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ORIGINAL CONTRIBUTION

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Context The rapid increase in multiresistant serotype 19A as a cause of invasive and respiratory pneumococcal disease has been associated in time with the widespread implementation of 7-valent pneumococcal conjugate vaccination (PCV-7) in several countries. Because spontaneous fluctuations in time and antibiotic selective pressure may have induced this serotype 19A increase, controlled studies are needed to assess the role of PCV-7.

Objective To examine the association of PCV-7 vaccination and nasopharyngeal acquisition of serotype 19A pneumococci, their clonal distribution, and antibiotic susceptibility.

Design, Setting, and Patients Post hoc per-protocol completer's analysis as part of a randomized controlled trial of nasopharyngeal *Streptococcus pneumoniae* carriage enrolling 1003 healthy newborns with follow-up to the age of 24 months in the Netherlands, which has low antibiotic resistance rates. The study was conducted before widespread PCV-7 implementation in infants, between July 7, 2005, and February 14, 2008. Nasopharyngeal swabs were obtained at the age of 6 weeks and at 6, 12, 18, and 24 months.

Intervention Infants were randomly assigned to receive 2 doses of PCV-7 at 2 and 4 months; 2 + 1 doses of PCV-7 at 2, 4, and 11 months; or no dosage (unvaccinated control group).

Main Outcome Measure Cumulative proportion of children with nasopharyngeal acquisition of a new serotype 19A strain from 6 through 24 months of age.

Results Nine hundred forty-eight children completed the study. Fifty-four nasopharyngeal serotype 19A carriage isolates from 318 in the 2-dose group, 66 isolates from 327 in the 2 + 1-dose group, and 33 isolates from 303 in the unvaccinated were collected from 6 weeks through 24 months. The cumulative proportion who tested positive for new nasopharyngeal serotype 19A acquisition from 6 through 24 months of age was significantly higher in those having received the 2 + 1-dose PCV-7 schedule (16.2%; 95% confidence interval [CI], 12.6%-20.6%) vs those who were unvaccinated (9.2%; 95% CI, 6.5%-13.0%; relative risk [RR], 1.75; 95% CI, 1.14-2.70) but not after a 2-dose schedule (13.2%; 95% CI, 9.9%-17.4%; RR, 1.43; 95% CI, 0.91-2.25). There were 28 different sequence types identified, including 6 new types. The proportion of children with new 19A acquisition who had used antibiotics in the last 6 months (18.7%) did not differ among groups. Five isolates were penicillin-intermediate susceptible and another 3 were nonsusceptible to erythromycin and azithromycin, all in the vaccine groups.

Conclusion A 2 + 1-dose PCV-7 schedule was associated with an increase in serotype 19A nasopharyngeal acquisition compared with unvaccinated controls.

Trial Registration clinicaltrials.gov Identifier: NCT00189020

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A RAPID INCREASE IN THE PRESence of pneumococcal serotype 19A strains that are often multiresistant to antibiotics has been observed over the last decade.[1-3] In the United States, serotype 19A is now the leading causative pneumococcal serotype of invasive[1,4,5] and respiratory pneumococcal disease[6,7] and the most frequently observed serotype in nasopharyngeal carriage.[8] In the United States and other countries, the increase in serotype 19A disease was associated in time with the widespread implementation of heptavalent pneumococcal conjugate vaccination (PCV-7) in routine infant immunization programs. The role of PCV-7 in the increase in serotype 19A is however debatable because increases in other countries without PCV-7 implementation have also been reported.[9,10]

Because increases are often serotype 19A strains resistant to antibiotic agents and found in countries with high antibiotic prescription and resistance rates,[3,11] antibiotic pressure is thought to be an important selection factor.[10] Furthermore, when studied over long periods, significant fluctuations in seroepidemiology have been shown in different populations such as those in Den-

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mark and Spain before the introduction of PCV-7.[12,13]

Because all data on the expansion of serotype 19A have been obtained from uncontrolled observational studies, the role of PCV-7 cannot be discriminated from other factors such as antibiotic selection pressure or natural fluctuations in time. Data from randomized controlled trials are crucial for assessing PCV-7's true contribution. A large randomized trial on the effect of PCV-7 on pneumococcal carriage was performed in the Netherlands before widespread introduction of PCV-7 in the national immunization program.[14] Antibiotic prescription rates in primary care and antibiotic resistance rates are relatively low in the Netherlands compared with regions like southern Europe.[15] We performed a post hoc analysis to investigate the association between PCV-7 vaccination and nasopharyngeal acquisition of serotype 19A pneumococci in the first 2 years of life, their clonal distribution, and their antibiotic susceptibility.

