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CASE REPORT

Next-generation sequencing-based clinical metagenomics identifies *Prevotella pleuritidis* in a diabetic adolescent with large parapneumonic effusion and negative growth of pleural fluid culture: a case report

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ABSTRACT

We report a 12-year-old diabetic boy with a right-sided parapneumonic effusion and pneumonia who failed initial empirical antibiotics. *Prevotella pleuritidis* was identified from the pleural fluid using next-generation sequencing-based clinical metagenomics with cultures of pleural fluid and blood resulting negative. The patient responded well to intravenous meropenem followed by oral metronidazole.

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Introduction

Community-acquired pneumonia is the leading cause of mortality in children. In developed countries, the incidence is 33 per 10,000 in children up to 5 years of age with a mortality rate of less than one per 10,000 children per year [1]. Parapneumonic effusions developed in about 2–12% of children with CAP with an incidence of 2 per 100,000 from 2011 to 2013 in the USA [2]. Commonly seen pathogens associated with paediatric parapneumonic effusions are *S. pneumoniae, S. aureus*, mycoplasma and viruses [1]. With the advent of molecular testing and next-generation sequencing, the incidence of anaerobes is found higher than previously reported [3].

Prevotella spp. is a Gram-negative, non-motile rod-shaped anaerobe found in oral, colonic and vaginal flora commensal flora. They are difficult to culture. *Prevotella spp*. has been isolated from patients with chronic sinusitis, brain abscesses and oral cavity infections. In adults, there have been case reports describing its identification in thoracic empyema and liver abscess [4,5]. Antimicrobial regimen for infections caused by *Prevotella spp*. include metronidazole, amoxicillin/clavulanate, carbapenems, cephalosporins, clindamycin and chloramphenicol [4].

Case history

A 12-year-old obese boy (BMI 36 kg/m^2) with poorly controlled type II diabetes (HbA1c 11.7, blood glucose 22.6 mmol/L) presented with a 24-hour history of right-sided chest pain, cough, dyspnoea and fever (maximum temperature 38.1 °C) (Day 1). His chest X-ray (CXR) was remarkable for a right lower lobe infiltrate (Figure 1) and multiplex respiratory polymerase chain reaction (PCR) was positive for Coronavirus OC43. He was admitted to the inpatient ward under the diagnostic impression of pneumonia and after his symptoms improved over a lapse of 24 hours he was discharged home on cefdinir (Day 2).

During the following 48 hours after his discharge, the patient had recurrence of the right-sided chest pain along with dyspnoea and fever (38.7 °C). Upon re-evaluation (day 4), his full blood count showed leukocytosis (15.1x10^9/L) with neutrophilia (76%) and CXR (Figure 2). Lung ultrasound demonstrated a small right-sided pleural effusion. Blood culture was drawn, the patient was readmitted and cefepime plus vancomycin were started.

The following day (Day 5), the patient's clinical status worsened leading into respiratory distress and hypoxaemia (SaO2 74%) requiring high flow nasal cannula at 10 litres per minute. Computed tomography of this chest (with contrast) showed interval worsening of the right-sided pleural effusion. Ultrasound-guided pleurocentesis and pigtail catheter placement into the right pleural space were performed with 600 ml of turbid sanguineous fluid removed. Pleural fluid analysis demonstrated glucose 255 mg/dL, protein 5.5 gm/dL, lactate dehydrogenase (LDH) 297 U/L. Serum total protein was 6.6 gm/dL and serum LDH was 169 U/L. Both aerobic and anaerobic cultures remained negative after 5 days. Pleural fluid was also sent to for next-generation sequencing-based clinical metagenomics analysis (IDbyDNA, Explify®). The patient sample was analysed with a clinical metagenomics test validated for detection of >900 respiratory pathogens, the Explify Respiratory® test. Briefly, RNA was extracted and treated with DNase I using a commercially available

