Prevalence of *Streptococcus dysgalactiae* and Microbial Co-detection in Patients Presenting with Pharyngitis

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Abstract

Background: *Streptococcus pyogenes* (GAS) is often the only pathogen tested for when a patient presents with symptoms of pharyngitis. Widespread use of rapid strep tests creates a diagnostic gap when pharyngitis is caused by other bacterial pathogens, such as *Streptococcus dysgalactiae* (GCGS). *S. dysgalactiae* often goes undetected in clinical specimens, resulting in incorrect diagnosis as viral infection, and potentially leading to downstream negative sequela. In this study, we investigated the incidence of GCGS in patients symptomatic for pharyngitis and its co-detection with other viral and bacterial pathogens responsible for pharyngitis.

Methods: Throat swabs (n=5014) from symptomatic patients were collected from February-March and July-September 2017. DNA from the specimens was extracted and tested using the Target Enriched Multiplex PCR (TEM-PCR™) Pharyngitis Panel. This panel tests for the presence of common microbial pathogens responsible for pharyngitis with high sensitivity and specificity. Detection was performed on a microarray platform with complementary target-specific detection probes.

Results: *S. dysgalactiae* was detected in 87 specimens out of 5014 tested. The prevalence rates by age groups was 1.39% in patients younger than 15 years (n=4185) and 3.5% in patients older than 15 years (n=829). Of the 87 GCGS detections, the most viral co-detections were observed with Enteroviruses (n=10). No significant co-detection with other bacterial pathogens including GAS (n=448), *Chlamydophila pneumoniae* (n=18) and *Mycoplasma pneumoniae* (n=40) were observed. Detection of *Fusobacterium necrophorum* (n=49) and *Arcanobacterium haemolyticum* (n=17) was also observed. GCGS and *F. necrophorum* were co-detected in 5 specimens which had no viral presence.

Conclusions: In this study, we show that GCGS prevalence in patients symptomatic for pharyngitis is second only to GAS and, in many cases, is not accompanied by viral co-detection. Since the pathogen often produces mild disease that mimics viral infection symptoms a misdiagnosis of the infection as viral and therefore non-treatable can result. The data presented here underscores the need to include the detection of an emerging cause of pharyngitis, *S. dysgalactiae,* despite suspected viral etiology, for more accurate therapeutic decisions.

Note: Abstract amended to include additional test sample data

Introduction

- Acute pharyngitis is one of the most common conditions encountered in the physician's office.
- Viruses are the predominant cause of pharyngitis, with patients presenting mild to moderate sore throat and hoarseness. Viral pharyngitis is often self-limiting.
- When bacterial pharyngitis is suspected, Group A *Streptococcus pyogenes* (GAS) is often the only potential causative agent considered, diagnosed by rapid antigen detection or culture.
- Traditional diagnostics are often limited to detection of GAS and most are unable to detect other bacterial causes of pharyngitis, including *Streptococcus dysgalactiae* (GCGS).[1,2]
- Clinical presentation of GCGS is often milder than GAS, mimicking that of viral pharyngitis, which combined with a negative rapid strep test result, may lead to misdiagnosis. Complications of untreated GCGS throat infections can include sepsis, pneumonia, and endocarditis.[3]
- Recent studies suggest the prevalence rate for GCGS could be as high as 10%, but this number could be biased due to insufficient detection methods.[4]
- Molecular methods, such as multiplex PCR, offer improved detection of viral and/or bacterial agents causing pharyngitis, advancing diagnosis and treatment measures.
- In this study, we report the prevalence rate of *S. dysgalactiae* in throat swab specimens collected from symptomatic patients, including viral and bacterial co-detection rates and age-related and seasonal detection patterns.

Materials & Methods

Specimen Collection. Throat swabs from patients symptomatic for pharyngitis (n=5014) were collected by clinicians and transported overnight to Diatherix Eurofins (Huntsville, AL) for routine testing. The specimens were collected in two phases: Phase I (n=1799; February-March 2017; age: 1 month-86 years, median age: 8 years) and Phase II (n=3215; July-September 2017; age: 1 month-86 years; median age: 7 years).

Nucleic Acid Extraction. Extractions from the clinical specimens were performed on the KingFisher™ Flex instrument (Thermo Fisher Scientific, Waltham, MA) using in-house methods with extraction reagents from Omega Bio-Tek (Norcross, GA) and Qiagen (Hilden, Germany).

