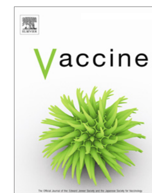
Vaccine 38 (2020) 5454–5463

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Antimicrobial susceptibility and clonality of *Streptococcus pneumoniae* isolates recovered from invasive disease cases during a period with changes in pneumococcal childhood vaccination, Norway, 2004–2016

Lotta Siira [a,b,1], Didrik F. Vestrheim [a], Brita A. Winje [a], Dominique A. Caugant [c,d], Anneke Steens [a,*]

[a] Department of Infection Control and Vaccines, Division of Infection Control and Environmental Health, Norwegian Institute of Public Health, Oslo, Norway
[b] European Program for Public Health Microbiology Training (EUPHEM), European Centre for Disease Prevention and Control, (ECDC), Stockholm, Sweden
[c] Division of Infection Control and Environmental Health, Norwegian Institute of Public Health, Oslo, Norway
[d] Department of Community Medicine and Global Health, Faculty of Medicine, University of Oslo, Oslo, Norway

ARTICLE INFO

Article history:
Received 16 January 2020
Received in revised form 25 May 2020
Accepted 15 June 2020
Available online 30 June 2020

Keywords:
Invasive pneumococcal disease
Pneumococcal conjugate vaccine
Surveillance
Streptococcus pneumoniae
Antimicrobial resistance

ABSTRACT

Changes in pneumococcal antimicrobial resistance (AMR) have been reported following use of pneumococcal conjugate vaccines (PCVs) in childhood vaccination programmes. We describe AMR trends and clonality in Norway during 2004–2016; we studied 10,239 invasive pneumococcal disease (IPD) isolates in terms of serotypes, antimicrobial susceptibility, and for a systematically collected subset of 2473 isolates, multilocus sequence types (ST). The IPD cases were notified to the Norwegian Surveillance System for Communicable Diseases and pneumococcal isolates were collected through the National Reference Laboratory for Pneumococci. The cases are sourced from the entire Norwegian population. We supplemented the IPD isolates with isolates from carriage studies in children attending day-care, performed in 2006 (before mass childhood vaccination with PCV7), 2008 (2 years after PCV7 introduction), 2013 (2 years after the transition to PCV13), and 2015. IPD cases were 0–102 years old; median 64 years. Carriage study participants were typically aged 1–5 years. Overall, AMR was low; a maximum of 7% of IPD isolates were resistant, depending on the antimicrobial. Erythromycin and trimethoprim/sulfamethoxazole resistant IPD (ERY-R and SXT-R, respectively) decreased in the PCV7 period (2006–2010). In the PCV13 period (2011–2016) however, we saw an indication of increased non-susceptibility among IPD isolates. This increase was mainly due to non-vaccine serotypes 15A-ST63 (multidrug resistant), 24F-ST162 (SXT-R), 23B-ST2372 (penicillin non-susceptible and SXT-R) and 33F (ERY-R and clindamycin resistant). Resistant or non-susceptible IPD isolates were often clones introduced into Norway during the study period. The exception was ERY-R isolates; initially, these largely consisted of an established serotype 14-ST9 clone, which disappeared after introducing PCV7. The carriage study results mostly resembled the changes seen in IPD with a maximum of 9% of the participants per study carrying resistant pneumococci. As actual PCVs are not fully limiting AMR, higher-valency vaccines and prudent use of antimicrobials are still needed to temper pneumococcal AMR.

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1. Introduction

Streptococcus pneumoniae, the pneumococcus, can cause mild infections, like otitis media, or severe invasive pneumococcal disease (IPD). Prior to the use of pneumococcal conjugate vaccines (PCVs) in childhood vaccination programmes, pneumococcal disease was estimated to cause ~11% of deaths in children <60 months, globally [1]. Asymptomatic nasopharyngeal carriage is frequent especially among young children, the main pneumococcal reservoir, and is a prerequisite for developing disease [2,3]. In addition to preventing IPD, PCVs decrease carriage of vaccine serotypes and therefore affect transmission in the population [4,5]. The 7-valent PCV (PCV7), covering serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, was included in the childhood immunisation programme in Norway in July 2006. It was replaced by the 13-valent vaccine (PCV13) covering six additional serotypes (1, 5, 3, 6A, 7F, 19A) in April 2011. Childhood PCV use resulted in a decline in the incidence of IPD caused by vaccine-serotypes as well as overall IPD, especially in the youngest and oldest age groups due to direct and indirect protection, respectively [6–8].

* Corresponding author.
E-mail address: anneke.steens@fhi.no (A. Steens).
[1] Current address: Expert Microbiology Unit, Department of Health Security, Finnish Institute for Health and Welfare (THL), Helsinki, Finland.

https://doi.org/10.1016/j.vaccine.2020.06.040
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Antimicrobial resistance (AMR) to several classes of agents developed in *S. pneumoniae* in the 1960s and 1970s; pneumococcal resistance to β-lactams and macrolides is often clonal in nature [9,10]. Resistance has been particularly pronounced in settings with high consumption of antimicrobials. However, high consumption is not a requirement for clonal spread of resistance; e.g., a macrolide-resistant clone spread successfully in Norway despite relatively low national consumption of macrolides [11,12]. PCV7 and PCV13 cover serotypes that account for the most common resistant isolates described globally. Since the introduction of PCV in childhood immunisation programmes, several studies have reported a decrease in pneumococcal resistance and sometimes reductions in antimicrobial prescriptions as a result of a decreased prevalence of vaccine serotypes. However, an increasing prevalence of non-susceptible non-vaccine serotypes (NVTs) has also been reported [13–20], resulting from serotype replacement. Such increases may be due to the spread of clones already present in the country, to clones novel to the country or to capsular switching.

Changes in serotype and genotype distribution and prevalence of AMR in the pneumococcal population are first detectable among carriage isolates, and later among IPD isolates. Surveillance combining antimicrobial susceptibility, serotype, and genotype data of both carriage and IPD isolates is therefore valuable in documenting potential changes in the circulating pneumococci following the use of conjugate vaccines. In this study, we aimed to describe trends in antimicrobial susceptibility in Norway during 13 years of IPD surveillance (2004–2016) when changes in pneumococcal childhood vaccination occurred. The IPD data included >10,000 IPD cases/isolates sourced from the entire Norwegian population. In addition, we present for comparison the characteristics of carried isolates collected from children attending day-care in the Oslo area in four cross-sectional studies during the same study period.