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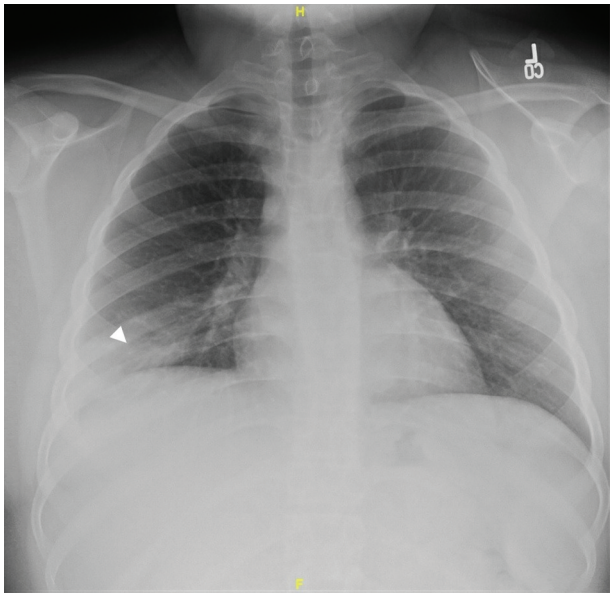

Figure 1. Chest X-Ray (CXR) on day 1. CXR showed increased consolidation (▲) on the right lower lobe.

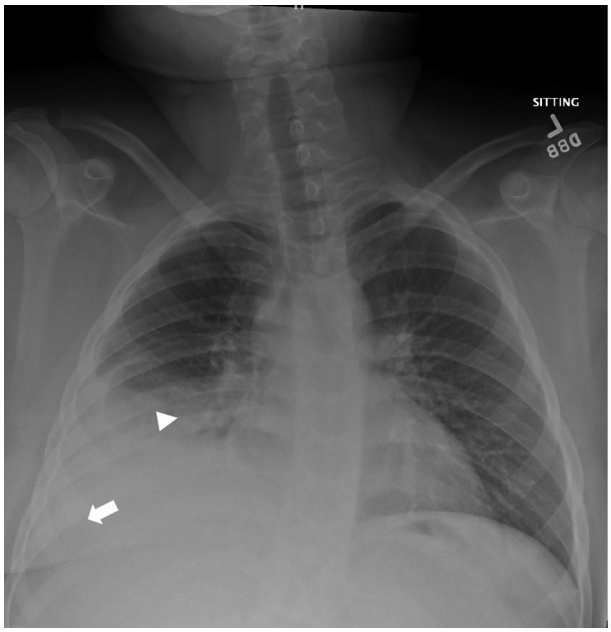

Figure 2. Chest X-Ray (CXR) on day 4. Mild interval worsening of right pleural effusion (➡) and patchy consolidation of the right middle and lower lobes (▲)

kit (Zymo Research, Irvine CA, USA) using manufacturer recommendations. Shotgun cDNA libraries were prepared using Nextera DNA Flex kits (Illumina, San Diego CA, USA) and sequenced using a NextSeq 500 instrument (Illumina) to a depth of 7.4×10^6 reads with appropriate internal and external controls, and sequencing data analysed with the Explify Platform.

Following effusion drainage, the patient had clinical improvement from a respiratory standpoint, his respiratory support was weaned off and antibiotics were switched to ceftriaxone and linezolid (Day 6). Unfortunately, 2 days after the effusion drainage the

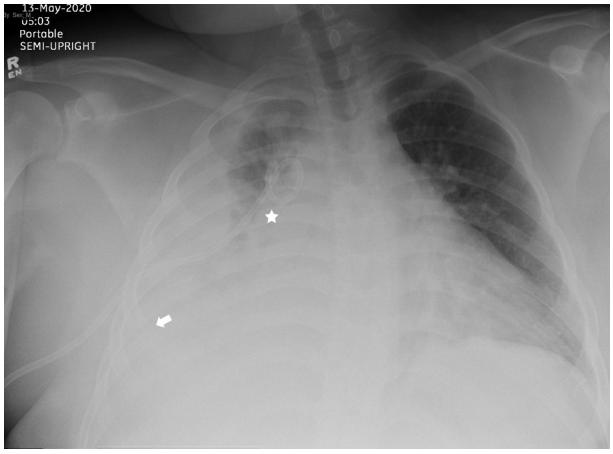

Figure 3. Chest X-Ray (CXR) on day 6. Moderate to large right pleural effusion (➡) with right chest tube placed (★)

chest tube output decreased significantly in the setting of a worsening pleural effusion on subsequent imaging studies (Figure 3) and persistent febrile spikes. Video-assisted thoracoscopy and right lung decortication were performed, and pleural fluid was again sent for culture (Day 6).

