

Multicentre surveillance of the prevalence and molecular epidemiology of macrolide resistance among pharyngeal isolates of group A streptococci in the USA

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Objectives: Rates of macrolide resistance in group A streptococci (GAS) were reported to be low in the US in the 1990s. However, we documented an unexpectedly high rate of macrolide resistance among GAS in Pittsburgh, PA, in 2001 and 2002. In an effort to define the current prevalence of macrolide-resistant GAS in the US, a multicentre surveillance project was initiated.

Methods: Between October 2002 and May 2003, 50 pharyngeal GAS isolates per month were requested from each of the nine participating sites representing a wide geographical distribution. Standard susceptibility testing was performed and the macrolide resistance phenotype was assessed using double-disc diffusion testing. Monthly and annual rates of macrolide resistance were calculated for each site. An adjusted overall rate of macrolide resistance was determined to account for differences in the numbers of GAS isolates sent from each centre.

Results: Overall, 171 of the 2797 collected isolates of GAS (6.1%) were resistant to erythromycin. The adjusted overall resistance rate was 5.2%. Rates of macrolide resistance varied by site (range 3.0–8.7%) and also by month (<2% to >10%). The M phenotype of macrolide resistance accounted for >60% of all macrolide-resistant isolates recovered in this study.

Conclusions: These data suggest an increasing prevalence and broad geographical distribution of macrolide-resistant GAS in the US, indicating the need for ongoing local and national longitudinal surveillance to define the extent of this problem.

Keywords: antibiotic resistance, *Streptococcus pyogenes*, *mef*(A)

Introduction

Although macrolide resistance among *Streptococcus pyogenes* has been recognized in Europe and Japan for many years,¹ rates in group A streptococci (GAS) in the US have remained low.² In the Spring of 2001, we documented the emergence of a

high level of macrolide resistance among pharyngeal isolates of GAS acquired in Pittsburgh.³ During this time the monthly prevalence of macrolide resistance ranged from 0 to 41%, averaging 9.6%.⁴ The persistence of increased macrolide resistance in GAS at our centre over 2 years raises important questions regarding the current rate of resistance in the remainder of the US. The purpose

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of the current study was to define the prevalence of macrolide-resistant GAS in the US by performing longitudinal surveillance in multiple geographically separated centres.

Materials and methods

Between October 2002 and May 2003, 50 pharyngeal isolates per month were requested from the clinical microbiology laboratory of each of the nine children's hospitals located in Ann Arbor, MI; Columbus, OH; Durham, NC; Houston, TX; Little Rock, AR; Nashville, TN; Newark, NJ; Pittsburgh, PA; and San Diego, CA. All GAS recovered from the Pittsburgh site were evaluated. Isolates were obtained from children presenting to emergency departments and ambulatory care centres with symptoms of acute pharyngitis. All isolates were sent to the clinical microbiology laboratory at the Children's Hospital of Pittsburgh. The Institutional Review Board of each participating centre approved the study.

In vitro susceptibility testing was performed against erythromycin and clindamycin using Kirby–Bauer discs (BBL Becton Dickinson, Sparks, MD, USA).⁵ MICs were determined using Etest (AB Biodisk, Piscataway, NJ, USA) for isolates demonstrating intermediate susceptibility or resistance to erythromycin or clindamycin. Breakpoints approved by the CLSI for GAS were used.⁵ Results of antimicrobial susceptibility testing have been reported in part in a separate manuscript.⁶

The monthly and annual rates of macrolide resistance in GAS were determined for each site by dividing the number of resistant isolates by the total number of GAS isolates received. The overall combined rate of macrolide resistance for the entire study was adjusted to account for differences in the actual number of isolates received from each centre by determining the corrected number of resistant isolates per site (multiplying the observed resistance rate for each site by 400, the number of isolates requested per site for the entire study period) and then dividing the sum of the corrected number of resistant isolates from all sites by 3600 (the total number of isolates requested from the nine sites over the study period).

