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## **Pneumococcal pneumonia among shipyard workers: inside the features of disease onset**

Nadim CASSIR<sup>1,2</sup>, Léa LUCIANI<sup>1</sup>, David FERRIEUX<sup>1</sup>, Anthony LEVASSEUR<sup>1,2</sup>, Pierre-Edouard FOURNIER<sup>1,3</sup>, Philippe PAROLA<sup>1,3</sup>

<sup>1</sup> IHU-Méditerranée Infection, Marseille, France.

<sup>2</sup> Aix Marseille Univ, IRD, AP-HM, MEPHI, Marseille, France.

<sup>3</sup> Aix Marseille Univ IRD, AP-HM, VITROME, Marseille, France

**Keywords:** *Streptococcus pneumoniae*, pneumonia, shipyard workers, whole-genome sequencing

**Running title:** Shipyard workers and pneumococcus

**Highlights:** The onset of pneumococcal infection among shipyard workers are attributable to both a person-to-person spread of single strains of *S. pneumoniae* and a shift of different strains from commensal to pathogen under favorable conditions (professional exposure, viral infections).

Correspondence:

Dr Nadim Cassir

*M.D., Ph.D.*

University Hospital Institute (IHU)-Méditerranée Infection

Marseille University, IHU Méditerranée Infection, Faculté de Médecine Bd Jean Moulin,

Marseille 13005, France

cassirnadim@gmail.com

04 13 73 20 21

## **Abstract**

### Background

Several outbreaks of pneumococcal pneumonia among shipyard workers have been described. In this study, following a previous report of grouped cases, we aimed to elucidate the features of disease onset.

### Methods

We compared the population characteristics of shipyard workers with a confirmed diagnosis of pneumococcal pneumonia (N=38) to those of workers without pneumonia (N=53). We compared nine *S. pneumoniae* strains isolated from patients with pneumonia by capsular serotyping, multi-locus sequence typing, and whole genome sequencing.

### Results

Shipyard workers with *Streptococcus pneumoniae* pneumonia were more frequently from Italy ( $P=0.016$ ), had at least one underlying condition ( $P=0.024$ ), lived on-board the ship ( $P=0.009$ ). None of these factors was independent by multivariate analysis. While capsular serotyping enabled us to identify four different serotypes: 4 (n = 5), 8 (n = 2), 9N (n = 1), and 3 (n = 1), by sequence typing, we distinguished five sequence types (STs): ST801 (n = 4), ST205 (n = 2), ST1220 (n = 1), ST1280 (n = 1), and ST66 (n = 1). Whole genome sequencing confirmed the results obtained by MLST. Genomes of isolates of the same sequence type were similar with  $\leq 80$  single-nucleotide polymorphisms.

### Conclusions

We confirmed that the onset of pneumococcal infection among shipyard workers was attributable to both a person-to-person spread of single strains of *S. pneumoniae* and a shift of different strains from commensal to pathogen under favourable conditions

(professional exposure, viral infections). Control measures should therefore be implemented by taking into account these features.

## Introduction

*Streptococcus pneumoniae*, which colonises the human upper respiratory tract, is an opportunistic pathogen that may cause invasive life-threatening infections including bacteraemia, pneumonia and meningitis. Although vaccine-preventable, *S. pneumoniae* remains leading microorganism causing lower respiratory infections, a major cause of mortality worldwide with an estimated 2.38 million deaths in 2016 [1]. Infant vaccination programmes alone may not be sufficient to protect vulnerable populations against invasive pneumococcal disease (IPD) outbreaks across the globe. Optimal population-specific vaccination strategies to control outbreaks are needed. For instance, vaccination with 23-PPV is recommended for welders in the United Kingdom [2].

Several risk-factors for IPD have been identified, including, age, poor nutritional status, several comorbidities, and immunosuppression [7]. Grouped cases of community-acquired pneumonia caused by *S. pneumoniae* have been described in recent years among shipyard workers [3–7]. Welders and those exposed to metal fumes are recognised as being at greater risk of developing IPDs [8]. In addition, person-to-person spread requires close contact and community-acquired IPD outbreaks have been described in crowded environments such as jails, military camps and households [3]. In particular, it has been reported that inadequate ventilation increases the risk of IPD [9]. In addition to over-crowding, the level of pre-existing immunity against the infecting strain also affects bacterial transmission [6].

