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Identity and Prevalence of Multilocus Sequence Typing-Defined Clones of Group A Streptococci within a Hospital Setting

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Between July and October 2003, 121 clinical isolates of group A streptococci (GAS) were collected from a London hospital and characterized by multilocus sequence typing (MLST) to determine the identity and prevalence of clones circulating within this setting. A total of 39 sequence types (ST), of which 20 were represented by a single isolate, were identified. The eight most prevalent clones among the 121 GAS were ST117/*emm81* (16%), ST39/*emm4* (9%), ST62/*emm87* (7%), ST28/*emm1* (6%), ST36/*emm12* (6%), ST46/*emm22* (5%), ST334/*emm82* (5%), and ST101/*emm89* (4%). Compared to those in the MLST database (http://spyogenes.mlst.net), 12 (31%) of the 39 STs had not been previously identified, although 7 of these differed from recognized STs at only a single locus, suggesting they were closely related to previously recognized strains. Resistance to erythromycin and tetracycline was seen in 7 and 20% of isolates, respectively, with four isolates resistant to both agents. GAS strains with higher (>80) *emm* types accounted for 45% of GAS isolates collected during this study. Continuing GAS surveillance, using easily comparable methods, is important for detecting changes in the character of disease-causing isolates.

Streptococcus pyogenes (group A streptococci [GAS]) is an important pathogen associated in humans with a variety of diseases, ranging from pharyngitis and impetigo to severe invasive disease, including streptococcal toxic shock syndrome and necrotizing fasciitis (10). In the late 1980s, concern about GAS disease was heightened in many countries, as outbreaks of more-severe clinical infection were reported (12). GAS infections remain a significant public health problem. In 2003, 1,870 cases of bacteremia due to GAS in England, Wales, and Northern Ireland were reported to the Health Protection Agency's Streptococcus and Diphtheria Reference Unit (20), an incidence of 3.5 per 100,000 population.

Infections are largely treatable with appropriate antimicrobial therapy, although significant morbidity and mortality are reported for invasive GAS disease (34). Penicillin, to which GAS are uniformly susceptible, is the drug of choice for most infections with this organism (6). Erythromycin is recommended for treatment of penicillin-hypersensitive patients (6); however, resistance to this agent is of increasing concern (9, 25), and tetracycline can be used as an alternative in areas where resistance levels remain low (18). The development of an effective vaccine to prevent throat infections, which have a substantial economic cost and may occasionally lead to postinfectious sequelae and invasive disease (27), is highly desirable, and a number of vaccine targets are being assessed, including the highly variable immunogenic amino-terminal region of the M protein (11, 22). However, more than 100 M protein serotypes exist (16) and antibodies against this region offer type-specific protection (23, 28), necessitating the construction of multivalent vaccines by the use of hybrid proteins containing epitopes from several different M serotypes.

To guide public health action policy and inform therapeutic and preventative strategies for GAS infections, active and continuing surveillance is required to provide an accurate assessment of disease burden and epidemiological data on the character of disease-causing isolates. There are a number of programs in place for surveillance and characterization of invasive GAS disease (34; A. Jafir, Abstr. 14th Eur. Congress Clin. Microbiol. Infect. Dis., abstr. S263, 2004). Comparable data on noninvasive isolates, which contribute a significant disease burden, are likewise important.

Multilocus sequence typing (MLST) is a highly discriminatory unambiguous method for identifying clusters of isolates with identical or closely related genotypes and is highly suitable for the analysis of bacterial populations for epidemiological purposes (38). An MLST scheme has been developed for GAS (14). In the present study, clones of GAS, within a collection of clinical isolates predominantly from noninvasive disease, were defined by MLST.

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Bacterial strains. A total of 121 GAS were analyzed in this study. Strains were isolated from routine clinical specimens submitted to the Diagnostic Bacteriology Laboratory of St. Mary's Hospital National Health Service Trust, London, United Kingdom, over 4 months (July to October) during the summer and fall of 2003. Only one isolate per patient was included in the study. Isolates were recovered from specimens taken from hospitalized patients ($n = 11$) and outpatients attending emergency rooms ($n = 38$), specialty outpatient clinics ($n = 7$), and general practitioners ($n = 48$). The origins of 17 isolates were not specified. The majority (114 of 121; 94.2%)

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of GAS in this study were isolated from noninvasive specimens, including throat ($n = 47$), wound ($n = 23$), skin ($n = 7$), genital ($n = 8$), eye, ear, nose, and pus swabs. The remaining seven isolates were recovered from blood. Of these, three were from patients with bacteremia secondary to wound infection, three were isolated from injecting drug users, and one was of unknown etiology. No patients presented with severe invasive infections or postinfectious complications such as necrotizing fasciitis, toxic shock-like syndrome, rheumatic fever, or poststreptococcal glomerulonephritis during the study.

