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Case Report

Nonencapsulated *Streptococcus pneumoniae* as a cause of chronic adenoiditis

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ABSTRACT

Streptococcus pneumoniae is an important human pathogen. To cause disease, it must first colonize the nasopharynx. The widespread use of pneumococcal-conjugate vaccines which target the capsular polysaccharide has led to decreased nasopharyngeal carriage of vaccine serotypes, but a concomitant increase in carriage of non-vaccine serotypes and nonencapsulated *S. pneumoniae* (NESp). Some NESp express pneumococcal surface protein K (PspK), a virulence factor shown to contribute to nasopharyngeal colonization.

We present the case of a child with chronic adenoiditis caused by a PspK$^+$ NESp. We tested the pneumococcal isolate, designated C144.66, for antimicrobial resistance, the presence of the *pspK* gene and the expression of PspK. Sequence typing and genome sequencing were performed. C144.66 was found to be resistant to erythromycin and displayed intermediate resistance to penicillin and trimethoprim/sulfamethoxazole. C144.66 has the *pspK* gene in place of the capsule locus. Additionally, PspK expression was confirmed by flow cytometry. NESp are a growing concern as an emerging human pathogen, as current pneumococcal vaccines do not confer immunity against them. An inability to vaccinate against NESp may result in increased carriage and associated pathology.

Introduction

Streptococcus pneumoniae is the most common bacterial cause of acute otitis media (AOM) and pneumonia in children [1,2]. The pneumococcus is an important cause of bacterial rhinosinusitis as well as invasive disease such as sepsis and meningitis [3]. Historically, pneumococci that cause disease are encapsulated. Therefore, the 13-valent pneumococcal conjugate polysaccharide vaccine (PCV) and the 23-valent pneumococcal polysaccharide vaccine (PPV) target the specific capsular serotypes known to cause the majority of human disease [4]. Introduction of the PCV and PPV has led to decreased nasopharyngeal colonization of pneumococcal vaccine serotypes in humans, with a concomitant increase in isolation of both non-vaccine capsular serotypes and nonencapsulated *S. pneumoniae* (NESp) [5,6]. There has, additionally, been an increase in prevalence of disease caused by NESp [7–15].

Some NESp isolates express pneumococcal surface protein K (PspK) [12]. PspK has been shown to aid colonization of the nasopharynx [16]. We have previously shown that PspK increases adhesion of NESp to human epithelial cells and enhances AOM in the chinchilla model [17]. Expression of PspK in an avirulent NESp was sufficient to induce pathogenicity, and deletion of PspK from a virulent strain decreased the bacterial burden in chinchillas [17]. We present a case of a child with chronic adenoiditis found to have PspK$^+$ NESp from a sinus culture at adenoidectomy. To our knowledge, this is the first report of a PspK$^+$ NESp isolate from the United States.

Case report

A 2-year-old male presented to otolaryngology clinic for evaluation of chronic adenoiditis. Patient had a 6 month history of nasal congestion with a clear mucosal discharge and was a chronic mouth breather since birth. Of note, patient was refractory to several trials of antimicrobials. Enlarged adenoids were appreciated on physical exam. The patient's family was counseled and elected to undergo adenoidectomy, which was completed without complication. During the procedure, bilateral maxillary

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Table 1
Antimicrobial susceptibility of *S. pneumoniae* C144.66.

Antibiotic	Resistance
Erythromycin	Resistant
Penicillin G	Intermediate
Trimethoprim/sulfamethoxazole	Intermediate
Levofloxacin	Susceptible
Tetracycline	Susceptible
Vancomycin	Susceptible
Cefotaxime	Susceptible
Ceftriaxone	Susceptible
Chloramphenicol	Susceptible

sinuses were aspirated, both revealing sanguineous fluid that was sent for aerobic and anaerobic culture. The sinuses were then irrigated with saline. The right sinus culture returned with no growth after 48 h, and the left sinus culture had moderate growth of *S. pneumoniae*. Anaerobic cultures showed no growth. After 24 h observation, the patient was discharged. After discharge, the patient was lost to follow up.

