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Bacterial and Viral qPCR Data in Chronic Sinusitis

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Abstract

Many studies have investigated the flora associated with chronic sinusitis, but few compare real-time quantitative polymerase chain reaction (qPCR) data with the medical literature. The purpose of this study is to explore our current qPCR data as well as conduct a literature review on the clinical importance of microbiologic identification techniques such as qPCR and culturing. This study uses qPCR data from a sample set of 333 patients to conduct a comparative analysis. The study found that the three most prevalent sinonasal bacteria in sinusitis are *Staphylococcus aureus* (Cumulative: 70 positive samples and 21.02% identification rate), *Klebsiella pneumoniae* (Cumulative: 28 positive samples 8.41% positive identification rate), *and Haemophilus influenzae* (Cumulative: 20 positive samples and 6.00% positive identification rate) in our patient population. These findings differ from the pathogens traditionally cited in the otolaryngology medical literature. Furthermore, 29.73% of the samples contained non-bacterial pathogens, supporting the use of identification techniques such as qPCR. Ultimately, qPCR identification has helped avoid the inappropriate use of antibiotics.

Keyword: Chronic Sinusitis; qPCR; PCR; Bacteria; Identification; Culture; Sinonasal; Prevalence

Abbreviations

qPCR: Quantitative Polymerase Chain Reaction; PCR: Polymerase Chain Reaction; *M. pneumoniae: Mycoplasma pneumoniae*; *K. pneumoniae: Klebsiella pneumoniae*; *H. influenzae: Haemophilus influenzae*; *S. pneumoniae: Streptococcus pneumoniae; S. aureus: Staphylococcus aureus; M. catarrhalis: Moraxella catarrhalis; P. jirovecii: Pneumocystis jirovecii*; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; EBV-HHV4: Epstein Barr Virus; RSV B: Human Respiratory Virus B; HHV5: Human Herpesvirus 5/Cytomegalovirus; HHV6: Human Herpesvirus 6; UTM: Universal Transport Media; DNA: Deoxyribonucleic Acid; RNA: Ribonucleic Acid; cDNA: Complementary Deoxyribonucleic Acid; rpm: Revolutions Per Minute

Introduction

Chronic Sinusitis is a common diagnosis. Approximately 4.1 million patients have been primarily diagnosed with chronic sinusitis according to the CDC's 2016 national summary. The term chronic sinusitis refers to a condition in which the nasal passages are inflamed, often by an infection, for a period longer than 12 weeks. The four cardinal symptoms associated with chronic sinusitis include: facial pressure, nasal drainage, loss of smell, and nasal obstruction. Chronic sinusitis may present with the aforementioned symptoms or it may present concomitantly with acute sinusitis [1,2].

There are many laboratory techniques that are used to detect pathogens. Quantitative polymerase chain reaction (qPCR) is a real

time polymerase chain reaction and lab technique that can test for multiple targets in a patient sample. qPCR has three principal steps: denaturation, annealing, and elongation. In the first step, denaturation, the double stranded DNA (dsDNA) extracted from the patient sample is denatured by high temperatures causing the dsDNA to become two single strands of DNA. In the next step, annealing, the temperature is accordingly lowered so that DNA primers attach to each of the single strands of DNA. The final step of PCR, elongation, involves the DNA polymerase (usually taq polymerase due to its ability to withstand high temperatures) extending the DNA. This causes the single stranded DNA to become a new dsDNA. This cycle of steps repeats in a thermocycler where the temperature and length of cycles can be modified according to protocol. In probe based qPCR, a fluorophore and quencher is utilized in order to measure the amount of target in the DNA extracted via the amount of fluorescence signal emitted during the reaction [3].