METHODS

Study Design and Population

This was a post hoc analysis from a randomized controlled trial in the Western region of the Netherlands studying the effect of reduced-dose PCV-7 schedules on pneumococcal nasopharyngeal carriage. Enrollment started on July 7, 2005, and was completed on February 9, 2006, before the introduction of PCV-7 in the Dutch national immunization program. Follow-up ended February 14, 2008. The trial's methods were previously described and results have been published for pneumococcal carriage efficacy.[14] In brief, after obtaining written informed consent from both parents or guardians, participants were randomly assigned to receive (1) PCV-7 at the age of 2 and 4 months (2-dose group), (2) PCV-7 at 2, 4, and 11 months (2 + 1-dose group), or (3) no PCV-7 (unvaccinated control group; FIGURE 1). Parents were aware of the child's vaccination schedule.

Deep nasopharyngeal swabs were taken transnasally at age 6 weeks and age 6, 12, 18, and 24 months with a flexible, sterile, dry cotton-wool swab (Transwab Pernasal Plain, Medical Wire & Equipment Co, Ltd, Corsham, Wiltshire, England) by nurses trained in World Health Organization standard procedures.[16] Transport, isolation, and identification of pneumococci were done by standard methods as previously described.[14] Briefly, identification of *Streptococcus pneumoniae* was based on colony morphology and conventional methods of determination (optochin susceptibility and bile solubility assays). One *S pneumoniae* colony per plate was then subcultured, harvested, and kept frozen at –70°C for further testing. Pneumococcal serotyping was performed by the capsular swelling method (Quellung reaction) using type-specific antisera from the Statens Seruminstitut (Copenhagen, Denmark).

At each visit, a questionnaire was obtained from the parents, including questions on antibiotic use in the preceding period. All parent-reported antibiotic use was verified with physician's records or correspondence, if available.

An acknowledged national ethics committee from the Netherlands, Stichting Therapeutische Evaluatie Geneesmiddelen, approved the study protocol.

The PCV-7 was introduced in the Dutch national immunization program for all infants born after March 31, 2006, without a catch-up campaign.[17] In the 2007-2008 period, the antibiotic resistance rates of nasopharyngeal *S pneumoniae* isolates obtained from Dutch newborns to children aged 4 years who were in day care was 0% for amoxicillin, 0.5% for penicillin, and 8% for clarithromycin.[18]

Selection of Isolates

Persistent carriage of a specific pneumococcal strain in a single child would inflate the frequency of that strain relative to other strains in the population. Therefore, we performed cumulative acquisition analyses including only the first swab that tested positive for serotype 19A per child. For the sequence type and clonal complex distribution analyses, all newly acquired serotype 19A strains were included.[19]

Multilocus Sequence Typing Analysis

Serotype identification is based on the capsule polysaccharide structure, but because genes encoding proteins involved in capsule biosynthesis may exchange between pneumococci, it does not describe genetic relatedness. Multilocus sequence typing (MLST) was performed to evaluate the genetic relatedness of the serotype 19A isolates and to investigate potential preferential outgrowth of particular strains. Groups of related genotypes or clonal complexes may differ in transmission, colonization, or virulence potential. Multilocus sequence typing was performed at the Netherlands Reference Laboratory for Bacterial Meningitis.[20] The assignment of alleles and sequence types was performed by the software available from the Multi Locus Sequence Typing Web site's pneumococcal page (http://www.mlst.net). Allelic combinations not already in the database were submitted and assigned new sequence type numbers. To identify clonal complexes, isolates were grouped with all isolates present in the *S pneumoniae* database using the eBURST algorithm (http://eburst.mlst.net) with the software provided by the Multi Locus Sequence Typing Web site.[21] Clonal complexes consisted of sequence types that shared 6 of 7 alleles with at least 1 other sequence type in the complex and named after the putative founder (ie, the sequence type that has the greatest number of single-locus variants) of the group or after the most frequent sequence type of the group. Sequence types that did not group with others in the database were defined as singletons. Strains not associated with serotype 19A were reserotyped to confirm identity. Laboratory personnel assessing pneumococcal carriage and performing the typing were unaware of treatment allocation.

