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Impact of identification of *Streptococcus dysgalactiae* subspecies *equisimilis* from throat cultures in an adult population☆,☆☆

Amanda T. Harrington, Jill E. Clarridge III *

VA Puget Sound Health Care System, Seattle, WA 98108, USA
University of Washington, Seattle, WA 98108, USA

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ABSTRACT

Streptococcus dysgalactiae subspecies *equisimilis* (SDSE) are isolated from the throat of patients with pharyngitis, although the clinical significance remains debated. We sought to determine the incidence and association with pharyngitis of SDSE in an adult veteran population. Organisms were phenotypically identified to subspecies and Lancefield group, with selective 16S rRNA gene sequencing. From 833 throat cultures, the overall frequency of SDSE was 3.4% (64% group C and 36% group G) as compared to 8.6% for *S. pyogenes* (GAS). SDSE was described as a large colony in only 29% of the original culture evaluations by bench technologists, and clinical symptoms were similar for GAS and SDSE. Laboratory algorithms that are limited to identification of only GAS or are based on Lancefield group or visual identification of "large-colony type" β hemolytic Lancefield group C and G streptococci may be missing or misidentifying SDSE along with Anginosus group streptococci.

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1. Introduction

Streptococcus pyogenes is considered the predominant bacterial cause of pharyngitis in both adults and children, and many microbiology laboratories identify and report only Lancefield group A streptococci *Streptococcus pyogenes* (GAS). However, β hemolytic Lancefield group C and G streptococci (BHCGS) are also frequently isolated from throat culture in association with pharyngitis. Although colony size and serological groups designed by Lancefield do not correspond to individual species, in much of the published literature regarding BHCGS associated with pharyngitis the identification methods are limited to Lancefield typing and colony size alone. Therefore, many of the studies potentially fail to distinguish accurately between *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE), *S. canis*, and *S. anginosus*/*S. constellatus*, equating SDSE and BHCGS. SDSE, which can be Lancefield group C or G, is the subspecies of *S. dygalactiae* to which the human bacterial isolates belong (Jensen and Kilian, 2012; Vandamme et al., 1996). Both *S. anginosus* and *S. constellatus*, which are part of the respiratory flora, can be β hemolytic, and can be either Lancefield group C or, less commonly, G. *S. intermedius*, the other member of the Anginosus group, is also respiratory flora but is not β hemolytic on sheep blood agar. Other BHCGS include *Streptococcus equi* subspecies *zooepidemicus* (Lancefield group C) and *Streptococcus canis* (Lancefield group G), which are animal pathogens in humans and have only been associated with intimate animal contact or consumption of unpasteurized dairy products (Barnham et al., 1983; Facklam, 2002; Lam et al., 2007).

Many of the studies assessing the incidence of BHCGS from throat cultures have been done in pediatric populations, and the majority of the published reports evaluating adults have comprised younger populations (young adults <30 years of age) and do not include older adult populations (>45 years) (Corson et al., 1989; Lindbaek et al., 2005; Meier et al., 1990; Turner et al., 1997; Zwart et al., 2000). Laboratory methods in several of the studies evaluating incidence in adult populations have been limited to: using only Lancefield group and colony size without species level identification (eg. Lindbaek et al., 2005); including only β hemolytic Lancefield group C *Streptococci* (eg. Meier et al., 1990; Turner et al., 1997); or evaluating all "non group A" Lancefield groups (including F and B) together (eg. Tiemstra and Miranda, 2009; Zwart et al., 2000). Because most of the studies did not clearly differentiate between streptococcal species or did not exclusively identify SDSE, they may not have accurately represented its association with pharyngitis, and it is difficult to assess the true incidence or significance based on the literature. The goals of our study were to determine the incidence of accurately identified SDSE isolated from throat culture as compared to *S. pyogenes* in an adult Veteran population and to document clinical symptoms of pharyngitis in patients from whom the organisms were recovered.

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* Corresponding author. Tel.: +1-206-277-4514; fax: +1-206-764-2001.
E-mail address: jill.clarridge@va.gov (J.E. Clarridge).

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2. Materials and methods

To determine the frequency of SDSE in adult patients (>18 years of age), an analysis of a 2-year period was performed at VA Puget Sound Health Care System in Seattle, WA, USA. Throat swab specimens were inoculated on trypticase soy agar with 5% sheep blood (BAP) and incubated overnight at 35°C in anaerobic conditions. From the BAP culture, all β-hemolytic colonies isolated in significant numbers (i.e. ≥10 colonies) that were catalase negative and consistent with streptococci by Gram stain were further typed by Lancefield group (PathoDx, Remel, Lenexa, KS). In addition, the colony size, hemolysis characteristics and quantity of organisms was noted. Organisms that were identified as Lancefield group C or G were identified to species level using the Vitek 2 ID-GPC card (bioMerieux, Inc., Durham, NC, USA). In addition, 16S rRNA gene sequence analysis was performed on isolates using selective criteria and methods as previously reported (Mahlen and Clarridge, 2011). A limited amount of clinical information was obtained for each patient with a confirmed SDSE or GAS isolate identification, including age, sex, reported symptoms, and receipt of antibiotics. Statistical analysis was performed using a Fisher exact 2-tailed test, and probability values less than 0.05 were considered significant.