When a patient presents with symptoms consistent with chronic sinusitis and/or acute sinusitis, an antibiotic can be empirically prescribed or testing can be performed to help identify the pathogen. However, the initial treatment of chronic sinusitis involves topical steroids, antibiotics, nasal irrigation, and analgesic medications. If symptoms persist, testing is performed to identify the specific pathogen [4]. Probe based qPCR allows for relatively rapid testing of patient samples which allows for directed antibiotic therapy. It also potentially decreases the use of antibiotics in cases where there is a viral pathogen. Currently, there are few studies which have used qPCR to examine pathogens in the setting of chronic sinusitis and compare it to established pathogens in the setting of chronic sinusitis.

Materials and Methods

A total of 333 patients, clinically diagnosed with chronic sinusitis between April 1st 2021 and July 7th 2021 at Princeton Eye and Ear, a community based ENT practice in New Jersey and Pennsylvania, USA were identified. Samples were obtained via intranasal swab directed to the affected sinus or nasal passage. Swabs were placed in a Universal Transport Medium (UTM) provided by Azer Scientific. In the PCR laboratory, the samples were stored at 2 - 8°C if they were being tested for in the next 48 hours. Samples that were not being tested in the next 48 hours were stored at -15°C to -25°C. Frozen samples were thawed and all samples were vortexed for 10 seconds to equilibrate to room temperature. The nucleic acid was extracted using the MagMAX Viral/Pathogen Ultra Nucleic Acid Isolation Kit. All reagents used in this kit were provided by ThermoFisher Scientific unless specified. A 96 deep well reaction plate contained the reagents used in the extraction process. 50 µL of the enzyme mix and 400 µL of the patient samples were dispensed in the 1st row (Row A) in each well. 1000 µL of a wash solution was dispensed in the 3rd Row (Row C) in each well. 1000 µL of an 80% ethanol solution was placed in the 5th row (Row E) in each well. 500 µL of an 80% ethanol solution was placed in the 7th row (Row G) in each well. The 9th row (Row H) contained just the tip comb. 60 µL of an elution buffer was then added to the 12 well elution strip. The 96 deep well reaction plate was placed in the KingFisher™ Duo Prime with a deep-well heat block to undergo 3 separate washes for nucleic acid extraction. During the enzyme treatment, a binding/bead mix which contained 530 µL of binding solution and 20 µL of nucleic acid binding beads plus 10% overage was prepared per well. When prompted by the instrument approximately 20 minutes after the start of the wash, the sample plate was removed from the instrument. 10 µL of Proteinase K was added to each sample in the sample plate. The binding/bead mix was then added to each well. The sample plate was then added back to the instrument, and the script was then resumed. Approximately 30 minutes after the addition of the binding/bead mix, the elution strip was removed from the instrument which contained the purified nucleic Acid.

After the nucleic acids were purified and extracted, they were prepped for qPCR via pre-amplification which helped boost the concentration of the 46 targets and 2 controls being tested for. During this step, a reverse transcriptase is used to convert RNA to cDNA for qPCR. The preamplification reaction mix consisted of 2.5 µL of TaqPath™ 1-Step RT-qPCR Master Mix, CG and 2.5 µL of TaqMan® PreAmp Pool, Respiratory Tract Microbiota, 4X. The components of the mix were prepped and mixed (plus 10% coverage) per sample. After the preamplification reaction mix was prepped, 5 µL of the mix and 5 µL of the extracted nucleic acid was added to each well on a MicroAmp Optical 96-Well Reaction Plate. The plate was sealed with an adhesive film and then gently vortexed for 10 seconds for mixing, and then centrifuged for 10 seconds to bring the contents to the bottom of each well. The plate was then placed in the 96-well standard block SimpliAmp Thermal Cycler for pre-amplification. After the pre-amplification occurred, the pre-amplified product was diluted for qPCR. The TaqMan Array Card used after pre-amplification was removed from storage and then allowed to

equilibrate to room temperature via vortex and centrifuge for 10 seconds each. The adhesive film was then removed from the plate. A 1:20 ratio dilution of the pre-amplified samples were then prepared in a new 96-well plate (e.x 2 µL preamplification solution, 38 µL nuclease-free water). The plates were then sealed with a new adhesive film and vortexed and centrifuged each for 10 seconds.