Antimicrobial Susceptibility Testing

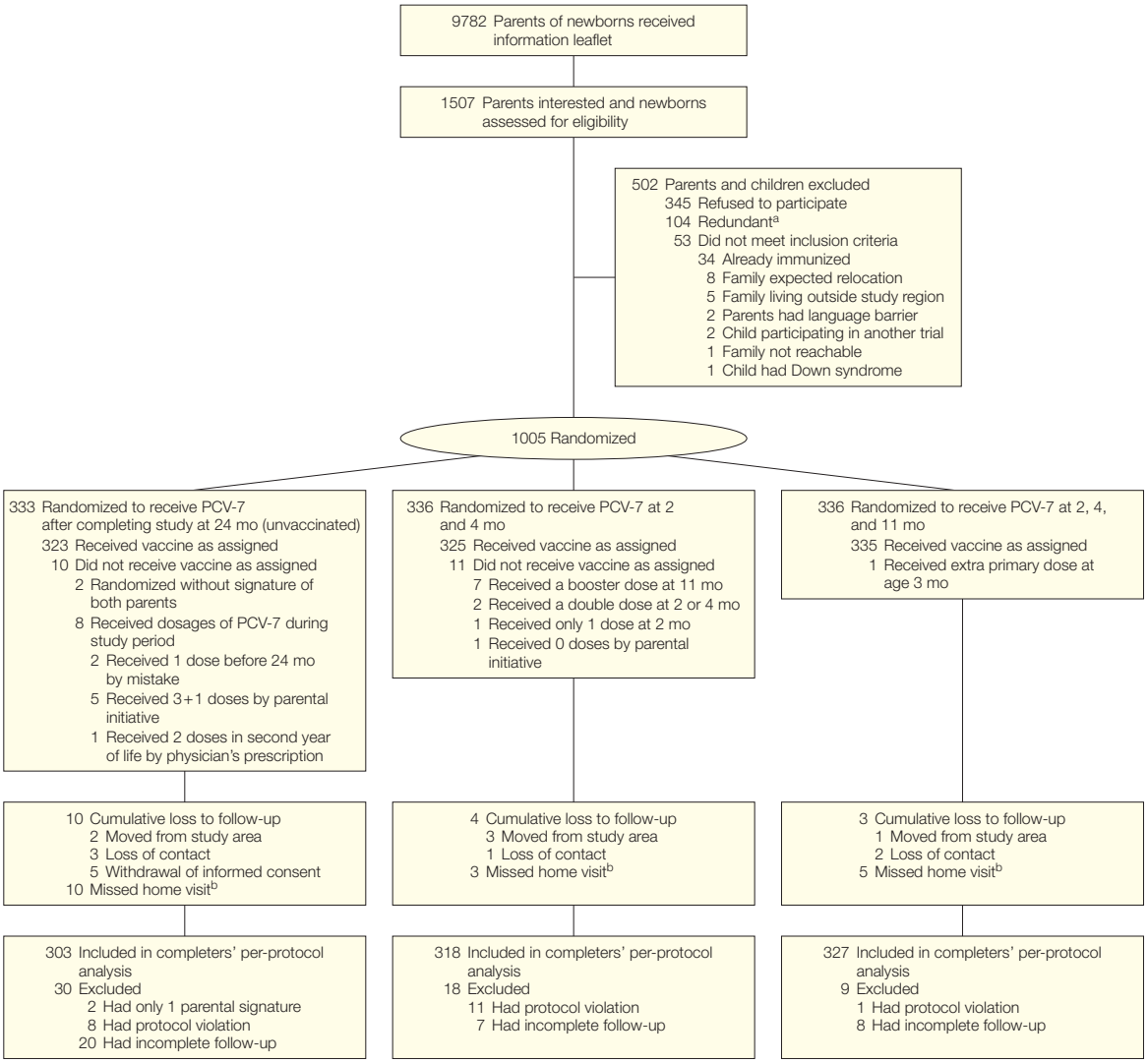
Susceptibility of *S pneumoniae* to penicillin, erythromycin, and azithromycin was determined by using the disk diffusion method. Isolates exhibiting inhibition zones less than 20 mm with a 1-µg oxacillin disk were further tested by Etest (PDM Epsilometer, AB Biodisk, Solna,

Sweden) for penicillin.[22] Isolates exhibiting inhibition zones 28 mm or more with an erythromycin 80-µg disk were considered susceptible to erythromycin, and those with an inhibition zone of less than 26 mm as nonsusceptible. Isolates with a penicillin minimum inhibitory concentration (MIC) of 0.06 µg/mL or less were considered penicillin susceptible. Isolates with a penicillin MIC of more than 0.06 µg/mL but 2.0 µg/mL or less were defined as penicillin-intermediate susceptible, and those with an MIC of more than 2.0 µg/mL were defined as resistant in accordance with European Committee on Antimicrobial Testing and as defined for oral penicillin by the Clinical and Laboratory Standards Institute because the treatment in our study was mainly oral.[23]