We recently reported an outbreak of pneumococcal pneumonia among shipyard workers in January and February 2020 in Marseille [7]. At that time, 37 cases were diagnosed, and one more has been reported since. Outbreak control measures were implemented, including a mass pneumococcal vaccination campaign.

In this study, we aimed to elucidate whether the onset of infection in these grouped cases was attributable to a propagated outbreak with person-to-person spread of a single strain of *S. pneumoniae* or whether it was attributable to a shift of different strains from commensal to pathogen under favourable conditions (professional exposure, viral infections), or both.

## Materials and Methods

### Case and control definition

A confirmed case was defined as a worker or crew member on the cruise liner which was undergoing renovation in the shipyard between January and February 2020 who was diagnosed with community-acquired *Streptococcus pneumoniae* pneumonia (clinical and radiological signs). Microbiological confirmation corresponded to *S. pneumoniae* isolated from blood or respiratory samples, a pneumococcal antigen detected in the urine, or a positive *S. pneumoniae* PCR on respiratory samples [10].

A control case was defined as a worker or crew member on the cruise liner undergoing renovation in the Marseille shipyard between January and February 2020 who had not been diagnosed with community-acquired *Streptococcus pneumoniae* pneumonia. Among the control cases for which we collected information as part of a systematic detailed survey, we differentiated patients with an acute upper respiratory infection (including rhinitis, pharyngitis, and bronchitis) from asymptomatic patients. All included patients underwent a medical examination, a chest radiography and further microbiological analysis. This study was approved by our local ethics committee.

### Epidemiological investigations

We performed a case-control study. A systematic survey was implemented in order to assess the age, sex, country of residence, medical conditions, occupational status, exposure to solvents, fumes or dust, and use of protective masks among the cohorts. We compared these characteristics and the results of the microbiological analysis between cases with and without a diagnosis of community-acquired *S. pneumoniae* pneumonia.

### Microbiological investigations

DNA/RNA from pharyngeal swabs were extracted using the QIAamp DNA Mini kit and Qiacube, both from Qiagen (Hilden, Germany) according to the manufacturer's recommendations. The real-time polymerase chain reaction (RT-PCR) specific for *S. pneumoniae* targeting the *LytA* gene was performed as described previously [11]. Respiratory viruses were detected using the FTD Respiratory pathogens 21 qPCR multiplex panel (Fast Track diagnostic - Siemens Healthineers, Erlanger, Germany). This commercial system makes direct typing of Influenza (A, H1N1 or B) and Coronaviruses (HKU1, 229E, NL63 and OC43) possible. Rhinovirus and enterovirus genotyping was performed by sequencing the VP2-VP4 and VP1 coding region, respectively, as previously describe [12,13]. The evolutionary analysis of Rhinovirus strains was inferred using the maximum likelihood method based on the Tamura-Nei model [14]. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with the higher log likelihood value (Figure 1). Finally, the genotyping of adenoviruses were performed by sequencing a partial hexon region as described previously [15].

For the detection of *S. pneumoniae* antigen in the urine, we used the Alere BinaxNOW test (Waltham, Massachusetts, United States) according to the manufacturer's recommendations.

Blood cultures were incubated using the BACT / ALERT® VIRTUO® automated microbial detection system (Biomérieux, Marcy l'Etoile, France). The subculture of the positive blood culture was performed using PolyViteX® and Columbia 5% sheep blood agars plate incubated at 37°C, 5% CO<sub>2</sub> (Biomérieux, Marcy l'Etoile, France). Sputum were cultivated using PolyViteX®, Columbia containing nalidixic

acid/colimycin and MacConkey agar plates incubated at 37°C, 5% CO<sub>2</sub>. For anaerobic incubation, we use an anaerobic jar (Mitsubishi) and an atmosphere generator, GasPak<sup>TM</sup> EZ Anaerobe Container System (Becton Dickinson Franklin Lakes, New Jersey, United States). Identification of bacteria grown was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry as described previously [16]. Antimicrobial susceptibility testing was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [17].

### Bioinformatic and evolutionary analyses

Nine strains of *S. pneumoniae* isolated from patients with community-acquired pneumonia were whole genome sequenced and their sequence typing was performed by multilocus sequence typing (MLST). The genomes were deposited in the European Nucleotide Archive under the bioproject accession number PRJEB42406.

