Clinical Infectious Diseases
BRIEF REPORT

Endocarditis Caused by Nontypeable *Streptococcus pneumoniae*

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The *Streptococcus pneumoniae* capsule is regarded as indispensable in bacteremia. We report an infant with a ventricular septal defect and infective endocarditis caused by nontypeable *S. pneumoniae*. In-depth investigation confirmed a deficient capsule yet favored pneumococcal fitness for causing infective endocarditis, rather than a host immune disorder, as the cause of infective endocarditis in this case.

Keywords. infective endocarditis; *Streptococcus pneumoniae*; bacterial polysaccharide capsule; pathogenesis.

We encountered a case of infectious endocarditis caused by a remarkable bacterial variant. For *Streptococcus pneumoniae* the most well-known virulence factor for causing invasive disease is its polysaccharide capsule. In this case however, the capsule of the pneumococcal blood isolate was nontypeable. Here we report on further investigation of host and bacterium to explain the pathogenesis of this exceptional case.

An 11-month-old girl with a history of a perimembranous cardiac ventricular septal defect endured a protracted upper respiratory tract infection. After 1.5 weeks she became more severely ill, with fever up to 40°C and decreased appetite. At clinical examination on referral to the hospital, she appeared septic. The blood culture collected before initiation of intravenous ceftriaxone treatment yielded *Streptococcus pneumoniae*. Echocardiography on day 4 of admission showed an 8 × 5-mm vegetation on the jet lesion of the right ventricular outlet tract's free wall. The fourth minor Duke's criterion that further confirmed infective endocarditis, was an ultrasound examination that demonstrated likely splenic infarction, aside from the predisposing heart condition, fever, and positive blood culture. Antibiotic therapy was changed to intravenous benzylpenicillin (100 000 IU/kg 4 times daily) plus intravenous gentamicin (7 mg/kg once daily). The latter was stopped when the pneumococcal isolate was confirmed to be penicillin susceptible (minimum inhibitory concentration, 0.008 mg/L). Fever ceased within 48 hours of antibiotic treatment, and follow-up blood cultures remained negative. The vegetation decreased in size and was cleared by the time intravenous antibiotic treatment was discontinued 4 weeks after the first negative blood culture.

Surprisingly, the pneumococcal blood culture isolate, serotyped by Quellung reaction to antisera at the Netherlands Reference Laboratory of Bacterial Meningitis, appeared nontypeable. Nontypeable pneumococci are seldom isolated from blood cultures; in Dutch national surveillance from 2007 to 2016, only 1 of 7157 pneumococcal isolates from blood or cerebrospinal fluid was nontypeable. In contrast, nontypeable isolates are frequently detected in nasopharyngeal carriage [1]. The pneumococcal capsule is presumed to be crucial for survival in the bloodstream [2]. This raised the question of whether the pneumococcal isolate of concern was truly devoid of a polysaccharide capsule, and if so, whether the infected child had an underlying immune disorder. The experimental study procedures performed are detailed in Supplementary File 1.

Pneumococcal Fitness Despite Defect in Capsule Biosynthesis

Whole-genome sequencing of the pneumococcal endocarditis isolate revealed a capsular locus sequence characteristic for serotype 38. All genes essential for encoding the serotype 38 capsule were intact and shared >99% identity with the reference serotype 38 sequence, except for a deletion resulting in a premature stop codon in *wcyV* (Figure 1A). This pneumococcal gene encodes the glycosyl transferase that sequentially links sugars to generate the capsular polysaccharide repeat unit. Compared with 2 additional serotype 38 blood culture isolates with intact wild-type *wcyV*, transmission electron microscopy confirmed significant capsular loss (Figure 1B–1G), resulting in a nontypeable phenotype. Although the endocarditis isolate was deficient in capsule, growth on blood agar and in nutrient-rich medium appeared equal to that of the wild-type isolates.