Statistical Analysis

The sample size for the trial was calculated for the primary outcome measure, vaccine serotype pneumococcal carriage, with the assumption of a vaccine serotype carriage rate of 35% in children in the second year of life based on previous experience.[24,25] The smallest clinically significant detectable difference was estimated as a 33% relative reduction in vaccine serotype carriage (25% vaccine serotype carriage rate) after a 2-dose schedule of PCV-7 compared with the unvaccinated group, with 80% power at a 5% significance level.

Figure 1. Enrollment Flow Diagram



PCV-7 indicates 7-valent pneumococcal conjugate vaccine.
[a] Parents of children interested in participating in the study were redundant because they were still in the information process of the study after enrollment target had already been achieved and informed consent procedure was cancelled.
[b] Incidentally missed home visit during follow-up due to illness, holiday, or another reason.

Table. Characteristics of the Children at Time of Enrollment and During Follow-up

	Group, No. (%) of Newborns		
	Unvaccinated (n = 303)	2-Dose (n = 318)	2 + 1-Dose (n = 327)
Male sex	150 (50)	166 (52)	166 (51)
Gestational age, mean (SD), wk	39.7 (1.8)	39.8 (1.7)	39.8 (1.7)
Premature birth (gestational age <37 wk at birth)	18 (6)	20 (6)	21 (6)
At home delivery	83 (27)	99 (31)	91 (28)
Birth weight, mean (SD), g	3491 (522)	3480 (571)	3511 (546)
Partially breastfed first 6 wk	237 (78)	256 (80)	265 (81)
No. of siblings, median (IQR)	1 (0-1)	0 (0-1)	0 (0-1)
Day care attendance[a]			
At 12 mo	180 (59)	200 (63)	200 (61)
At 24 mo	207 (68)	218 (69)	228 (70)
Use of oral or intravenous antibiotics during mo before swab			
At 12 mo	19 (6)	22 (7)	23 (7)
At 24 mo	10 (3)	18 (6)	11 (3)
Passive tobacco smoke exposure indoors			
At 12 mo	20 (7)	25 (8)	19 (6)
At 24 mo	25 (8)	27 (9)	21 (6)

[a]Defined as more than 4 hours per week with at least 1 child from a different family.

This resulted in a sample size of 330 infants per group, including a 10% dropout rate.[14]

Because our hypothesis was etiological and protocol violations and loss to follow-up minimal, we performed a post hoc completer's per-protocol analysis including all children who completed the follow-up and adhered to the allocated vaccination schedule. However, an intention-to-treat analysis was also performed and compared with the completer's per-protocol analysis. Missing data was less than 1.5%. We did not correct for multiple testing. Adjustments for multiple testing are mostly used when the assumption is that all null hypotheses are true simultaneously, and this is not true for the outcome comparisons in this study.

The main outcome measure was the cumulative proportion of children with nasopharyngeal acquisition of a new serotype 19A clone from the age of 6 months (after finishing primary series) through 24 months of age and was compared for both vaccine groups separately to the PCV-7 unvaccinated control group. As secondary outcomes, we investigated cumulative proportions of serotype 19A acquisition at each intermediate sampling point (6, 12, and 18 months) to evaluate differences over time. Proportional differences in acquisition between the treatment groups and the unvaccinated group were analyzed by using the χ^2 test or a 2-sided Fisher exact test, where appropriate.

Multinomial logistic regression analysis was used to assess the influence of vaccination schedule on the risk of acquisition of the most frequently isolated sequence types. With more than 2 mutually exclusive categories, a multinomial logistic regression model uses information contained in differences within categories, in differences between nonreference categories, and in ordering among categories. Five groups were included in the model based on relative frequencies of sequence types: 199, 3009, 3017, a group consisting of all other sequence types, and a reference group including all children without serotype 19A acquisition.

P value <.05 was considered statistically significant. All *P* values are 2-sided. Data were analyzed with SPSS version 17.0 (SPSS Inc, Chicago, Illinois) and Episheet (October 6, 2002, version).

RESULTS

A total of 1005 children were enrolled and randomly assigned to 1 of the 3 study groups; 2 children were excluded because 1 of the approval signatures from the parents could not be obtained. Of these 1003 children, 948 children completed the follow-up and adhered to the assigned vaccination schedule and were included in the analyses (Figure 1). There were no differences in baseline characteristics between the 3 study groups (TABLE).