After genome sequencing, reads were assembled using SPAdes version 3.14.1 with default parameters [18]. All genomes were annotated using Prokka with default parameters [19]. Core and accessory genes were identified using Roary [20] with BLASTP identity of 95% and the percentage of isolates in the core genome taken as 99%. A phylogenetic tree was reconstructed by using MEGA 7 [14] using the Maximum-Likelihood (ML) method for molecular evolutionary analysis. SNP-sites were then used to detect SNP in the aligned core genome [21]. A Roary plot was used to generate the figure of the pangenome with the gene presence and absence of each sample.

### Statistics

PASW Statistics 17.0 (SPSS, Inc., Armonk, USA) was used for the statistical analysis. Mean +/- SD or median with minimum and maximum was used to describe

continuous variables. The percentage and number of events were used for quantitative variables. A Student's t-test or Mann-Whitney U-test was used to perform 2-group comparisons for quantitative variables. The  $\chi^2$  test was used to perform 2-group comparisons for qualitative variables, or the Fisher exact test was used when the expected count was  $<5$ . A multivariate analysis using logistic regression was performed to identify independent risk factors for community-acquired *S. pneumoniae* pneumonia. Clinically relevant variables and variables associated ( $P < .10$ ) with community-acquired *S. pneumoniae* pneumonia in univariate analysis were used in the model. A significance threshold of 0.05 was adopted for all statistical analyses.

## Results

Overall, 3,200 workers had daily access to the ship which was undergoing renovation between January and February 2020. Of the 38 confirmed cases of community-acquired pneumococcal pneumonia (an attack rate of 4.6/1,000/month), 18 (47.3%) were hospitalised, including five (13.1%) in the intensive care unit. All survived. The patients were all shipyard workers, with the exception of one crew member of the cruise liner. Bacteriological documentation was distributed as follows: 9 (23.7%) positive blood cultures, 16 (42.1%) positive urinary antigens, 2 (5.3%) positive sputum cultures, and 24 (63.1%) positive nasopharyngeal RT-PCRs. There were 10 patients with a combined detection of *S. pneumoniae* with more than one positive result obtained by a different method of identification. All isolates were susceptible to amoxicillin, clindamycin, erythromycin, tetracycline and vancomycin, with the exception of one which was resistant to erythromycin. By capsular serotyping, we identified four different serotypes: 4 (n = 5), 8 (n = 2), 9N (n = 1), and 3 (n = 1). By sequence typing, we identified five sequence types (STs): ST801 (n = 4), ST205 (n = 2), ST1220 (n = 1), ST1280 (n = 1), and ST66 (n = 1). Whole genome sequencing confirmed the results obtained by MLST. Genomes of isolates of the same sequence type were similar, with  $\leq 80$  single-nucleotide polymorphism (SNP) (see Supplementary Figure 1).

Of the 38 confirmed cases of community-acquired pneumococcal pneumonia, co-infection with respiratory viruses was identified in 19 (50%) cases. Rhinovirus was the most commonly identified virus (N=8), followed by Influenza (N=6), Coronavirus (N=4), Enterovirus (N=1), Respiratory Syncytial Virus (N=1), and Adenovirus (N=1). In two patients, more than one virus was detected. Of the strains of Rhinovirus, only three were revealed to be similar through sequence typing (Figure 2). In

asymptomatic patients, only one (9.1%) tested positive for a respiratory virus (Enterovirus) (see Supplementary Table 1). When comparing patients with and without *S. pneumoniae* detection, we found that Rhinovirus detection was strongly associated with *S. pneumoniae* detection (13/48 vs. 0/43;  $p=0.0001$ ) (see Supplementary Table 2).

When compared with control patients using univariate analysis, case patients with community-acquired *Streptococcus pneumoniae* pneumonia were more frequently from Italy ( $p=0.016$ ), had at least one underlying condition ( $p=0.024$ ), and lived on board the vessel ( $p=0.009$ ) (Table 1). Using multivariate analysis, we did not identify any independent risk factors for pneumococcal pneumonia in the studied population.

