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M Protein Gene (*emm* Type) Analysis of Group A Beta-Hemolytic Streptococci from Ethiopia Reveals Unique Patterns

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The genetic diversity of group A streptococcal (GAS) isolates obtained in 1990 from Ethiopian children with various streptococcal diseases was studied by using *emm* gene sequence analysis. A total of 217 GAS isolates were included: 155 and 62 isolates from throat and skin, respectively. A total of 78 different *emm*/*st* types were detected among the 217 isolates. Of these, 166 (76.5%) belonged to 52 validated reference *emm* types, 26 (11.9%) belonged to 16 already recognized sequence types (*st* types) and 25 (11.5%) belonged to 10 undocumented new sequence types. Resistance to tetracycline (148 of 217) was not correlated to *emm* type. Isolation rate of the classical rheumatogenic and nephritogenic strains was low from cases of acute rheumatic fever (ARF) and acute glomerulonephritis (AGN), respectively. Instead, the recently discovered *st* types were overrepresented among isolates from patients with ARF (3 of 7) and AGN (9 of 16) ($P < 0.01$) compared to isolates from subjects with tonsillitis and from healthy carriers (10 of 57 and 16 of 90, respectively). In contrast to rheumatogenic strains from the temperate regions, more than half of the isolates from ARF (four of seven) carried the genetic marker for skin preference, *emm* pattern D, although most of them (six of seven) were isolated from throat. Of 57 tonsillitis-associated isolates, 16 (28%) belonged to *emm* pattern D compared to <1% in temperate regions. As in other reports *emm* patterns A to C were strongly associated with throat, whereas *emm* pattern D did not correlate to skin. This first large-scale *emm* typing report from Africa has demonstrated a heterogeneous GAS population and contrasting nature of GAS epidemiology in the region.

Accurate identification and typing of group A hemolytic streptococci (GAS) is an essential part of epidemiological and pathogenetic studies of streptococcal diseases. A serotyping system based on antigenic variation of a surface exposed M protein and developed by Rebecca Lancefield has been in use since 1928. Although additional serotyping systems, the T and OF typing, were developed as valuable and practical substitutes, the M typing system was considered the gold standard. However, a limited supply of antisera and the high nontypeability rate among isolates, in particular those from the tropics, challenged its continued usage. In recent years, several molecular typing systems have been reported as alternatives for M typing (4, 19, 25). Most of the knowledge that has been accumulated concerning GAS epidemiology is based on M typing system; hence, a molecular system that correlates with this system has practical significance. The *emm* typing system (4, 25) which is based on sequence analysis of PCR products of the N-terminal hypervariable region of the M protein gene, concurs with M serotyping almost 1:1 (17). In addition to its simplicity, this typing system has allowed the detection of several previously unknown GAS types from different geographic regions (5, 24, 32, 36).

Adequate knowledge of the dynamics of *emm* types in a region may shed light on the pathogenesis of GAS infections and is crucial for selecting appropriate vaccine candidates. One of the possible candidates for a streptococcal vaccine is the N-terminal region of M protein, which is known to provoke protective immunity. Antibodies to this region are not known to cross-react with human tissue. Nevertheless, the existence of a large number of *emm* types causes an obstacle in developing effective vaccines. To date, a total of 117 validated *emm* types have been documented (18). In addition, new ones are being reported continuously so that the number has now reached more than 225 distinct types (see http://www.cdc.gov/ncidod/biotech/infotech_hp.html). Hence, a vaccine targeting this highly variable region of M protein needs to be multivalent, consisting of several types. Currently, such multivalent vaccines composed of the most common *emm* types in Australia and United States are in development (9, 20, 23).

Streptococcal diseases and their complications are endemic in the tropics (10, 34). In Ethiopia, up to 60% of all hospital admissions of cardiac cases were reported to have a rheumatic origin (14). It has been noted for several years that a difference in M-type distribution exists between the tropics and the temperate regions, since antisera produced against strains from Europe and United States did not recognize large proportions of isolates from the tropics. This implies that a multivalent vaccine targeted against the most common *emm* types in other part of the world may not be effective in Africa. We sought here to survey the genetic diversity of GAS from Ethiopia by using *emm* gene sequence analysis and to understand better the epidemiology of this important pathogen in a country where complications resulting from streptococcal infections are still common. A total of 217 GAS isolates obtained from Ethiopian children with various streptococcal diseases and healthy throat carriers during a 1-year period in 1990 (38) were examined. This was the first large-scale *emm* typing investigation from Africa. The findings demonstrated divergent features of GAS epidemiology in this region.

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TABLE 1. New M protein gene sequence types detected in the present study

Provisional sequence type	EMBL accession no.	No. of isolates (% of total)	Disease association(s) (no. of isolates)	Tissue of isolation (no. of isolates)	T type	Opacity reaction	Tetracycline resistance (no. of resistant isolates/total no.)	Nucleotide sequence similarity (%)[a]
*st*62	AJ606468	9 (4.19)	ARF (1), AGN (1), tonsillitis (1) impetigo (3), carrier (3)	Skin (4), throat (5)	T2/B3264	Negative	6/9	*st*C3852 (80)
*st*463	AJ586330	5 (2.33)	Tonsillitis (3), carriers (2)	Throat (5)	B3264	Positive	5/5	*st*369 (33)
*st*206	AJ586326	1 (0.47)	AGN	Throat	B3264/13	Positive	0/1	*emm*96 (37)
*st*212	AJ586327	3 (1.40)	AGN (2), impetigo (1)	Skin (3)	B3264/27/44	Positive	0/3	*emm*2 (51)
*st*221	AJ586328	1 (0.47)	AGN	Skin	NT	Negative	0/1	*emm*36 (78)
*st*414	AJ586329	1 (0.47)	Tonsillitis	Throat	T2/12	Negative	1/1	*emm*83 (92)
*st*5	AJ586332	1 (0.47)	Impetigo	Skin	T3/13/B	Positive	1/1	*emm*104 (32)
*st*794	AJ586331	2 (0.93)	Impetigo (1), carrier (1)	Skin (1), throat (1)	T2/NT	Negative	1/2	*emm*11 (36)
*st*430	AJ864510	1	Tonsillitis	Throat	T2	Negative	0/1	*emm*102 (33)
*st*689	AJ864511	1	Carrier	Throat	NT	Negative	0/1	*emm*34 (37)

[a] Similarity at the *emm* type-specific 150 bases.