Of all nonvaccine serotype carriage isolates, serotype 19A was the second most frequently identified after serotypes 6A and 6C, which were indistinguishable at the time of the microbiological analyses. The proportion of serotype 19A carriage isolates of all nonvaccine serotype carriage isolates collected during the study period was 8.6% (33 of 381) from the 303 participants in the unvaccinated group, 10.8% (54 of 501) from the 318 in the 2-dose group, and 12.2% (66 of 539) from 327 in the 2 + 1-dose group. Of all 948 children, 12 children tested positive for 19A carrying the same serotype 19A strain at 2 consecutive sampling points with a 6-month interval and 3 children at 3 consecutive sampling points over a period of at least 12 months.

Nasopharyngeal Acquisition of Serotype 19A

At baseline, when the participants were 6 weeks old and before they had received any vaccination, 1% of those in the unvaccinated group (3 of 303; 95% confidence interval [CI], 0%-2.9%), 0.9% in the 2-dose group (3 of 318; 95% CI, 0%-2.7%), and 0.3% in the 2 + 1-dose group (1 of 327; 95% CI, 0%-1.7%) tested positive for serotype 19A. Among infants older than 6 months who had finished the primary vaccine series, 123 newly acquired 19A strains were found. Forty-two isolates were found in the 2-dose group, 53 in the 2 + 1-dose group, and 28 in the unvaccinated control group.

At 24 months and after having completed the vaccine series, the cumulative proportion of participants with acquisition of a new serotype 19A clone in the 2 + 1-dose group was 16.2% (95% CI, 12.6%-20.6%; 53 of 327; relative risk [RR], 1.75; 95% CI, 1.14-

2.70) vs 9.2% (95% CI, 6.5%-13.0%; 28 of 303) in the unvaccinated control group (FIGURE 2). The cumulative proportion in the 2-dose group was also higher than in the unvaccinated group but did not reach statistical significance (13.2%; 95% CI, 9.9%-17.4%; 42 of 318 children; RR, 1.43; 95% CI, 0.91-2.25).

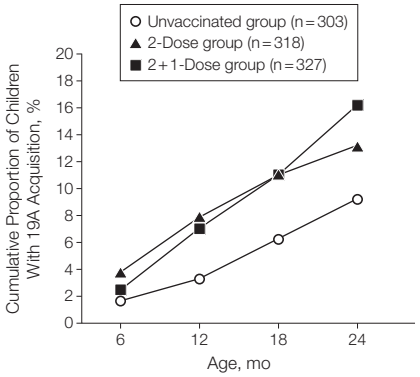
At the intermediate follow-up points, the cumulative proportion of children with serotype 19A was significantly higher at the age of 12 and 18 months in both the 2-dose and 2 + 1-dose groups than in the unvaccinated group but not at 6 months (Figure 2). The results of the intention-to-treat analysis were very similar (eTable1 available at http://www.jama.com).

Clonal Distribution

Among the 123 newly acquired serotype 19A nasopharyngeal strains obtained from all participants, 28 different sequence types were identified. The 6 new types found in vaccinated children were added to the multilocus sequence typing database. The 28 sequence types belonged to 10 clonal complexes and 3 were singletons (FIGURE 3 and FIGURE 4). Clonal complexes 15, 138, 124, and 177 are named after a founder sequence type not present among the isolates in this study. Seventeen of the 42 newly acquired serotype 19A isolates in the 2-dose group and 19 of 53 in the 2 + 1-dose group were different sequence types, whereas 11 of the 28 sequence types in the unvaccinated group were different sequence types (Figure 3). Of the sequence types in vaccinated children, 17 were not observed in the unvaccinated children; 4 sequence types were exclusively observed among children in the unvaccinated control group. The most frequently acquired sequence types were 199, 3009, and 3017, which were present in all groups (Figure 3).

A multinomial logistic regression model showed a higher risk (but not statistically significant) of acquisition of sequence type 199 in 17 children from the 2 + 1-dose group than in 7 children in the unvaccinated group (odds ratio [OR], 2.29; 95% CI, 0.95-5.55). This was not the case for the other main sequence types: 3009 (OR, 1.18; 95% CI, 0.46-3.00) and 3017 (OR, 0.94; 95% CI, 0.27-3.27). Furthermore, the risk of acquiring the remaining other sequence types was higher in the 2-dose (n = 21; OR, 2.77; 95% CI, 1.23-6.21) and in the 2 + 1-dose group (n = 21; OR, 2.48; 95% CI, 1.09-5.61) than in the unvaccinated group (n = 8). Six isolates with sequence types not previously described for serotype 19A were found, of which 2 were observed in the 2-dose group and 4 in the 2 + 1-dose group but none in the unvaccinated group. Two of these isolates were sequence type 179, which has been associated only with vaccine serotype 19F according to the multilocus sequence typing database and was also present in our study in combination with a serotype 19F capsule; 3 isolates were sequence type 432, previously associated with nonvaccine serotype 21 only; and 1 isolate was sequence type 3863, previously only associated with nonvaccine serotype 15C.