## Discussion and conclusion

In addition to our series of 38 grouped cases of confirmed community-acquired pneumococcal pneumonia among shipyard workers [7], four other similar grouped cases have been reported in Europe since 2015 [3–6]. In these reports, the most commonly pneumococcal capsular serotype identified was serotype 4, although several other serotypes have been identified within grouped cases (Table 2). Moreover, we found that strains belonging to the same capsular serotype corresponded in part to different multi-locus sequence-types (MLSTs), confirmed by whole genome sequencing that provides additional resolution (Figure 1). The two major pneumococcal capsular serotypes identified in this study (*i.e.*, 4 and 8) have been associated with a high attack rate [22]. However recent studies have demonstrated that genetic lineage also contributes towards invasiveness. For instance, clade specific clustering linked to either carriage or invasive disease have been found among serotype 1 strains from West Africa [10]. However, because we did not analyse isolates from control cases, we cannot draw any conclusions with regard to causality. This remains, however, the first report of a case series of pneumococcal pneumonia among shipyard workers for which whole-genome sequencing allowed a high-resolution comparison of strains. In addition, most of the Rhinovirus strains co-detected with *S. pneumoniae* were different by sequence typing. Overall, these results call into question the hypothesis of a single strain responsible for an outbreak with person-to person spread as has been described in prison settings [9,23].

Although there is evidence that droplet transmission occurs for *S. pneumoniae* [24], the onset of pneumococcal infections may also be due to the shift of *S. pneumoniae* from a commensal to a pathogen through interaction with its host under favourable conditions such as exposure to airway irritants [25]. Previous investigations into pneumococcal pneumonia outbreaks among shipyard workers revealed that many

parallel tasks involving exposure to inorganic dust and metal fumes in poorly-ventilated conditions were performed in close proximity and that some of the individuals exposed were not using respiratory masks as recommended [6]. In this study, we found no statistically significant differences between patients with and without community-acquired pneumococcal pneumonia regarding their age, exposure to airway irritants, and their use of protective masks (Table 1).

The grouped case of acute respiratory infections among shipyard workers that we report here occurred in winter. Using PCR, we detected several respiratory viruses from naso-pharyngeal samples, especially in symptomatic patients with either an acute upper respiratory infection or pneumococcal pneumonia. It is well known that seasonal invasive pneumococcal disease (IPD) is more frequent during drier, colder months when airway secretions are more abundant and is more likely to occur in conjunction with respiratory viral infections [26]. An increase in the density of *S. pneumoniae* in the nasopharynx has been associated with the onset of acute respiratory illness and might also play a role in person-to-person transmission [27]. Notably, it has recently been shown that total pneumococcal densities (encompassing all present serotypes) during asymptomatic periods were significantly higher when a respiratory virus was detected compared to periods when no virus was detected [27]. Pneumococcal infection severity has been observed to increase with influenza co-infection [28]. Other respiratory viruses such as rhinovirus and adenovirus have been associated with increased pneumococcal colonisation [28]. In outbreak settings, therefore, interventions aimed at preventing co-infections such as influenza vaccination and measures to reduce person-to-person transmission such as hand hygiene and protective mask wearing, together with the rapid detection and isolation of symptomatic patients who test positive for respiratory viruses and/or *S. pneumoniae*, may have the potential to interrupt transmission and mitigate

pneumococcal infection [29]. However, complementary interventions specifically targeting pneumococcus, such as vaccinating the population of shipyard workers are required to prevent pneumococcal outbreaks [30].

Although the number of patients studied and the number of typed strains are low, and our systematic survey has been hampered by missing data, this study contributes further information about the features of the onset of pneumococcal pneumonia among shipyard workers. Indeed, we confirmed that the onset of pneumococcal infection among shipyard workers was attributable to both person-to-person spread of single strains of *S. pneumoniae* and a shift of different strains from commensal to pathogen under favourable conditions (professional exposure, viral infections). Control measures should therefore be implemented by taking these features into account.

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## **Conflicts of interest**

NC, LL, DF, PEF, AL, PP declare no conflicts of interest.

## **Authors’ contribution**

Interviewing and treating the patients: NC, DF

Laboratory investigations: LL, PEF

Genomic investigation: AL

Manuscript drafting: NC, LL, AL, PP

All authors reviewed the paper.

