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Published in:
Acta Oto-Laryngologica

DOI:
[10.3109/00016489.2014.950326](https://doi.org/10.3109/00016489.2014.950326)

2015

[Link to publication](#)

Citation for published version (APA):

Gisselsson-Solén, M., Henriksson, G., Hermansson, A., & Melhus, Å. (2015). Effect of pneumococcal conjugate vaccination on nasopharyngeal carriage in children with early onset of acute otitis media - a randomized controlled trial. *Acta Oto-Laryngologica*, 135(1), 7-13. <https://doi.org/10.3109/00016489.2014.950326>

Total number of authors:
4

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PO Box 117
221 00 Lund
+46 46-222 00 00

Effect of Pneumococcal Conjugate Vaccination on Nasopharyngeal Carriage in Children with Early Onset of Acute Otitis Media – a Randomised Controlled Trial

Marie Gisselsson Solén^{1*}, Gunnel Henriksson², Ann Hermansson¹, Åsa Melhus³

¹Department of Otorhinolaryngology, Head and Neck Surgery, Lund University Hospital, Lund, ²Department of Laboratory Sciences/Section of Clinical Bacteriology, Malmö University Hospital, Malmö, and ³Department of Medical Sciences/Section of Clinical Bacteriology, Uppsala University, Uppsala, Sweden

Running title: Otitis pathogens and PCV7 in otitis-prone children

*Corresponding author: Marie Gisselsson Solén, Department of Otorhinolaryngology, Head and Neck Surgery, Lund University Hospital, S-22185 Lund, Sweden.

Tel: +44 756 427 9917. Fax: +46 46 171758. E-mail: marie.gisselsson-solen@med.lu.se

Abstract

Conclusion: Although children vaccinated with heptavalent pneumococcal conjugate vaccine (PCV) had fewer episodes of acute otitis media (AOM), this trial was unable to prove a simultaneous decrease in nasopharyngeal carriage.

Objective: Carriage rates of AOM pathogens in the nasopharynx are high among children, and colonization is the first step towards infection. The possible impact of PCV on carriage is therefore of interest, particularly in children with recurrent AOM. The aims of this study were to examine the effect of heptavalent PCV on carriage of AOM pathogens in children at high risk of developing recurrent disease, and to monitor carriage of resistant pathogens in vaccinated and unvaccinated children.

Methods: 109 children with an onset of AOM before six months of age, 89 of whom developed recurrent disease, were enrolled in a trial. Fifty-two children were vaccinated and all were closely monitored for three years.

Results: There was no difference statistically between vaccinated children and controls concerning the carriage of any of the major AOM pathogens. There was evidence of within-child clustering for *S. pneumoniae* ($p=0.002$) and *H. influenzae* ($p<0.001$), indicating that children continued to carry either species over time. Resistance rates were generally low and comparable with national levels.

Key words

Recurrent acute otitis media; nasopharyngeal carriage; PCV; *Streptococcus pneumoniae*; *Haemophilus influenzae*; *Moraxella catarrhalis*

Introduction

Acute otitis media (AOM) is the most common bacterial infection in children, with a peak incidence during the first two years of life[1]. It is usually caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* or *Streptococcus pyogenes*[2].

Bacteria enter the middle ear via the Eustachian tube from the nasopharynx, which is considered to be the reservoir of upper respiratory tract pathogens. At birth, the nasopharynx is sterile, however, it is soon colonized by one or more of the AOM pathogens[3, 4], and asymptomatic carriage of these bacteria is very common in young children[5].

During the last decade, vaccination with conjugate pneumococcal vaccines, the first of which was the heptavalent vaccine (PCV7), has become part of the immunization programmes in most developed countries. Several studies have indicated that a bacterial shift occurs in the nasopharynx after vaccination, with pneumococcal vaccine serotypes being replaced by non-vaccine serotypes[6, 7].

Resistance among AOM pathogens has increased in many countries since the turn of the millennium. It is not known whether this increase is just co-incidental, or whether it is associated with the introduction of the new vaccine. Although some authors have described an increase in non-susceptible strains after the introduction of PCV7[8], authors from other countries have reported similar findings before vaccine introduction[9].