The preamplified samples were then tested for the 46 targets and 2 controls using probe based qPCR. Firstly, TaqMan Array Cards were allowed to equilibrate to room temperature. The bottle of TaqMan Fast Advanced Master Mix was then gently mixed. 20 µL of Diluted Pre Amplified Product, 50 µL of TaqMan Fast Advanced Master Mix, and 30 µL of Nuclease-free Water were then placed into a well, cortex, and centrifuged. 100 µL of the mixture then went into each port of the TaqMan Array Card. The card was then centrifuged at 1200 rpm (301 x g) for 1 minute two times. The card was then sealed using a TaqMan® Array Card Sealer. Lastly, the card was loaded into the QuantStudio™ 12K Flex Real-Time PCR System where the qPCR was performed. Data was then automatically generated and exported to an excel file for review and analysis.

The data from the qPCR lab was then sorted based on initial diagnosis as well as test site. A total of 468 patient samples were obtained from the time period of April 2021 to July 2021. Of those 468 patients samples, 333 were selected for the study as they were associated with chronic sinusitis. Of those 468 patient samples, a total of 187 viable patient samples that were diagnosed with chronic sinusitis were used to collect data on the bacterial and viral rates in the nasopharyngeal flora, and a total of 146 viable samples were used to collect data on the bacterial and viral rates in the sinus flora.

Results

Out of 333 samples from patients identified as having chronic sinusitis, 187 were sampled from the nasopharynx and 146 were from the sinus.

	Sample Size	*M. pneumoniae*	*K. pneumoniae*	*H. influenzae*	*S. pneumoniae*	*S. aureus*	*M. Catarrhalis*
Nasopharyngeal	187	0.53% (1)	8.56% (16)	6.42% (12)	0.53% (1)	18.72% (35)	1.07% (2)
Sinus	146	1.37% (2)	8.22% (12)	5.47% (8)	2.05% (3)	23.97% (35)	3.42% (5)
Sinonasal (combined)	333	0.90% (3)	8.41% (28)	6.00% (20)	1.20% (4)	21.02% (70)	2.10% (7)

Table 1: Bacterial pathogen positivity rates.

	Sample Size	*P. jirovecii*	SARS-CoV-2	Adenovirus	EBV-HHV4	Rhinovirus	Coronavirus OC43	Parainfluenza 3	RSV B	Bocavirus	HHV5	HHV6
Nasopharyngeal	187	0.53% (1)	0.53% (1)	2.67% (5)	6.42% (12)	5.88% (11)	0.53% (1)	1.07% (2)	0.00% (0)	1.07% (2)	1.07% (2)	3.74% (7)
Sinus	146	0.68% (1)	0.68% (1)	4.11% (6)	10.87% (15)	7.53% (11)	4.11% (6)	1.37% (2)	0.68% (1)	0.68% (1)	1.37% (2)	6.16% (9)
Sinonasal (combined)	333	0.60% (2)	0.60% (2)	3.30% (11)	8.11% (27)	6.60% (22)	2.10% (7)	1.20% (4)	0.30% (1)	0.90% (3)	1.20% (4)	4.80% (16)

Table 2: Non-bacterial pathogen positivity rate.

Table 1 and 2 demonstrate the most common bacterial and non-bacterial pathogens (respectively) identified by qPCR broken down by sample site (i.e. sinus or nasopharynx) The three most prevalent bacteria in the nasopharyngeal flora were *Staphylococcus aureus* with 35 positive samples and a positive identification rate of 18.72%, *Klebsiella pneumoniae* with 16 positive samples and a positive identification rate of 8.56%, and *Haemophilus influenzae* which had 12 positive samples and a positive identification rate of 6.42%. The three most prevalent bacteria in the sinus flora were also *Staphylococcus aureus* with 35 positive samples and a positive

identification rate of 23.97%, *Klebsiella pneumoniae* with 12 positive samples and a positive identification rate of 8.22%, and *Haemophilus Influenzae* with 8 positive samples and a positive identification rate of 5.47%. The three most prevalent bacteria across all sites were *Staphylococcus aureus* (Cumulative: 70 positive samples and 21.02% identification rate), *Klebsiella pneumoniae* (Cumulative: 28 positive samples 8.41% positive identification rate) and *Haemophilus influenzae* (Cumulative: 20 positive samples and 6.00% positive identification rate).