The genetic relatedness of the erythromycin-resistant isolates was determined initially using field-inversion gel electrophoresis (FIGE) of *Apa*I-digested genomic DNA.⁶ *emm* typing and subtyping, as well as T-typing, were performed at the Streptococcal Laboratory of the Centers for Disease Control and Prevention (CDC) on representative

isolates within each FIGE type, including those expressing different macrolide resistance phenotypes.⁷

Macrolide resistance isolates were categorized into those expressing an M phenotype, an inducible MLS (MLS_i) phenotype or a constitutive MLS (MLS_c) phenotype using the double-disc diffusion test (D-test).⁶ The presence of *mef*(A), *erm*(B) and *erm*(A) resistance genes was detected by PCR amplification.³

Results

Overall, 171 of the 2797 collected isolates of GAS (6.1%) were resistant to erythromycin. The adjusted rate of macrolide resistance for all sites during this study period was 5.2%. The 'projected' annual rate of macrolide resistance by site varied from 3.0% to 8.7%. Although 'projected' annual rates of resistance were <5% at six of the nine sites during 2002–2003, the monthly rate of macrolide resistance exceeded 10% for at least 1 month during the study period at five sites. Remarkably, the rate of macrolide resistance exceeded 20% for at least 1 month at two sites. The 'projected' annual rate and range of monthly rates of GAS resistance for each site are shown in Table 1.

The macrolide resistance phenotype was determined for 171 resistant isolates, of which 117 (68%) expressed the M resistance phenotype, 45 (27%) expressed the MLS_i phenotype and 9 (5%) expressed the MLS_c resistance phenotype. Although the M phenotype was expressed in more than two-thirds of resistant isolates overall, it accounted for ≤50% of the resistant isolates recovered from five of the participating centres (Table 1).

Molecular epidemiological analysis was performed to determine the relative prevalence of clones of resistant GAS recovered during this study. Among the 171 resistant isolates, 12 *emm* types were identified. *emm*75 accounted for 47% of the resistant GAS and was recovered from seven of the nine participating centres. *emm*12 accounted for 26% of all resistant isolates and was recovered from eight of the nine sites. Table 2 shows the relative prevalence and geographical distribution of *emm* types within each macrolide resistance phenotype. Eight different *emm* types were identified among the 117 macrolide-resistant GAS isolates expressing the M phenotype. *emm* type 75 accounted for 62% of the isolates and was recovered from seven of the nine sites. Eight *emm* types were identified among the 45 MLS_i

Table 1. Prevalence, phenotype and *emm* type of macrolide-resistant GAS recovered during 2002–2003 at nine US centres

Study site (number of isolates tested)	Overall rate of resistance (%)	Range of monthly rate of macrolide resistance (%)	% M phenotype resistance among all resistant isolates	Number of different <i>emm</i> types	Predominant <i>emm</i> type
AR (421)	3.8	0–8.5	50	4	12.0
CA (164)	3.0	0–10.3	80	3	12.0
MI (334)	4.5	0–9.1	45	6	12.0
NC (250)	6.4	0–13.2	21	3	75.0
NJ (76)	3.9	0–7.7	100	2	12.0
OH (450)	8.7	0–20.4	93	4	75.0
PA (769)	7.8	0–23.3	74	5	75.0
TN (267)	6.0	0–12.2	27	3	75.0
TX (67)	3.0	0–11.1	50	2	75.0 ^a /1.0 ^a

^aThere were only two macrolide-resistant isolates recovered from this site.

Macrolide-resistant group A streptococci in the US

Table 2. Relative prevalence of clones of resistant group A streptococci within each macrolide resistance phenotype and their geographic distribution

Resistance phenotype	<i>emm</i> type	Total isolates	Sites with <i>emm</i> type
M (117)	75.0	71	AR, CA, NC, OH, PA, TN, TX
	12.0	28	AR, MI, OH, PA, TN
	1.0	9	MI, PA
	4.0	3	MI, OH, NJ
	6.11	1	PA
	6.44	2	MI
	2.0	1	MI
	73.0	1	TN
	76.0	1	OH
	12.0	9	MI, NC, NJ, OH
MLS _i (45)	4.0	8	MI, NC, OH
	58.0	8	AR, PA
	75.0	8	NC, PA, TN
	1.0	3	TN, TX
	28.0	1	AR
	77.0	1	TN
	94.1	1	TN
MLS _c (9)	12.0	6	AR, CA, NC
	12.25	1	AR
	12.7	1	MI
	73.3	1	MI

phenotype isolates, while two *emm* types accounted for the 9 MLS_c resistance phenotype isolates. *emm* type 12 was the most common type among both MLS_i and MLS_c phenotypes.