2. Materials and methods

2.1. Data sources and collection

This is an observational retrospective population-based cohort study using IPD surveillance data from 2004 to 2016, covering the PCV7 introduction in 2006 and the transition to PCV13 in 2011. It is mandatory to report all IPD cases (patients with isolation of *S. pneumoniae* from a normally sterile site) to the Norwegian Surveillance System for Communicable Diseases (MSIS). Corresponding isolates are submitted to the National Reference Laboratory for Pneumococci at the Norwegian Institute of Public Health (NIPH). Isolate and patient data were linked using the national personal identifier.

In addition to the IPD data, we included data from carriage studies performed in the autumns of 2006 (before mass childhood vaccination with PCV7 started), 2008 (2 years after PCV7 introduction), 2013 (2 years after the change to PCV13), and 2015, which have previously been published [7,21,22]. The carriage studies included isolates sampled from children typically aged 1–5 years attending day-care centres in and around Oslo, the capital of Norway. See for more details the following references: [7,21–23].

2.2. Laboratory methods

IPD isolates were serotyped using the Quellung reaction using capsule specific antisera (SSI Diagnostica, Denmark). Carriage isolates were identified by using the commercial Pneumotest-Latex kit (SSI); Quellung was used for confirmation and serotyping. Serotypes were categorised as PCV7, serotypes only included in PCV13 (PCV13-7), or NVT.

Antimicrobial susceptibility was determined for penicillin G (PEN), ceftriaxone (CRO), cefotaxime (CTX), erythromycin (ERY), clindamycin (CLI), tetracycline (TET), and trimethoprim/sulfamethoxazole (SXT). In 2004–2005, doxycycline was used as a proxy for TET resistance. For IPD isolates from 2009 to 2016 and all carriage isolates, minimum inhibitory concentrations (MICs) were determined using antimicrobial gradient strips (Etest, Biomérieux, France). Pure cultures of pneumococci, suspended in Mueller-Hinton broth at 0.5 McFarland (or 1.0 for mucoid colonies such as serotype 3), were subsequently used to inoculate Mueller-Hinton agar with 5% horse blood. Agar plates with gradient strips were incubated overnight at 35 °C, with 5% CO_2 before reading MIC values. During 2004–2008, antimicrobial susceptibility of IPD isolates was performed by disk diffusion method (AB Biodisk, Sweden); MICs were determined only for isolates with decreased susceptibility as observed in the disk diffusion method. Isolates were characterized as belonging to the susceptible, standard dosing regime (S), susceptible, increased exposure (I) or resistant (R) categories using EUCAST breakpoints, v 9.0 [24]. Non-susceptibility was defined as the I or R categories. Multidrug resistance (MDR) was defined as non-susceptibility to PEN, combined with resistance to $\geq$ 2 non-β-lactam antimicrobials.

All IPD isolates from the first 6 months of every second year (2005–2015) and all carriage isolates were genotyped using multilocus sequence typing (MLST) [25]. Sequence types (STs) were determined using the MLST database (http://pubmlst.org/spneumoniae/).

2.3. Data analysis

We described antimicrobial non-susceptibility/resistance among IPD isolates as annual incidence (number of cases / 100,000 population) and as percentage of the cases. Population denominators were obtained from Statistics Norway (http://www.ssb.no). Incidences were corrected for missing antimicrobial susceptibility and serotype data proportionally to the distribution among those with complete data. We calculated incidence rate ratios (IRRs) with 95% confidence intervals [95%CI] using Poisson regression. The IRR describes the average gradient of the incidence-over-time-plot, i.e., the amount the incidence changes on average per year since a change in vaccination was introduced. IRRs were calculated overall and by serotype category. For the analysis, we included the year of introduction/transition of the vaccine as the baseline, and otherwise included all years when the vaccine selection pressure was present; i.e. for PCV7 serotypes years 2006–2016, for PCV13 and PCV13-7 serotypes years 2011–2016 and for NVTs years 2006–2016. Observed changes in individual serotypes were not tested for statistical significance.

To assess the origin of the non-susceptible and resistant clones over the years, we categorised each clone in whether it I: was already established in Norway prior to the studying year, II: was novel to Norway but defined in the MLST database, or III: potentially had originated from capsular switching (i.e., the serotype/ST combination was new in Norway and for the MLST database). For this categorisation, MLST results of all IPD and carriage isolates sampled in the previous or same years were used to determine previous existence in Norway. This included STs of IPD isolates from the first 6 months of 2000. We assumed potential capsular switching if the ST/serotype combination was not in the MLST database prior to the year our isolate was found, except if, I: the ST was novel, II: only the serogroup was registered and matched our isolate, or III: our ST was 6C, while the ST described in the database was 6A, with an entry date in 2007 or before [26].

We used Stata 15 for data analysis.

3. Results

3.1. Description of the data

Overall 10,239 IPD cases were available for this study; for 92% both, serotype and antimicrobial susceptibility data was available from the isolate (n = 9438; supplementary Table 1). STs were available for 24% (n = 2473) of isolates. The median age of the IPD cases was 65 years (interquartile range: 50–78; 6% was younger than 5 years old) and 51% was male. Through the carriage studies, 2146 isolates were available (range by study, 463–583 isolates [21,22]). The median age of the carriage-study participants was 3.5 years and 52% was male.

3.2. Changes coinciding with vaccine introduction

The overall IPD incidence decreased from 24/100,000 population in the pre-PCV period (average 2004/2005) to 15/100,000 in the PCV7 period (in 2010) and further to 11/100,000 in the PCV13 period (in 2016).

3.2.1. Multidrug resistance

The incidence of IPD caused by MDR isolates slightly increased from 0.11/100,000 population in 2004 to 0.38/100,000 in 2016 (Fig. 1); IRRs for the separate periods were not significantly different from 1 meaning that the change was too small to be statistically significant (Fig. 2A). There were 158 MDR-IPD isolates (1.7% of IPD isolates; Supplementary table 2), representing 22 serotypes and one non-capsulated isolate. PCV13 serotypes accounted for 59% (n = 93). After PCV introduction, IPD caused by MDR-NVTs increased as can be seen in Fig. 2B, where the IRR of the NVTs was significantly larger than 1. The most frequent MDR-serotype was NVT serotype 15A; it accounted for 28% of these isolates (n = 45) and was the main driver of the increase in MDR-IPD in the PCV13 period. Nearly all (12 of 13) genotyped 15A-MDR IPD isolates were ST63 (Table 1); this clone was also present in all carriage studies since 2008, with increasing prevalence after PCV13 introduction, and covered 52% (13 of 25) of all carried MDR isolates. Serotype 19A was the second most common MDR-serotype, accounting for 27% of the MDR IPD isolates (n = 42), but this one was not found among the carriage isolates. Seven of the MDR-serotype 19A IPD isolates were genotyped and represented six different STs (Table 1). MDR-serotype 19A IPD increased during the PCV7 period and decreased again in the PCV13 period.