At the time of introduction, PCV7 covered less than 50% of invasive serotypes in Sweden, and it was not part of the national vaccination programme until 2009. To evaluate the effect of PCV7 on the number of AOM episodes in very young Swedish children at risk of developing rAOM, a randomised study was performed prior to vaccine introduction. A reduction of AOM episodes by 26% was noted in the vaccinated group[10]. The present study

was a secondary outcome in this trial. Its purpose was to investigate the carriage rates of major AOM pathogens in vaccinated and unvaccinated children, and to monitor the presence of resistant AOM pathogens in the two groups.

Materials and methods

Study design

The study was a randomised, prospective, single-blinded trial performed at the ENT Department at Lund University Hospital, Lund, Sweden. Patients were recruited between March 2003 and June 2007. For inclusion, the child had to have had at least one AOM episode confirmed by an otorhinolaryngologist before the age of six months. Previous research has shown such children to have a 60-80% risk of developing rAOM[11], which was confirmed in the present cohort[10]. Exclusion criteria were allergy to the vaccine, anatomical or chromosomal abnormalities, previously diagnosed immune deficiencies, prematurity, prior administration of gammaglobulin or pneumococcal vaccine and a history of idiopathic thrombocytopenic purpura. The study was approved by the Ethics Committee at Lund University, and written consent was obtained from the parents.

At inclusion, a questionnaire with information about the family history of rAOM, siblings in public day-care, breast-feeding, parental smoking, and the number of AOM prior to inclusion was completed. Half of the children were randomised to vaccination with PCV7 (Prevenar®, Wyeth Lederle, now Pfizer) as recommended by the manufacturer. An external supervisor generated the randomisation list in advance by the drawing of lots. Each included child received a consecutive study identification number, which was reported to the external supervisor, who then revealed the child's allocation to the nurse administering the

vaccine. This nurse took no other part in the study. The Ethics Committee did not approve of the use of an unrelated control vaccine, such as a hepatitis B vaccine, so only the study personnel but not the parents were blinded to the allocation of the children. During the first year after inclusion, all children were examined by one of the two study doctors at regularly scheduled visits every other month. In addition, during the entire three-year follow-up, the children were examined whenever the parents suspected a new episode of AOM. A final scheduled visit to one of the study doctors was carried out three years after inclusion. Otomicroscopy and nasopharyngeal cultures were performed at all doctors' visits, and questionnaires monitoring health status and antibiotic use were filled in.

Definitions and treatment of AOM

AOM was defined as a bulging ear drum and opaque fluid in the middle ear in a child with symptoms of an acute infection. A new episode of AOM was diagnosed if the child, after completing treatment with antibiotics and having a period with no clinical signs of AOM, had new symptoms and otomicroscopic findings as described above. Recurrent AOM was defined as three episodes during six months, or four in a year.

All episodes of AOM were treated in accordance with Swedish guidelines at the time[12], i.e. children under two years of age were always treated with antibiotics, as were older children with an affected general condition and/or rAOM. The primary drug-of-choice was penicillin V followed by amoxicillin in difficult-to-treat AOM or in cases of quick relapses. Otherwise, antibiotics were chosen according to the results of earlier nasopharyngeal/middle ear cultures.

Bacterial cultures

Nasopharyngeal samples were collected with the M40 Transystem (Copan Diagnostics, Corona, CA, USA) and transported to the Department of Clinical Microbiology at Malmö University Hospital during 2003-2006, and to the Department of Clinical Microbiology at Uppsala University Hospital from 2007 onwards. The samples were cultured on blood and chocolate agar plates (Becton, Dickinson and Company, Sparks, MD, USA) and were incubated for 48 h in a moist environment with 5% CO₂. Bacteria were identified using standard laboratory procedures. All isolates were frozen at -70°C.