No Overt Immune Disorder

Until the endocarditis event, the patient had appeared healthy, without symptoms of her ventricular septal defect. Antibiotic prophylaxis to prevent endocarditis had not been indicated. She

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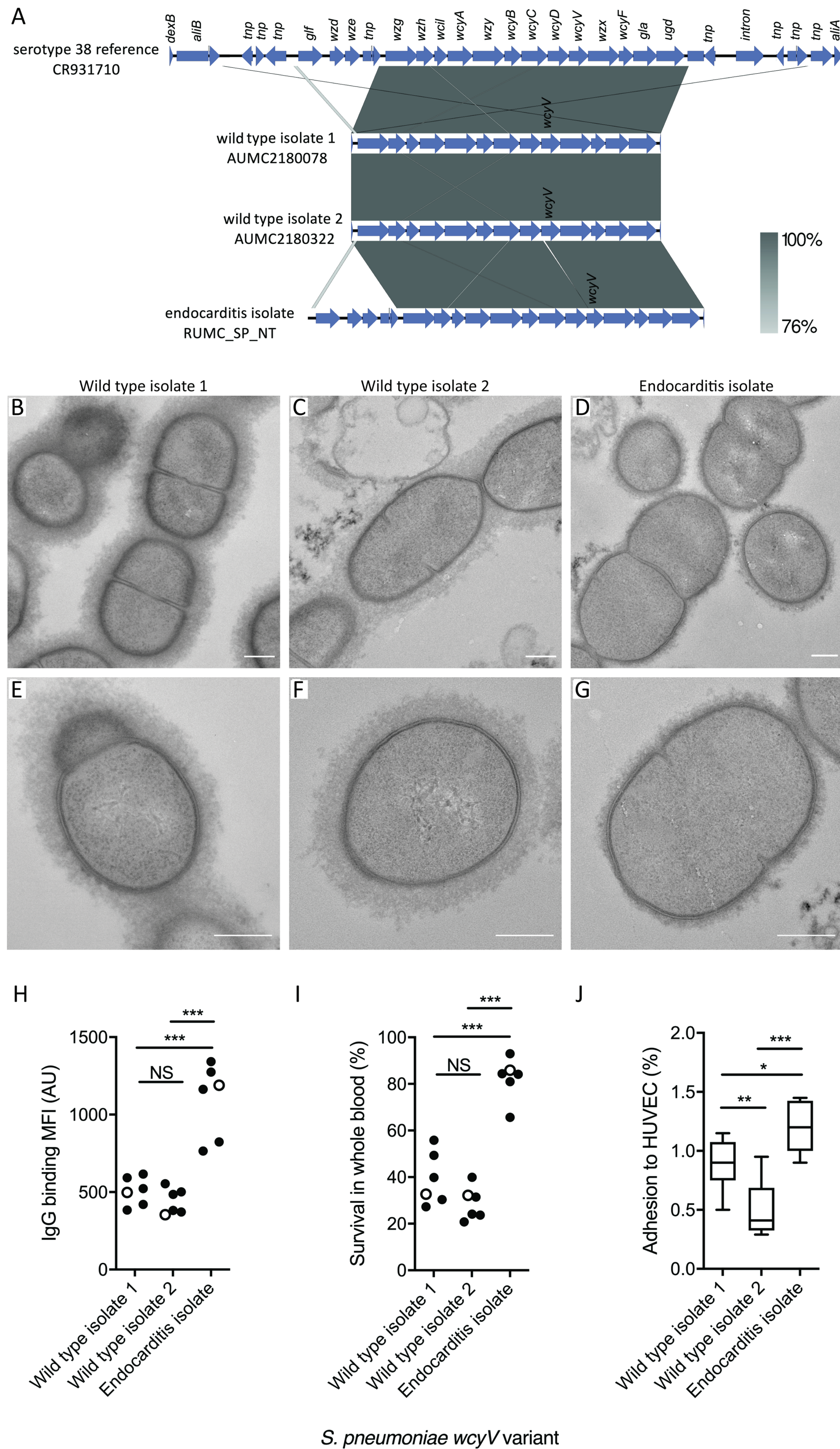