3.2.2. Penicillin-non-susceptible pneumococci (PNSP)

Overall, 3.3% of IPD patients were infected with PNSP (n = 309). The most common serotypes were 19A (21%; n = 64), 15A (15%; n = 46), 23B (9%; n = 28), 9V (7%; n = 22) and 14 (7%; n = 22); Table 1. The incidence of PNSP-IPD increased from 0.31/100,000 in 2004, peaked at 0.76/100,000 in 2012 and decreased to 0.59/100,000 in 2016 (Fig. 1). Overall, PNSP-IPD caused by PCV7 serotypes decreased since 2006 as indicated by an IRR below 1 (IRR 0.88 [CI95% 0.81–0.94]; several serotypes and STs; Fig. 2B). In the PCV13 period, PNSP-IPD decreased further as a result of a decrease of PCV13-7 serotypes (IRR 0.74 [0.62–0.89]), mainly 19A. Serotype 19A PNSP-IPD increased from 1 to 2 cases/year pre-PCV to 12 cases in 2012, followed by a decrease in the PCV13 period (≤4 cases/year). Six of 16 genotyped isolates were ST199; the others belonged to nine other STs (Table 1). The majority of the serotype 19A-PNSP isolates met the MDR definition (66%; n = 42/64). Carriage results showed a very similar trend, with the maximum number of carried serotype 19A-PNSP isolates in 2012 and a subsequent decrease. The incidence of PNSP-NVT IPD showed an increase (IRR 1.2 [1.1–1.3]) after 2006, mainly related to MDR-serotype 15A-ST63 and PNSP-serotype 23B (mainly ST2372). Although the latter isolates were not MDR, 26/28 serotype 23B-PNSP were also non-susceptible to SXT. Serotype 23B-PNSP were present among the IPD isolates since 2010 and serotype 23B-ST2372 PNSP was present in the 2013 and 2015 carriage studies.

Six isolates (2% of PNSP) were fully resistant to PEN; all were PCV13 serotypes and fulfilled the MDR definition.

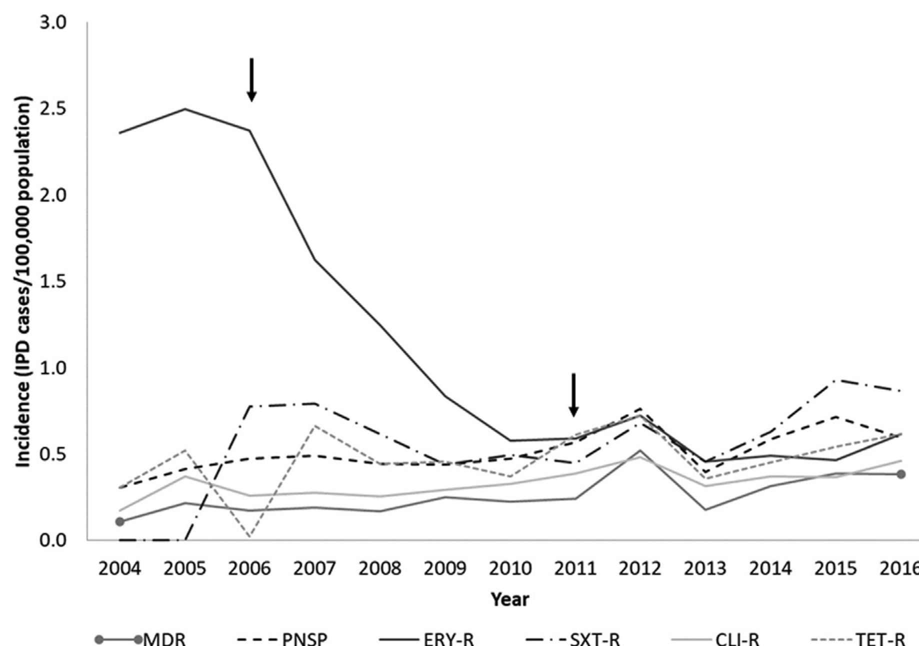

Fig. 1. Incidence of invasive pneumococcal disease (IPD) caused by resistant or non-susceptible isolates in Norway, 2004–2016, by antimicrobial. Footnote for Fig. 1: For 2008, data on non-susceptibility to trimethoprim/sulfamethoxazole (SXT) was missing; we therefore show the average of 2007 and 2009. MDR, multidrug resistant; PNSP, penicillin-non-susceptible pneumococci; ERY-R, erythromycin resistant; SXT-R, trimethoprim/sulfamethoxazole resistant; CLI-R, clindamycin resistant; TET-R, tetracycline resistant. The arrows indicate the introduction of PCV7 (July 2006) and subsequent transition to PCV13 (April 2011).