Antibiotic susceptibility

In cultures obtained during AOM, susceptibility testing to appropriate antibiotics was performed using the IsoSensitest agar (Oxoid Ltd., Basingstoke, UK). The disc diffusion method and testing of beta-lactamase production was performed as recommended by the Swedish Reference Group for Antibiotics (SRGA)[13]. MIC-determination by Etest (AB Biodisk, Solna, Sweden) was performed when indicated and according to the manufacturer's instructions. The species-related breakpoints defined by the SRGA were used for the categorization of isolates into susceptible, indeterminate, or resistant.

Typing of *S. pneumoniae*

Pneumococci from the first 30 participants were further analysed with serotyping and genetic fingerprinting. The first pneumococcal isolate from each patient was serotyped at Statens Serum Institut (SSI), Copenhagen, Denmark. The DNA of the following isolates was analysed together with the DNA of the initial isolate, using arbitrarily primed (AP)-PCR and BOX-A

PCR as earlier described[14, 15]. Whenever a new DNA-pattern appeared, the isolate was serotyped at SSI.

Statistics

Sample size calculation for the trial was based on the main outcome, being the number of AOM episodes during the first two years of life in the two treatment groups. This resulted in a sample size of 110 infants including a 10% dropout rate. The present analyses were planned as secondary outcomes. All statistical analyses were carried out according to the intention-to-treat principle. Cultures obtained during antibiotic treatment were excluded in order not to bias the results towards bacteria not susceptible to the commonly given antibiotics. The vaccine and control groups were compared with respect to the presence of *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and *S. pyogenes*, but also with respect to *S. pneumoniae* with decreased susceptibility/resistance, to beta-lactamase-producing *H. influenzae* and to beta-lactamase negative *H. influenzae* with ampicillin resistance (BLNAR). The statistical package Stata 13, College Station, Texas, USA was used for all analyses. To account for the multiple measurements within each individual, data were analysed using a random effects Poisson regression model. The first follow-up period started at birth and ended at the date of the first culture. Each following period lasted from the date of the previous culture until the date of the next. The vaccine and control groups were compared using univariate regression.

Results

Patients and cultures

A total of 109 children were enrolled in the study, 52 of whom were vaccinated and 57 of whom were not. Less than 5% of eligible children refused to participate. Four children

discontinued their participation in the study, four were diagnosed with immune deficiencies and were prescribed extra vaccinations and/or immunoglobulins by paediatricians, one presented with a chromosomal abnormality, and another four showed violations with vaccine protocol. However, the latter nine patients were all followed up according to study protocol (Fig 1). Though parents were not blinded to their child's allocation, in only one case did parents reveal their child's allocation. For baseline characteristics of the patients, see Table 1.

Cultures were analysed according to the intention-to-treat principle, so available data for patients who discontinued their participation in the study were used until the date when the patients were censored. Data from immune-deficient and insufficiently vaccinated patients were analysed in the group to which they belonged at the start of the study. From the 109 children, 1,318 nasopharyngeal cultures were obtained. Antibiotic treatment excluded 125 cultures, leaving 1,193 cultures (563 from the vaccine group and 630 from the control group) from 107 children for statistical analyses. The total follow-up time was 101,972 person-days or 279 person-years.

Nasopharyngeal carriage and impact of vaccination

Nasopharyngeal carriage rates were generally high. *S. pneumoniae* grew in 540 cultures (45%), *H. influenzae* in 380 (32%), *M. catarrhalis* in 615 (52%) and *S. pyogenes* in 21 cultures (2%) (Fig 2). Two-hundred and thirty-one cultures (19%) did not contain any AOM pathogens. There was strong evidence of within-child clustering for *S. pneumoniae* and *H. influenzae* (p -values for the test of $\theta=0$ was 0.002 and <0.001 , respectively), indicating that children tended to carry the same species over time. The average number of cultures per person was similar in the two groups, and there was no evidence of a difference in the overall

carriage rates of *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* or *S. pyogenes* between vaccinated and unvaccinated children (Table 2).