MATERIALS AND METHODS

Bacterial isolates. The GAS isolates included in the present study were obtained in Ethiopia during January-December in 1990 from children with various streptococcal disease manifestations and from healthy school children as described earlier (38). A total of 217 GAS isolates were included: 155 and 62 isolates were obtained from throat and skin, respectively. These isolates were associated with cases of acute rheumatic fever or chronic rheumatic heart disease (ARF; $n = 7$), acute poststreptococcal (i.e., occurring after streptococcal infection) glomerulonephritis (AGN; $n = 16$), tonsillitis ($n = 57$), and impetigo ($n = 47$) and carriers ($n = 90$). Consecutive cases of rheumatic fever, glomerulonephritis, and tonsillitis were from the Ethio-Swedish Children's Hospital located at the Black Lion Hospital, Addis Ababa, whereas impetigo and healthy throat carriers were from three elementary schools located close to the Black Lion Hospital. Forty to forty-eight children below the age of 12 years of age and without any signs of upper respiratory infection were randomly selected every month from January to December 1990 for throat cultures. An individual child was included in the study only once. About 44% of the GAS isolates were T nontypeable with T antigen antisera from Prague (38). M serotyping of 107 of these isolates was performed at Public Health Laboratory Services, London, United Kingdom, and only 49 (45.7%) strains were M typeable (37). GAS isolates with undocumented new or atypical sequences were subjected to biochemical classification using the Strep 20 API kit (bioMérieux) to further verify that they belonged to *Streptococcus pyogenes*. This collection of isolates was tested earlier for antibiotic resistance for commonly used antibiotics using the disk diffusion method (38).

***emm* typing.** *emm* typing was performed according to the protocol described by the Centers for Disease Control and Prevention (CDC; http://www.cdc.gov/ncidod/biotech/strep/protocols.html), with some modifications. Enzymatic digestion of bacterial cells to prepare chromosomal DNA as recommended by CDC was omitted. Instead, DNA templates were prepared by boiling bacterial suspensions in distilled water. Briefly, about half a loopful of bacterial cells were picked from an overnight growth on blood agar plates and suspended in 100 μl of distilled water. The suspension was then boiled for 2 min and used for PCR immediately or stored at −20°C until used. Primers 1 and 2 as described by the CDC (5′-TAT TCG CTT AGA AAA TTA A-3′ and 5′-GCA AGT TCT TCA GCT TGT TT-3′) were used for amplifying the N-terminal region of *emm* gene. PCR reagents were from Ampli*Taq* Gold (Applied Biosystems), and the PCR mixture was according to the manufacturer's instructions. Amplification was performed on a PCR thermal cycler (GeneAmp*, PCR System 9700; Applied Biosystems) using the cycle parameters given on the CDC website. Amplification products were purified with JetQuick spin column technique (Germond) according to the manufacturer's instructions. PCR products were then run on the gel to ensure the quality and to estimate the concentration of the product for sequencing reaction.

Primers emmseq2 (TATTCGCTTAGAAAATTAAAAACAGG) or primer 1 were used for sequencing the PCR products using BioDye terminator cycle sequencing ready reaction kit (version 2.0; BioSystems). Primer 1 was used when sequencing with emmseq2 did not give good results. Sequencing reaction mixtures were prepared according to the instructions provided with the kit, and the cycle parameters were 96°C for 1 min for initial denaturation, followed by 25 cycles of 96°C for 10s, 50°C for 5s, and 60°C for 4 min and a holding temperature of 4°C. Sequencing products were purified by using the ethanol sodium acetate precipitation and subjected to automated sequence analysis on an ABI Prism 310 genetic analyzer. The Sequencher genetic software program version 3.0 was used to edit the sequences. The 5′ end of the sequences were compared to sequences in the database available at the CDC website (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm). Sequences that did not find a match in this database were checked for similarity with the published sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/). Sequences that were unidentifiable through these databases were sent to the CDC for verification and were assigned new sequence types or subtypes.

***emm* pattern.** *emm* pattern for the isolates was predicted from the published information of McGregor et al. (30). The *emm* pattern, which is based on differences in the chromosomal arrangement of the *emm* and *emm*-like gene family, has been implicated as a genetic marker for tissue site tropism of GAS strains (6, 33). Since isolates of a given *emm* type usually display the same *emm* pattern (30), it is possible to make inferences with a high degree of reliability from the published data.

Tests for significance. The level of significance was determined by calculating the *P* value using the chi-square test.

Nucleotide sequence accession number. The *emm* gene sequences for the new types can be found in the EMBL database under the respective accession numbers given in Table 1, as well as at the CDC website. The sequences for the new subtypes were deposited in the CDC streptococcal database (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm).

RESULTS

***emm* type distribution.** Table 2 presents the *emm*/*st* types, tissue of isolation, and disease association of the GAS isolates examined. "*emm*" refers to type-specific sequences of one of the standard reference GAS strains, and "*st*" stands for sequence types discovered later, including new ones in the present study and which await validation by international committee (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm) (18). A total of 78 different *emm*/*st* types were detected among the 217 GAS isolates. Of these 217 isolates, 166 (76.5%) belonged to 52 validated standard reference *emm* types (17, 18), 26 isolates (11.9%) belonged to 16 already recognized sequence types (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm) (*st* types) and 25 (11.5%) belonged to 10 undocumented new *st* types from the