Antimicrobial sensitivities of the isolate, complete with minimum inhibitory concentrations, showed erythromycin resistance, as well as intermediate resistance to both penicillin G and trimethoprim/sulfamethoxazole (Table 1).

The clinical isolate was designated C144.66. Genomic DNA was isolated from C144.66 using Qiagen DNeasy kit. Polymerase chain reaction amplification was performed with primers for *pspK*, LSM826 (5′-CCCGGGGCATGAATAATAAGAATATCA-3′) and LSM827 (5′-GAATTCGCCTAATTTTTATGTTTAACAAATG-3′) and for the conserved capsule gene *cpsA* (forward: 5′-GCAGTACAGCAGTTTGTTGGACTGACC-3′ and reverse: 5′-GAATATTTTCATTATCAGTCCCAGTC-3′). PCR, Western blot analysis, and flow cytometry were completed through standard methods [16,18]. Polymerase chain reaction analysis compared to an encapsulated strain showed that C144.66 lacked the highly conserved first gene in the capsule locus, *cpsA* (Fig. 1). Further PCR analysis demonstrated the presence of *pspK*. Expression of PspK was confirmed through Western blot (data not shown), and flow cytometry comparing C144.66 to a known PspK positive strain (Fig. 2).

To further characterize C144.66, multilocus sequence typing was performed as previously described [19]. Additionally, genome sequencing was carried out on C144.66. Multiplexed paired-end libraries (2× 150 bp) were prepared using a Nextera XT DNA sample preparation kit (Illumina). Sequencing was done with an Illumina MiSeq. CLC Genomics Workbench v6.0 software was used for quality trimming of the reads and *de novo* assembly. Rapid Annotations using Subsystems Technology (RAST) pipeline

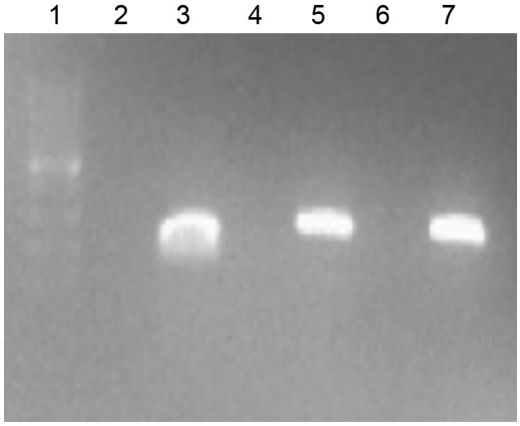
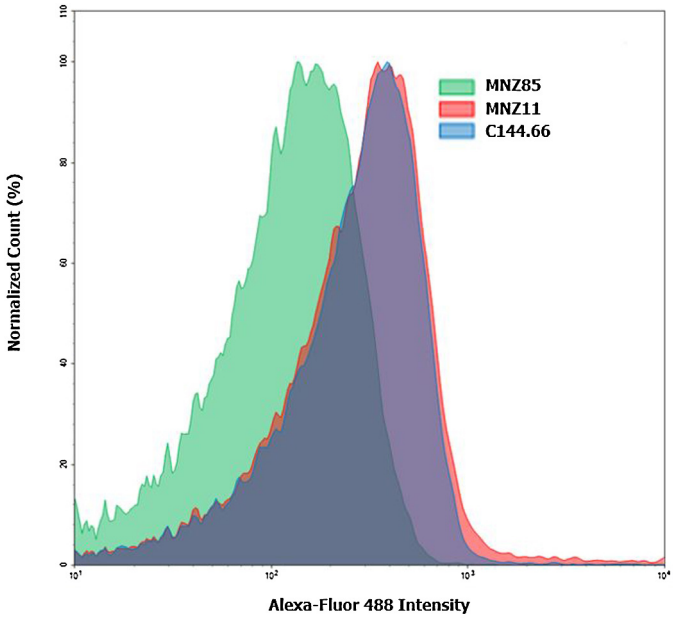


Fig. 1. PCR analysis of C144.66. PCR analysis of strains MNZ11 (*cpsA*$^-$, *pspK*$^+$) D39 (*cpsA*$^+$, *pspK*$^-$), and C144.66 (*cpsA*$^-$, *pspK*$^+$), respectively, for *cpsA* (lanes 2–4) and *pspK* (lanes 5–7). Lane 1 is a 1-Kb DNA ladder.