MLST. Internal fragments of seven housekeeping genes were amplified by PCR and subjected to nucleotide sequence determination as previously described (14). Distinct allele numbers were assigned to every unique gene sequence, and each isolate was given a seven-integer allelic profile. Isolates with identical allelic profiles were assigned to the same sequence type (ST). A database containing the allelic profiles of GAS isolates and associated epidemiological data is maintained at http://spyogenes.mlst.net. Isolates with STs differing at only one of the seven loci (single-locus variants; [SLVs]) were considered to be closely related (17).

***emm* sequence typing.** The DNA sequence of the 5′ end of the *emm* gene was obtained as previously described (5). An *emm* type and a subtype were assigned according to exact 150-bp sequences as described at a Centers for Disease Control website (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.html). A unique *emm* type is defined as having <92% sequence identity to any other known *emm* type over the 90 bases encoding the N-terminal 30 residues of the mature M protein.

Antimicrobial susceptibility testing. All isolates were tested by disk diffusion using 5-μg erythromycin and 10-μg tetracycline disks (Oxoid, Basingstoke, United Kingdom) on Iso-Sensitest agar with 5% defibrinated horse blood in accordance with British Society for Antimicrobial Chemotherapy guidelines (3).

Results and discussion. We report the results of *emm* sequence typing and MLST of a collection of GAS isolates recovered from clinical specimens at a London hospital. From the 121 GAS isolates, representing 36 distinct *emm* types, 39 STs were resolved (Table 1). Eight STs account for 57.9% of GAS isolates, while 20 STs are represented by a single isolate. The isolation of multiple GAS of a single clone did not appear to be the result of an outbreak, as isolates were generally recovered from specimens taken at various times throughout the study and from patients presenting in different locations. As a result of comparisons performed using the present *S. pyogenes* database at http://spyogenes.mlst.net (identification numbers 1 to 682), 12 new STs were described in this study (although 7 of these are SLVs of previously identified STs) (Table 2).

There was a strong correlation between *emm* and ST within this set of isolates, with the majority (84.6%) of STs associated with a single and distinct *emm* type. Three *emm* types were found in association with multiple STs (Table 1). *emm22* was associated with a pair of SLVs (ST46 and ST328). *emm82* was associated with two STs (ST314 and ST334), which are SLVs of ST26, and both are therefore likely to be descended from the latter ST. Only *emm103* was found in association with two highly divergent STs (ST311 and ST327, which differ at six of the seven loci). Similar concordance has been noted in other local epidemiological studies using both *emm* sequence typing and MLST (30, 35, 40).

The most prevalent clone isolated in the summer of 2003 was ST117/*emm81*. The 19 ST117/*emm81* isolates were recovered from a variety of specimens, including blood ($n = 4$), wound ($n = 5$), skin ($n = 2$), eye ($n = 2$), ulcer ($n = 2$), throat ($n = 1$), and unspecified ($n = 3$) swabs. A study of *emm* types present in the United Kingdom during the 1980s reported *emm81* as an important cause of skin disease (8). In this study, 30 isolates (represented by 18 STs), having been recovered from skin and wound swabs, were associated with skin infections. ST117/*emm81* was the most prevalent (23.3%; 7 of 30) clone associated with skin isolates, suggesting that this strain continues to be common in United Kingdom GAS skin infections. ST117/*emm81* GAS have also been recovered from aboriginal Australians (30), with whom skin infection is common. The finding of ST117/*emm81* as an important clone in United Kingdom GAS infections may be reflective of the time period in which this study was conducted. It would be of interest to determine whether this clone was similarly prevalent during winter months.

In the United Kingdom, invasive GAS infection associated with injecting drug use appears to be increasing (13). Three isolates in this study, two ST117/*emm81* clones and one ST62/*emm87* clone, were obtained from the blood of injecting drug users. It is probable that skin or mucosal lesions related to drug injection served as a portal of entry to the bloodstream in these instances (29). There is evidence to suggest that high-risk behavior such as sharing of needles and the resultant multiple handling of drug paraphernalia, rather than contaminated batches of drug, is contributing to the increase in GAS infection in this group (13). That infections in users in this study were caused by commonly circulating clones is supportive of this suggestion.