TABLE 2. M protein gene (*emm*) type and *emm* pattern distribution in relation to disease and tissue of isolation[a]

emm type	*emm* pattern[b]	Total no. of isolates	%	Site of isolation		Sequelae		Disease status			Tetracycline[c]		Comment
				Skin	Throat	AGN	ARF	Carrier	Impetigo	Tonsillitis	R	S	
*emm*1	A-C	8	3.69		8			2		6	1	7	
*emm*1.2.b	NA	2	0.92	2		1			1		2		
*emm*100	D	4	1.84	3	1			1	3		3	1	
*emm*102.4	E	1	0.46	1					1		1		New subtype
*emm*103	E	4	1.84	2	2				2	2	4		
*emm*105	D	2	0.92		2			1		1	2		
*emm*106.2	E	2	0.92		2			2			2		New subtype
*emm*11.2	E	1	0.46	1					1		1		New subtype
*emm*112.2	E	5	2.30	2	3			2	2	1	5		New subtype
*emm*114.2	E	5	2.30	1	4		1	1	1	2	5		
*emm*116.1	D	3	1.38		3			2		1	1	2	
*emm*118.1	E	1	0.46		1					1	1		New subtype
*emm*118.2	E	1	0.46		1			1			1		New subtype
*emm*12	A-C	15	6.91		15			9		6	1	14	
*emm*18	A-C	1	0.46	1		1						1	
*emm*18.8	A-C	6	2.76		6		1	4		1	5	1	New subtype
*emm*19.4	A	1	0.46		1			1				1	
*emm*22	E	1	0.46		1			1			1		
*emm*22.4	E	2	0.92		2			2			2		New subtype
*emm*22.5	E	2	0.92	1	1			1	1		1	1	New subtype
*emm*25.1	E	8	3.69	1	7	1		3	1	3	6	2	
*emm*28	E	4	1.84	3	1			1	3		4		
*emm*30.1	A-C	2	0.92		2					2		2	New subtype
*emm*36.1	D	1	0.46		1			1				1	New subtype
*emm*41.2	D	1	0.46		1			1				1	
*emm*43.6	D	2	0.92		2			2			1	1	New subtype
*emm*43.7	D	2	0.92	1	1			1	1		1	1	New subtype
*emm*44/61.0	E	3	1.38	1	2			1	1	1		3	
*emm*49.1	E	3	1.38		3			1		2	3		
*emm*5.31	A-C	1	0.46		1			1				1	New subtype
*emm*55.1	A-C	1	0.46	1		1					1		New subtype
*emm*57.0	A-C	1	0.46		1			1				1	
*emm*58.0	E	1	0.46		1					1		1	
*emm*6.26	A-C	3	1.38		3			3			3		New subtype
*emm*64.4	D	1	0.46	1					1			1	New subtype
*emm*65.0	D	1	0.46		1					1	1		
*emm*65.1	D	2	0.92	1	1				1	1	2		New subtype
*emm*66.0	E	1	0.46		1			1			1		
*emm*67.0	D	2	0.92		2			1		1	2		
*emm*68.0	E	1	0.46		1			1				1	
*emm*71.0	D	6	2.76		6			2		4	5	1	
*emm*73.0	E	2	0.92	1	1			1	1		2		
*emm*74.0	D	10	4.61	4	6	1	2	2	3	2	9	1	
*emm*75.1	E	5	2.30		5			3		2	4	1	
*emm*77.0	E	5	2.30	2	3			3	2		5		
*emm*78.0	E	1	0.46		1			1			1		
*emm*8.1	E	4	1.84		4			4			3	1	New subtype
*emm*80.1	D	3	1.38	3					3		1	2	
*emm*81.1	D	1	0.46		1			1			1		
*emm*81.2	D	4	1.84		4			2		2	3	1	New subtype
*emm*84.1	E	1	0.46		1			1				1	New subtype
*emm*85.0	D	1	0.46		1			1			1		
*emm*85.1	D	1	0.46		1			1			1		New subtype
*emm*89.8	E	2	0.92	1	1	1				1	2		New subtype
*emm*9.0	E	1	0.46	1					1		1		
*emm*90.3	E	2	0.92	1	1			1	1		2		New subtype
*emm*92.0	E	2	0.92		2			1		1	2		
*emm*95.0	D	1	0.46	1		1					1		
*emm*97.1	D	3	1.38		3			2		1	1	2	
*emm*99.2	D	3	1.38	2	1				2	1	2	1	New subtype
*st*11014.0	E	1	0.46	1					1		1		
*st*1207.0	E	3	1.38	1	2			2	1		2	1	
*st*1731.1	E	4	1.84	2	2				2	2	4		New subtype
*st*1759.0	NA	2	0.92		2			1		1	2		
*st*206.0	NA	1	0.46		1	1					1		New type

Continued on following page

TABLE 2—*Continued*

emm type	*emm* pattern[b]	Total no. of isolates	%	Site of isolation		Sequelae		Disease status			Tetracycline[c]		Comment
				Skin	Throat	AGN	ARF	Carrier	Impetigo	Tonsillitis	R	S	
*st*211.1	REA	1	0.46	1					1			1	New subtype
*st*212.0	NA	3	1.38	3		2			1		3		New type
*st*221.0	NA	1	0.46	1		1						1	New type
*st*2904.1	E	1	0.46		1			1			1		New subtype
*st*2917.0	D	1	0.46	1					1		1		
*st*2940.1	D	1	0.46	1			1					1	New subtype
*st*3757.0	D	2	0.92	1	1	1		1			2		
*st*414.0	NA	1	0.46		1					1	1		New type
*st*430	NA	1	0.46		1					1		1	New type
*st*463.0	NA	5	2.30		5			3		2	5		New type
*st*5.0	NA	1	0.46	1					1		1		New type
*st*62.0	NA	9	4.15	4	5	1	1	3	3	1	6	3	New type
*st*6735.0	E	3	1.38	1	2	1		1		1	3		
*st*689	NA	1	0.46		1			1				1	New type
*st*794.0	NA	2	0.92	1	1			1	1		1	1	New type
*st*809.0	D	1	0.46		1					1	1		
*st*854.1	A-C +D	1	0.46	1					1		1		New subtype
*st*D432.0	D	2	0.92		2		1	1				2	
*st*g1750.0	NA	1	0.46	1					1			1	
*st*g653.1	NA	1	0.46	1		1					1		New subtype
*st*L1376.1	NA	1	0.46	1		1						1	New subtype
Total		217	100.00	62	155	16	7	90	47	57	148	69	

[a] Numbers in table body refer to the number of isolates except as noted in the column headings.
[b] NA, *emm* pattern is not available in the literature; REA, rearranged *emm* pattern, a rare occurrence not belonging to the regular categories.
[c] R, resistant; S, susceptible.

present studies. Table 1 shows the provisional designations of the 10 new *st* types discovered in the present study, along with their properties. The type-specific 150 bases sequences of these new types were compared against the sequences in the CDC website (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm) for similarity. Sixty-three of the isolates (63 of 217 [29.0%]) had new subtype sequences belonging to 32 *emm*/*st* types (Table 2). Three of the isolates had atypical GAS *st* sequences: two of them had sequences reported earlier in group G streptococci, *st*g1750 and *st*g653, and the third one, *st*L1376, had a sequence reported earlier among group L streptococci.