Sample	Gate	Count	% Single Cells	% All	Mean X
MNZ85	Single Cells	47,857	100.00 %	95.71 %	127
MNZ11	Single Cells	48,857	100.00 %	97.71 %	509
C144.66	Single Cells	49,069	100.00 %	98.14 %	375

Fig. 2. Flow cytometry for the presence of PspK. For analysis 1×10^7 log phase pneumococci were incubated with a mouse anti-PspK antiserum. A biotinylated anti-mouse secondary antibody was detected by streptavidin conjugated to Alex Flour 488. A representative histogram is shown. The negative control is MNZ85 which lacks PspK.

determined gene prediction and annotation for the assembled contigs.

The sequence type (ST) of C144.66 was 9570. A draft genome sequence of C144.66 was generated to provide further information about the strain. Both approaches confirmed that C144.66 was a PspK$^+$ NESp.

We previously demonstrated that C144.66 can cause AOM in the chinchilla model [17]. To extend our observation, we used a mouse model of colonization. C57/BL6 mice were anesthetized with isoflurane and infected by inoculation with 1×10^7 colony forming units of C144.66 into the nasal passage. After 5 days, the mice were euthanized. Nasopharyngeal washes as well as nasal

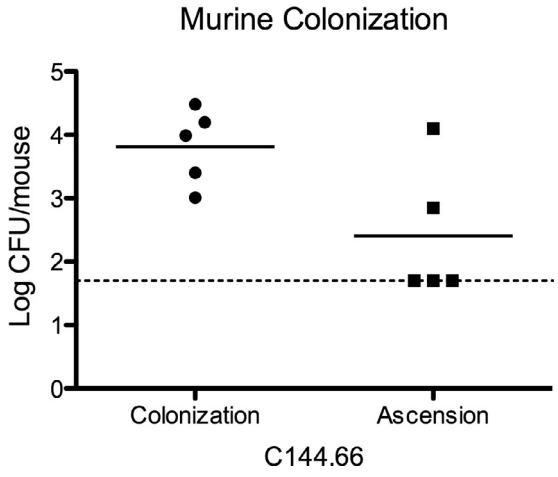


Fig. 3. Colonization and ascension of C144.66 in a murine model. After intranasal inoculation of C144.66, colonization was achieved in all mice. Ascension into the middle ear occurred in two of the five mice. The dash line is the lower limit of detection.

passages and middle ears were collected to estimate the bacterial load by plating on blood agar with 200 μg/mL gentamicin. All mice were found to have nasopharyngeal colonization by C144.66; additionally, C144.66 was found to have ascended to the middle ear in a subset of the mice (Fig. 3).

Conclusion

This case of chronic adenoiditis caused by NESp reinforces the growing concern of infection caused by pneumococcal strains not covered by the current vaccines. With widespread use of PCV and PPV, human nasopharyngeal colonization by NESp will likely continue to increase.

Since pneumococcal colonization is a prerequisite for disease, it is likely that with increased carriage of NESp there will be increased disease burden. A recent study from Japan found that 6.4% of AOM isolates were NESp with 4.7% of the NESp being PspK positive [20]. AOM is the most common indication for antimicrobial prescription for children in the United States, and has a financial burden in excess of $2.8 billion, a number likely to grow with increasing infections with non-vaccine serotypes and NESp [21]. After widespread use of the 7-valent PCV (prior to the introduction of the 13-valent PCV), new serotypes, including capsular serotype 19A, emerged; some strains were multidrug resistant [22,23]. Studies have shown that NESp can contain multiple antimicrobial resistance genes [24–26]. Thus, co-colonization of NESp with encapsulated pneumococci could lead to increased antimicrobial resistance among encapsulated pneumococci. Additionally, there is concern for the emergence of novel serotypes as well as proliferation of NESp with novel proteins, such as PspK, which are not covered by currently available pneumococcal vaccines.

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