While blood cultures and two sets of pleural fluid cultures remained negative, the Explify® test was positive for *Prevotella pleuritidis* and *Human Pegivirus*, reported within 36 hours after receiving the specimen (Day 8). Subsequently, intravenous meropenem was initiated after 4 days of antibiotics (cefepime and vancomycin for 2 days (Day 4–6), ceftriaxone and linezolid for 2 days (Day 6–8)). Although blood glucose improved over days 4–10, red blood cell indices deteriorated (Table 1) possibly due to the effects of the chemotherapy on the bone marrow. Serial white blood cell counts and temperature are shown in Figure 5. Throughout the rest of his admission (Day 14), the patient improved clinically and radiographically (Figure 4), and chest tube was removed 6 days after lung decortication (Day 13). Meropenem (Day 8–13) was switched to oral metronidazole after 5 days on which he was ultimately discharged home to complete 2 weeks of therapy (Day 13–27). Symptoms and red cell indices had completely resolved on his follow-up appointment 3 weeks after discharge.

Discussion

This is the first reported case of *Prevotella pleuritidis* causing pneumonia associated with parapneumonic effusion in children. Our study is important as it demonstrated the utility of next-generation sequencing-based metagenomics to identify pathogens difficult to grow using traditional methods. Furthermore, it confirms *Prevotella pleuritidis* as a pathogen contributing to pneumonia and parapneumonic effusions.

Table 1. Blood chemistry panel and complete blood count with differential during the hospital course.

	Reference Range	Day 1	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Sodium	132–144 mmol/L	138	139	134	140	139	142	142	N/A
Potassium	3.3–4.7 mmol/L	4.1	3.8	4.2	4.0	3.7	4.5	4.1	N/A
Chloride	97–107 mmol/L	100	100	99	106	103	107	106	N/A
Carbon dioxide	16–25 mmol/L	26	23	24	24	25	24	28	N/A
Glucose	4.2–5.6 mmol/L	22.6	18.9	17.2	16.1	10.6	15.3	6.4	N/A
Blood urea nitrogen	2.1–6.1 mmol/L	<1.8	2.9	<1.8	2.1	2.9	<1.8	2.1	N/A
Creatinine	53.0–88.4 µmol/L	34.5	43.3	39.8	35.4	31.8	30.9	35.4	N/A
Calcium	2.20–2.65 mmol/L	2.28	2.2	2.18	2.05	2.13	2.03	2.1	N/A
CRP	<8 mg/L	96	149	N/A	N/A	296	N/A	N/A	N/A
RBC count	4.1–5.5 x 10^{12}/L	4.7	4.9	4.3	4.2	4.1	3.6	3.7	3.6
Haemoglobin	128–160 g/L	125	129	113	110	106	95	97	93
Haematocrit	37–47%	38	40	35	35	33	30	31	30
Platelets	150–450 x 10^{9}/L	241	298	208	215	231	223	267	278
WBC count	3.5–10.5 x 10^{9}/L	11.0	12.0	15.1	14.4	12.2	12.2	8.9	9.0
Differential count									
Neutrophils %	35.0–75.0%	61.8	59.0	75.7	67.9	75.5	82.7	60.9	65.0
Lymphocyte %	28.0–48.0%	25.1	28.0	10.7	16.4	13.5	8.4	28.2	22.0
Monocyte %	2.0–8.0%	10.6	5.0	12.2	13.8	9.0	7.8	8.7	10.0
Eosinophil %	0.0–8.0%	1.5	3.0	0.5	0.6	0.9	0.1	0.9	2.0
Basophils %	0.0–4.0%	0.5	0	0.3	0.3	0.4	0.2	0.6	0

CRP: c-reactive protein; WBC: white blood cell; RBC: red blood cell; N/A: not available