Figure 2. Cumulative Proportions of Children With New Acquisition of Serotype 19A After Finishing Primary Series of 7-Valent Pneumococcal Conjugate Vaccine vs Unvaccinated Children



Proportional differences in acquisition were analyzed by χ^2 test or 2-sided Fisher exact test, where appropriate. Acquisition of serotype 19A was statistically significant for both treatment groups at 12 and 18 months ($P < .05$) vs the unvaccinated group and at 24 months for those in the 2 + 1-dose group vs the unvaccinated group.

Figure 3. Sequence Type Distribution for Newly Acquired Serotype 19A Strains in Children Between 6 and 24 Months of Age After a 2-Dose or 2 + 1-Dose PCV-7 Schedule and in Unvaccinated Control Children

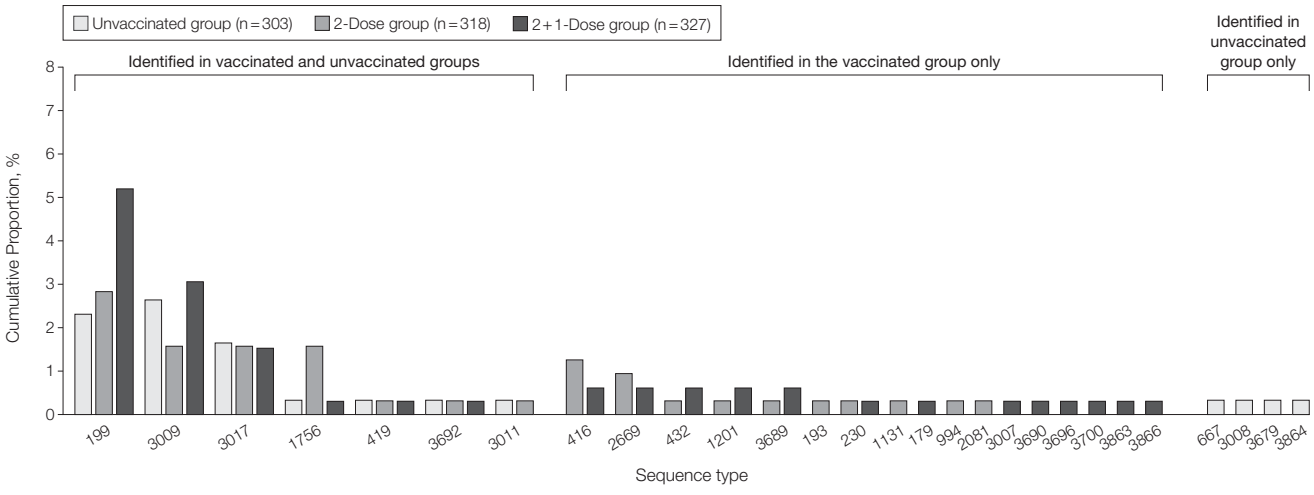
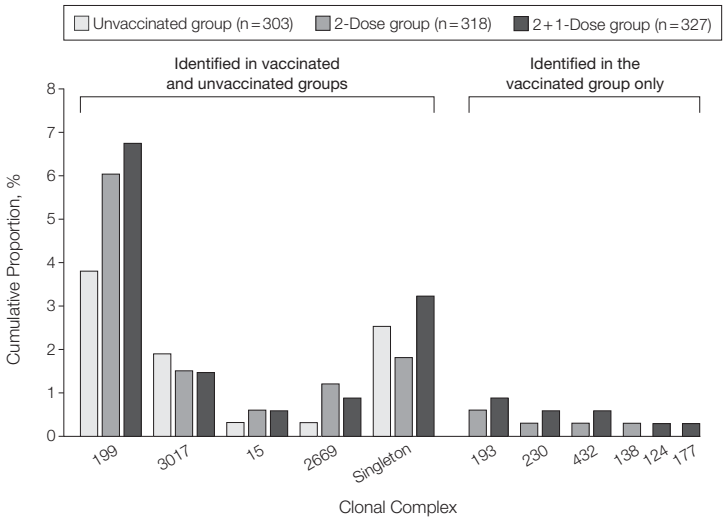