Materials & Methods *(continued)*

Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR™). The nucleic acids extracted from clinical specimens were tested on the TEM-PCR™ Pharyngitis Panel. TEM-PCR™ is a highly multiplexed, nested, end-point PCR technique, in which the primer mix contains two pairs of gene-specific primers for each target. Inside nested primers have a unique tag sequence complementary to proprietary superprimers, also included in the primer mix. TEM-PCR™ cycling includes initial enrichment and tagging of each target, followed by traditional PCR amplification (Figure 1).

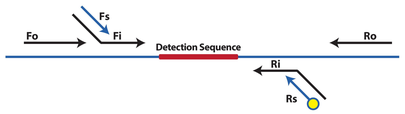

Figure 1. TEM-PCR™ Scheme.
Low concentration nested gene-specific primers are (Fo – forward out; Fi – forward in; Ri – reverse in; and Ro – reverse out) designed to enrich the targets during the initial PCR cycles. Later in the procedure, a pair of universal SuperPrimers (Fs and Rs) is used to amplify all targets. The Rs primer is labeled with biotin for subsequent detection.

Table 1. TEM-PCR™ Pharyngitis Panel Targets.

Bacterial Targets	Viral Targets
Arcanobacterium haemolyticum *Chlamydophila pneumoniae* *Fusobacterium necrophorum* *Mycoplasma pneumoniae* *Streptococcus dysgalactiae* (GCGS) *Streptococcus pyogenes* (GAS)	Adenovirus groups B/E Enterovirus Human coronavirus Influenza A Influenza A pdmH1N1 Influenza B Parainfluenza virus types 1, 2, 3, 4 Respiratory Syncytial Virus A/B Rhinovirus

Hybridization and Detection. Amplified TEM-PCR™ products were hybridized to target detection probes on custom microarray plates (Microarrays, Inc., Huntsville, AL). Streptavidin-phycoerythrin conjugate (Moss, Inc., Pasadena, MD) was added to the hybridization reaction, followed by a series of wash steps. Fluorescence signals were analyzed on a SensoSpot Fluorescence Low Density Microarray Analyzer (Sensovation AG, Radolfzell, Germany). Results for each target were reported as "Detected" or "Not Detected" based on signal intensity above background.

Data Analyses. The results obtained from the study were evaluated for bacterial and viral co-detections, patient age, and the seasonal pattern of pathogen detection. P-values, used for indicating probability of dependency between two variables, were determined using Fisher's exact test for pathogen detection rates.

Results

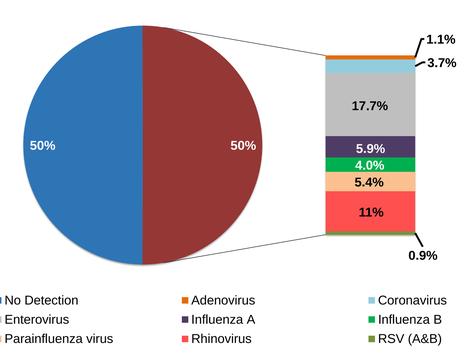

Figure 2. Prevalence Rates of Viral Pathogens.
Throat swab specimens from symptomatic patients (n=5014) were evaluated by the TEM-PCR™ Pharyngitis Panel.

Results *(continued)*

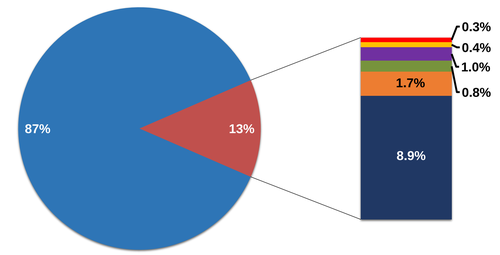

Figure 3. Prevalence Rates of Bacterial Pathogens.
Throat swab specimens from symptomatic patients (n=5014) were evaluated by the TEM-PCR™ Pharyngitis Panel.

Table 2. Viral Co-detections with *S. dysgalactiae* and *S. pyogenes*.

Viral Detection	*S. dysgalactiae* (n=87)	*S. pyogenes* (n=448)
Adenovirus groups B/E	0 (0%)	0 (0%)
Enterovirus	10 (11.5%)	80 (17.9%)
Human coronavirus	1 (1.1%)	11 (2.5%)
Influenza A	4 (4.6%)	46 (10.3%)
Influenza A pdmH1N1	0 (0%)	0 (0%)
Influenza B	0 (0%)	0 (0%)
Parainfluenza virus	6 (6.9%)	7 (1.6%)
Respiratory Syncytial Virus A/B	0 (0%)	0 (0%)
Rhinovirus	7 (8.0%)	38 (8.5%)

Table 3. Bacterial Co-detections with *S. dysgalactiae* and *S. pyogenes*.