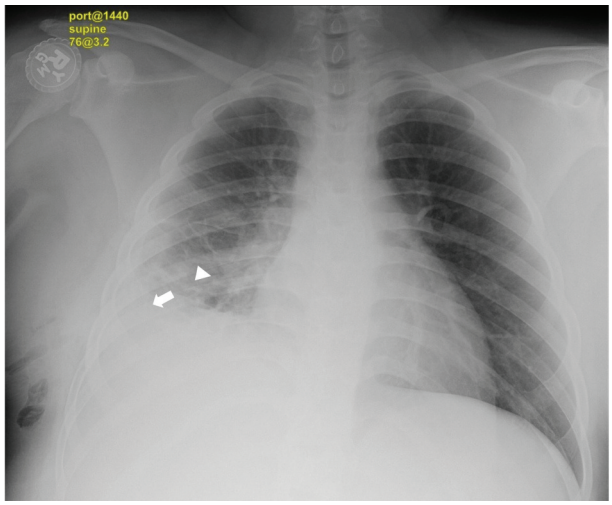

Figure 4. Chest X-Ray (CXR) on day 13. Interval improvement of pleural effusion (➡) and consolidation (▲) of the right lower lung with chest tube removed

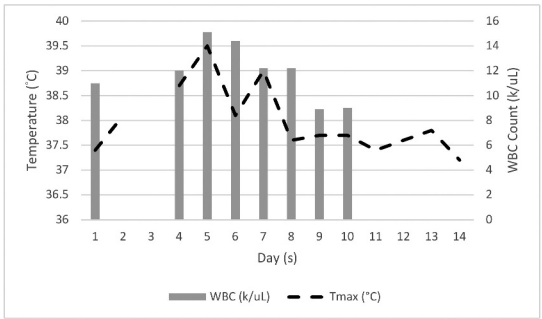

Figure 5. Trend of white blood cell count and temperature of the patient during hospitalisation.

Previously categorized as Bacteroides species, *Prevotella species* was reclassified into a new genus, *Prevotella*, with the initial species *Prevotella melaninogenica* proposed by Shah and Collins in 1990 sharing moderate saccharolytic activity with predominance in oral flora [6]. Our knowledge of *Prevotella spp*. taxonomy and antimicrobial susceptibility remained limited due to the difficulty of culture secondary to the fastidious natures of anaerobes, and that species of the tested *Prevotella* isolates were not differentiated or only certain isolates representing different species were tested [7]. Thanks to the recent introduction of molecular biology techniques (e.g. 16S rRNA gene sequencing), our understanding of *Prevotella spp*. taxonomy has significantly improved with several new species have been discovered [8]. This includes *Prevotella pleuritidis*, a newly discovered strain isolated from an adult with suppurative pleuritis in 2007 [9]. *Prevotella pleuritidis* was also reported in an adult with liver abscess in 2017 [10]. However, to our best knowledge, infections associated with *Prevotella pleuritidis* have not been reported in children. This case report regarding the diagnosis and management of complicated pneumonia secondary to *Prevotella pleuritidis* in an adolescent will deepen our understanding of this pathogen.

Several risk factors might be associated with parapneumonic effusion [2], such as previous empyema, immunocompromised status and delayed initiation of appropriate antibacterial treatment. Diabetes is a known risk factor for infection, possibly due to hyperglycaemia-related impairment of immune response, vascular insufficiency, and increased skin and mucosal colonization. Furthermore, the composition of enteral microbial flora is altered with a marked reduction in *Prevotella* species in diabetics as compared to healthy people [11]. However, the impact of the altered enteral flora on susceptibility to infection among diabetics remains unclear. The presence of diabetes and delayed diagnosis and initiation of appropriate antibiotics might partially explain the development of parapneumonic effusions in this case.

The treatment of pneumonia and parapneumonic pleural effusion is early and appropriate antimicrobial therapy. However, culture-based methods are limited

Table 2. Comparison between next-generation sequencing-based clinical metagenomics and culture in the diagnosis of infection.