Figure 4. Clonal Complex Distribution for Newly Acquired Serotype 19A Strains in Children Between 6 and 24 Months of Age After a 2-Dose or 2 + 1-Dose PCV-7 Schedule and in Unvaccinated Control Children



Clonal complexes, ie, groups of related genotypes, consisted of sequence types that shared 6 of 7 alleles with at least 1 other sequence type in the complex and named after the putative founder of the group or after the most frequent sequence type of the group. Clonal complexes 15, 138, 124, 177 are named after a founder sequence type not present among the isolates in this study.

Antibiotic Use and Resistance

In the total study population, 5.8% (95% CI 5.1%-6.6%) had used antibiotics (mostly amoxicillin with or without clavulanate) within the month preceding the nasopharyngeal sampling and 17.8% (95% CI 16.6%-19.0%) within the preceding 6 months (eTable 2 available at http://www.jama.com). The proportion of children with newly acquired serotype 19A after receiving either oral or intravenous antibiotics within the 6 months before swab specimen collection was 18.7% (95% CI, 12.8%-26.5%; 23 of 123) and did not differ among the groups: 5 of the 28 children (17.8%) in the unvaccinated group, 10 of 42 (23.8%) in the 2-dose group, and 8 of 53 (15.1%) in the 2 + 1-dose group. Of these 23 children, 18 received broad-spectrum penicillin; 6, macrolides; and 1, sulfonamides, with 1 prescription unknown. The remaining 100 children had not used antibiotics within 6 months prior to the swab collection. With respect to the 19A strains, 5 (4.1%, 95% CI, 1.7%-9.2%) of the 123 newly acquired serotype 19A-isolates were penicillin-intermediate susceptible (MIC >0.06 µg/mL), of which 3 were found in the 2-dose group, 2 in the 2 + 1-dose, and none in the unvaccinated group. None of these isolates was penicillin resistant (MIC >2.0 µg/mL).Three isolates (2.4%, 95% CI, 0.8%-6.9%) were nonsusceptible to erythromycin and azithromycin and were all found in the 2 + 1-dose group.

COMMENT

To the best of our knowledge, this is the first study showing an association between a 2 + 1-dose PCV-7 schedule and nasopharyngeal acquisition of serotype 19A in children in the first 2 years of life in a randomized controlled study. This increase in serotype 19A nasopharyngeal acquisition in vaccinated children was associated with a diffuse proliferation of several serotype 19A strains plus the appearance of new strains. Furthermore, an increase in sequence type 199 that also predominated in unvaccinated control infants was observed. Antibiotic resistance or antibiotic consumption could not account for the observed increase.

The debate about the role of PCV-7 in the increase in serotype 19A disease has been driven by observed increases in serotype 19A disease following PCV-7 implementation in several countries, in particular countries with high antibiotic use,[3,26-28] but also over time in countries before PCV-7 implementation.[9,10] It is plausible that the origin of the expansion of serotype 19A as observed in diverse populations and settings is multifactorial. Several contributing factors have been described, such as the baseline prevalence of serotype 19A. Indeed, serotype 19A was already a frequent colonizer of the nasopharynx in unvaccinated children in our study population,[14] specifically in the second year of life, and this serotype seems a likely transmissible serotype and an obvious candidate for nasopharyngeal serotype replacement. Apparently, the reduction in colonization of vaccine serotypes following PCV-7 vaccination creates a vacant nasopharyngeal niche where other nonvaccine serotypes, in particular certain 19A clones, may expand. The increase in serotype 19A acquisition particularly after administration of the booster dose at 11 months coincides with the previously reported substantial decrease in vaccine serotype carriage at 18 months in the group that had received a booster dose.[14]

Another possible explanation for why serotype 19A, in particular, has emerged may be found in the intrinsic biochemical properties of the pneumococcal capsule. According to the model of Weinberger et al,[29] strains expressing capsular polysaccharides that are metabolically less costly are likely to dominate in carriage. Serotype 19A has one of the metabolically cheapest polysaccharides, which would provide 19A pneumococci with selective advantages during competition for an ecological niche. The abundance of different 19A sequence types identified in vaccinated children supports the capsule-dependent mechanism of the 19A emergence.[29]