Distribution of pneumococcal serotypes

Pneumococcal isolates from the first 30 patients were serotyped. Twenty different serotypes were identified, of which the most predominant were 23F and 19F (see Table 3). Only twice did a certain serotype (types 6A and 6B) re-occur after a colonization period with another pneumococcal strain. Simultaneous carriage of two different serotypes was recorded at one occasion (11A+6B). Five of the vaccine serotypes were found at least once (6B, 14, 18C, 19F and 23F). Only in three cases were vaccine serotypes found in vaccinated children after more than two vaccine doses, and never after administration of the booster dose. Changes of pneumococcal serotype took place in 28 of the 30 patients during the study period (mean 2 ± 1 ; median 2, range 0-5). In addition, 18 pneumococcal isolates could not be typed (non-typeable or dead during storage). There was no difference in the number of serotype changes between rAOM and non-rAOM children. Both patient groups were, however, very small ($n=24$ and $n=6$, respectively).

Resistant bacteria

Overall, carriage of resistant bacteria was rare. *S. pneumoniae* with some sort of decreased susceptibility was detected in 24 patients (12 vaccinated and 12 unvaccinated). Most patients only acquired a resistant pneumococcal strain once, but three patients acquired them twice, and one patient five times, resulting in a total of 31 episodes. Reduced susceptibility to beta-lactams was most common, occurring in 13 episodes, followed by resistance to

trimetoprimsulphametazole (6 episodes), erythromycin (6 episodes) and tetracyclines (2 episodes). Strains resistant to more than one antibiotic were found in four episodes.

Beta-lactamase-producing *H. influenzae* was found in 15 patients (8 vaccinated and 7 unvaccinated), each patient only having one episode of carriage, and BLNAR in 15 patients (9 vaccinated and 6 unvaccinated), three patients having two episodes of carriage with long *H. influenzae*-free intervals in between. No difference between vaccinated and unvaccinated children in the risk of carrying resistant bacteria could be proved (Table 2). Though the risk ratios for carrying pneumococci with some sort of decreased susceptibility and for BLNAR were seemingly higher for the vaccinated group, the confidence intervals were wide and the p-values low. For baseline information on patients/cultures with resistant bacteria, see Table 4.

Discussion

In the present study of 109 children with early onset of AOM, the carriage rates of major AOM pathogens in the nasopharynx were explored in children vaccinated or not vaccinated with PCV7. Furthermore, the susceptibility to antibiotics of the isolated bacteria was investigated. The trial did not give evidence for a difference in nasopharyngeal carriage rates between vaccinees and controls, although the number of AOM episodes was decreased in the vaccinated children according to the primary endpoint[10]. Carriage of resistant strains was rare and comparable to national levels in an unvaccinated population.

Only two other studies[16, 17], both based on the same Dutch vaccination trial with 1,005 children, have reported the effect of PCV7 on all major AOM pathogens. Those studies did, however, not focus on children with rAOM. The children in the Dutch trial were

of a similar age as those in the present study (< 3 years old), so the children should be comparable. In contrast to the results presented here, the Dutch authors noted a slight reduction in pneumococcal carriage (OR 0.68, 95% CI 0.51-0.91) among vaccinated children, however no changes in *H. influenzae* or *M. catarrhalis* carriage. A possible explanation for the differing results concerning *S. pneumoniae* could be that the present study was too small to detect minor differences in pneumococcal carriage. However, a large decline in overall pneumococcal carriage could hardly be expected after vaccination with a vaccine that only covers about 50% of the present serotypes.

Serotyping was only performed on a subset of patient isolates. The most commonly identified serotypes were 23F and 19F. Though data were sparse, the observations that colonization with the same serotype rarely occurred twice, and that colonization with vaccine serotypes did not occur in vaccinated children after completion of the vaccination scheme, are in accordance with earlier findings, i.e. colonization or vaccination confers some strain-specific immunity[18, 19]. Interesting in this context was the observation of within-child clustering of *S. pneumoniae* and *H. influenzae*; some children tended to be colonized with either *S. pneumoniae* or *H. influenzae* for longer time periods. This finding fits well with clinical observations, although it has not earlier been documented: Since the carried species is often also involved in the middle ear infections[20], this might, or rather should, in turn influence the physician's choice of antibiotic.

