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First Two Cases of Infected Aortic Aneurysm Caused by Non-Vaccine *Streptococcus pneumoniae* Serotype 23A

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Dear Editor,

Infected aortic aneurysm (IAA) is an uncommon, but life-threatening condition. Identification of the causative pathogen is essential for accurate diagnosis and effective treatment. However, 14–40% of IAA cases are culture-negative [1]. IAA due to *Streptococcus pneumoniae* is rare, and reports of the involvement of *S. pneumoniae* capsular serotypes and sequence types (STs) in IAA are even rarer [2-5]. We identified *S. pneumoniae* from culture-negative IAA by genetic analysis. To the best of our knowledge, as of 2019, only 59 cases of pneumococcal IAA have been reported in France, the United Kingdom (UK), the Netherlands, Germany, Switzerland, Belgium, Denmark, the United States (USA), Canada, Chile, Japan, Hong Kong, Korea, and Austria since 1977 [2-5]. In the previous cases of IAA due to *S. pneumoniae*, capsular serotype analysis was reported only for seven: 10A and 23F in the UK, 4 and 8 in Denmark, 19F in Hong Kong, 4 in Belgium, and 23 in USA [2-5]. We report the first two cases of culture-negative IAA due to non-vaccine *S. pneumoniae* serotype 23A, ST338. The study protocol was approved by the Institutional Ethics Committees of Tohoku University, Sendai, Japan (No. 2018-1-456).

Case 1 was of a 68-year-old female treated in 2017 for three aneurysms in the thoracic aorta. Case 2 was of a 70-year-old male treated in 2014 for a ruptured pararenal aortic aneurysm. Informed consent was obtained from the patient in case 1, and in case 2, research information has been disclosed in accordance with the ethical guidelines for Medical and Health Research Involving Human Subjects established by the Japanese Ministry of Health, Labor and Welfare (https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/hokabunya/kenkyujigyou/i-kenkyu/index.html). Both patients had no history of disease-causing immune deficiency or pneumococcal vaccination and underwent surgical treatment. In case 1, the two larger aneurysms were resected during staged surgeries. In case 2, the aneurysm ruptured, leading to abscess formation in the anterior cavity of the iliopsoas muscle, and left renal artery was reconstructed owing

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to occlusion.

As the blood and tissue cultures were negative in both cases, 16S ribosomal RNA gene analysis of tissue was performed as described previously [1]. In both cases, the sequence similarity was the highest with *S. pneumoniae*, and species identification was further confirmed by amplification of the *lytA* region, which is specific to *S. pneumoniae* [6]. In case 1, *S. pneumoniae* was identified in two separate aneurysms. Oral antibiotics were prescribed for both patients at discharge, and no recurrence has been reported.

The serotype of the *S. pneumoniae* isolated from each patient was identified as 23A following the US Centers for Disease Control and Prevention protocols for conventional PCR (https://www.cdc.gov/streplab/pneumococcus/resources.html) [7]. Multilocus

Table 1. Clinical and molecular characteristics of two cases with *Streptococcus pneumoniae*-infected aortic aneurysms