In contrast to most reports wherein a large proportion of the isolates belonged to a few *emm* types, dominance by certain types was not obvious in the present study. Twelve different *emm* types (listed in a descending order)—*emm*12, *emm*74.0, *st*62, *emm*1, *emm*25.1 and *emm*18, *emm*71, *emm*112, *emm*114, *emm*75, *emm*77, and *st*463—made up 40.1% of the isolates (Table 2). The most common type, *emm*12, comprised only 6.9% of the total isolates (15 isolates). Three of the types—*emm*12, *emm*1, and *emm*18—were among the most prevalent types in the temperate regions. Interestingly, types *emm*3 and *emm*4, which have remained among the most frequent types for decades in the Western world (8, 12, 39), were absent in this collection. On the other hand, types *emm*74 and *emm*25, which have been rare in Europe and the United States, were among the commonly isolated types in the Ethiopian collection. It is also important to note that two of the previously undocumented sequence types—*st*62 and *st*463—were among the most common types in this collection.

***emm* type versus acute rheumatic fever.** Six different *emm*/*st* types were detected among the seven ARF cases in the present study. These GAS types were *st*2940, *emm*18, *st*D432, *st*62, *emm*74 (two isolates), and *emm*114. Interestingly, three of them (42.9%) belonged to *st* types. All except type *st*2940 were isolated from throat samples. It is not possible to discriminate whether these isolates were in the carrier state or whether they were actually responsible for triggering the rheumatic fever. However, it is possible that *st*2940 could have triggered rheumatic fever because it was isolated from a skin lesion in a patient who had healing lesions all over her body when admitted to the hospital. In temperate regions rheumatic fever is usually associated with throat infection, but available data indicate that GAS skin infection could be more important in causing rheumatic fever in the tropics (5, 10, 29). Based on decades of epidemiological observations, GAS serotypes M1, 3, 5, 6, 14, 18, 19, and 24 were known to be associated with rheumatic fever in the temperate regions (34). Of these rheumatogenic types, only type *emm*18 was represented among *emm* types isolated from the rheumatic fever cases in the present study. It is worth mentioning that types *emm*1 and *emm*18 were among the 12 most common *emm* types, and three more of the classical rheumatogenic types—*emm*6, *emm*5, and *emm*19—were also represented among the present GAS collection. Therefore, despite the circulation of the so-called rheumatogenic strains among the general GAS population in Addis Ababa, *emm* types not implicated with rheumatic fever in temperate regions, were isolated from rheumatic fever cases in the present study.

***emm* type versus AGN.** Among the 16 GAS isolates from AGN patients, 15 different *emm*/*st* types were detected (Table 2). These were *emm*1.2, *emm*18, *emm*25, *emm*55, *emm*74, *emm*89, *emm*95, *st*206, *st*212, *st*221, *st*3757, *st*62, *st*6737, *st*g653,

TABLE 3. Predicted *emm* pattern distribution among isolates[a]

emm pattern[a]	No. of isolates (%)	No. (%) of isolates from:						
		Skin	Throat	AGN	ARF	Tonsillitis	Carrier	Impetigo
A-C	39 (17.9)	2 (3.2)	37 (23.9)	2 (12.5)	1 (14.3)	15 (26.3)	21 (23.3)	0 (00.0)
D	61 (28.1)	19 (30.6)	42 (27.1)	3 (18.8)	4 (57.1)	16 (28.0)	23 (25.5)	15 (31.9)
E	83 (38.2)	24 (38.7)	59 (38.1)	3 (18.8)	1 (14.3)	20 (35.1)	37 (41.1)	22 (46.8)
Others	2 (0.9)	2 (3.2)	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	2 (4.3)
NA	32 (14.7)	15 (24.2)	17 (11.0)	8 (50.0)	1 (14.3)	6 (10.5)	9 (10.0)	8 (17.0)
Total	217 (100)	62 (100)	155 (100)	16 (100)	7 (100)	57 (100)	90 (100)	47 (100)

[a] *emm* pattern according to McGregor et al. (30). Others, *emm* patterns of two skin isolates, *st*845 and *st*211, do not fit any of the three categories; NA, *emm* types for which *emm* pattern information is not available.

and *st*L1376. Most of them, 87.5% (14 of 16) were obtained from skin lesions. Nine of the sixteen isolates (56.3%) belonged to *st* sequence types. Epidemiological surveys from Europe, the United States, and the Caribbean islands have associated M serotypes 2, 4, 12, 15, 25, 49, 55, 56, 59, 60, and 61 with AGN (7, 13). Of these, only types *emm*25 and *emm*55 were represented among the AGN isolates in the present study. It is interesting that *emm*12, which is the most common isolate in our collection, was not associated with AGN. Moreover, type M49, which is commonly referred to as nephritogenic, was not isolated from the AGN cases in the present study. Two of the three GAS isolates with atypical GAS sequence type, *st*g653 and *st*L1376, were associated with AGN. The findings clearly indicate that types other than what have been reported elsewhere as nephritogenic are more important in causing AGN in this region of the world.

***emm* type versus tonsillitis and healthy carrier state.** Thirty-three distinct *emm*/*st* types were detected among the 57 GAS strains isolated from tonsillitis cases (Table 2). Thirteen of these were represented by 2 or more isolates, and they comprised 64.9% (37 of 57) of the tonsillitis isolates. The most frequent *emm* types isolated from tonsillitis cases were *emm*12, *emm*1, *emm*71, and *emm*25. Three of these—*emm*1, *emm*12, and *emm*25—have been among the most frequently isolated GAS strains from tonsillitis cases in Europe and the United States (1, 8, 15). A total of 49 *emm*/*st* types were detected among the 90 isolates from throat cultures of healthy carriers. Twelve of these were represented by three or more isolates and comprised 50% (45 of 90) of the total isolates. Two new sequence types, *st*62 and *st*463, were among these 12 isolates. The four most common types among isolates from the throat of healthy carriers were *emm*12, *emm*18, *emm*8, and *emm*22. Of the 57 GAS isolates from tonsillitis cases and the 90 isolates from the throats of healthy carriers, 10 (17.5%) and 16 (17.7%) isolates, respectively, belonged to *st* types. These levels are significantly lower ($P < 0.01$) compared to the proportion of *st* types among AGN (9 of 15 [56.3%]) and ARF (3 of 7 [42.9%]) isolates.

