



Case report

Spontaneous pneumococcal peritonitis diagnosed by qPCR

Daniel Jarovsky^{a,*}, Thais Grodzicki Ambrus^a, Mariana Galvão Gurgel^a,
 Marcelo Jenné Mimica^a, Mariana Volpe Arnoni^b, Flávia Jacqueline Almeida^a,
 Marco Aurélio Palazzi Sáfy^a, Eitan Naaman Berezin^a

^aPediatric Infectious Diseases Unit - Santa Casa de São Paulo, São Paulo, Brazil

^bHospital Infection Control Unit - Santa Casa de São Paulo, São Paulo, Brazil

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ABSTRACT

Spontaneous bacterial peritonitis is an uncommon manifestation of invasive pneumococcal disease and frequently occurs when an underlying hepatic disease is present. Bacterial identification through culture can be particularly challenging in patients with prior or concurrent antimicrobial use. DNA amplification detects very few copies of target DNA under ideal conditions in CSF or pleural effusion and, therefore, can be useful in selected infections. A culture-negative spontaneous pneumococcal peritonitis without preexisting peritoneal disease diagnosed by qPCR is herein described.

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Introduction

Invasive pneumococcal disease (IPD) is a major cause of morbidity and mortality worldwide despite the availability of antimicrobial therapy and vaccines. Spontaneous bacterial peritonitis (SBP) is an uncommon manifestation of IPD and frequently occurs when an underlying hepatic disease is present [1]. Although *S. pneumoniae* has long been a well-recognized gram-positive organism causing SBP in adults, very few recent reports describe the disease in children. Moreover, bacterial identification through culture and serotyping can be particularly challenging in patients with prior or concurrent antimicrobial use. Despite the growing use of real-time polymerase chain reaction (also called quantitative PCR or qPCR) to increase detection of *Streptococcus pneumoniae* (*Spn*) in CSF and pleural effusion, there are few descriptions of its use in peritoneal fluid. A culture-negative spontaneous pneumococcal peritonitis without preexisting peritoneal disease diagnosed by qPCR is herein described.

Presentation of case

A 2-year old girl was admitted to our service for a 3-day history of abdominal distension, high and recurrent fever (39.5 °C),

vomiting and anorexia. She was severely dehydrated and hypoactive, presenting with tachycardia (CR = 160 bpm), tachypnea (RR = 60 ipm), and a distended and painful abdomen at examination, without peritoneal inflammation signs. The additional parameters of the clinical examination were unremarkable. She had no underlying conditions or previous hospitalization. However, she took a vegan diet, presented with moderate underweight (8.7 kg - Z score = -2.47; BMI index = 12 kg/m² - Z score = -3.38), and an incomplete 2-dose (2 primary doses without booster) pneumococcal immunization series (the 10-valent pneumococcal conjugate vaccine was in use in Brazil at that moment as a 3 + 1 dose schedule - at 2, 4 and 6 months of age plus a booster at 12–18 months). Initial laboratory evaluation revealed severe leukopenia (1150 WBC/mm³), C-reactive protein = 40.1 mg/dL (reference <0.5 mg/dL), bowel edema at abdominal X-ray and ultrasound, without free liquid in the abdominal cavity. Urinalysis and lipid profiles were normal.

As a refractory septic shock of abdominal origin was evidenced, high dose ceftriaxone (100 mg/kg/day), metronidazole and ampicillin were empirically initiated and she was transferred to the intensive care unit. Within 48 h from admission, exploratory laparotomy was performed, which identified dilated and an inflamed small bowel and purulent secretions in the abdominal cavity (Fig. 1). No signs of bowel perforation, acute appendicitis or any other apparent focus for the pyogenic infection were identified. Appendectomy was performed, and its histological examination subsequently revealed no inflammatory process.

* Corresponding author at: Alameda Jau 585/121, Jardim Paulista, São Paulo, SP, 01420001, Brazil.

E-mail address: daniel@jarovsky.com.br (D. Jarovsky).

