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Identification, clinical aspects, susceptibility pattern, and molecular epidemiology of beta-haemolytic group G *Streptococcus anginosus* group isolates from central Taiwan ☆

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ABSTRACT

No literature is available on the prevalence and clinical aspects of beta-haemolytic group G *Streptococcus anginosus* group in central Taiwan. In this study, we used 16S rRNA gene sequencing and 16S-23S rDNA intergenic spacer sequencing (where necessary) as the gold standard for molecular identification. Twenty-seven *S. anginosus* group isolates were identified from 273 beta-haemolytic GGS isolates collected from patients in central Taiwan between February 2007 and August 2011. Of the 27 isolates, 22 were *S. anginosus* and 5 were *Streptococcus constellatus*. The 3 commercial methods, Rapid ID 32 Strep, API 20 Strep, and Vitek 2 GP card, identified 77.8%, 40.7%, and 37.0% of *S. anginosus* group isolates, respectively, with acceptable %ID or probability level. All the *S. constellatus* isolates possessed the *lmb* gene (encoding laminin-binding protein); however, none of the *S. anginosus* isolates possessed this gene. All the 27 isolates were susceptible to penicillin. Five *S. anginosus* group isolates (18.5%) were resistant to erythromycin. The resistance genes, *ermB* and *mefA*, were detected in 3 (2 *S. anginosus* and 1 *S. constellatus*) and 2 (2 *S. anginosus*) isolates, respectively. Pulsed field gel electrophoresis showed that most *S. anginosus* group isolates were genetically diverse. This is the first study to evaluate 3 commercial methods for the identification of beta-haemolytic group G *S. anginosus* group species, and only the Rapid ID 32 Strep system showed considerable ability. The clinical aspects, susceptibility pattern, and molecular epidemiology of beta-haemolytic group G *S. anginosus* group isolates from central Taiwan were also first presented.

1. Introduction

The clinical importance of beta-haemolytic group G streptococci (GGS) is increasing. In general, *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) is the most clinically relevant GGS (Takahashi et al., 2010). However, other beta-haemolytic GGS, especially *Streptococcus anginosus* group (also called *Streptococcus milleri* group), are also associated with human infections.

The *S. anginosus* group includes 3 species, namely, *S. anginosus*, *Streptococcus constellatus*, and *Streptococcus intermedius*. According to the literature, most strains of *S. anginosus* group give no or partial hemolysis and are either serologically ungroupable or belonged to Lancefield group A, C, F, or G (Whiley and Beighton, 1991). These bacteria cause pyogenic infections and are strongly associated with abscess formation (Claridge et al., 2001; Weightman et al., 2004). A population-based surveillance study on invasive pyogenic streptococcal infections in Canada showed that the annual incidence of *S. anginosus* group infection was higher than that of group A and B streptococcal infections (Laupland et al., 2006). However, only a few studies have focused on beta-haemolytic group G *S. anginosus* group. A survey in southern India on species causing beta-haemolytic group C and G streptococcal infections showed that group G *S. anginosus* represented 19% of the organisms causing these infections and no *S. intermedius* and *S. constellatus* were found (Reissmann et al., 2010). Although the clinical significance of *S. anginosus* group is growing worldwide, information on infections caused by beta-haemolytic GGS other than SDSE is obscure in general. This might result from the fact that in many clinical laboratories, routine GGS identification is merely based on Lancefield serogrouping and does not reach species level.

The notable contribution of the *S. anginosus* group to human infections suggests that this group of pathogens deserves more attention. In this study, a total of 273 clinical beta-haemolytic GGS isolates were collected from several regional hospitals in central Taiwan between February 2007 and August 2011. We used 16S rRNA gene sequencing and 16S-23S rDNA intergenic spacer sequencing

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(where necessary) as the gold standard for molecular identification. *S. anginosus* group isolates were then identified using API 20 Strep, Rapid ID 32 Strep, and Vitek 2 systems (bioMérieux, Marcy l'Etoile, France) to evaluate the feasibility of these systems for differentiating between beta-haemolytic *S. anginosus* group members. Clinical aspects, virulence factors distribution, drug susceptibility, and epidemiology of these isolates were also investigated.

2. Materials and methods

2.1. Bacterial isolates

A total of 273 consecutive, non-duplicate beta-haemolytic GGS isolates collected between February 2007 and August 2011 from the Central Laboratory of the Central Region Hospital Alliance (including Taichung Hospital, Fongyuan Hospital, Changhwa Hospital, Nantou Hospital, and Tsaotun Psychiatric Center) and the Clinical Bacteriology Laboratory of the Lin Shin Hospital (all located in central Taiwan) were investigated. Grouping was performed using the Streptococcal Grouping Kit (Oxoid, Basingstoke, Hampshire, UK). The bacteria were cultured at 37 °C in CO_2 incubator using trypticase soy agar supplemented with 5% sheep blood (Becton, Dickinson and Company, Sparks, MD, USA) and stocked at Protect Bacterial Preservers (Technical Service Consultants, Heywood, Lancashire, UK).