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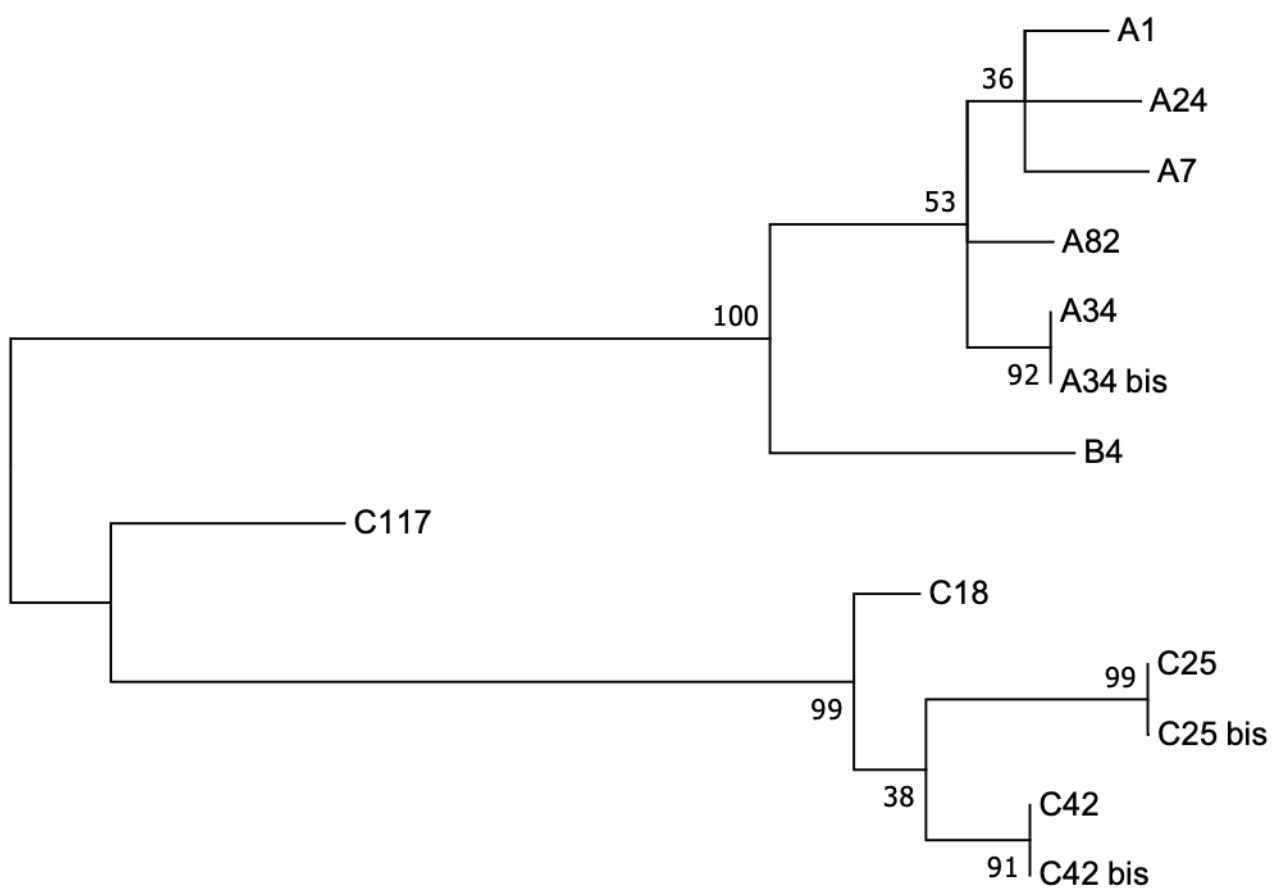
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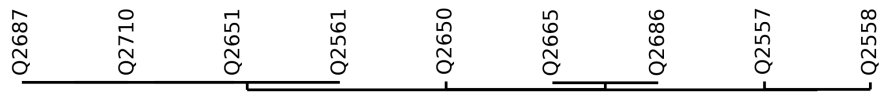


0.20

Roary matrix  
(2805 gene clusters)



Tree  
(9 strains)