Serotypes and strains already predominating in the nasopharynx are subject to high antibiotic pressure in coun-

tries with high antibiotic consumption and hence are likely to become resistant. In an environment with high use of antibiotics, these strains then may have a selective advantage for filling a vacant niche in the nasopharynx, eg, as a result of vaccine serotype elimination in the nasopharynx following PCV-7. A recent study by Dagan and coworkers[10] illustrated the potential promoting role of antibiotic use, especially azithromycin, in the increase of multidrug–resistant serotype 19A strains in otitis media independent of PCV-7 vaccination. Together with the shown disease potential of serotype 19A for invasive and respiratory disease, this may explain the increase in 19A disease, in particular resistant strains. In our study, however, antibiotic use and resistance was relatively low. Although azithromycin use was somewhat higher among vaccinees in our study than among children in the unvaccinated group, antibiotic use within 6 months of nasopharyngeal sampling could not account for the observed increase in 19A acquisition.

Several underlying mechanisms for the increase in resistance have been suggested: proliferation of 1 or more genotypes; introduction of new clones into the community; capsular-switch events, in which clones previously identified as other serotypes switch their polysaccharides to become 19A; and acquisition of new resistance mechanisms.[10]

In the United States, most of the serotype 19A disease is due to proliferation of preexisting sequence type 199 and a new multidrug–resistant strain 320.[30-34] In our study, we also observed an increase in 199 that was already a predominant sequence type in unvaccinated control children. None of the 33 newly acquired sequence type 199 isolates were nonsusceptible to penicillin and therefore antibiotic pressure as the driving force for this increase in our study seems unlikely. Furthermore, we observed the emergence of several 19A strains of which some were already present in children in the unvaccinated group but others were not. With respect to capsular-switch events, the vaccine-to-nonvaccine serotype switch is of concern in the PCV-7 era because it facilitates emergence of vaccine escapees.[35] We found 6 isolates from 3 clones that had not been previously associated with serotype 19A, all from PCV-7 vaccinated children. Only 2 of these were sequence types previously associated with vaccine serotype 19F, and this sequence type was also present in several 19F isolates in our study. Therefore, the observed incongruence between sequence type and serotype could have been the result of capsular switch under vaccine pressure. Lipsitch et al[36] also reported limited evidence of capsular switching in colonization under vaccine pressure.

Some limitations of our study need to be recognized. First, these results are derived from a post hoc analysis, and we did not correct for multiple testing; therefore, the data need to be interpreted with caution. Second, although pneumococcal disease is always preceded by nasopharyngeal colonization, colonization will not necessarily progress to disease. However, the clonal distribution as observed in our study resembles that of serotype 19A invasive isolates recovered from children with invasive pneumococcal disease in the Netherlands in the same period, illustrating the disease potential of these 19A clones. Furthermore, several studies have shown the relatively high disease potential of serotype 19A compared with other serotypes.[37,38] Third, we investigated the effect of reduced-dose schedules and not the full 3 + 1-schedule. This study may underestimate the role of PCV-7 when given in 4 doses. Last, due to the 6-month sampling intervals in our study, we may have missed several carriage episodes. Therefore, our figure of nasopharyngeal serotype 19A acquisition may be underestimated.

Major strengths of this study are the randomized controlled study setting in an environment with a largely PCV-7 unvaccinated population with low antibiotic pressure and low antibiotic resistance rates. Furthermore, we gathered information on preceding antibiotic use and evaluated susceptibility and were therefore able to investigate the role of antibiotic pressure at the individual level.

In addition to the contributing role of antibiotic selective pressure as previously described by others, we now have demonstrated, to our knowledge for the first time, the facilitating role of PCV-7 in nasopharyngeal acquisition of serotype 19A. In view of the proven disease potential of serotype 19A for otitis media and invasive pneumococcal disease and the observed association with antibiotic resistance, vaccines of broader coverage including protection against serotype 19A may further aid to pneumococcal disease prevention. However, we need to be aware that other serotypes with similar characteristics and disease potential may be the next in line to proliferate and therefore pneumococcal surveillance remains important after introduction of expanded pneumococcal conjugate vaccines.

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Author Contributions: Drs van Gils and Sanders had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs van der Ende and Sanders contributed equally to the study.
Study concept and design: Sanders, Hak, van Alphen, van der Ende.
Acquisition of data: Van Gils, Veenhoven, Rodenburg, Bruin, Keijzers, Van der Ende.
Analysis and interpretation of data: Van Gils, Sanders, van der Ende, Veenhoven, Hak, van Alphen, Bogaert, Trzcinski.
Drafting of the manuscript: Van Gils, Sanders, van der Ende.
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