Figure 1. Experimental examination of the pneumococcal endocarditis isolate compared with 2 *Streptococcus pneumoniae* serotype 38 *wcyV* wild-type blood isolates. *A,* Pneumococcal capsule encoding sequence. Capsular biosynthesis genes of the pneumococcal study isolates compared with the *S. pneumoniae* serotype 38 *cps* reference sequence. Genes are represented as arrows on the forward and reverse strands, with functional annotation indicated above. Gray blocks indicate regions of sequence similarity, and the white gap (ie, no similarity) within *wcyV* indicates a 92–base pair deletion in the *wcyV* gene of the endocarditis isolate compared with the wild-type *wcyV* sequence present in the 2 selected comparator blood isolates. *B–G,* Pneumococcal capsular phenotype. Polysaccharide capsule surrounding the cells of the pneumococcal study isolate as visualized by transmission electron microscopy of ultrathin sections of chemically fixed and resin-embedded cells; scale bars represent 200 nm. The endocarditis isolate (*D, G*) demonstrates loss of capsule compared with wild-type isolates 1 (*B, E*) and 2 (C, *F*). *H–J,* Host-pathogen interaction ex vivo. *H,* Immunoglobulin G (IgG) deposition to the bacterial surface determined by flow cytometry. *I,* Percentage survival of the *S. pneumoniae* isolates in whole blood. *J,* Adhesion of *S. pneumoniae* isolates to human umbilical vein endothelial cells (HUVECs) (n = 8). Closed circles represent plasma specimens from healthy controls; open circles, plasma from the patient with endocarditis. For each plasma sample, the average of 4 experiments was used to determine statistical significance. Abbreviations: AU, arbitrary units; NS, not significant. *$P < .05$; **$P < .01$; ***$P < .001$ (1-way analysis of variance with Tukey post hoc test).

had received both doses of the 10-valent pneumococcal conjugate vaccine at 2 and 4 months of age, according to the National Immunisation Program schedule. Her total leukocyte and neutrophil counts were within normal ranges, her circulating antibody levels were within the normal range for her age at the time of diagnosis (immunoglobulin [Ig] G, 10.57 g/L; IgA, 0.70 g/L; and IgM, 1.98 g/L) and remained stable during the 2-year follow-up period. She had an adequate antibody response to a booster dose of 10-valent pneumococcal conjugate vaccine at 11.5 months of age. Complement screening showed normal alternative pathway activity (100%; reference values, 67%–133%) and classic pathway (80%; reference, 67%–149%). Screening for cellular immunity disorders revealed normal T-cell, B-cell, and natural killer cell subsets (cell counts: $CD3^+$ T cells, 3.3×10^9/L; $CD4^+$ T cells, 1.4×10^9/L; $CD8^+$ T cells, 0.5 × 109/L; total $CD19^+$ B cells, 0.8×10^9/L; and $CD3^+CD16^+CD56^+$ natural killer cells, 0.214×10^9/L) compared with children 1–9 years of age. Furthermore, whole-exome sequencing (gene package version DG 2.15) could not identify any known primary immunodeficiency. To summarize, no overt immune disorder was identified, and a 4-year follow-up remained completely uneventful.

Ex Vivo Host-Pathogen Interaction

To determine immune recognition of this pneumococcal isolate by the patient, we measured the degree of antibody deposition on the bacterial surface and bacterial survival in blood using plasma samples from the recovered patient and 5 healthy donors. We observed no deficit in the patient's IgG deposition on the endocarditis isolate or wild-type isolates, compared with controls (Figure 1H). Although the endocarditis isolate attracted more antibody deposition than the wild-type isolates (Figure 1H*)*, it showed consistently better survival in blood (Figure 1I). In addition, adhesion to endothelial cells was increased for the endocarditis isolate compared with the 2 wild-type isolates (Figure 1J). These results further favor bacterial fitness in blood, rather than a host immune disorder, as an explanation for this case of infective endocarditis by nontypeable *S. pneumoniae*.

DISCUSSION

To our knowledge this is the first description of endocarditis caused by nontypeable *S. pneumoniae*. Beyond a minor congenital heart anomaly, we found no underlying immune deficiency explaining the course of this infection caused by a pneumococcus lacking its presumed major virulence factor for invasive disease: a thick polysaccharide capsule. This case demonstrates how an exceptional bacterial variant turns out to be well equipped for seeding a particular site of infection.