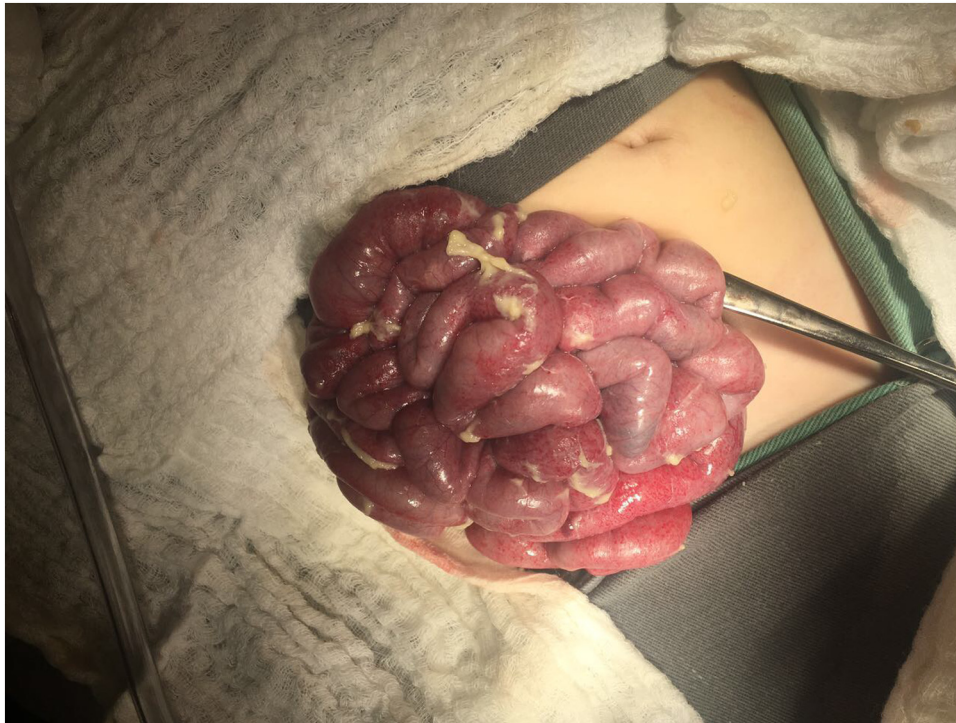


Fig. 1. Exploratory laparotomy revealing dilated and inflamed small bowel and purulent secretions in the abdominal cavity.

She required ventilatory support for 10 days, multiple vasoactive drugs (noradrenaline, adrenaline, dobutamine, and milrinone), hemodialysis for 7 days and several blood transfusions. Sustained hemodynamic and ventilatory parameters improved on the 15th day of the antimicrobial course. The patient was discharged after 25 days of combined treatment, with no residual sequelae. An immune evaluation was not fully performed since the family never attended the outpatient clinic after discharge.

Blood cultures (collected before the introduction of empirical antimicrobial therapy and thrice during the feverish period) and ascitic fluid cultures (collected during the surgical procedure and twice during the following days) yielded no bacterial growth. From our experience of the use of a molecular technique for the diagnosis of presumptive invasive bacterial infections, *Streptococcus pneumoniae* was identified by qPCR ($C_T=28$) in the abdominal fluid collected five days after the laparotomy (the 7th day of antibacterial treatment). Bacteria were not observed in this sample and polymorphonuclear cell count could not be evaluated due to degenerate neutrophils. The TaqMan® qPCR multiplex assay was developed, validated and performed at Instituto Adolfo Lutz [2] and comprises 3 sets of primers targeting meningococcal capsular transport gene (*ctrA*), pneumococcal autolysin gene (*lytA*), and *H. influenzae* polysaccharide capsular expression gene (*bexA*). The detection threshold of such molecular approach is 200 fg (± 80 target DNA copies) [3].

Discussion

Streptococcus pneumoniae is a rare etiology of severe intra-abdominal infection, occurring as three clinical patterns: in patients with preexisting peritoneal disease [1], in patients without underlying peritoneal disease (presumably originated from colonization or infection of the female genital organs) or as an infection involving an intraabdominal organ with or without concurrent peritonitis. Since *Spn* is considered a non-enteric bacterium, it has been hypothesized to cause SBP following a

bacteremic respiratory tract infection and subsequent bacteremia [4]. Even in the era of routine pneumococcal vaccination, *Spn* remains a significant organism causing SBP in children with underlying diseases, particularly nephrotic syndrome, chronic liver disease, and immunodeficiency [5].