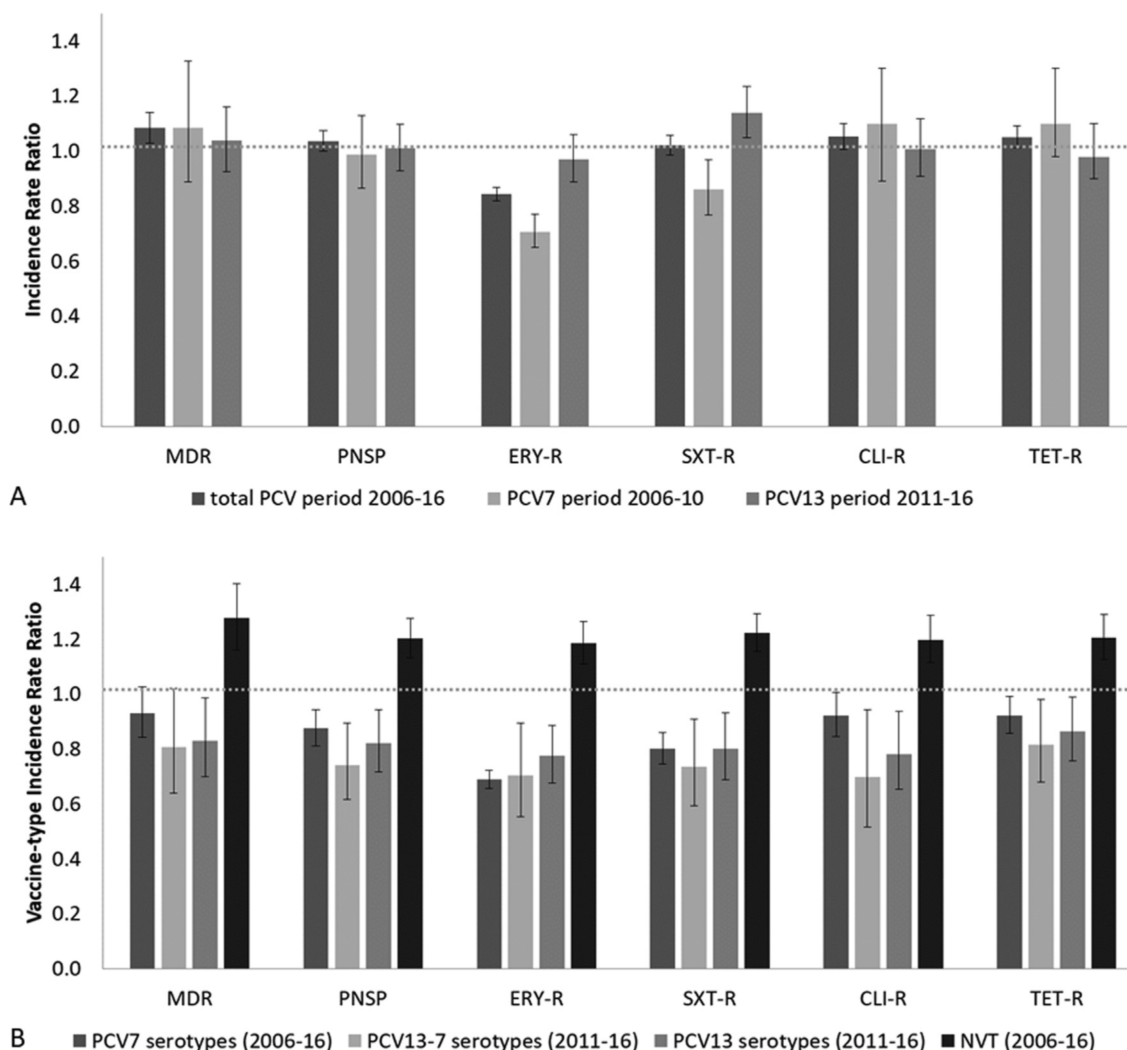

Fig. 2. Incidence rate ratios (IRRs) of invasive pneumococcal disease caused by non-susceptible or resistant invasive pneumococcal (IPD) isolates. (A) IPD caused by all serotypes, by time period. (B) IPD caused by vaccine serotype group. Note that the included years differ between serotype categories, as those cover the period when vaccine selection pressure was present. IRRs describe the average gradient of the incidence-over-time-plot; i.e., the amount the incidence changes on average per year since a change in vaccination was introduced. The horizontal dotted line marks IRR 1.0, which indicates no change over time. The black vertical lines indicate the 95% confidence interval around the estimated IRR. Footnote for Fig. 2: MDR, multidrug resistant; PNSP, penicillin-non-susceptible pneumococci; ERY-R, erythromycin resistant; SXT-R, trimethoprim/sulfamethoxazole resistant; CLI-R, clindamycin resistant; TET-R, tetracycline resistant; PCV, pneumococcal conjugate vaccine; PCV7, 7-valent PCV; PCV13, 13-valent PCV; PCV13-7 serotypes, i.e. the six serotypes covered by PCV13 but not by PCV7; NVT, non-vaccine serotypes, i.e. serotypes not included in the 7- or 13-valent pneumococcal conjugate vaccines.

The 151 non-MDR-PNSP isolates represented 31 serotypes and 26 STs, with PCV13 serotypes accounting for 50% of them (mainly serotypes 19A and 9V).

3.2.3. Erythromycin resistant (ERY-R) pneumococci

Overall, 6.7% of IPD cases were infected with ERY-R pneumococci (n = 633), representing 38 different serotypes and two non-capsulated isolates. IPD caused by ERY-R isolates decreased in the PCV7 period (IRR 0.71 [0.65–0.77], Fig. 2A), mainly due to a sharp decline in ERY-R serotype 14. The majority (98%) of these were ST9 (n = 82) or its single locus variants (SLVs) ST3102 (n = 7) and ST3190 (n = 1). Serotype 14-ST9 isolates were not identified in the latest genotyped sample from 2015, and were only present in the 2006 carriage study. Serotype 19A ERY-R increased after PCV7 introduction, followed by a decrease after 2012 (several STs; Table 1). In the carriage study, serotype 19A ERY-R was only found once (in 2015). ERY-R IPD caused by NVTs increased since 2006 (IRR 1.2 [1.1–1.3], Fig. 2B), mainly due to MDR-serotype 15A-ST63 as discussed above. In the pre-PCV period, only two ERY-R NVT isolates were present (serotypes 15A and 38). Of the 51 ERY-R NVTs that did not meet the MDR definition, the most frequent serotype was 33F, covering 39% of those isolates (n = 20). Of the three serotype 33F isolates that were genotyped, two were ST717 and one ST9583, a SLV of 717. ERY-R serotype 33F IPD isolates were first present in 2006 and slightly increased over time. Serotype 33F was only found once among carriage isolates (in 2008; ST9583).

3.2.4. Trimethoprim/sulfamethoxazole resistant (SXT-R) pneumococci

Overall, 3.6% of IPD cases were infected with SXT-R pneumococci (n = 307); these isolates represented 43 serotypes, of which the most common were 24F (covering 20%; n = 61), 19A (14%; n = 43), 9V (10%; n = 32) and 23B (7%; n = 21). There was a decrease in the incidence of SXT-R IPD in the PCV7 period from 0.78/100,000 to 0.49/100,000 and an increase during the PCV13 period to 0.86/100,000 (Fig. 1 and Fig. 2A). The former reflected a decrease in SXT-R PCV7 serotypes (IRR 0.80 [0.75–0.86], Fig. 2B), mainly due to a decrease in serotypes 9V(genotype data was available

Table 1
Serotype/sequence type (ST) combinations and their numbers among invasive pneumococcal disease isolates for serotypes with at least 10 non-susceptible (to penicillin) or resistant (to the other antimicrobials) isolates found during the study period. The highlighted serotype/ST combinations indicate clones that represented more than 50% of the genotyped isolates of that serotype.