As a rule, Sweden is a country with low antibiotic resistance rates. This was true also in this trial, despite an overall high consumption of antibiotics among the otitis-prone children. To be able to draw reliable inferences of any differences in carriage rates of resistant bacteria between vaccinated and unvaccinated children in a country with such low background rates, the study would have had to be huge.

The greatest drawback with this study is that the microbiological questions were secondary outcomes for which the study was not powered. This problem becomes even greater when there does not seem to be a difference between the compared groups. Is this because there is, in fact, no difference, or is it because the power of the study is too low? Unless you include the entire population in your study, it is never going to be possible to “prove” a situation of no difference between two groups, and with only 109 participants, we can only claim not being able to prove a difference.

Although the size was problematic, the study had its strengths. These included the very close follow-up by two well-trained physicians who used strict diagnostic criteria for AOM. Though small from the start, the drop-out rate from the trial was low, which would decrease the risk of selection bias. The children were followed during both sickness and health, yielding a large amount of cultures. The trial also focused on a vulnerable subgroup on which information on colonization patterns following vaccination is very limited.

Unfortunately, the Ethics Committee would not allow the use of a control vaccine, wherefore the trial was not double-blinded. However, the blinding of the study doctors worked well, which decreased the risk of observer bias. For the actual culture results, the lack of parental blinding was probably not a problem, but one might suspect that parents of an unvaccinated child would seek care more readily. This could introduce bias, but the number of cultures per person was similar in the two groups, so this kind of bias should be less of a problem.

Though rAOM children vaccinated with PCV7 had fewer AOM episodes, we were not able to confirm a simultaneous decrease in nasopharyngeal carriage of AOM pathogens in this trial. New vaccines with broader coverage have been introduced recently. The impact of these on children in general and on rAOM children in particular will need to be

investigated. There are over 90 known serotypes of *S. pneumoniae*, and in years to come, it will be necessary to monitor the long-term effects of vaccination on pneumococcal nasopharyngeal carriage to establish to what extent presently rare serotypes will replace vaccine serotypes, and to what extent will they cause disease.

Acknowledgement

This study was supported by the Swedish Association of Local Authorities and Regions.

References

1. Alho OP, Koivu M, Sorri M, Rantakallio P. The occurrence of acute otitis media in infants. A life-table analysis. *Int J Pediatr Otorhinolaryngol*. 1991 Feb;21(1):7-14.
2. Kilpi T, Herva E, Kaijalainen T, Syrjanen R, Takala AK. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. *Pediatr Infect Dis J*. 2001 Jul;20(7):654-62.
3. Aniansson G, Alm B, Andersson B, Larsson P, Nylen O, Peterson H, et al. Nasopharyngeal colonization during the first year of life. *J Infect Dis*. 1992 Jun;165 Suppl 1:S38-42.
4. Faden H, Waz MJ, Bernstein JM, Brodsky L, Stanievich J, Ogra PL. Nasopharyngeal flora in the first three years of life in normal and otitis-prone children. *Ann Otol Rhinol Laryngol*. 1991 Aug;100(8):612-5.
5. Ingvarsson L LK, Ursing J. The bacterial flora in the nasopharynx in healthy children. *Acta Otolaryngol (Suppl)*. 1982;Suppl 386:94-6.
6. Huang SS, Hinrichsen VL, Stevenson AE, Rifas-Shiman SL, Kleinman K, Pelton SI, et al. Continued impact of pneumococcal conjugate vaccine on carriage in young children. *Pediatrics*. 2009 Jul;124(1):e1-11.
7. van Gils EJ, Veenhoven RH, Hak E, Rodenburg GD, Bogaert D, Ijzerman EP, et al. Effect of reduced-dose schedules with 7-valent pneumococcal conjugate vaccine on nasopharyngeal pneumococcal carriage in children: a randomized controlled trial. *JAMA*. 2009 Jul 8;302(2):159-67.
8. Hoberman A, Paradise JL, Shaikh N, Greenberg DP, Kearney DH, Colborn DK, et al. Pneumococcal resistance and serotype 19A in Pittsburgh-area children with acute otitis media before and after introduction of 7-valent pneumococcal polysaccharide vaccine. *Clin Pediatr (Phila)*. 2011 Feb;50(2):114-20.

