), and were confirmed as Lancefield Group A by latex agglutination grouping. T-typing was performed using the slide agglutination method with rabbit anti-T sera (Sevapharma, Praha, Czech Republic), while the serum opacity factor was determined using the

agar method with sterile filtered horse serum (TCS Biosciences, Buckingham, UK) as described previously [3].

All GAS isolates were tested by the disk-diffusion method for their sensitivity to penicillin, tetracycline, erythromycin and clindamycin. MICs were determined by Etest (AB Biodisk Solna, Sweden) for isolates that showed resistance to any of the antibiotics tested. The three erythromycin resistance phenotypes were differentiated by the double-disk test, using erythromycin and clindamycin disks as described previously [10]. Culture conditions, as well as interpretation of the inhibition zones and MICs, were according to CLSI criteria.

Information concerning consumption of antibiotics in Cyprus was provided by the Pharmaceutical Service of the Cypriot Ministry of Health. Data were collected using anatomical therapeutic classification/daily defined doses (ATC/DDD) methodology, with results expressed in DDD/1000 inhabitants/day (DID).

During the study period, 88 GAS isolates were obtained from 88 children (aged 9 months to 15 years; mean age 6.6 years) presenting with pharyngitis or scarlet fever. Forty-six of the children were males and 42 were females.

Table 1 summarises the T-types and the antibiotic sensitivity patterns for the isolates. Only one (1.1%) GAS isolate was resistant to erythromycin (M-phenotype). No resistance to either penicillin or clindamycin was detected. However, 16

(18.2%) GAS isolates were resistant to tetracycline, with MICs ranging from 12 to 48 mg/L. Macrolide consumption in Cyprus was estimated to be 1.7 DID.

To the best of our knowledge, this is the first published study describing the circulating T-serotypes and antimicrobial resistance patterns of *S. pyogenes* isolates from Cyprus. A diverse population of GAS serotypes was revealed, together with a low frequency of erythromycin resistance (1.1%) and a much higher frequency of tetracycline resistance (18.2%). Of the 88 GAS isolates, 86 (97.7%) were T-typeable, which is a high figure in comparison with other countries, e.g., Greece (96%), the USA (89%), Thailand (60%) and Malaysia (40%) [11–13]. Eleven T-serotypes were detected during the study, the most common of which was T28. A similar diversity among T-serotypes was revealed in a study undertaken in Athens, Greece, where 36 different T-types were isolated over a 3-year period [11].

Resistance to erythromycin (1.1%) appeared to be one of the lowest levels in Europe and worldwide, with other studies reporting frequencies of 6% in France, 3–7% in the USA, 5–24% in Greece, 25–>30% in Italy, and 22% in Spain [7–9,11,14–16].

Several limitations of the present study should be acknowledged. Most importantly, there was a limited number of GAS isolates, and M/*emm*-typing was not performed. Nevertheless, the results obtained were similar to those reported from other European countries with respect to the diversity among T-serotypes. The low level of macrolide resistance may be related to the low consumption of macrolides in Cyprus during the study period compared with other European countries [17]. Reports from several countries have correlated decreased consumption of antibiotics with reduced resistance, or vice versa [18–20].

**Table 1.** T-serotypes and erythromycin and tetracycline susceptibility of group A streptococci isolated from paediatric patients in Cyprus

T-type	OF	Antibiotic sensitivity pattern			No. of isolates
		Fully sensitive	Erythromycin-resistant	Tetracycline-resistant	
28	+	20	0	2	22
8/25/Imp19	+	19	0	1	20
12	–	3	0	5	8
3	–	7	0	0	7
12	+	0	0	6	6
25	+	6	0	0	6
1	–	5	0	0	5
3/13/B3264	–	4	0	0	4
B3264	+	1	0	1	2
6	–	2	0	0	2
3/13/B3264	+	1	0	0	1
13	+	0	0	1	1
25	–	1	0	0	1
14	+	1	0	0	1
Non-typeable	–	0	1	0	1
Non-typeable	+	1	0	0	1
Total		71	1	16	88

OF, opacity factor.

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