Bacterial Detection	*S. dysgalactiae* (n=87)	*S. pyogenes* (n=448)
A. haemolyticum	1 (1.1%)	0 (0%)
C. pneumoniae	0 (0%)	0 (0%)
F. necrophorum	5 (5.7%)	5 (1.1%)
M. pneumoniae	0 (0%)	7 (1.6%)
S. dysgalactiae	NA	1 (0.2%)
S. pyogenes	1 (1.1%)	NA

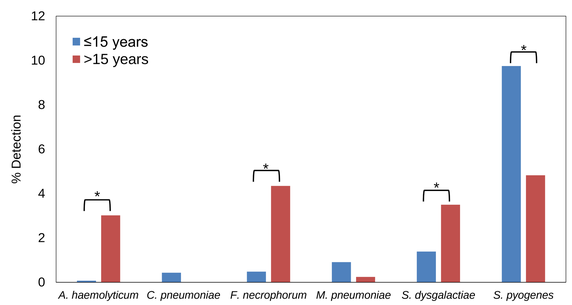

Figure 4. Pharyngitis Pathogen Prevalence By Age Group.
Prevalence rates of bacterial pharyngitis pathogens were evaluated for age groups ≤15 years (n=4185) and >15 years (n=829). *p<0.001

Results *(continued)*

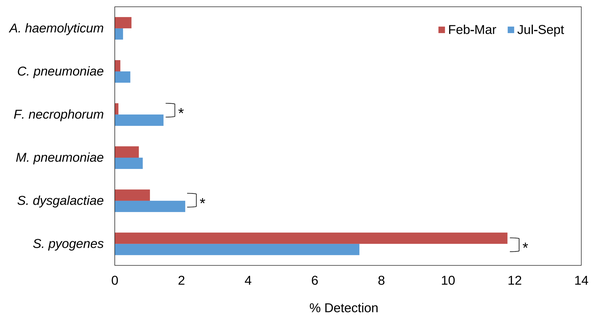

Figure 5. Prevalence Data of Pharyngitis Pathogens in Winter and Summer 2017.
Prevalence rates of bacterial pharyngitis pathogens were evaluated for specimens collected in winter (n= 1799) and summer (n= 3215). *p<0.01

Discussion

- Viral and bacterial detections were made in 50% and 13% of clinical pharyngitis specimens, respectively.
- *S. dysgalactiae* was the second most prevalent bacterial pathogen (1.7%; n=87) detected after *S. pyogenes* (8.9%; n=448).
- Of the 87 *S. dysgalactiae* positives, no co-detections were observed with *S. pyogenes* or viruses in 59 (68%) of specimens.
- An age-related difference in detection rates was observed for *S. pyogenes*, *S. dysgalactiae*, *F. necrophorum*, and *A. haemolyticum.* Detection of *S. pyogenes* in patients ≤15 years was higher compared to those >15 years of age. *S. dysgalactiae, F. necrophorum*, and *A. haemolyticum* detection rates were higher in patients >15 years of age.
- Seasonality of *S. pyogenes*, *S. dysgalactiae,* and *F. necrophorum* detection was suggested from this study. Detection of *S. pyogenes* was more prevalent in winter than in summer, while the reverse was observed for *S. dysgalactiae* and *F. necrophorum.*

Conclusions

- *S. dysgalactiae* was the second most common bacterial pathogen detected in pharyngitis specimens, predominately identified in the summer months from patients over the age of 15.
- Although detection of *S. pyogenes* and *S. dysgalactiae* was not mutually exclusive, the rate of co-detection was low.
- Traditional diagnostics fail to identify GCGS in most cases. With symptoms that can mimic viral etiologies, *S. dysgalactiae* is often misdiagnosed and untreated which can lead to serious downstream complications.
- Further research is needed to investigate the prevalence of *S. dysgalactiae*, *F. necrophorum,* and *A. haemolyticum* from patients symptomatic for pharyngitis.
- The data presented in this study underscore the need to include the detection of non-GAS pathogens causing pharyngitis, especially *S. dysgalactiae*, for better therapeutic decisions and patient outcomes.

References

[1] Fine, Andrew M., Victor Nizet, and Kenneth D. Mandl. "Large-scale validation of the Centor and McIsaac scores to predict group A streptococcal pharyngitis." *Archives of internal medicine* 172.11 (2012): 847-852.

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[4] Lindbæk, Morten, et al. "Clinical symptoms and signs in sore throat patients with large colony variant β-hemolytic streptococci groups C or G versus group A." *Br J Gen Pract* 55.517 (2005): 615-619.

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