The three most prevalent non-bacterial pathogens in the nasopharyngeal flora were EBV-HHV4 with 12 positive samples and a positive identification rate of 6.42%, Rhinovirus with 11 positive samples and a positive identification rate of 5.88%, and HHV6 which had 7 positive samples and a positive identification rate of 3.74%. The three most prevalent non-bacterial pathogens in the sinus flora were also EBV-HHV4 with 15 positive samples and a positive identification rate of 10.87%, Rhinovirus with 11 positive samples and a positive identification rate of 7.53%, and HHV6 which had 9 positive samples and a positive identification rate of 6.16%. The total percentage of non-bacterial pathogens found of the 333 samples was 29.73% (Cumulative: 99 positive non-bacterial positive samples). The three most prevalent non-bacterial pathogens found in both flora were EBV-HHV4 (Cumulative: 27 positive samples and 8.11% identification rate), Rhinovirus (Cumulative: 20 positive samples and 6.60% positive identification rate), and HHV6 (Cumulative: 16 positive samples and 4.80% positive identification rate). Furthermore, a total of 99 samples were found to be non-bacterial indicating that a total of 29.73% of the samples were either viral or fungal.

Discussion

Traditionally, the most common bacterial pathogens implicated in chronic sinusitis have been *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pyogenes* as reported by Brooks and Wald [5,6]. These studies used traditional culture plating techniques. Our study is unique in that we used qPCR data from chronic sinusitis patients to determine which pathogens were present. In contrast to Brooks and Wald and others, our qPCR data identified a different grouping of top three pathogens. This difference may indicate that certain broad-spectrum antibiotics used to empirically treat sinusitis may not be appropriate. Furthermore, many cases of suspected bacterial infections were actually due to non-bacterial illnesses. According to the qPCR data, a total of 99 samples (or 29.73%) were either viral or fungal, indicating that the use of an identification technique such as qPCR allowed for 29.73% of the patients used in this study to potentially avoid taking an unnecessary dose of antibiotics. Furthermore, both Epstein-Barr Virus and Rhinovirus had a higher prevalence rate of all the flora than the third most prevalent bacteria, *H. influenzae*. This indicates that some of the infections that seem as bacterial may in fact be viral and non-bacterial.

Treating a viral infection with antibiotics can lead to negative consequences for the patients such as bacteria developing antibiotic resistance. According to the CDC, the majority of acute sinus infections occur from viral pathogens indicating that the majority of patients that have sinusitis require a treatment for viral, and not bacterial infections [7]. The prime treatment options for viral infections associated with acute and chronic sinusitis are supportive or adjunctive treatment that often include: bed rest, decongestants, and analgesic medications if the patient is experiencing severe pressure due to congestion [1].

When treating viral infections, distinguishing the cause of the infection becomes even more vital as there is a clear correlation between antimicrobial resistant bacteria and antibiotic usage [8]. Therefore, if proper identification of bacteria or viral pathogens is not done, improper antibiotic usage may lead to an increase in antibiotic resistance in bacteria associated with chronic sinusitis. This phenomenon is more problematic considering that one of the recent bacteria that has been identified as increasingly resistant to antimicrobial treatments is *Streptococcus pneumoniae* [8]. Proper identification of pathogens may also help decrease pain induced by antibiotic treatment as there has been an established correlation between long-term antibiotic in chronic rhinosinusitis and adverse side effects such as diarrhea, headaches, changes to systemic microbiomes, nausea, and the development of resistant bacteria [9].