Resistance pattern (overall number of isolates with that resistance pattern)	Vaccine-serotype category[a]	Serotype (number of non-susceptible or resistant isolates)	Number with ST available from systematic sampling	ST (number of non-susceptible or resistant isolates)
MDR (*n* = 158)	NVT	**15A** (*n* = 45)	13	**63** (*n* = 12), 3777 (*n* = 1)
	PCV13-7	19A (*n* = 42)	7	2013 (*n* = 2), 276 (*n* = 1), 3710 (*n* = 1), 3772 (*n* = 1), 8640 (*n* = 1), 9387 (*n* = 1)
	PCV7	14 (*n* = 14)	2	143 (*n* = 1), 554 (*n* = 1)
	PCV7	19F (*n* = 10)	3	63 (*n* = 1), 81 (*n* = 1), 236 (*n* = 1)
PNSP (*n* = 309)	PCV13-7	19A (*n* = 64)	16	199 (*n* = 6), 2013 (*n* = 2), 172 (*n* = 1), 276 (*n* = 1), 3710 (*n* = 1), 3772 (*n* = 1), 8517 (*n* = 1), 8640 (*n* = 1), 9387 (*n* = 1), 11,340 (*n* = 1)
	NVT	**15A** (*n* = 46)	13	**63** (*n* = 12), 3777 (*n* = 1)
	NVT	**23B** (*n* = 28)	6	**2372** (*n* = 4), 8518 (*n* = 1), 8959 (*n* = 1)
	PCV7	9V (*n* = 22)	2	156 (*n* = 1), 1269 (*n* = 1)
	PCV7	14 (*n* = 22)	3	143 (*n* = 1), 156 (*n* = 1), 554 (*n* = 1)
	PCV7	19F (*n* = 15)	3	63 (*n* = 1), 81 (*n* = 1), 236 (*n* = 1)
	PCV7	23F (*n* = 14)	5	81 (*n* = 2), 277 (*n* = 1), 342 (*n* = 1), 9320 (*n* = 1 s)
	PCV7	6B (*n* = 12)	4	95 (*n* = 1), 315 (*n* = 1), 3207 (*n* = 1), 3614 (*n* = 1)
ERY-R (*n* = 633)	PCV7	**14** (*n* = 376)	92	**9** (*n* = 82), 3102 (*n* = 7), 143 (*n* = 1), 554 (*n* = 1), 3190 (*n* = 1)
	NVT	**15A** (*n* = 46)	13	**63** (*n* = 12), 3777 (*n* = 1),
	PCV13-7	19A (*n* = 42)	8	3546 (*n* = 2), 199 (*n* = 1), 276 (*n* = 1), 416 (*n* = 1), 3772 (*n* = 1), 8640 (*n* = 1), 9387 (*n* = 1)
	PCV7	**19F** (*n* = 30)	13	**179** (*n* = 7), 236 (*n* = 2), 63 (*n* = 1), 81 (*n* = 1), 462 (*n* = 1), 9328 (*n* = 1)
	PCV7	6B (*n* = 27)	9	176 (*n* = 2), 469 (*n* = 2), 8573 (*n* = 2), 95 (*n* = 1), 138 (*n* = 1), 315 (*n* = 1)
	NVT	**33F** (*n* = 21)	3	**717** (*n* = 2), 9583 (*n* = 1)
	PCV7	9V (*n* = 18)	2	162 (*n* = 1), 5960 (*n* = 1)
	PCV7	23F (*n* = 11)	5	81 (*n* = 2), 242 (*n* = 1), 342 (*n* = 1), 9320 (*n* = 1)
SXT-R (*n* = 307)	NVT	**24F** (*n* = 61)	7	**162** (*n* = 6 [b]), 644 (*n* = 1)
	PCV13-7	19A (*n* = 43)	12	199 (*n* = 4), 2013 (*n* = 2), 3546 (*n* = 2), 3710 (*n* = 1), 8517 (*n* = 1), 9387 (*n* = 1), 11,340 (*n* = 1)
	PCV7	9V (*n* = 32)	4	162 (*n* = 2), 156 (*n* = 1), 5960 (*n* = 1)
	NVT	**23B** (*n* = 21)	3	**2372** (*n* = 3)
	NVT	**33F** (*n* = 18)	6	**100** (*n* = 6)
	PCV7	6B (*n* = 12)	4	8573 (*n* = 2), 3614 (*n* = 1), 8711 (*n* = 1)
	PCV13-7	**1** (*n* = 11)	2	**217** (*n* = 2)
	NVT	**23A** (*n* = 11)	1	**338** (*n* = 1)
	PCV7	23F (*n* = 11)	5	311 (*n* = 2), 81 (*n* = 1), 277 (*n* = 1), 342 (*n* = 1)
CLI-R (*n* = 199)	NVT	**15A** (*n* = 46)	13	**63** (*n* = 12), 3777 (*n* = 1)
	PCV13-7	19A (*n* = 32)	6	3546 (*n* = 2), 416 (*n* = 1), 3772 (*n* = 1), 8640 (*n* = 1), 9387 (*n* = 1)
	PCV7	**19F** (*n* = 25)	10	**179** (*n* = 7); 63 (*n* = 1), 462 (*n* = 1), 9328 (*n* = 1)
	NVT	**33F** (*n* = 18)	3	**717** (*n* = 2), 9583 (*n* = 1)
	PCV7	14 (*n* = 17)	2	143 (*n* = 1), 554 (*n* = 1)
	PCV7	6B (*n* = 15)	6	176 (*n* = 2), 8573 (*n* = 2), 95 (*n* = 1), 315 (*n* = 1)
TET-R (*n* = 282) [c]	PCV13-7	19A (*n* = 55)	11	2013 (*n* = 2), 3546 (*n* = 2), 276 (*n* = 1), 416 (*n* = 1), 847 (*n* = 1), 3710 (*n* = 1), 3772 (*n* = 1), 8640 (*n* = 1), 9387 (*n* = 1)
	PCV7	19F (*n* = 54)	21	179 (*n* = 6), 177 (*n* = 5), 462 (*n* = 5), 63 (*n* = 1), 81 (*n* = 1), 236 (*n* = 1), 3100 (*n* = 1), 9328 (*n* = 1)
	NVT	**15A** (*n* = 43)	12	**63** (*n* = 11), 3777 (*n* = 1)
	NVT	12F (*n* = 12)	1	3377 (*n* = 1)
	PCV7	23F (*n* = 12)	6	81 (*n* = 2), 242 (*n* = 1), 342 (*n* = 1), 2031 (*n* = 1), 9320 (*n* = 1)
	PCV7	6B (*n* = 12)	7	8573 (*n* = 2), 95 (*n* = 1), 315 (*n* = 1), 3207 (*n* = 1), 5862 (*n* = 1), 11,205 (*n* = 1)
	PCV13-7	3 (*n* = 10)	5	180 (*n* = 2), 260 (*n* = 1), 271 (*n* = 1), 11,341 (*n* = 1)

MDR, multidrug resistance (i.e., non-susceptibility to penicillin, combined with resistance to $\geq$ 2 non-β-lactam antimicrobials); PNSP, penicillin non-susceptible pneumococci; ERY-R, erythromycin resistant; SXT-R, trimethoprim/sulfamethoxazole resistant; CLI-R, clindamycin resistant; TET-R, tetracycline resistant.