The most common GAS clones in this study, in order of decreasing prevalence, were ST117/*emm81*, ST39/*emm4*, ST62/*emm87*, ST28/*emm1*, ST36/*emm12*, ST46/*emm22*, ST334/*emm82*, and ST101/*emm89* (Table 1). The Health Protection Agency Streptococcus and Diphtheria Reference Unit has recently reported preliminary results from the first year of enhanced surveillance of invasive GAS infections in the United Kingdom (19). While MLST analysis has not been performed, M serotyping results indicate that M1, M3, M87, M89, M12, M83, and M5 were important causes of invasive disease in the United Kingdom in 2003. It is clear that, as in this study, strains with higher (>80) *emm* types account for a significant proportion of isolates.

The emergence of GAS strains with higher *emm* types as a significant cause of disease may have important ramifications for *emm*-directed vaccine strategies. The hexavalent vaccine, containing N-terminal M protein fragments of serotypes 1, 3, 5, 6, 19, and 24, presently in phase 1 clinical trials (26) would be active against only 13.2% of noninvasive isolates in this study and none of the invasive isolates. A 26-valent M protein vaccine under development includes N-terminal peptides covering 39.7% of isolates in this study (Table 1), although it may provide protection against additional M types (22). The serotypes in this expanded-multivalent vaccine were selected, in part, for their frequency in uncomplicated pharyngitis (22) and

TABLE 1. Prevalence of MLST-defined clones within 121 GAS from a London hospital

Sequence type	*emm* type	*emm* subtype(s)	No. of isolates (%)	No. of isolates resistant to:	
				Erythromycin	Tetracycline
ST117	81	81.0	19 (15.7)		5
ST39	4	4.0	11 (9.1)		
ST62	87	87.0	9 (7.4)		
ST28	1[b]	1.0	7 (5.8)		1
ST36	12[b]	12.0	7 (5.8)	1[c]	1
ST46	22[a,b]	22.0, 22.2	6 (5.0)	1	
ST334	82[a]	82.0	6 (5.0)		
ST101	89[b]	89.0	5 (4.1)		
ST52	28[b]	28.0	4 (3.3)		
ST63	77[b]	77.0	4 (3.3)	1[c]	3
ST99	5[b]	5.3, 5.37, 5.38, 5.39	4 (3.3)		
ST315	3[b]	3.1	4 (3.3)		
ST253	78	78.0	3 (2.5)		
ST5	83	83.1	2 (1.6)		2
ST55	2[b]	2.0	2 (1.6)		
ST89	94	94.1	2 (1.6)	2[c]	2
ST150	75[b]	75.0	2 (1.6)	2	
ST247	68	68.1, 68.3	2 (1.6)		1
ST328	22[a,b]	22.0	2 (1.6)		
ST3	33[b]	33.0	1 (0.8)		1
ST75	9	9.0	1 (0.8)		
ST100	55	55.0	1 (0.8)		1
ST166	st463	st463.0	1 (0.8)		1
ST167	118	118.0	1 (0.8)		
ST168	120	120.0	1 (0.8)		
ST176	58	58.0	1 (0.8)		
ST284	stNS1033	stNS1033.0	1 (0.8)		1
ST285	95	95.0	1 (0.8)		1
ST297	63	63.3	1 (0.8)		1
ST307	100	100.0	1 (0.8)		
ST308	27G	27G.0	1 (0.8)		
ST311	103[a]	103.0	1 (0.8)		1
ST314	82[a]	82.0	1 (0.8)		
ST327	103[a]	103.0	1 (0.8)		
ST331	73	73.0	1 (0.8)		
ST333	8	8.0	1 (0.8)		1
ST336	85	85.1	1 (0.8)	1	
ST337	66	66.0	1 (0.8)		
ST364	93	93.0	1 (0.8)		1

[a] *emm* types associated with multiple STs.
[b] *emm* types represented in the 26-valent vaccine (22) which includes the specific 5′ sequences of *emm* types 1, 1.2, 2, 3, 5, 6, 11, 12, 13, 14, 18, 19, 22, 24, 28, 29, 33, 43, 59, 75, 76, 77, 89, 92, 101, and 114.
[c] Also resistant to tetracycline.

include serotypes for 86% of North American pharyngeal isolates (37). When only the isolates recovered from throat swabs in this study ($n = 47$) are considered, the 26-valent vaccine contains epitopes for 26 (55.3%) of the throat GAS. Variability in N-terminal M protein sequence, as determined by the presence of multiple *emm* subtypes, was noted within three MLST-defined clones (ST46, ST99, and ST247) in this study (Table 1). It is unknown what, if any, impact this variability might have on vaccine efficacy and whether M-generated antibodies provide protection to all subtypes of a given *emm* type.

