

Emergence of the Same Successful Clade among Distinct Populations of *emm89 Streptococcus pyogenes* in Multiple Geographic Regions

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The emergence of clades within *emm89 Streptococcus pyogenes* isolates that rapidly became the dominant lineages expressing this *emm* type was recently reported in the United Kingdom (1) and in a study that included isolates from the United States, Finland, and Iceland (United States/FI/IC) (2). In the United Kingdom, the emerging clade was associated with the absence of the *hasABC* locus, responsible for the synthesis of the hyaluronic acid capsule (1). The study from the United States/FI/IC (2) highlighted the strict association of the emerging clade with an *nga* promoter variant, also found in contemporary *emm1* isolates, which results in increased expression of the *nga* locus. The study from the United Kingdom also examined this region and found that the *nga-ifs-slo* locus and surrounding sequences of the emerging clade shared 99% DNA identity with that of contemporary *emm1* and *emm12* strains, but the authors do not offer any information on the *nga* promoter (1). The acquisition of this region by *emm1* isolates is currently considered the major molecular event triggering the success and enhanced virulence of this clone (3).

Given that the two studies characterized *emm89* strains recovered in overlapping time periods, it was possible that the two were documenting the dissemination of the same clade in different geographic areas, although the information presented did not allow this conclusion since the papers analyze different aspects of the strains. We set out to test this hypothesis by reanalyzing the publicly available data, and we also characterized *emm89* isolates recovered in Portugal to investigate if this clade could be also emerging in southern Europe. While analyzing this data, an additional paper was published focusing on the interplay between expression of the *nga-ifs-slo* locus and that of capsule in the virulence of the *emm89* strains of the United States/FI/IC study (4).

We analyzed the sequencing reads deposited in public databases from the strains included in the two papers in order to determine the presence of the *hasABC* locus, the sequence types (STs), the variant of the *nga* promoter, and possible variations in the *nga* coding sequence. Briefly, the raw sequencing reads were mapped using Bowtie2 to exemplar sequences of each of the loci together with at least 600 bp of upstream and downstream sequence. A total of 907 strains met the quality standards that we required in our analysis in both the *has* and *nga* loci. Multilocus sequence typing (MLST) alleles could be confidently determined for 886 of these strains. A total of 79 isolates presented novel STs due to the presence of new alleles or allelic profiles, which were submitted to the *S. pyogenes* MLST database (<http://pubmlst.org/spyogenes/>) and assigned ST791 to ST804. In addition, we analyzed 125 *emm89* isolates recovered in Portugal between 2000 and 2009, including 26 pharyngitis isolates and 37 invasive isolates which have been previously characterized (5, 6), as well as 62 isolates recovered from skin and soft tissue infections (SSTI) (unpublished data). The isolates were screened for the presence of the *has* locus using previously described primers (7). The *nga* gene

and its promoter region were amplified and sequenced in a subset of 95 isolates for which MLST data were available.

In the three datasets (United Kingdom, United States/FI/IC, and Portugal), all isolates carrying *nga* promoter variant 3 (2) lacked the *hasABC* locus (Table 1). Confirming our analysis, the absence of the *hasABC* locus in strains carrying *nga* promoter variant 3 in the United States/FI/IC strains was also independently reported (4). In contrast, all isolates harboring the capsular locus presented variant 1 or 2 of the *nga* promoter. Variant 3 isolates were predominantly of ST101 in all datasets (94%), although 4 United Kingdom isolates and 31 United States/FI/IC isolates presented single-locus variants (SLVs) of ST101. These results indicate that the same clade lacking the hyaluronic acid capsule and carrying an altered *nga* promoter region (designated *emm89-new* here) is present in the five countries. In Portugal, *emm89-new* was first detected among pharyngitis isolates (in 2004) followed by SSTI isolates (2006) and invasive isolates (2007) (Fig. 1) and also rapidly became dominant among *emm89* strains, being associated with a significant increase in the incidence of this *emm* type among isolates from SSTI (data not shown).

A total of 12 distinct nucleotide sequences were identified for the *nga* gene, corresponding to 8 protein variants. Since none included the G330D polymorphism, all variants are predicted to result in NADase proteins with detectable activity (2, 8). Regardless of geographic origin, each promoter variant was largely associated with a specific *nga* allele, with less than 5% of the isolates of each variant presenting other alleles (Table 1). However, differences were found between the dominant *emm89* populations prior to the emergence of *emm89-new* in the three datasets. Variant 1 of the *nga* promoter was not found among United Kingdom strains and was identified in a single isolate from Portugal and in a minority of isolates from Finland and Iceland, while it was the dominant variant among the United States strains. All *has*⁺ *emm89* United Kingdom isolates belonged to ST101, while in the United States/FI/IC and Portugal isolates, eight and four other STs were identified, respectively. ST407 and ST408, both SLVs of ST101, were the most frequent STs among the *has*⁺ *emm89* isolates in the United States/FI/IC study and in Portugal, respectively. The difference between the *has*⁺ *emm89* populations of the United Kingdom and Portugal is further sup-

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TABLE 1 Characteristics of 1,002 *emm89* strains isolated in the United States/FI/IC ($n = 778$), United Kingdom ($n = 129$), and Portugal ($n = 125$)