[a] Vaccine-serotype category PCV7 = serotypes covered by the 7-valent pneumococcal conjugate vaccine (PCV), PCV13-7 = serotypes only included in the 13-valent PCV but not in PCV7, NVT = non-vaccine serotypes.

[b] In addition to the systematically sampled isolates for MLST, we determined the ST of all available 24F isolates up to 2015 because of an increase in its incidence. Of the extra analysed isolates (*n* = 55), 30 were ST162; all occurred from 2012 onwards and with increasing incidence, and all were SXT-R. In the period 2012–2015, only 10 non-ST162 serotype 24F isolates were observed (ST177: *n* = 6, ST72: *n* = 3, ST11618: *n* = 1). The ST177 and ST72 were susceptible to all tested antimicrobials, while the ST11618 isolate was MDR.

[c] In 2004–2005, TET susceptibility was inferred from doxycycline susceptibility.

for only a few isolates: serotype 9V isolates were ST162 or its SLVs). In the PCV13 period, SXT-R IPD caused by the PCV13-7 serotypes decreased (IRR 0.73 [0.59–0.91]) as a result of decreases in serotype 1 and 19A (MDR-ST199 and other STs). These SXT-R PCV7 and PCV13-7 serotypes were uncommon in all carriage studies. The IPD incidence of SXT-R NVTs increased during the PCV13 period (IRR 1.2 [1.2–1.3]), driven by a sharp increase in SXT-R serotype 24F-ST162, as well as an increase in SXT-R serotype 23B

(ST2372). Serotype 24F-ST162 was also present in the 2013 and 2015 carriage studies; all isolates were SXT-R. The serotype 23B isolates have been described in the PNSP section above.

3.2.5. Clindamycin resistant (CLI-R) pneumococci

Overall, 2.1% of IPD cases were infected with CLI-R pneumococci (n = 199), representing 52 serotypes. All but one isolate were also ERY-R. The overall incidence of CLI-R IPD did not change over time (Fig. 2A); the average annual incidence was 0.33/100,000 (Fig. 1). However, CLI-R PCV13-7 serotypes decreased in the PCV13 period (IRR 0.70 [0.52–0.94], Fig. 2B), due to the decline of serotype 19A. Two-thirds of the CLI-R serotype 19A isolates were MDR (n = 21; several STs). This decline was levelled off by an increase in serotype 15A-MDR isolates. Serotype 15A covered 23% of all CLI-R IPD isolates (n = 46); 92% of those genotyped were ST63. A similar trend, with an increase over time in serotype 15A (ST63) CLI-R was seen among the carried isolates. PCV7 serotype 19F was prevalent among CLI-R IPD isolates (13% of total; n = 25), although the numbers never exceeded five cases per year. Of the genotyped CLI-R serotype 19F IPD isolates, 8/of 10 were ST179 or its SLV ST9328; this clone was also present in all carriage studies. Of the 35 non-MDR CLI-R NVTs, the most frequent serotype was 33F with 49% (n = 17) isolates. They appeared in 2007 at 0–1 isolates/year in the PCV7 period, and increased to 1–4 isolates/year in the PCV13 period. The three genotyped isolates were ST717 and a SLV, ST9583; the latter was also present in the 2013 carriage study.

3.2.6. Tetracycline resistant (TET-R) pneumococci

Overall, 3.0% of IPD cases were infected with TET-R pneumococci (n = 282), representing 42 serotypes and a non-encapsulated isolate. The overall incidence of TET-R IPD did not change in any of the three analysed periods (Fig. 2A). The average annual incidence was 0.47/100,000. In the PCV13 period, IPD caused by TET-R NVTs increased (IRR 1.2 [1.1–1.3], Fig. 2B), mainly due to MDR-serotype 15A-ST63. PCV13 serotypes covered 61% of the TET-R isolates; the most frequent were serotypes 19A (20% of total; n = 55) and 19F (19%; n = 54). Serotype 19A TET-R IPD (several STs; Table 1) was particularly prominent in the PCV7 and early PCV13 period and declined during the PCV13 period, while serotype 19F TET-R was present throughout our study. Of the genotyped TET-R serotype 19F isolates, 12/21 were ST177 or its SLV ST179 or related STs. Serotype 19F TET-R isolates with these STs were also present in the carriage studies. Interestingly, TET-R ST177 was susceptible to ERY, while TET-R ST179 was ERY-R and CLI-R.

3.2.7. Other antimicrobials

Non-susceptibility to CRO and CTX was rare (<0.4% of total; n = 22 and n = 38, respectively) and none of the isolates was resistant to CRO, while two were resistant to CTX (Supplementary table 2).

3.3. The origin of non-susceptible/resistant clones

As presented in bold in Table 1, for some serotypes, the majority of non-susceptible/resistant isolates for each antimicrobial belonged to one ST or closely related variants, indicating a clonal nature of antimicrobial non-susceptibility. This was the case particularly for serotype 14-ST9 (ERY-R), serotype 24F-ST162 (SXT-R), serotype 15A-ST63 (MDR), serotype 23B-ST2372 (PNSP and SXT-R), serotype 19F-ST179 (ERY-R and CLI-R; some were also TET-R), serotype 33F-ST100 (SXT-R) and serotype 33F-ST717 (ERY-R and CLI-R). However, as can be seen in Table 1, many serotypes with non-susceptible/resistant isolates were not dominated by large clones in Norway. Compared to antimicrobial susceptible isolates, i.e., those that did not fit any of the AMR non-susceptibility/resistance patterns we studied, the non-susceptible/resistant isolates were slightly less often dominated by one specific clone (data not shown).

The origin of the non-susceptible and resistant clones in Norway were estimated using the three categories described in the Material & Methods section. Overall, for all genotyped IPD isolates, 84% had a serotype/ST combination that was already established in Norway, 15% were novel to Norway and 0.8% had a novel serotype/ST combination indicating potential capsular switching (Table 2). Nine of the latter were NVTs (6C, 10F, 18F, 23A, 23B, 24F, 33F, 35A, 35C); three of them (serotype 23B-ST440, serotype 24F-ST664 and serotype 6C-ST1135) were potential switches from a vaccine serotype and were identified in the PCV13 period. Among the genotyped isolates that were PNSP, ERY-R, CLI-R, TET-R or SXT-R (n = 248), 66% were part of established Norwegian clones, 33% were novel to Norway and 1.2% had a novel serotype/ST combination indicating potential capsular switching. All three AMR isolates with novel serotype/ST combinations were identified in the PCV13 period; these were serotype 3-ST271 (MDR), serotype 24F-ST644 (SXT-R) and serotype 6B-ST8711 (SXT-R).