***emm* types versus skin and throat.** The concept of distinct throat and skin *emm* types has been widely accepted. M protein serotypes, such as M types 1, 3, 4, 5, 6, 12, 14, 18, 19, and 24 of *S. pyogenes*, were found to be associated with throat infection (34), while M serotypes such as 2, 49, 57, 59, 60, and 61 are associated with impetigo (13). The number of isolates of each *emm* type in the present study is not sufficient enough to make statistical correlations on tissue specificity. However, it is worthy to note that a number of *emm* types with multiple isolates (≥3)—*emm*1, *emm*6, *emm*8, *emm*12, *emm*18, *emm*25, *emm*26, *emm*49, *emm*71, *emm*75, *emm*81, *emm*116, and *st*463—were isolated from the throat only. Two *emm* types with three isolates each, *emm*80 and st212, were associated with skin origin only.

***emm* pattern versus disease.** The GAS population was also analyzed for *emm* pattern distribution, a genetic marker for tissue preference (6, 33). Patterns A to C have been associated with throat, pattern D is associated with skin, and pattern E is known to have no tissue preference (6). Furthermore, pattern D strains have caused higher skin pathology compared to strains with *emm* patterns A to C in the human mouse skin model (33). The *emm* pattern of 183 of 217 (84.3%) isolates in the present study was predicted (Table 3) based on the published information from McGregor et al. (30). These 183 isolates belonged to 63 different *emm*/*st* types. The *emm* pattern for 32 (14.7%) of the isolates, belonging to 15 *emm*/*st* types, was not available, because these types were not included in the report of McGregor et al. In the overall GAS population in the present study, *emm* pattern E was isolated more frequently (38.2%), followed by pattern D (28.1%) and the least common were patterns A to C (17.9%). Patterns A to C were strongly associated with throat ($P < 0.01$), whereas the frequency of *emm* pattern D strains did not differ significantly among our skin and throat isolates (Table 3). The strong association of *emm* pattern A-C strains with the throat is in agreement with a previous report from temperate regions (6). On the other hand, our observation of the lack of association of *emm* pattern D with skin is in contrast to what has been previously reported (5, 6). The distribution of the three patterns among isolates from symptomatic throat infection and asymptomatic throat carrier state was not statistically significant. Of 57 isolates from tonsillitis cases, 16 carried the genetic marker for skin preference, the *emm* pattern D. Among the ARF isolates the *emm* pattern D was overrepresented, with 57% (4 of 7) of the isolates. This is in contrast to so-called rheumatogenic strains from the temperate regions, which belonged to *emm* patterns A to C (6). Evaluation of AGN isolates with respect to *emm* pattern could not be made because *emm* patterns of 50% of the isolates from the AGN had not been determined.

***emm* type versus tetracycline resistance.** The present collection of GAS was earlier tested for antibiotic resistance (38). The strains were sensitive to the most commonly used antibiotics other than tetracycline, with 68.2% (148 of 217) being resistant to tetracycline. It was of interest to examine the re-

lationship of tetracycline resistance to *emm* type. Tetracycline resistance was detected in 59 of the 78 *emm*/*st* types, indicating that genes encoding resistance to tetracycline could be acquired by a wide variety of GAS types. Reports from the United States and Europe have indicated an association between erythromycin resistance and certain *emm* types (1, 2, 22). The number of isolates of each *emm*/*st* in our study does not permit extensive statistical analysis. However, it is interesting that only 1 of 8 *emm*1 and 1 of 15 *emm*12 types were resistant. On the other hand, a higher proportion of *emm*74 (9 of 10), *st*463 (6 of 9), and *emm*25 (6 of 8) types were resistant to tetracycline. Similar to our findings, Ho et al. (21) from the United States found *emm*1 isolates to be significantly less likely to have resistance to tetracycline. In another study from the United States, *emm*1 isolates were associated with resistance to tetracycline (2). The acquisition of tetracycline resistance in GAS *emm*1 strains is therefore completely independent of the *emm* gene. In Europe and the United States a strong association was reported between type *emm*75 and erythromycin resistance (1, 2). Interestingly, resistance to tetracycline among the *emm*75 isolates in the present study was high (four of five isolates).

DISCUSSION

The findings described here demonstrated that the GAS population among children in Addis Ababa is highly heterogeneous, with 78 distinct *emm*/*st* types detected among 217 isolates collected over a 1-year period. Earlier attempts to serotype this collection of GAS isolates using M typing sera from Public Health Laboratory Services, London, United Kingdom, revealed that only 45.7% of them were typeable (37). With the *emm* sequence typing method it was possible to type 100% of the 217 GAS isolates obtained from Ethiopian children. The low serotypeability rate among this collection of GAS is not surprising because 38 (48.7%) of 78 types detected belonged to types for which M typing sera were not available. These types belonged to the lately validated types *emm*94 to *emm*124 (18) and to several recently recognized sequences (*st*), including the new types discovered here.

The number of distinct *emm*/*st* types, 78, detected among the present collection of GAS strains is much higher than has been found in several *emm* typing reports from other countries, where larger numbers of isolates collected over a longer period of time were screened (5, 12, 16, 31, 35, 39). In Japan only 29 *emm*/*st* types were detected among 906 clinical isolates collected from 1990 to 1999 (35). In Mexico, only 31 *emm* types were detected among 423 isolates collected from symptomatic pharyngeal specimens from 1991 to 2000 (16). In a population-based study among the Australian aboriginal population, only 31 distinct types were reported among 141 isolates collected from the skin and throats of asymptomatic cases over a 25-month period (5). Among 400 isolates collected over 2 years period from 24 hospitals in Israel, 59 types were detected (31). The more than 16,000 isolates collected from various clinical specimens over a period of 11 years in the United Kingdom (12) were not as diversified as the GAS collection from Ethiopia. In a nationwide survey in Canada, 54 M serotypes were reported among 4,760 GAS isolates submitted to the national center for streptococci from 1993 to 1999 (39). In Spain only 30 different *emm* types were detected among 614 pharyngeal isolates collected from eight different hospitals over a 4-year period (1). Although a clear reason for the relatively higher heterogeneity observed among the Ethiopian isolates is not apparent, it may partially be explained by the diversity in the sources of the specimens. In contrast to many reports on *emm* typing surveys in which the isolates were obtained from either clinical samples or asymptomatic cases, the isolates in the present study were obtained from both clinical and asymptomatic cases, as well as from throat and skin sources. It may also be possible that the GAS population in this region is under some strongly diversifying natural selection pressure.