PCR was performed to confirm the genetic basis of resistance for each of the macrolide resistance phenotypes. The *mef*(A) gene was detected in each of the eight *emm* types expressing the M phenotype; *erm*(A) was detected in seven of the eight *emm* types expressing the MLS_i phenotype, and *erm*(B) was found in both isolates of the eighth *emm* type and also found in the single isolate of *emm*73.3 expressing the MLS_c phenotype. No PCR products were obtained for eight MLS_c isolates belonging to *emm* types 12, 12.7, and 12.25; an additional analysis has identified identical dual mutations (A2058G and U2166C) in domain V of 23S rRNA in these isolates.⁸

Discussion

We recently documented the emergence and persistence of relatively high rates of macrolide resistance in pharyngeal isolates of GAS recovered from children in Pittsburgh.^{3,4} This multicentre study was initiated to determine the prevalence of macrolide resistance among GAS in the US. We found an overall adjusted rate of macrolide resistance in GAS of 5.2%. Although this rate is relatively low compared with much of the rest of the world, it represents a nearly twofold increase from rates reported in the US in the 1990s. Our results are consistent with three recently published studies evaluating GAS isolates from the US which reported rates of 6.2–6.8% for isolates obtained between 1998 and 2003^{9–11} but differ from observations by Tanz *et al.*¹² who reported a stable rate of macrolide resistance of <5% for 2000–2003.

We observed a high degree of month-to-month variability in the rate of macrolide-resistant GAS in many of our participating centres. Five of the nine centres had a rate of macrolide resistance in excess of 10% for at least 1 month during the surveillance period. This temporal variability is consistent with previous reports^{4,10} and may account for the differences in reported rates of macrolide resistance. Results reported by Tanz *et al.*¹² were obtained during 2 week intervals from each site on three separate occasions. The narrow timeframe studied may have missed periods of increased prevalence, leading to an underestimation of the prevalence of macrolide resistance.

A fairly wide variation in rates of macrolide resistance between participating centres was observed during the current study (3.0–8.7%) and in reports by Barrozo *et al.*¹⁰ (<1–29%), Richter *et al.*¹¹ (2.7–11%) and Tanz *et al.*¹² (0–9.0%). In contrast to these observations, Critchley *et al.*⁹ found that the rate of resistance was fairly constant throughout the nine Census Regions of the US. The presence of geographical variability implies that overall rates of macrolide resistance in GAS cannot be extrapolated to individual geographical locations within the US. Accordingly, while national surveillance is necessary, results of regional analyses are needed to guide specific antibiotic choices for penicillin-allergic patients requiring treatment for GAS pharyngitis.

Twelve different *emm* types accounted for all macrolide-resistant GAS recovered during the study period; *emm*75 accounted for nearly half and *emm*12 accounted for one-quarter. These two *emm* types were recovered from the majority of the participating sites in our study and were also the most frequently recovered macrolide-resistant GAS types during the surveillance carried out by others.^{11,12} While *emm*12 and *emm*75 accounted for the majority of resistant isolates, macrolide resistance was found in numerous other *emm* types in all three studies. Of interest, we identified *emm*75 GAS among isolates expressing either the M or MLS_i resistance phenotypes; *emm*12 GAS isolates were found to express M, MLS_i or MLS_c resistance phenotypes. Taken together, these results suggest that macrolide resistance in GAS in the US is being spread both by dissemination of specific clones and by spread of macrolide resistance genes [*mef*(A), *erm*(A) or *erm*(B)] from resistant isolates into previously susceptible strains of GAS. This latter phenomenon probably occurs by way of a transposon, as has been reported for both *mef*(A) and *erm*(B).^{13,14}

In conclusion, these data suggest an increasing prevalence and broad geographical distribution of macrolide resistance in GAS in the US. Ongoing surveillance is needed to confirm these observations.

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Transparency declarations

Dr M. D. G. has previously served on an advisory board with Aventis-Pasteur, but does not currently have any active grant

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