S. pneumoniae is infrequently identified as the cause of infective endocarditis compared to other streptococcal species [3]. Its frequency may be somewhat underestimated owing to a lack of typical cues for suspecting infective endocarditis. Pneumococcal endocarditis affects native valves and mostly occurs together with more expected infection foci, like meningitis or pneumonia [4]. In addition, the autolytic properties of pneumococcus prevent blood cultures from continuing to be positive once administered antibiotics are present in the culture medium [5]. Still, for *S. pneumoniae* a clearly lower proportion (1%) of bacteremic episodes is associated with infective endocarditis [3], compared with 21% in community-acquired *Staphylococcus aureus* bacteremia [6]. Another distinct rarity in this clinical case was a bloodstream infection caused by nontypeable *S. pneumoniae*, as the polysaccharide capsule is thought to be indispensable for avoiding immunological recognition and rapid clearance from the bloodstream [7]. After multidisciplinary consultation, we were convinced that it was in this young patient's interest to perform nonroutine microbiological examinations, to estimate the likelihood of an underlying immune disorder that allowed this infection to occur.

The molecular basis of nontypeable pneumococci that have been isolated from patients with invasive disease mostly involved present but defective capsule genes. In several cases the *wchA* gene was affected, which codes for the transferase that couples the initial sugar to a lipid carrier [7]. In the current case, there was a defect in *wcyV*, whose encoded glycosyl transferase adds a sequential sugar to the precursor chain within the bacterial cytoplasm [8]. Alterations in this polysaccharide repeat unit generally form noncompatible substrates for downstream enzymes. Our imaging of the endocarditis isolate indeed suggests that the altered repeat unit was still translocated to the extracellular space but no longer polymerized. A striking analogy has been reported for porcine endocarditis, where 32% of *Streptococcus suis* blood isolates appeared to be unencapsulated, mainly owing to various modifications in the glycosyl transferase gene *cps2F* the functional homologue of *wcyV* [9]. The global context of the pneumococcal capsule variant studied here is described in Supplementary File 1. Although one similar variant was captured in The Gambia, seeding invasive disease probably requires an encounter with a distinct host.

The pathogenesis of infective endocarditis involves damaged endocardium that is colonized by bacteria from the bloodstream, and subsequent development of an infected vegetation that can potentially disseminate and cause septic emboli [10]. However, from a bacteriological perspective, these transitions between host tissues are not that straightforward. Interaction with human epithelial cells requires *S. pneumoniae* to expose proteins from underneath the capsule [11], whereas in the bloodstream pneumococci need their polysaccharide capsule to protect themselves from human complement deposition and rapid clearance. This parallel need for and peril of decapsulation in establishing endocarditis also holds true for other bacteria, like *S. aureus* and *S. suis* [9, 12, 13].

In this report, we confirm enhanced adhesion of the sparsely encapsulated pneumococcus to endothelial cells. However,

while human endocarditis cases due to unencapsulated variants of other bacteria have been described before, it is still unsure how these endocarditis isolates manage to survive the blood circulation. One possible explanation could be the occurrence of variable encapsulation states during infection, which have been observed in vivo [14]. Mechanisms that mediate differential capsular expression in *S. pneumoniae* comprise genotypic variants, epigenetic regulation, and introns—a particular feature in the capsule operons of serotype 38 pneumococci [8, 15]. This remarkable case reinforces attention to capsule density in gram-positive bacteria as a mediator of survival in blood as well as interaction with cardiac endothelium, which are both required for the establishment of infective endocarditis.

Clinicians are trained to identify assumed nonvirulent pathogens in invasive disease as warning sign for host defects or primary immunodeficiencies. The current case highlights the growing evidence that in a unique host-pathogen interaction the specifics of the host are as important as those of the pathogen. It underscores the efforts needed to broaden our knowledge of microbial variants that mediate altered pathogenicity.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Note

Potential conflicts of interest. N. M. v. S. reports that patent WO 2013/020090 A3 has been licensed by the company Vaxcyte, generating royalties when milestones are reached and royalties from a patent on vaccine development against *Streptococcus pyogenes,* not part of the work submitted here (licensee University of California San Diego inventors, N. M. v. S. and Victor Nizet). N. M. v. S. also reports consulting fees from MSD (fee for service paid directly to the institution, related to pneumococcal invasive disease, and consulting fee for an expert panel) and GlaxoSmithKline (fee for service paid directly to the institution, related to pneumococcal invasive disease); payment or honoraria from MSD for expert meeting contribution on pneumococcal disease); and personal stocks from GenMab, Bank of America, and exchange-traded funds. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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