The GAS collection included in the present study was obtained from a single city: Addis Ababa. It should be noted, however, that Addis Ababa is the capital city of Ethiopia, a cosmopolitan city where a high influx of people from the countryside takes place. In addition, during the sampling period there was an increased migration of people toward Addis Ababa mainly from the northern part of the country due to the civil war that was taking place. Thus, isolates in the present study may represent GAS populations from a larger geographic area in Ethiopia.

Since the introduction of the sequence typing method of GAS based on the heterogeneity of the 5′ end of the *emm* gene (4, 25), several previously undocumented new types have been detected. The number of distinct *emm*/*st* types has more than doubled in the last few years, and currently the CDC database contains over 225 distinct *emm*/*st* types. In the present study 25 isolates (11.5%) of the isolates belonged to 10 previously unrecognized sequence types. The detection rate of undocumented types varies from country to country. In general, more new types were reported from Malaysia (24), Thailand (32), Brazil (36), the United States (3), and Australia (5) compared to those reported from European countries. It is interesting that no new *emm* type was detected among 133 isolates that were nontypeable by serotyping in Israel (31). The number of newly discovered types among the Ethiopian isolates is comparable to findings reported from Malaysia, Brazil, and Thailand (24, 32, 36).

Poststreptococcal sequelae, ARF and AGN, remain frequent in the tropics, but epidemiological data on GAS dynamics here are still inadequate compared to the temperate regions. From the scanty information available it has been speculated that there is a contrasting feature of GAS epidemiology between the temperate and tropical climate (5, 10, 28, 29). Based on a 1-year study, our findings depict a distinctive pattern of GAS types associated with poststreptococcal complications. The predominance of *st* types among ARF (42.9%) and AGN (56.3%) isolates in the present study despite the presence of the classical rheumatogenic and nephritogenic types in the general GAS population clearly implies the different nature of GAS types involved in poststreptococcal complications in the region. Moreover, in contrast to what was observed in the temperate regions, most (57.3%) isolates from ARF cases in the present study belonged to *emm* pattern D, a genetic marker for skin preference (6, 33). The classical rheumatogenic strains from the temperate regions belonged to *emm* pattern A-C and were throat isolates. Most (six of seven [85.7%]) ARF isolates in the present study were obtained from throat cultures. However, since more than half of them carry

the genetic marker for skin preference, *emm* pattern D, it is likely that the primary site of colonization could be the skin, with the throat being a secondary site of infection. In temperate regions it has been widely accepted that rheumatic fever follows throat infection by *emm* pattern A-C GAS strains. On the other hand, in the tropics skin infection rather than throat infection was linked to rheumatic fever based on the fact that GAS skin infection rates were far more common and throat carrier rates were lower in the tropics (5, 10, 29). The throat healthy carrier rate among children in Ethiopia (38) was, however, comparable (i.e., 17%) to what has been reported for the temperate regions. Although our results could not substantiate the link between skin infection and rheumatic fever, they strongly suggest the importance of GAS strains with the genetic marker for skin preference. Whether rheumatic fever follows infection of the skin or the throat by the pattern D strains remains to be explored.

A considerable proportion (16 of 57 [28%]) of tonsillitis-associated GAS isolates in the present study carry the skin preference genetic marker, *emm* pattern D. This figure is much higher compared to reports from Italy (15), Spain (1), and Germany (8), where fewer than 1% of tonsillitis-associated strains had the *emm* pattern D. It has been noted for several years that the symptoms of tonsillitis in tropical and subtropical climates are relatively milder or subclinical compared to tonsillitis in temperate regions (10, 28). The difference in the clinical manifestation of streptococcal tonsillitis between the tropics and temperate regions may be due to the higher prevalence of *emm* pattern D strains causing tonsillitis in the tropics, as demonstrated in the present study.

The highly diversified nature of the GAS population in Ethiopia challenges the application of a multivalent vaccine composed of the N-terminal region of the most common *emm* types. A multivalent vaccine composed of 26 M protein N-terminal region was anticipated to protect ca. 90% invasive GAS infections in the United States (23). Of the 26 *emm* types included in this multivalent vaccine, only 15 (57.7%) were detected among the GAS collection in the present study (Table 4). In theory, this vaccine would prevent only 31% (68 of 217) of infections in Addis Ababa. In addition, 6 (50%) of the 12 most frequently isolated *emm* types—*emm*74, st62, *emm*25, *emm*71, *emm*112, and *st*463—in the present study are not included in the vaccine. Thus, the 26 *emm* multivalent vaccine, which is basically designed for the United States population, will be ineffective in preventing GAS infection in the Ethiopian population. Designing an effective multivalent vaccine based on the N-terminal region of M protein for regions such as Ethiopia, where the GAS population is highly heterogeneous, the dynamics of streptococcal infections is poorly understood, and skin infections may be more important than throat infection would be challenging. Based on the observations made in the present study, a vaccine carrying at least 43 different *emm* type-specific epitopes would be required to cover ca. 80% of GAS infections in Addis Ababa. There is also the concern that upon the introduction of such a vaccine the once-rare *emm* types may eventually replace the dominant *emm* types and may become important in causing infections. Thus, there will be a need for active surveillance, and a change in the composition of a vaccine may be required on a regular basis. In view of these facts, alternative broad-spectrum GAS vaccines using part of conserved region of M protein or other antigens (11, 26, 27) need to be promoted. GAS vaccine research thus far has mainly aimed at attaining protective immunity at the mucosal level, so as to prevent GAS colonization of the throat. With the possible link between skin infection by GAS and ARF in the tropics, consideration should be given to antigens that have significance in skin colonization and that would induce protective immunity at the portal of entry.