Promoter variant (n)	<i>nga</i> allele (n) ^a	NADase allele	<i>has</i> locus	ST(s) (n) ^b
1 (195)	3 (1)	2	+	407 (1)
	4 (2)	2	+	380 (2)
	5 (190)	2	+	407 (135), 803 (44), 795 (2), 799 (1)
	6 (1)	3	+	407 (1)
	11 (1)	8	+	407 (1)
2 (168)	7 (160)	4	+ ^c	101 (122), 408 (32), 568 (2), 553 (1), 797 (1)
	8 (1)	5	+	101 (1)
	9 (2)	6	+	142 (2)
	10 (2)	7	+	382 (1)
	12 (3)	4	+	101 (3)
3 (639)	1 (638)	1	–	101 (593), 801 (22), 580 (3), 791 (2), 792 (1), 793 (1), 794 (1), 796 (1), 798 (1), 800 (1), 802 (1), 804 (1)
	2 (1)	1	–	101 (1)

^a Nucleotide sequences of the *nga* alleles can be found at <http://dx.doi.org/10.6084/m9.figshare.1573013>.

^b Sequence type information was obtained for a subset of 981 isolates.

^c The *hasB* gene was not detected in two isolates (one each from references 1 and 2).

ported by differences in superantigen profiles, with the majority (49/69, 71%) of the isolates in Portugal carrying the *speC* gene, in contrast to the situation reported in the United Kingdom (10/48, 21%) (1). Isolates representing *emm89*-new presented the same dominant profile, including *speC*, *speG*, and *smeZ* in both countries, although other superantigen combinations were also identified, both in the United Kingdom (12/83, 14%) and in Portugal (7/56, 13%).

Taken together, these observations suggest that, in spite of the differences in the structures of the *S. pyogenes emm89* populations previously circulating in different countries, the same clade disseminated and outcompeted other *emm89* lineages over the first decade of the 21st century in all countries studied and possibly throughout North America and Europe. This successful clade is characterized by the absence of the *hasABC* locus and the presence

of an *nga-ifs-slo* locus variant associated with increased expression of NADase and SLO, possibly acquired from *emm1* or *emm12* strains by horizontal gene transfer (1, 2). While virulence studies using animal models indicate an increased virulence of the *emm89*-new strains (2, 4), other *in vitro* studies suggest that the novel *emm89*-new phenotype may be advantageous for environmental persistence and transmission, resulting in an increased number rather than severity of *emm89* infections (1). Indeed, epidemiological data from the United Kingdom and Portugal show that an increase in the incidence of disease-associated *emm89* isolates has occurred since the emergence of *emm89*-new. Furthermore, the detection of *emm89*-new in Portugal occurred sooner among milder infections than in more severe disease, also consistent with an advantage of this lineage in persistence and transmission rather than increased virulence. Additional surveillance will

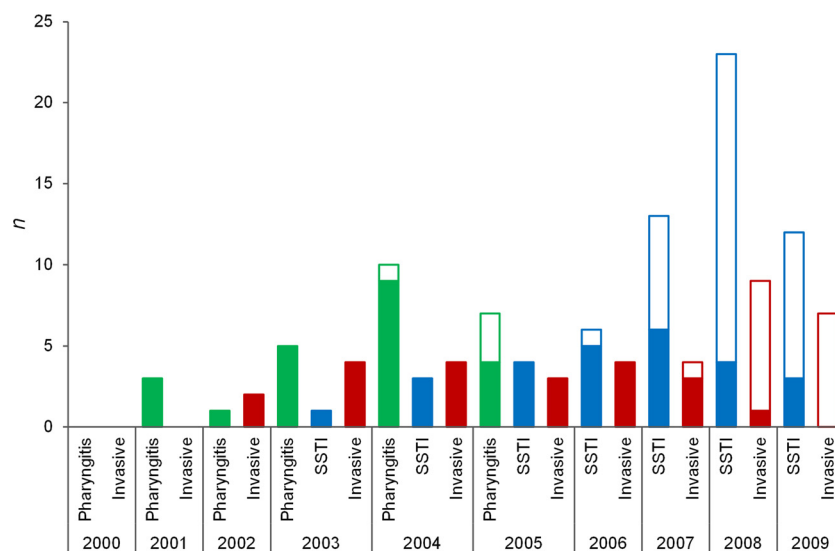


FIG 1 Numbers of *emm89* isolates with (filled bars) and without (open bars) the *has* locus recovered in Portugal between 2000 and 2009, classified by infection type. Pharyngeal isolates were studied only in the period of 2000 to 2005; skin and soft tissue infection (SSSI) isolates were studied only in the period of 2003 to 2009.

contribute to clarify the relative importance of *emm89*-new in different types of infection. The *emm89*-new clade has most probably emerged in an ST101 genetic background, although a minority of isolates presenting SLVs of ST101 were identified. This possibly occurred a limited number of times or even on a single occasion given the extremely limited genetic diversity found among *emm89*-new isolates. From this one event or from a limited number of events occurring in an unidentified geographic location, *emm89*-new rapidly disseminated worldwide, displacing a number of other *emm89* lineages. Still, the detection of SLVs of ST101, together with the diverse superantigen profiles detected, suggests that *emm89*-new is already undergoing limited diversification in each of the geographic locations, which may lead to further increases in virulence or transmissibility of this lineage.

Nucleotide sequence accession numbers.

Nucleotide sequences of the *nga* alleles can be found at <http://dx.doi.org/10.6084/m9.figshare.1573013>.

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