Characteristics	Pneumococcal pneumonia N=38	No pneumococcal pneumonia N=53	HR [CI 95%]	p
Age, mean (SD)	42 (13.3)	39.6 (11.6)	-	0.359
Male	38 (100)	49 (92.4)	-	0.109
<b>Country of residence, No. (%)</b>				
Bulgaria	0 (0)	11 (25)	-	0.003*
Estonia	2 (7.1)	5 (11.4)	0.600 [0.108 - 3.328]	0.439
France	3 (10.7)	11 (25)	0.360 [0.091 - 1.429]	0.116
Italia	8 (28.6)	3 (6.8)	5.47 [1.308 - 22.856]	0.016*
Lithuania	3 (10.7)	10 (22.7)	0.410 [0.102 - 1.638]	0.165
Poland	2 (7.1)	0 (0)	-	0.148
Romania	3 (10.7)	0 (0)	-	0.055
Russia	1 (3.6)	1 (2.3)	1.590 [0.096 - 26.640]	0.630
Serbia	2 (7.1)	0 (0)	-	0.148
<b>Underlying medical condition, No. (%)</b>				
None	22 (73.3)	40 (93)	0.210 [0.050 - 0.858]	0.024*
<b>Smoking status, No. (%)</b>				
Yes	14 (63.6)	21 (50)	1.750 [0.607 - 5.044]	0.219
<b>Occupation at the shipyard, No. (%)</b>				
Plumber	1 (4.8)	2 (5)	0.950 [0.081 - 11.128]	0.730
Electrician	2 (9.5)	2 (5)	2.000 [0.261 - 15.320]	0.427
Welder	2 (9.5)	3 (7.5)	1.300 [0.200 - 8.447]	0.567
Manager	5 (23.8)	9 (22.5)	1.080 [0.309 - 3.752]	0.574
Security	2 (9.5)	12 (30)	0.250 [0.049 - 1.224]	0.064
Others	9 (42.9)	13 (32.5)	1.560 [0.525 - 4.626]	0.300
<b>Work, No. (%)</b>				
Mainly Inside	12 (100)	28 (84.4)	-	0.194
Mainly Outside	6 (50)	25 (75.8)	0.320 [0.080 - 1.277]	0.101
Both	6 (50)	20 (60.6)	1.530 [0.410 - 5.820]	0.500
<b>Exposure, No. (%)</b>				
Solvent	5 (33.3)	13 (37.1)	0.850 [0.237 - 3.024]	0.530
Metal fume	13 (86.7)	26 (74.3)	2.25 [0.423 - 11.959]	0.283
Dust	8 (53.3)	26 (74.3)	0.400 [0.112 - 1.403]	0.131
<b>Use of protective mask, No. (%)</b>				
Yes	13 (86.7)	30 (83.3)	1.300 [0.231 - 7.315]	0.565
<b>Accommodation, No. (%)</b>				
On boat	18 (90)	21 (56.8)	6.860 [1.386 - 33.934]	0.009*
<b>Respiratory virus detection, No. (%)</b>				
Yes	19 (50)	21 (60.37)	0.650 [0.280 - 1.530]	0.500

General features				Clinical characteristic described No. (%)				Microbiological features of confirmed cases		Control measures	
Date	Location	Number of case	Attack rate /1000/month	Pneumonia	Meningitis	Isolated bacteriemia	Death	Serotypes	Genotypes MLST	Prophylactic antibiotherapy	Vaccination
Arp-May 2015	Ireland	4		4 (100)	0 (0)	0 (0)	0 (0)	4 (n=3) 3 (n=1)	801 (n=2) 205 (n=1)	Azithromycin	PPV23
Apr-Jun 2017	Singapore	3		3 (100)	0 (0)	0 (0)	0 (0)				
Jan-Apr 2019	Norway	21		21 (100)	0 (0)	0 (0)	0 (0)	4 (n=17)	801 (n=8)	No	PCV13
May-Nov 2019	Finland	31	0,63	30 (97)	1 (3)	0 (0)	1 (3)	12F (n=12) 4 (n=11) 8 (n=1)	6202 (n=9) 801 (n=7) 1480 (n=1)	No	PPV23
Jan-Feb 2020	France	38	4,5	38 (100)	0 (0)	0 (0)	0 (0)	4 (n=5) 8 (n=2) 3 (n=1) 9N (n=1)	801 (n=4) 205 (n=2) 1220 (n=1) 1460 (n=1) 66 (n=1)	No	PPV23