When looking at the specific AMR groups, the majority of MDR isolates were novel to Norway (54%, n = 20; Table 2); this was more frequent than for isolates that were susceptible to the tested antimicrobials (13%, n = 292; not statistically tested). Similarly, among the PNSP, 58% were novel to Norway (n = 42), 41% were part of established Norwegian clones (n = 30) and one isolate had a novel serotype/ST combination. Similar patterns were observed for CLI-R (47% novel to Norway), SXT-R (39% novel) and TET-R (54% novel). The proportion of clones novel to Norway was lower (23%) for ERY-R isolates.

Focusing on the origin of the main resistant clones, either from IPD or from carriage, ERY-R serotype 14-ST9 was already established in Norway in 2004, while MDR-serotype 15A-ST63 was first seen in 2005, so, also before PCV7 introduction. Similarly, the ERY-R/CLI-R serotype 19F-ST179 clone was introduced in Norway in 2005 and was present during the rest of the study period. The serotype 33F-ST100 clone was introduced in Norway in 2005, but its SXT-R clone was first found in 2007. SXT-R serotype 24F-ST162 was first found in 2012, so after the transition to PCV13, and became increasingly dominant over the study period. SXT-R serotype 23B IPD isolates first appeared in 2012 and were present every year since then. The ERY-R/CLI-R serotype 33F-ST717 clone was first seen in Norway in 2013.

4. Discussion

Our study covering 13 years of antimicrobial susceptibility surveillance in whole Norway showed that AMR is low, but the incidence changed following changes in pneumococcal childhood vaccination. In the PCV7 period, we observed a decrease in ERY-R and SXT-R IPD. The decrease of PCV7 serotype 14-ST9 played an important role, as this clone had been responsible for most ERY-R IPD in Norway [12]. In the PCV13 period, NVT resistance/non-susceptibility increased for all studied antimicrobials. The introduction and expansion of MDR-serotype 15A-ST63, SXT-R serotype 24F-ST162, PNSP/SXT-R serotype 23B-ST2372 and ERY/CLI-R serotype 33F contributed to this. An increase in MDR-serotype 19A in the PCV7 period and a subsequent decrease in the PCV13 period slightly restrained some of the abovementioned trends. The carriage studies results mostly resembled the changes seen in the IPD data despite the fact that they were performed in children and in a relative small geographic area.

While for several serotypes the non-susceptible/resistant isolates were dominated by specific clones, overall, these isolates were genetically more diverse than the susceptible isolates. This

Table 2
The origin of invasive pneumococcal disease clones, overall and for the non-susceptible or resistant isolates, Norway, years 2004–2016. Isolates have been systematically selected for genotyping (n = 2473).

Characteristic	Origin of the clones	Time period									
		Pre-PCV (2004–2005)		Introduction year (2006) [a]		PCV7 (2007–2010)		Vaccine transition period (2011)		PCV13 (2012–2016)	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
All	Norwegian clone	431	83	–	–	818	85	319	82	512	86
	Introduction of existing or novel clone	87	17	–	–	143	15	68	17	75	13
	Potential capsular switch	3	0.6	–	–	4	0.4	2	0.5	11	1.8
Antimicrobial susceptible [b]	Norwegian clone	388	85	–	–	738	87	301	83	476	88
	Introduction of existing or novel clone	68	15	–	–	108	13	60	17	56	10
	Potential capsular switch	3	0.7	–	–	4	0.5	2	0.6	8	1.5
MDR	Norwegian clone	0	0	–	–	3	27	6	60	10	67
	Introduction of existing or novel clone	6	100	–	–	8	73	2	40	4	27
	Potential capsular switch	0	0	–	–	0	0	0	0	1	7
PNSP	Norwegian clone	0	0	–	–	7	29	7	54	16	59
	Introduction of existing or novel clone	9	100	–	–	17	71	6	46	10	37
	Potential capsular switch	0	0	–	–	0	0	0	0	1	3.7
ERY-R	Norwegian clone	40	74	–	–	55	85	11	79	15	60
	Introduction of existing or novel clone	14	26	–	–	10	15	3	21	9	36
	Potential capsular switch	0	0	–	–	0	0	0	0	1	4
SXT-R	Norwegian clone	0	0	–	–	14	45	3	60	18	72
	Introduction of existing or novel clone	0	0	–	–	17	55	2	40	4	16
	Potential capsular switch	0	0	–	–	0	0	0	0	3	12
CLI-R	Norwegian clone	2	22	–	–	6	50	6	67	12	63
	Introduction of existing or novel clone	7	78	–	–	6	50	3	33	7	37
	Potential capsular switch	0	0	–	–	0	0	0	0	0	0
TET-R	Norwegian clone	3	20	–	–	12	40	7	70	15	54
	Introduction of existing or novel clone	12	80	–	–	18	60	3	30	12	43
	Potential capsular switch	0	0	–	–	0	0	0	0	1	3.6

MDR, multidrug resistant; PNSP, penicillin-non-susceptible pneumococci; ERY-R, erythromycin resistant; SXT-R, trimethoprim/sulfamethoxazole resistant; CLI-R, clindamycin resistant; TET-R, tetracycline resistant.
[a] No systematically selected isolates were analysed by MLST for the year 2006, so no information on the origin of the clones is available.
[b] Antimicrobial susceptibility was defined as not being PNSP, MDR, ERY-R, SXT-R, CLI-R, TET-R, ceftriaxone resistant or cefotaxime resistant.

matches well with the fact that many of the MDR, PNSP, TET-R, CLI-R and SXT-R isolates were more often newly introduced clones compared to susceptible isolates, although their proportion decreased over time as these clones established themselves in Norway. With nasopharyngeal niches becoming vacant due to vaccination, competition is absent and less fit clones have a chance to establish themselves [27]. Travel to areas where AMR is more prevalent than in Norway after vaccine introduction/transition in vaccination may therefore have contributed to the observed increase in NVT resistance/non-susceptibility. It should, however, be noted that vaccine serotypes still persisted in low numbers after vaccine introduction, with the majority of the serotype 19F and 14 IPD isolates in the PCV13 period being non-susceptible to at least one antimicrobial.