Resistance to erythromycin and tetracycline was seen in 6.6 and 19.8% of isolates, respectively, with four isolates resistant to both agents (Table 1). This is similar to levels reported among invasive United Kingdom isolates (20). In this study the appearance of resistant isolates was not associated with particular clones. Indeed, resistant isolates included strains with 19 distinct STs. Furthermore, for a number of erythromycin resistance-associated STs, susceptible clones (ST36, ST46, and

TABLE 2. Allelic profiles of new STs associated with this report

Sequence type	Allelic profile							SLV in MLST database[a]
	gki	*gtr*	*murI*	*mutS*	*recP*	*xpt*	*yqiL*	
ST247	11	9	1	7	2	8	3	No
ST284	2	66	1	52	75	2	12	No
ST285	86	6	8	3	9	3	1	ST14
ST307	1	31	61	40	60	8	42	No
ST311	4	2	2	51	1	59	1	No
ST314	4	65	21	16	17	3	1	ST26
ST315	2	6	8	5	2	3	53	ST15
ST328	9	8	58	1	1	3	4	ST46
ST333	51	3	8	25	33	3	27	No
ST334	84	2	21	16	17	3	1	ST26
ST337	8	7	8	49	9	3	1	ST44
ST364	2	2	9	13	2	14	55	ST10

[a] SLVs (single-locus variants) differ from another ST at only one of the seven MLST loci and are considered to be closely related. The current *S. pyogenes* MLST database (http://spyogenes.mlst.net) includes 678 isolates, representing 273 STs.

ST63) were also identified in this study. These findings suggest that the emergence of erythromycin resistance in London GAS resulted from acquisition of resistance genes by susceptible isolates present in the circulating population rather than by the introduction of a resistant clone into the population and clonal expansion of the resistant clone. Similar findings were noted for tetracycline-resistant isolates.

Erythromycin-resistant MLST-defined clones of ST36, ST46, ST63, and ST150 GAS have previously been reported in Germany (35) and Poland (40). At this point it is unclear whether these resistant clones are spreading throughout Europe or whether this represents multiple acquisitions of resistance genes by the same prevalent STs. That scenario is supported by the finding of ST36 clones carrying either *mefA* or *ermB* resistance genes and ST63 GAS harboring *mefA*, *ermB*, or *ermTR* genes (35, 40).

Surveillance data suggest that a small number of GAS clones account for the majority of disease, although country-to-country and year-to-year fluctuation in the relative proportions of the most common types occur (1, 7, 15, 19, 21, 24, 31, 34, 37, 41). The emergence of strains with higher *emm* types has, in part, reshaped the overall picture of GAS infection in the United Kingdom in recent years. The introduction of a vaccine, particularly if it targets only a subset of GAS isolates (for example, the most common M serotypes), may have an impact on the bacterial population (39). The potential effects of vaccination on the incidence of non-vaccine serotype disease are unknown. Ongoing surveillance is required to monitor epidemiological changes in GAS character that may result from the appearance of more-virulent or -resistant clones. It has been proposed that emergence and global dissemination of highly virulent strains may be responsible for the notable increase in severe M1 disease in 1980s (32), although the factors mediating the increased virulence are unclear. Phage-encoded Sda1 streptodornase is present in post-1980s M1 GAS, but not in older isolates (4), and likewise, phage-encoded SpeA exotoxin is apparently more frequently associated with post-1980s isolates (32, 33). The acquisition of resistance genes by susceptible clones is of concern, as proliferation of resistant clones may result from selection imposed by antibiotic use (2, 36). Studies to examine the long-term consequences of resistance on population structure should ideally include both susceptible and resistant isolates.

In summary, the present study describes the GAS clones circulating in London during the summer of 2003. This work provides useful comparative data for future studies.

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