TABLE 4. *emm* types included in the multivalent vaccine composed of 26 *emm* types (see reference 23) versus the *emm* types represented in the present study

emm type	Represented	No. of isolates
1	Yes	8
1.2	Yes	2
2	No	0
3	No	0
5	Yes	1
6	Yes	3
11	Yes	1
12	Yes	15
13	No	0
14	No	0
18	Yes	7
19	Yes	1
22	Yes	5
24	No	0
28	Yes	4
29	No	0
33	No	0
43	Yes	4
59	No	0
75	Yes	5
76	No	0
77	Yes	5
89	Yes	2
92	No	0
101	No	0
114	Yes	5
Total		68

We sought here to survey the genetic diversity of GAS isolates from Ethiopia by using *emm* gene sequence analysis. Our GAS collection contained isolates from the skin and throats of both symptomatic and asymptomatic individuals, as well as from ARF and AGN cases, thus allowing us to examine the correlation of *emm* type to tissue of isolation or type of disease. Our findings demonstrated the highly heterogeneous nature of the GAS population in Ethiopia during the study period. Strains with the genetic marker for skin preference were associated with ARF, which is in contrast to strains from the temperate region, where rheumatogenic strains carry the genetic marker for throat preference. Overrepresentation of recently recognized *st* types among isolates from rheumatic fever and poststreptococcal glomerulonephritis compared to isolates from tonsillitis and healthy throat carriers indicate the different nature of GAS strains associated with poststreptococcal complications. This report is the first large-scale study of its kind from Africa, and it underscores the uniqueness of GAS epidemiology in the region. To better understand the dynamics of GAS epidemiology in the tropics, further surveillance in the region is needed, which may also help elucidate the pathogen-

esis of this important pathogen, as well as help in designing an appropriate vaccine.