9. Dagan R, Givon-Lavi N, Leibovitz E, Greenberg D, Porat N. Introduction and proliferation of multidrug-resistant *Streptococcus pneumoniae* serotype 19A clones that cause acute otitis media in an unvaccinated population. *J Infect Dis.* 2009 Mar 15;199(6):776-85.
10. Gisselsson-Solen M, Melhus A, Hermansson A. Pneumococcal vaccination in children at risk of developing recurrent acute otitis media - a randomized study. *Acta Paediatr.* 2011 Oct;100(10):1354-8.
11. Harsten G, Prellner K, Heldrup J, Kalm O, Kornfalt R. Recurrent acute otitis media. A prospective study of children during the first three years of life. *Acta Otolaryngol.* 1989 Jan-Feb;107(1-2):111-9.
12. STRAMA. Treatment for Acute Inflammation of the middle Ear, Consensus Statement 2000. 2000; Available from: <http://en.strama.se/dyn//,87,14.html>.
13. SRGA. <http://www.srga.org>.
14. Nilsson P, Laurell MH. Several different clones present during the penetration phase of resistant *Streptococcus pneumoniae* in the city of Malmö, Sweden. *Microb Drug Resist.* 1999 Spring;5(1):37-43.
15. van Belkum A, Sluijter M, de Groot R, Verbrugh H, Hermans PW. Novel BOX repeat PCR assay for high-resolution typing of *Streptococcus pneumoniae* strains. *J Clin Microbiol.* 1996 May;34(5):1176-9.
16. van Gils EJ, Veenhoven RH, Rodenburg GD, Hak E, Sanders EA. Effect of 7-valent pneumococcal conjugate vaccine on nasopharyngeal carriage with *Haemophilus influenzae* and *Moraxella catarrhalis* in a randomized controlled trial. *Vaccine.* 2011 Oct 13;29(44):7595-8.
17. van den Bergh MR, Biesbroek G, Rossen JW, de Steenhuijsen P, Pitsers WA, Bosch AA, van Gils EJ, et al. Associations between pathogens in the upper respiratory tract of young children: interplay between viruses and bacteria. *PLoS One.* 2012;7(10):e47711.

18. Weinberger DM, Dagan R, Givon-Lavi N, Regev-Yochay G, Malley R, Lipsitch M. Epidemiologic evidence for serotype-specific acquired immunity to pneumococcal carriage. *J Infect Dis.* 2008 Jun 1;197(11):1511-8.
19. Black S, Shinefield H, Fireman B, Lewis E, Ray P, Hansen JR, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatr Infect Dis J.* 2000 Mar;19(3):187-95.
20. Syrjanen RK, Herva EE, Makela PH, Puhakka HJ, Auranen KJ, Takala AK, et al. The value of nasopharyngeal culture in predicting the etiology of acute otitis media in children less than two years of age. *Pediatr Infect Dis J.* 2006 Nov;25(11):1032-6.

Tables

	Vaccinated	Controls
Number of patients	52	57
Mean age at inclusion (months)	5.0	5.1
Male (%)	32 (62)	38 (67)
rAOM (%)	41 (79)	48 (84)
Siblings in daycare (%)	36 (69)	37 (65)
Breast-feeding < 4 months (%)	11 (21)	16 (28)
Parental smoking (%)	5 (10)	9 (16)
Mean no of cultures/patient	10.8	11.1

Table 1. Baseline characteristics of children included in the study.

	RR	95% CI	p
All <i>S. pneumoniae</i>	1.09	0.85-1.41	0.50
All <i>H. influenzae</i>	1.12	0.75-1.68	0.58
All <i>M. catarrhalis</i>	0.99	0.79-1.23	0.91
All <i>S. pyogenes</i>	0.52	0.18-1.54	0.24
<i>S. pneumoniae</i> with decreased susceptibility	2.39	0.83-6.90	0.11
Beta-lactamase-producing <i>H. influenzae</i>	1.08	0.39-3.00	0.89
BLNAR	2.54	0.72-8.97	0.15

Table 2. Univariate analysis of the effect of vaccination on nasopharyngeal carriage.