2.2. Molecular identification

Bacterial DNA extraction was performed using the Genomic DNA Purification kit (Biokit, Miaoli, Taiwan) according to the manufacturer's instruction. The primer pair 5′-CGGGTGAGTAACGCGTAGG-TAAC-3′ and 5′-TGACGTCATCCCCACCTTCCTC-3′ was designed and used for 16S rDNA fragment amplification. The polymerase chain reaction (PCR) mixture (20 μL) contained 10 mmol/L Tris–HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 1 U *Taq* DNA polymerase, 0.2 mmol/L deoxynucleoside triphosphate, 0.2 μmol/L of each primer, and 1 μL of DNA sample. The target DNA was amplified using MyCycler™ thermal cycler (Bio-Rad, Hercules, CA, USA) under following conditions: initial denaturation at 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 61 °C for 30 s, and 72 °C for 70 s; and final extension at 72 °C for 10 min. DNA sequencing was performed using BigDye® Terminator v3.1 kit and 3730 DNA Analyzer (Applied Biosystems, ABI, Foster, CA, USA). NCBI database was accessed and searched using the nucleotide BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Any uncertainty of identification after 16S rRNA gene sequencing was resolved using 16S–23S rDNA intergenic spacer sequencing as previously described (Chen et al., 2004).

2.3. Phenotypic testing

API 20 Strep, Rapid ID 32 Strep, and Vitek 2 systems (bioMérieux) were used to identify non-SDSE isolates according to the manufacturer's instructions. For the API 20 Strep system, results were interpreted after incubation for 4 and 24 h, respectively. Isolates were identified using the corresponding identification software (API 20 STREP V7.0), and a %ID ≥ 80 was considered acceptable. For the Rapid ID 32 Strep system, isolates were identified using apiweb™ identification software (rapid ID 32 STREP V3.0), and a %ID ≥ 80 was considered acceptable. For the Vitek 2 system, Vitek 2 GP colorimetric identification card was used. The results were interpreted automatically by using bioMérieux Vitek 2 system, and a probability of ≥85% was considered acceptable.

2.4. Detection of virulence factors

The genes encoding M protein (*emm*), streptolysin O (*slo*), laminin-binding protein (*lmb*), streptolysin S (*sagA*), streptokinase (*skc*), glyceraldehyde 3-P dehydrogenase (*gapC*), and superantigens including streptococcus pyrogenic exotoxins A (*speA*), C (*speC*), G (*speG*), G^{dys} (*spegg*), H (*speH*), I (*speI*), J (*speJ*), K (*speK*), L (*speL*) and M (*speM*), mitogenic exotoxin Z (*smeZ*), and streptococcal superantigen A (*ssa*) were amplified by PCR (Brandt et al., 2005).

Table 1
Comparison of the 3 commercial methods with 16S rRNA gene sequencing for the identification of 27 beta-haemolytic group G *S. anginosus* group isolates.

16S rRNA gene sequence (No.)	No. (%) of positive isolate		
	Rapid ID 32 Strep	API 20 Strep	Vitek 2
S. anginosus (22)	17 (77.3)	7 (31.8)	8 (36.4)
S. constellatus (5)	4 (80.0)	4 (80.0)	2 (40.0)
Total (27)	21 (77.8)	11 (40.7)	10 (37.0)

2.5. Antimicrobial susceptibility testing

Drug susceptibility was tested using broth microdilution and disc diffusion methods as recommended by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2011). *Streptococcus pneumoniae* ATCC 49619 was used as a reference strain. Susceptibilities of these isolates to penicillin, vancomycin, erythromycin, clindamycin, levofloxacin, and cefotaxime were examined and interpreted according to the CLSI guideline. The double-disc diffusion test (D test) and detection of erythromycin resistance genes were performed as reported (De Azavedo et al., 1999).

2.6. Pulsed field gel electrophoresis analysis

Bacterial suspension with an OD_{610} of 1.6–1.8 was used for plug preparation, and DNA was digested with *Sma*I at 37 °C for 4 h. *Xba*I-digested chromosomal DNA of *Salmonella enterica* serovar Braenderup H9812 was used as the molecular weight marker (Hunter et al., 2005). Pulsed field gel electrophoresis (PFGE) was performed using CHEF-DR III system (Bio-Rad), and electrophoresis conditions were set as previously described (Bert et al., 1997). The data were analysed using GelComparII software (Applied Maths NV, Sint-Martens-Latern, Belgium). One PFGE group was defined as a group containing at least 3 isolates with a similarity coefficient of ≥75%.

3. Results and discussion

Based on 16S rRNA gene sequencing, 246 of the 273 (90.1%) GGS clinical isolates collected from central Taiwan were identified as SDSE, indicating that SDSE is the most prevalent beta-haemolytic GGS pathogens in this region. All the remaining 27 GGS isolates belonged to the *S. anginosus* group, with 22 being *S. anginosus* isolates and 5 being *S. constellatus* isolates (data not shown). No *S. intermedius* was found. In general, *S. constellatus* and *S. anginosus* are more frequently isolated from clinical samples than *S. intermedius* (Weightman et al., 2004). A previous study in northern Taiwan showed that SDSE was the major cause of GGS bacteraemia (93.5% cases), followed by *S. anginosus* (5.4% cases) (Liao et al., 2008). In this study, 5 *S. anginosus*

Table 2
Susceptibilities of *S. anginosus* and *S. constellatus* isolates to antimicrobial agents.

Antimicrobial agent	*S. anginosus* (n = 22)		*S. constellatus* (n = 5)	
	MIC range	% Susceptible	MIC range	% Susceptible
Vancomycin	0.25–1	100	0.5–1	100
Penicillin	≤0.015–0.03	100	≤0.015–0.06	100
Erythromycin	≤0.015 to >16	81.8	0.03 to >16	80.0
Clindamycin	≤0.015 to >16	90.9	≤0.015 to >16	80.0
Cefotaxime	0.03–0.5	100	0.06–0.25	100
Levofloxacin	0.12–2	100	0.25–8	80.0