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REFERENCES

1. **Alberti, S., C. Garcia-Rey, M. A. Dominguez, L. Aguilar, E. Cercenado, M. Gobernado, and A. Garcia-Perea.** 2003. Survey of *emm* gene sequences from pharyngeal *Streptococcus pyogenes* isolates collected in Spain and their relationship with erythromycin susceptibility. J. Clin. Microbiol. **41:**2385–2390.
2. **Barrozo, C. P., K. L. Russell, T. C. Smith, A. W. Hawksworth, M. A. K. Ryan, and G. C. Gray.** 2003. National Department of Defense surveillance data for antibiotic resistance and *emm* gene types of clinical group A streptococcal isolates from eight basic training military sites. J. Clin. Microbiol. **41:**4808–4811.
3. **Beall, B., R. Facklam, T. Hoenes, and B. Schwartz.** 1997. Survey of *emm* gene sequences and T-antigen types from systemic *Streptococcus pyogenes* infection isolates collected in San Francisco, California; Atlanta, Georgia; and Connecticut in 1994 and 1995. J. Clin. Microbiol. **35:**1231–1235.
4. **Beall, B., R. Facklam, and T. Thompson.** 1996. Sequencing *emm*-specific PCR products for routine and accurate typing of group A streptococci. J. Clin. Microbiol. **34:**953–958.
5. **Bessen, D. E., J. R. Carapetis, B. Beall, R. Katz, M. Hibble, B. J. Currie, T. Collingridge, M. W. Izzo, D. A. Scaramuzzino, and K. S. Sriprakash.** 2000. Contrasting molecular epidemiology of group A streptococci causing tropical and nontropical infections of the skin and throat. J. Infect. Dis. **182:**1109–1116.
6. **Bessen, D. E., C. M. Sotir, T. L. Readdy, and S. K. Hollingshead.** 1996. Genetic correlates of throat and skin isolates of group A streptococci. J. Infect. Dis. **173:**896–900.
7. **Bisno, A. L.** 1991. Group A streptococcal infections and acute rheumatic fever. N. Engl. J. Med. **325:**783–793.
8. **Brandt, C. M., B. Spellerberg, M. Honscha, N. D. Truong, B. Hoevener, and R. Lutticken.** 2001. Typing of *Streptococcus pyogenes* strains isolated from throat infections in the region of Aachen, Germany. Infection **29:**163–165.
9. **Brandt, E. R., K. S. Sriprakash, R. I. Hobb, W. A. Hayman, W. Zeng, M. R. Batzloff, D. C. Jackson, and M. F. Good.** 2000. New multi-determinant strategy for a group A streptococcal vaccine designed for the Australian Aboriginal population. Nat. Med. **6:**455–459.
10. **Carapetis, J. R., B. J. Currie, and E. L. Kaplan.** 1999. Epidemiology and prevention of group A streptococcal infections: acute respiratory tract infections, skin infections, and their sequelae at the close of the twentieth century. Clin. Infect. Dis. **28:**205–210.
11. **Cleary, P. P., Y. V. Matsuka, T. Huynh, H. Lam, and S. B. Olmsted.** 2004. Immunization with C5a peptidase from either group A or B streptococci enhances clearance of group A streptococci from intranasally infected mice. Vaccine **22:**4332–4341.
12. **Colman, G., A. Tanna, A. Efstratiou, and E. T. Gaworzewska.** 1993. The serotypes of *Streptococcus pyogenes* present in Britain during 1980–1990 and their association with disease. J. Med. Microbiol. **39:**165–178.
13. **Cunningham, M. W.** 2000. Pathogenesis of group A streptococcal infections. Clin. Microbiol. Rev. **13:**470–511.
14. **Daniel, E., and B. Abegaz.** 1993. Profile of cardiac disease in an out-patient cardiac clinic. Trop. Geogr. Med. **45:**121–123.
15. **Dicuonzo, G., G. Gherardi, G. Lorino, S. Angeletti, M. De Cesaris, E. Fiscarelli, D. E. Bessen, and B. Beall.** 2001. Group A streptococcal genotypes from pediatric throat isolates in Rome, Italy. J. Clin. Microbiol. **39:**1687–1690.
16. **Espinosa, L. E., Z. Li, D. G. Barreto, E. C. Jaimes, R. S. Rodriguez, V. Sakota, R. R. Facklam, and B. Beall.** 2003. M protein gene type distribution among group A streptococcal clinical isolates recovered in Mexico City, Mexico, from 1991 to 2000, and Durango, Mexico, from 1998 to 1999: overlap with type distribution within the United States. J. Clin. Microbiol. **41:**373–378.
17. **Facklam, R., B. Beall, A. Efstratiou, V. Fischetti, D. Johnson, E. Kaplan, P. Kriz, M. Lovgren, D. Martin, B. Schwartz, A. Totolian, D. Bessen, S. Hollingshead, F. Rubin, J. Scott, and G. Tyrrell.** 1999. *emm* typing and validation of provisional M types for group A streptococci. Emerg. Infect. Dis. **5:**247–253.
18. **Facklam, R. F., D. R. Martin, M. Lovgren, D. R. Johnson, A. Efstratiou, T. A. Thompson, S. Gowan, P. Kriz, G. J. Tyrrell, E. Kaplan, and B. Beall.** 2002. Extension of the Lancefield classification for group A streptococci by addition of 22 new M protein gene sequence types from clinical isolates: *emm103* to *emm124.* Clin. Infect. Dis. **34:**28–38.
19. **Gardiner, D. L., A. M. Goodfellow, D. R. Martin, and K. S. Sriprakash.** 1998. Group A streptococcal Vir types are M-protein gene (*emm*) sequence type specific. J. Clin. Microbiol. **36:**902–907.
20. **Hall, M. A., S. D. Stroop, M. C. Hu, M. A. Walls, M. A. Reddish, D. S. Burt, G. H. Lowell, and J. B. Dale.** 2004. Intranasal immunization with multivalent group A streptococcal vaccines protects mice against intranasal challenge infections. Infect. Immun. **72:**2507–2512.
21. **Ho, P. L., D. R. Johnson, A. W. Y. Yue, D. N. C. Tsang, T. L. Que, B. Beall, and E. L. Kaplan.** 2003. Epidemiologic analysis of invasive and noninvasive group A streptococcal isolates in Hong Kong. J. Clin. Microbiol. **41:**937–942.
22. **Hoe, N. P., K. E. Fullerton, M. Liu, J. E. Peters, G. D. Gackstetter, G. J. Adams, and J. M. Musser.** 2003. Molecular genetic analysis of 675 group A streptococcus isolates collected in a carrier study at Lackland Air Force Base, San Antonio, Texas. J. Infect. Dis. **188:**818–827.
23. **Hu, M. C., M. A. Walls, S. D. Stroop, M. A. Reddish, B. Beall, and J. B. Dale.** 2002. Immunogenicity of a 26-valent group a streptococcal vaccine. Infect. Immun. **70:**2171–2177.
24. **Jamal, F., S. Pit, R. Facklam, and B. Beall.** 1999. New *emm* (M protein gene) sequences of group A streptococci isolated from Malaysian patients. Emerg. Infect. Dis. **5:**182–183.
25. **Kaufhold, A., A. Podbielski, D. R. Johnson, E. L. Kaplan, and R. Lutticken.** 1992. M protein gene typing of *Streptococcus pyogenes* by nonradioactively labeled oligonucleotide probes. J. Clin. Microbiol. **30:**2391–2397.
26. **Kotloff, K. L., and J. B. Dale.** 2004. Progress in group A streptococcal vaccine development. Pediatr. Infect. Dis. J. **23:**765–766.
27. **Lei, B., M. Liu, G. L. Chesney, and J. M. Musser.** 2004. Identification of new candidate vaccine antigens made by *Streptococcus pyogenes*: purification and characterization of 16 putative extracellular lipoproteins. J. Infect. Dis. **189:**79–89.
28. **Markowitz, M.** 1991. Streptococcal disease in developing countries. Pediatr. Infect. Dis. J. **10:**S11–S14.
29. **Martin, D. R., L. M. Voss, S. J. Walker, and D. Lennon.** 1994. Acute rheumatic fever in Auckland, New Zealand: spectrum of associated group A streptococci different from expected. Pediatr. Infect. Dis. J. **13:**264–269.
30. **McGregor, K. F., B. G. Spratt, A. Kalia, A. Bennett, N. Bilek, B. Beall, and D. E. Bessen.** 2004. Multilocus sequence typing of *Streptococcus pyogenes* representing most known *emm* types and distinctions among subpopulation genetic structures. J. Bacteriol. **186:**4285–4294.
31. **Moses, A. E., C. Hidalgo-Grass, M. Dan-Goor, J. Jaffe, I. Shetzigovsky, M. Ravins, Z. Korenman, R. Cohen-Poradosu, and R. Nir-Paz.** 2003. *emm* Typing of M nontypeable invasive group A streptococcal isolates in Israel. J. Clin. Microbiol. **41:**4655–4659.
32. **Pruksakorn, S., N. Sittisombut, C. Phornphutkul, C. Pruksachatkunakorn, M. F. Good, and E. Brandt.** 2000. Epidemiological analysis of non-M-typeable group A *Streptococcus* isolates from a Thai population in northern Thailand. J. Clin. Microbiol. **38:**1250–1254.
33. **Scaramuzzino, D. A., J. M. McNiff, and D. E. Bessen.** 2000. Humanized in vivo model for streptococcal impetigo. Infect. Immun. **68:**2880–2887.
34. **Stollerman, G. H.** 2001. Rheumatic fever in the 21st century. Clin. Infect. Dis. **33:**806–814.
35. **Tanaka, D., Y. Gyobu, H. Kodama, J. Isobe, S. Hosorogi, Y. Hiramoto, T. Karasawa, and S. Nakamura.** 2002. *emm* typing of group A streptococcus clinical isolates: identification of dominant types for throat and skin isolates. Microbiol. Immunol. **46:**419–423.
36. **Teixeira, L. M., R. R. Barros, A. C. Castro, J. M. Peralta, M. Da Gloria S. Carvalho, D. F. Talkington, A. M. Vivoni, R. R. Facklam, and B. Beall.** 2001. Genetic and phenotypic features of *Streptococcus pyogenes* strains isolated in Brazil that harbor new *emm* sequences. J. Clin. Microbiol. **39:**3290–3295.
37. **Tewodros, W.** 1994. Beta-hemolytic streptococcal infections and their complications among Ethiopian children: epidemiology and virulence factors. Karolinska Institute, Stockholm, Sweden.
38. **Tewodros, W., L. Muhe, E. Daniel, C. Schalén, and G. Kronvall.** 1992. A one-year study of streptococcal infections and their complications among Ethiopian children. Epidemiol. Infect. **109:**211–225.
39. **Tyrrell, G. J., M. Lovgren, B. Forwick, N. P. Hoe, J. M. Musser, and J. A. Talbot.** 2002. M Types of group A streptococcal isolates submitted to the National Centre for *Streptococcus* (Canada) from 1993 to 1999. J. Clin. Microbiol. **40:**4466–4471.