Very few pediatric studies have focused on detection of bacterial DNA in peritoneal fluid. In To our knowledge, this is the first report of a culture-negative community pneumococcal peritonitis diagnosed by qPCR in a child without a preexisting peritoneal disease. While our patient did not present with any of the classic IPD high-risk comorbidities [6], chronic undernutrition could significantly increase IPD risk due to the development of persistent systemic inflammation and immunosuppression [7]. Lower pneumococcal invasiveness may be sufficient to cause severe disease to a weak host, posing challenges when high-risk conditions are present. Unvaccinated or incompletely vaccinated individuals (such as our patient) and those with established risk factors for IPD may not be benefited as much from the herd protective effects of widespread childhood immunization as healthy persons [8]. Likewise, the efficacy of pneumococcal vaccines is likely to be lower among persons with such conditions when compared to healthier ones, probably due to lower antibody responses and relatively short-lived protection [9].

Culture-based, conventional tests for the detection of *Spn* (bile solubility, optochin sensitivity, and the GenProbe AccuProbe *Pneumococcus* identification test) [10], as well as the capsular swelling (Quellung reaction) – the current gold standard method for *Spn* serotyping – are expensive, require proficiency and most importantly, lack sensitivity in most sterile fluids. Also, they cannot discern serotypes in culture-negative specimens [11]. These limitations have serious implications for IPD diagnosis in children, where culture-negative IPD and use of antimicrobials before specimen collection are frequent.

To overcome these barriers, rapid and sensitive diagnostic tests like latex agglutination test and immuno-chromatographic tests have substantially improved knowledge of the burden of

pneumococcal infection [12]. Likewise, nucleic acid amplification tests are culture growth independent, rapid and usually accurate diagnostic methods for infectious diseases. Although not available in our setting, broad-range 16S ribosomal DNA gene-based molecular techniques are generally highly sensitive and specific for detecting the presence of eubacterial DNA and can increase detection of culture-negative peritoneal samples in up to 50% [13–15].

Polymerase chain reaction (PCR) amplifying genes encoding autolysin (*LytA*), pneumococcal surface adhesin A (*PsaA*) and the *cps* loci for all 90 known pneumococcal serotypes have been used for diagnostic and serotyping in culture-negative specimens in cerebrospinal fluid, pleural effusion and blood [16]. While viable organisms are not required for DNA amplification and PCR detects as little as one copy of target DNA under ideal conditions in CSF or pleural effusion, the sensitivity of PCR in blood and peritoneal samples can be substantially reduced. Among the possible factors include the low specimen volume used during DNA amplification, the presence of porphyrin inhibitors (generated from the breakdown of hemoglobin), pneumococcal autolysis, DNA degradation (from suboptimal storage conditions), reduced bacterial loads, and time of sample collection after the initiation of antimicrobial treatment [14,17–19]. Furthermore, *Spn* DNA can be detected in the bloodstream of healthy children without clinical disease, presumably due to direct bacterial invasion of the blood from the nasopharynx or by killed organisms within phagocytes and antigen-presenting cells. Such situation makes PCR a problematic assay to distinguish between carriage and disease in patients with a high prevalence of pneumococcal nasopharyngeal carriage [17,20].

A definite source of infection could not be identified, but a C_T value of 28 is not consistent with a low-level DNA contamination, suggesting a true infection. Additionally, no PMNs were recovered from the qPCR-positive sample, which is in accordance with other studies were more than 75% of true positives had no PMNs visible by smear [15]. Despite not evaluated in our case, uncommon asymptomatic colonization sites with *S. pneumoniae* (such as the vaginal tract) can lead to invasive disease and cause unusual clinical manifestations. The frequency of young children vaginally colonized and the duration of such colonization are still unclear, since the well described data on nasopharyngeal colonization cannot be extrapolated to other body sites.

Conclusion

For evaluation of culture-negative peritoneal fluid aspirates, as the case described, qPCR is likely to have a very high specificity in peritoneal fluid since it is uncontaminated by upper respiratory tract secretions. Applying molecular assays in community SBP can improve diagnosis of pneumococcal peritoneal infection, especially in settings of late sample collection and prior use of antimicrobial agents.

Disclosures

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.idcr.2019.e00489>.

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