Interestingly, the resistance or non-susceptibility to all studied antimicrobials peaked in 2012. This seems to reflect changes in the number of resistant PCV13-7 and NVT serotype isolates in the PCV7 period, before PCV13 decreased the occurrence of the PCV13 serotypes. For example, the number of non-susceptible or resistant serotype 19A isolates remained at the same level or slightly higher in 2012 as in 2011, although the number of susceptible serotype 19A IPD isolates had already started to decrease. The number of non-susceptible and resistant serotype 19A IPD isolates decreased sharply in 2013. Overall, the incidence of IPD caused by antimicrobial non-susceptible pneumococci in Norway was low compared to many other (Western) countries [28,29,30,47], which may reflect the low antimicrobials consumption in the population [31].

The greatest reduction in resistance following PCV7 implementation was seen for ERY as a consequence of the decrease in serotype 14-ST9, also known as the PMEN-9 England[14]ST9 clone, and its SLVs. By contrast, PNSP isolates and isolates resistant to other antimicrobials were more diverse before PCV introduction, which in turn did not allow for a sharp decrease. Other studies have indicated similar decreasing trends in ERY-R and increasing or stable trends in PNSP in the PCV7 and PCV13 period [32].

The incidence of IPD caused by resistant NVTs increased after PCV introduction; similar changes have been described elsewhere for IPD [10,20], as well as in carriage [30], partly also in similar serotypes. The most important resistant NVT in our study was MDR-serotype 15A. This serotype 15A-ST63 clone, also known as PMEN-25 Sweden[15A]ST63, emerged in the UK and Japan following PCV introduction [33,34] and has been described in the US [35], while a serotype 8-ST63 recombinant has spread in Spain [36]. Additionally, serotype 33F emerged in Norway, as a serotype displaying resistance to several antimicrobials (ERY, SXT, CLI, and intermediate susceptibility to TET), although it was not MDR. Serotype 33F has been emerging in USA in the PCV13 period, some of these isolates display non-susceptibility to antimicrobials [20,37]. Replacement of PCV13 serotype isolates by NVT isolates, was observed especially among SXT-R isolates. Serotype 24F was the most prominent SXT-R NVT; this serotype has also been seen elsewhere; e.g. in France, a study of meningitis isolates described an increase of this serotype, including some PNSP isolates [38]. Other resistant NVTs present in our study that may need follow up, are serotypes 12F (TET-R, SXT-R), 23B (PNSP, SXT-R) and 23A (SXT-R). An increased consumption of SXT in Norway in the period 2011–2016 [31] may have contributed to the selection pressure of SXT-R pneumococci. The increases or persistence of these non-susceptible NVT serotypes indicate the need for vaccines with broader serotype ranges or even serotype-independent vaccines. The PCVs that are currently being tested (PCV15 [39], cPCV7 [40], 20vPnC [41], PCV24 [42]) do unfortunately not include the main non-susceptible serotypes circulating in Norway. Serotype-independent vaccines [43–45], if proven effective, could therefore contribute to prevent IPD caused by non-susceptible or resistant isolates.

In this study, we used data from a 13-year period systematically collected through the well-established surveillance systems in Norway. Serotyping and antimicrobial susceptibility data were available for nearly all isolates. The fact that genotyping data were available for only a quarter of the IPD isolates may eschew some clonal patterns and fail to reveal all present clones. However, the serotype distribution of the isolates selected for MLST and those not selected was very similar. Although rarely identified in this study, we might have overestimated capsular switching, as it is unlikely that all previously existing serotype and genotype combinations are represented in the MLST database. Additionally, we may have misclassified new serotype-genotype combinations as novel clones or capsular switching. These clones may have been present undetected before vaccine introduction, and became detectable through unmasking [46]. Method changes in susceptibility testing may have some bearing on results; however, this is mitigated by use of consistent cut-offs developed by EUCAST for all interpretations.

In conclusion, we report a decrease in ERY-R and STX-R in the PCV7 period, and an increase in resistance and non-susceptibility in the PCV13 period. Overall, our study revealed low AMR in IPD in Norway; this was supported by the data from the carriage studies. Resistant or non-susceptible isolates were more often novel clones that were introduced into Norway during the study period, with the exception of ERY-R which was initially dominated by the established serotype 14-ST9 clone. We observed an increase in resistant or non-susceptible NVTs. There has been optimism for limiting AMR by PCVs, however, as our study shows, the result is not straight forward. We need new vaccines against a broader range of serotypes or independent of serotype, combined with continued prudent use of antimicrobials to limit emergence and spread of pneumococcal AMR. Higher-valency vaccines alone cannot stop AMR from evolving. We recommend maintaining a good surveillance system that encompasses both serotype/clonal and antimicrobial susceptibility data to enable understanding changes in the pneumococcal population and to inform future vaccination strategies.

5. Compliance with ethical standards

IPD was studied within the legal framework for surveillance activities at NIPH. The carriage studies were approved by the Regional Committee for Medical Research Ethics, South-Eastern Norway; parents/guardians gave written informed consent after the nature and possible consequences of the studies had been fully explained and before enrolment of their child. All the work has been carried out in accordance with the declaration of Helsinki.

CRediT authorship contribution statement

Lotta Siira: Conceptualization, Data curation, Methodology, Writing - original draft, Writing - review & editing. **Didrik F. Vestrheim:** Conceptualization, Writing - review & editing. **Brita A. Winje:** Conceptualization, Writing - review & editing. **Dominique A. Caugant:** Conceptualization, Writing - review & editing. **Anneke Steens:** Conceptualization, Data curation, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lotta Siira is a co-investigator in an unrelated study, for which the National Institute for Health and Welfare, Finland, has

received research funding from GlaxoSmithKline Biologicals SA. The other authors report no conflicts of interests.

Acknowledgements

We gratefully acknowledge the clinical microbiology laboratories for submitting IPD isolates to the national reference laboratory, the reference laboratory staff Anne Ramstad Alme, Gunnhild Rødal and Lene Kolstad for performing serotyping and antimicrobial susceptibility, and Torill Alvestad for performing MLST. We thank Kirsten Konsmo for MSIS data entry and delivery. We extend our gratitude to the children participating in the carriage studies, their parents, day care staff, and to Ingvild Essén, Line Tyskø and Kristine Hartmark for collecting the swabs. We also gratefully acknowledge local and international EUPHEM coordinators for guidance during this study and Loredana Ingrosso for reviewing the manuscript.

Appendix A. Supplementary material

The supplementary material presents tables with the description of the IPD data included in the study.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.06.040.

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