When discussing the importance of proper identification in chronic sinusitis treatment, it is also important to identify the differences among different types of identification techniques as some may be more sensitive or produce more consistent results. A previous study was conducted which compared the sensitivity and specificity differences among traditional PCR and qPCR in identifying Pseudomonas aeruginosa in cystic fibrosis patients. The results of the study indicated that qPCR was just as effective as traditional PCR and there is no significant difference in specificity between PCR and qPCR [10]. However, due to the lack of current studies that focus on different identification techniques for acute and chronic sinusitis, further research should be conducted on the differences in sensitivity between traditional culturing methods, PCR, and qPCR in the context of acute and chronic sinusitis.

Furthermore, it is important to note that bacterial infections associated with chronic sinusitis often contain both aerobic and anaerobic bacteria [2]. The qPCR data does not discriminate between aerobic and anaerobic Streptococcus pneumoniae. There may be a difference in bacterial infections that contain anaerobic bacteria and those that contain aerobic bacteria. Further research is required as it would elucidate more about the best treatment options available for chronic sinusitis infections.

Lastly, there is little research on quantified patient satisfaction and different identification techniques. In an effective treatment of chronic sinusitis, it is imperative that the patient understand and trust the diagnosis of the physician. Through the use of an identification technique such as qPCR, the patient can more easily trust the diagnosis as well as treatment options. This has significant implications as a positive correlation has been established between trust in the healthcare professional and treatment outcomes [11]. Further research should be conducted on the possible relationship between different clinical identification techniques and patient satisfaction.

Conclusion

Ultimately, the results obtained from qPCR data indicates that infections in chronic sinusitis may not be caused by microbiology thought to be commonly associated with chronic sinusitis. Specifically, the three most prevalent bacterial pathogens according to the qPCR data did not correspond with the three most prevalent bacterial pathogens in medical literature. Viral pathogens were very prevalent (29.73%) which supports the importance of identification techniques as viral infections associated with chronic sinusitis should be treated differently than bacterial infections. qPCR is just as effective as standard culturing techniques as a literature review revealed no significant difference in the amount of sensitivity between the two identification techniques. Lastly, because qPCR provides the patient and the physician with empirical data that can support the diagnosis, a correlation between the use of identification techniques such as qPCR and patient satisfaction may exist, but further research is required.

Conflict of Interest

Princeton Eye and Ear has an in-house qPCR laboratory that is CLIA certified by the FDA.

Bibliography

1. Bull PD. "Diseases of the Ear, Nose and Throat". 9th edition, Blackwell Science (2002).
2. Dhingra PL., *et al*. "Diseases of Ear, Nose, and Throat". 6th edition., Elsevier (2014).
3. Peirson Stuart N and Jason N Butler. "Quantitative polymerase chain reaction". *Methods in Molecular Biology* 362 (2007): 349-362.
4. Piromchai Patorn., *et al*. "Chronic rhinosinusitis and emerging treatment options". *International Journal of General Medicine* 6 (2013): 453-464.
5. Brook Itzhak. "Microbiology of sinusitis". *Proceedings of the American Thoracic Society* 8.1 (2011): 90-100.
6. Wald ER. "Microbiology of acute and chronic sinusitis in children". *The Journal of Allergy and Clinical Immunology* 90.3-2 (1992): 452-456.
7. "Sinus Infection (Sinusitis)". Centers for Disease Control and Prevention, Centers for Disease Control and Prevention (2019).
8. Furuya E and Lowy F. "Antimicrobial-resistant bacteria in the community setting". *Nature Reviews Microbiology* 4 (2006): 36-45.
9. Barshak Miriam Baron and Marlene L Durand. "The role of infection and antibiotics in chronic rhinosinusitis". *Laryngoscope Investigative Otolaryngology* 2.1 (2017): 36-42.
10. Deschaght Pieter., *et al*. "Comparison of the sensitivity of culture, PCR and quantitative real-time PCR for the detection of *Pseudomonas aeruginosa* in sputum of cystic fibrosis patients". *BMC Microbiology* 9.244 (2009).
11. Birkhäuer Johanna., *et al*. "Trust in the health care professional and health outcome: A meta-analysis". *PloS one* 12.2 (2017): e0170988.