

First Detection of Trimethoprim Resistance Determinant *dfrG* in *Streptococcus pyogenes* Clinical Isolates in India

Streptococcus pyogenes is capable of causing a wide spectrum of diseases, ranging from throat and skin infection to life-threatening invasive diseases (2). The sequelae of streptococcal infections, such as acute rheumatic fever, represent a big health hazard (1). Recently, the emergence of invasive streptococcal diseases in India has been reported (5). Streptococcal *emm* types of the Indian subcontinent differ from those of other countries (7, 8). *emm1*, which is one of the most prevalent invasive types of Western countries, is rare in India. Instead, another type, designated *emm1-2*, is prevalent in India (8), and a subtype, *emm1-2.2*, is associated with invasive disease (5). The development of antibiotic resistance of *S. pyogenes* in India is a serious problem. High resistance rates were observed for trimethoprim sulfamethoxazole, which is commonly prescribed in rural settings of India (3, 6). In this study, we performed whole-genome sequencing of a clinical isolate of type *emm1-2* and detected for the first time the trimethoprim resistance determinant dihydrofolate reductase gene, *dfrG*. The invasive *emm1-2.2 S. pyogenes* strain A1085 was collected during a survey in India. We identified an integrated sequence, not present in the genomes of *S. pyogenes* reference strains available in the NCBI database. BLAST analysis of a 3.3-kb integration element showed 99% to 100% identity to genomic DNA of *Staphylococcus aureus* TW20 (GenBank accession no. FN433596.1) and SAV0404 (GenBank accession no. AB205645.1). Within the integration sequence, three open reading frames (ORFs) were predicted (Fig. 1). ORF_01 was identified as *dfrG*, encoding the trimethoprim-resistant dihydrofolate reductase. ORF_02 and ORF_03 encode hypothetical proteins identical to annotated proteins in *Staphylococcus aureus* TW20. Flanking regions of the *S. pyogenes emm1-2.2* integration element were compared with regions of strain SF370 (*emm1*), which led to identification of SPy_1769 as the integration site, which was confirmed by PCR with primers specific for conserved regions. The amplification product of strain A1085 was sequenced, which confirmed the results of the genome sequencing. The *dfrG* gene was amplified and sequenced (Fig. 2; Table 1). The integration element of 3.3 kb and the specific 500-bp PCR product of *dfrG* were detected in all tested *emm1-2.2* strains from different sites. Strain SF370 (4) showed no integration element, and *dfrG* could not be amplified (Fig. 2; Table 1).

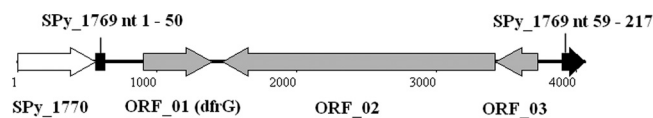


FIG 1 Genetic map of the *dfrG* gene locus in *S. pyogenes emm1-2* isolates. Genome sequencing of the invasive *emm1-2.2 S. pyogenes* strain A1085 identified an integrated sequence of 3.3 kb, not present in the genomes of *S. pyogenes* reference strains. Within the integration sequence, three open reading frames were predicted, which are indicated by gray arrows, with the direction of transcription shown by the arrowhead. ORF_01 was identified as *dfrG*, encoding the trimethoprim-resistant dihydrofolate reductase. ORF_02 and ORF_03 encode hypothetical proteins. Flanking regions of the integration element were compared with *S. pyogenes* SF370 (*emm1*), and SPy_1769 was identified as the integration site. The first 50 nucleotides (nt) of SPy_1769 are shown as a black box, followed by the integration element. Nucleotides 59 to 217 of SPy_1769 are upstream of the integration element, depicted as a black arrow. The white arrow corresponds to the gene *SPy_1770*.

The resistance to trimethoprim of the *emm1-2.2* strains was demonstrated by the disk diffusion test. The MIC of trimethoprim was determined by the agar dilution method. The MIC was recorded as the lowest concentration of trimethoprim that inhibited visible growth after 18 h of incubation at 37°C (Table 1). MIC values for *emm1-2.2* strains were high (>512 µg/ml), in contrast to those for the susceptible control strain SF370 (MIC ≤ 2 µg/ml).

Although trimethoprim resistance has been observed with beta-hemolytic streptococci, the underlying molecular mechanisms were not elucidated. *S. pyogenes emm1-2.2* is an important *emm* type in northern India associated with skin, throat, and invasive infections. The detection of the trimethoprim resistance determinant *dfrG* in all clinical isolates belonging to *emm1-2.2* is a cause of serious concern that underlines the need for longitudinal surveillance of isolates from different parts of India.

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TABLE 1 Trimethoprim resistance in *S. pyogenes emm1-2.2*

Isolate	Relevant properties	MIC of trimethoprim (µg/ml)	Reference
SF370	<i>emm1</i> Tmp ^s , <i>dfrG</i> mutant	≤2	4
A1038	Skin isolate, <i>emm1-2.2</i> Tmp ^r <i>dfrG</i> ⁺	>512	8
A1039	Skin isolate, <i>emm1-2.2</i> Tmp ^r <i>dfrG</i> ⁺	>512	8
A1040	Skin isolate, <i>emm1-2.2</i> Tmp ^r <i>dfrG</i> ⁺	>512	8
A1041	Throat isolate, <i>emm1-2.2</i> Tmp ^r <i>dfrG</i> ⁺	>512	8
A1042	Throat isolate, <i>emm1-2.2</i> Tmp ^r <i>dfrG</i> ⁺	>512	8
A1043	Throat isolate, <i>emm1-2.2</i> Tmp ^r <i>dfrG</i> ⁺	>512	8
A1085	Invasive isolate, <i>emm1-2.2</i> Tmp ^r <i>dfrG</i> ⁺	>512	5
A1095	Invasive isolate, <i>emm1-2.2</i> Tmp ^r <i>dfrG</i> ⁺	>512	5

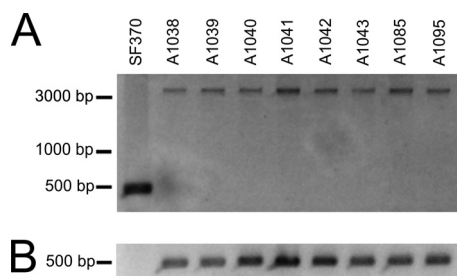


FIG 2 PCR detection of the insertion sequence (A) and *dfrG* (B). (A) The presence of the integration element was analyzed by PCR with primers specific for conserved regions flanking the integration site. *S. pyogenes emm1-2* strains showed an ~3.8-kb amplification product, indicating the integration element. As expected, *S. pyogenes* SF370 (negative control) showed no integration element, which is indicated by a 500-bp PCR product. (B) Specific PCR products of *dfrG* (500 bp) were detected in all tested *emm1-2.2* strains, whereas SF370 was negative for these products.

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