Variables	Case 1	Case 2
Age (yr), Sex	68, Female	70, Male
Underlying diseases	Sigmoid colon cancer (postoperative), hypertension	Bronchial asthma, hypertension, hyperuricemia, hyperlipidemia
Risk factors for infection	None	None
Pneumococcal vaccination history	None	None
Clinical presentation	Fever, back pain	Fever, lumbar pain
Site of aneurysm (size in mm; by CT)	Ascending aorta (58), descending aorta (34, <30)	Pararenal (40)
Inflammation around aneurysm (CT)	Positive (observed in the two larger aneurysms)	Positive
Maximum temperature before surgery (°C)	37.5	38.4
Maximum white blood cell count ($\times 10^9$/L) before surgery	8.4	15.5
Maximum C-reactive protein (nmol/L) before surgery	1,104.8	1,695.3
Surgical management	*In situ* grafting	*In situ* grafting
Microbiological diagnosis		
Blood culture	Negative	Negative
Tissue culture	Negative	Negative
16S rRNA gene sequence similarity*	99.64%	99.86%
lytA (specific to *S. pneumoniae*)	Positive	Positive
Serotype	23A	23A
MLST	ST338 (CC 156)	ST338 (CC 156)
Alterations in PBP genes	Positive (*pbp2x* and *pbp2b*)	Positive (*pbp2x* and *pbp2b*)
Amino acid substitutions in QRDR (GyrA and ParC)	None	None
Anti-microbial therapy		
Before admission	None	Ceftriaxone
After admission	Vancomycin (0.75→2.5 g/day†) and gentamicin (150 mg/day)	Piperacillin-tazobactam (9 g/day) and vancomycin (1 g/day), then teicoplanin (400→200 mg/day‡)
After identification of the pathogen	Sulbactam-ampicillin (6 g/day) and levofloxacin (500 mg/day)	Sulbactam-ampicillin (9 g/day)
At discharge	Oral amoxicillin (750 mg/day) and levofloxacin (500 mg/day)	Oral amoxicillin (1,500 mg/day)
Hospital treatment period (days)	60	61
Postoperative complications	None	Mild decline in renal function
Outcome	Alive	Alive

*Compared with *S. pneumoniae* type strains NCTC 7465^T (case 1) and ATCC 33400^T (case 2); †Vancomycin started at 0.75 g/day and increased to 2.5 g/day; ‡Teicoplanin started at 400 mg/day and decreased to 200 mg/day.
Abbreviations: rRNA, ribosomal RNA; CC, clonal complex; CT, computed tomography; MLST, multilocus sequence typing; QRDR, quinolone resistance-determining region; ST, sequence type; PBP, penicillin binding protein; NCTC, National Collection of Type Cultures; ATCC, American Type Culture Collection.

sequence typing (MLST) analysis was performed according to the *S. pneumoniae* MLST Database (https://pubmlst.org/spneumoniae/); in both cases, *S. pneumoniae* was identified as ST338, clonal complex 156. Alterations in *pbp2x* and *pbp2b*, genes encoding penicillin-binding protein that mediate β-lactam resistance in *S. pneumoniae*, were identified in both cases [8]. Mutations in the quinolone resistance-determining region of *gyrA* and *parC*, which are important determinants of levofloxacin resistance [9], were not detected in either case. The clinical and molecular characteristics of both cases are detailed in Table 1.

In Japan, the 7-valent pneumococcal conjugate vaccine (PCV7) was introduced for children younger than five yrs in 2010 and was replaced with PCV13 in 2013. In 2014, the 23-valent pneumococcal polysaccharide vaccine was introduced for vaccination in adults over 65 years. Depending on the effect of vaccination, reduced carriage and incidence of invasive pneumococcal disease (IPD) and increased prevalence of non-vaccine serotypes (NVTs) in IPD cases have been reported [10]. According to recent Japanese surveillance studies, the proportion of IPD cases attributed to serotype 23A (an NVT) has been increasing since the introduction of PCVs [10]. More than 90% of 23A isolates from adult IPD cases showed alterations in *pbp2x* and *pbp2b*, and approximately a half of them were reported as ST338, similar to the isolates in the current cases. Genetic analysis of the isolates in our cases indicated susceptible to levofloxacin, as reported in previous IPD cases in Japan [10]. Although the current cases were successfully treated, the isolates were NVTs and harbored *pbp2x* alterations, making them resistant to cephems. As cephem antibiotics are used frequently in Japan, there is a concern that antibiotic selection pressure will lead to an increase in the proportion of drug-resistant isolates. Moreover, with the introduction of PCVs, there is also a concern regarding the relative increase in NVTs and drug-resistant isolates.

In conclusion, we identified 23A *S. pneumoniae*, ST338, in culture-negative IAA for the first time in two independent cases, raising the concern that NVT and drug-resistant pneumococci may exist in culture-negative IAA cases. Further epidemiological studies and investigation of preventive measures for IPD, including IAA, are required.

AUTHOR CONTRIBUTIONS

RK wrote the manuscript; TM, NM, SK, and YS treated patients and collected samples; RK, RN, HY, and DO conducted the laboratory work, described the associated process, and interpreted the results; NO, YK, and MK contributed to the writing of the manuscript.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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