	Pros	Cons
Next-generation sequencing-based clinical metagenomics	• Shot-gun approach free of hypothesis or bias • Accurate and relatively timely identification of pathogens • Ability to discover new species or variants of known species • Less affected by previous antimicrobial use	• High cost • Requires preparation of sequencing library and computational power for analyses of metagenomics data • Interpretation may be affected by pathogens from contamination or colonization
Culture	• Readily affordable and accessible • Gold standard for diagnosis of most infectious diseases	• Time-consuming • Low positive rate • Sensitivity affected by previous antimicrobial use • Limited use in viral infection • Limited use in pathogens unsuitable for culture (e.g. fastidious organisms)

for sub-optimal sensitivity and relatively long turnaround times, requiring up to 48 to 72 hours to actionable results [12]. The multiplex respiratory PCR help overcome the problem of long turnaround times with high diagnostic accuracy and taxonomic resolution. However, the utility is limited by the pre-determined set of pathogens and thus are not comprehensive enough to capture less commonly seen pathogens [12]. For instance, it is reported that only 40–80% of respiratory pathogens were detected in with pneumonia using multiplex respiratory PCR [13]. Therefore, the aforementioned methods might result in delay or failure to identify causative pathogens, creating a clinical dilemma. This dilemma was seen in our case where repeated cultures of pleural fluids and blood as well as multiplex respiratory PCR failed to identify the causative pathogen, *Prevotella pleuritidis*. This resulted in delayed initiation of appropriate antibiotics. The gap in making a timely and accurate diagnosis of the causative pathogen could be bridged by culture-independent next-generation sequencing-based clinical metagenomics. The 48-hour turnaround time seen in our case and high sensitivity resulted in timely treatment and improvement of the patient's symptoms. This could prevent severe complications of parapneumonic effusion, such as empyema and bronchopulmonary fistula.

Next-generation sequencing-based clinical metagenomics is able to provide culture-independent unbiased detection, quantification and genetic profiling of pathogens through a shotgun approach, and the timely and accurate identification of pathogens [14]. Our clinical experience with the results of next-generation sequencing-based clinical metagenomics leading to change of patient care resonated with prior literature. Zhou et al. identified *Prevotella spp*. in a diabetic adult presented with thoracic empyema who was initially received prolonged treatment for tuberculosis [4]. Another strength of next-generation sequencing is its ability to identify pathogens that are species unrecognized before or are genetically divergent strains not detectable using PCR. With next-generation sequencing-based metagenomics, there are a few limitations that must be considered. First of all, upon identifying a pathogen in a sample, it can be difficult to determine the clinical significance in identifying whether the pathogen is causing an active infection, contaminant or host normal flora. For instance, in this case, in addition to *Prevotella pleuritidis*, next-generation sequencing-based clinical metagenomics reported the identification of *Human Pegivirus*, a virus of unclear pathogenicity [15]. The interpretation of the presence of this virus raises the question of its relevance in its pathogenicity of the parapneumonic effusion. Secondly, as compared to multiplex respiratory PCR, next-generation sequencing-based metagenomics has a relatively long turnaround time from library preparation and sequencing. Moreover, the cost of next-generation sequencing-based metagenomics and the need for computational power may be a prohibitory factor preventing its generalization. However, metagenomic testing may potentially shorten the length of hospitalization and unnecessary antibiotics exposure, reducing the total medical expenditure. There are also exciting applications in development which are able to mine antimicrobial resistance data as well as host response (transcriptome) information from clinical metagenomic data. Given the strengths and limitations of next-generation sequencing-based metagenomics, we considered it a reasonable alternative for immunocompromised patients and when routine therapeutic approaches and traditional diagnostic methods (e.g. culture-based method) fail. Table 2 compared the pros and cons between next-generation sequencing-based metagenomics and culture for diagnosing infectious diseases.

One major limitation of this case is that no cell count analysis was performed on the initial pleural fluid analysis, which limits the true identification of parapneumonic effusion and empyema. However, the surgical inflammatory ring found during the Video-assisted thoracoscopy procedure and that Light's criteria for exudates was met (i.e. the ratio of protein in pleural fluid and plasma ≥0.5 g/L) suggested that empyema was likely present.

Next-generation sequencing-based metagenomics could guide the diagnosis and treatment of causative pathogens for pneumonia and parapneumonic

effusion when traditional culturing methodologies fail. Furthermore, next-generation sequencing-based clinical metagenomics could shed light on pathogens that were not classified or identified using traditional methods associated with certain diseases.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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