3. Results

From a total number of 833 throat cultures, 28 isolates were identified as SDSE and 72 were identified as GAS, with overall frequencies of 3.4% and 8.6% respectively. Beta hemolytic Lancefield group F/G/C *S. anginosus* or S. *constellatus* were isolated from approximately 3% of throat cultures, and were not included in this evaluation. Species level identification was confirmed for selected isolates using 16s rRNA gene sequencing; 5 isolates from throats identified as *S. dysgalactiae* by Vitek 2 were confirmed as SDSE. In contrast to group G streptococci isolated from other sites (i.e., wound specimens), there were no *S. canis* isolated from throat culture.

The gender distribution between patients with SDSE and GAS was not significantly different. The age distribution for patients with SDSE was 21–63 years of age, with a median age of 36 years of age. The age distribution was slightly wider in patients with GAS (22–83 years of age), with a higher median age of 39 years. Of patients with SDSE, 92% were noted to have symptoms of sore throat, 41% were noted to have difficulty swallowing, 37% were noted to have lymphadenopathy, and 19% were noted to have a cough. None of these demographics or clinical symptoms were noted at significantly different rates for patients with GAS (Table 1). However, 47% of patients with GAS isolated had clinical symptoms of fever as compared to only 7% patients with SDSE ($P = 0.002$). Of patients with SDSE isolated, 63% were noted to have pharyngeal exudates as compared to 40% of patients with GAS ($P = 0.06$) (Table 1). Of patients with SDSE, 70% were given treatment by their physician, and 3 patients who were not given treatment returned with similar symptoms (Table 1). Data regarding receipt of antibiotics prior to specimen collection for culture were not available, which is a limitation of the study.

In 82% of the culture evaluations, SDSE was described as "moderate" or "many," correlating semi-quantitatively with growth in the second and third quadrants of the culture plate. The isolated organisms were described as "few" in 5 culture evaluations, and in one was noted as "few, but predominant". Although SDSE are categorized as "large-colony-forming" BHCGS, in our study this organism was described as a large colony in only 29% of the original culture evaluations by the bench technologist. Colony descriptions by the bench technologist were also variable regarding description of color (Table 2).

4. Discussion

One goal of our study was to determine the incidence of accurately identified SDSE associated with pharyngitis in an older adult population; we found it isolated at a frequency of approximately 1/3 of that of GAS. In our study, we specifically excluded β hemolytic *S. anginosus* and *S. constellatus*, which are typically isolated in lower quantities and often considered commensals (Clarridge et al., 1999; Turner et al., 1993). It should be noted that *S. constellatus* subsp. *pharyngis* (both β hemolytic and group C) has been associated with clinical symptoms of acute pharyngitis (Whiley et al., 1999). A second goal was to document clinical symptoms in patients from whom SDSE and GAS were isolated. While the many of the symptoms were similar, we did find some significant differences. Only 7% of patients with SDSE were noted to have fever compared to 47% of patients with GAS, but 63% of patients with SDSE were noted to have pharyngeal exudates compared to only 40% of patients with GAS (Table 1).

Review of the initial culture plate descriptions revealed two notable points. SDSE was usually recovered from throat specimens in large quantities (>10 colonies in the second quadrant). However, in 5 culture evaluations, SDSE was recovered in low quantities (~10–20 colonies in the primary quadrant). Zwart et al. (2000) found that low

Table 1
Characteristics of patients from whom β-hemolytic Lancefield group C/G *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) or Lancefield group A *Streptococcus pyogenes* (GAS) were identified.

Characteristics	Number (%)		
	SDSE	GAS	
Median age (range in years of age)	36 (21–63)	39 (22–83)	
Gender			
Male	18 (64)	52 (72)	
Female	10 (36)	20 (28)	
Symptoms reported[a]	n = 27	n = 70	
Sore throat	25 (92)	67 (95)	
Exudate	17 (63)	28 (40)	$P = 0.06$
Lymphadenopathy	10 (37)	31 (44)	
Difficulty swallowing	11 (41)	23 (33)	
Cough	5 (19)	11 (16)	
Fever	2 (7)	33 (47)	$P = 0.002$
Given treatment	19 (70)	64 (91)	
Not treated and returned with similar symptoms	3 (11)	0 (0)	

[a] Records not available for all patients.

Table 2
Descriptive characteristics of organisms identified as *Streptococcus dysgalactiae* subspecies *equisimilis* on original isolation plates.

Characteristics[a]	Number (%)
Lancefield group (n = 28)	
Group C	18 (64)
Group G	10 (36)
Macroscopic description[b]	
Size (n = 14)	
Large	4 (29)
Medium	1 (7)
Small	5 (35)
Tiny	4 (29)
Color (n = 15)	
White	8 (53)
Grey	7 (47)
Quantity (n = 28)	
Many	14 (50)
Moderate	9 (32)
Few	5 (18)

[a] Descriptive characteristics not available for all isolates. n = 28 total isolates.
[b] Descriptions were based on the primary evaluation of the culture plate.