Serotypes	No of cultures	No of patients	Vaccinees/ controls
3	1	1	1/0
6A	7	5	4/1
6B*	10	5	3/2
10A	2	2	0/2
11A	12	4	2/2
14*	14	5	1/4
15C	4	3	2/1
17F	1	1	1/0
18C*	6	3	0/3
19A	6	4	2/2
19B	1	1	0/1
19F*	25	9	5/4
22F	1	1	1/0
23A	2	1	1/0
23B	8	1	1/0
23F*	21	9	1/8
33F	2	1	1/0
35B	3	1	0/1
35F	2	1	1/0
38	3	1	1/0

Table 3. Pneumococcal serotypes in nasopharyngeal cultures obtained during both healthy periods and AOM episodes.

*Serotype included in the vaccine.

	No. of patients	of which had rAOM (%)	of which had daycare siblings (%)	No of cultures	of which age<2 (%)	with recent antibiotics (%)
<i>S. pneumoniae</i> with decreased susceptibility	24	22 (92)	16 (67)	59	56 (95)	24 (41)
Beta-lactamase producing <i>H. influenzae</i>	15	14 (93)	13 (87)	18	15 (83)	7 (39)
BLNAR	15	15 (100)	10 (67)	33	30 (91)	15 (45)

Table 4. Characteristics of patients/cultures with bacteria with decreased susceptibility/resistance

Figures

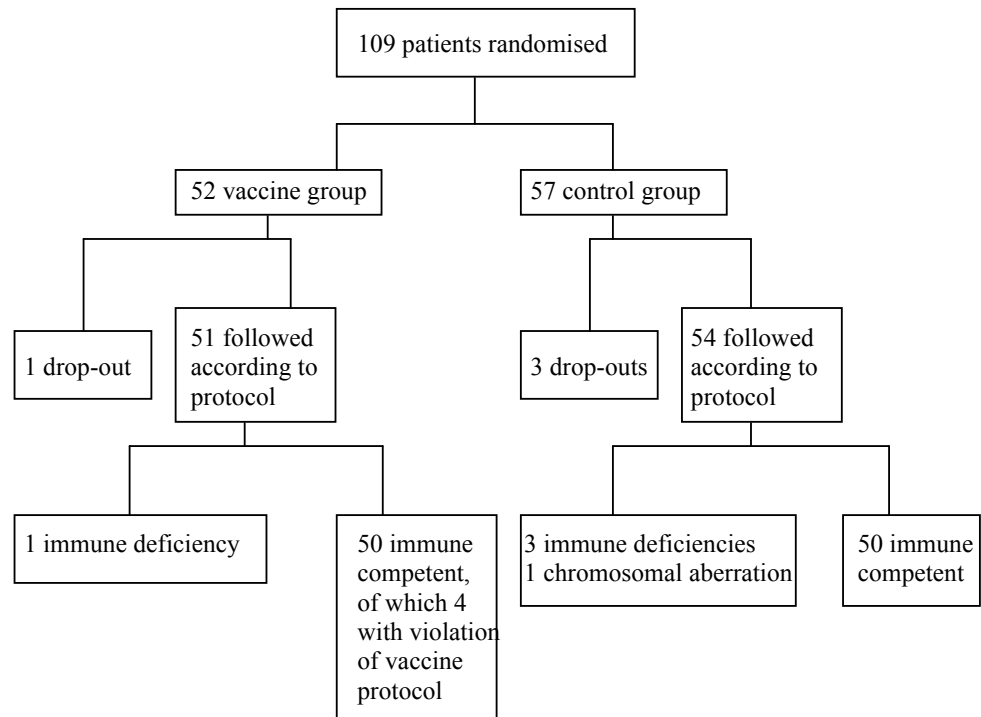


Figure 1

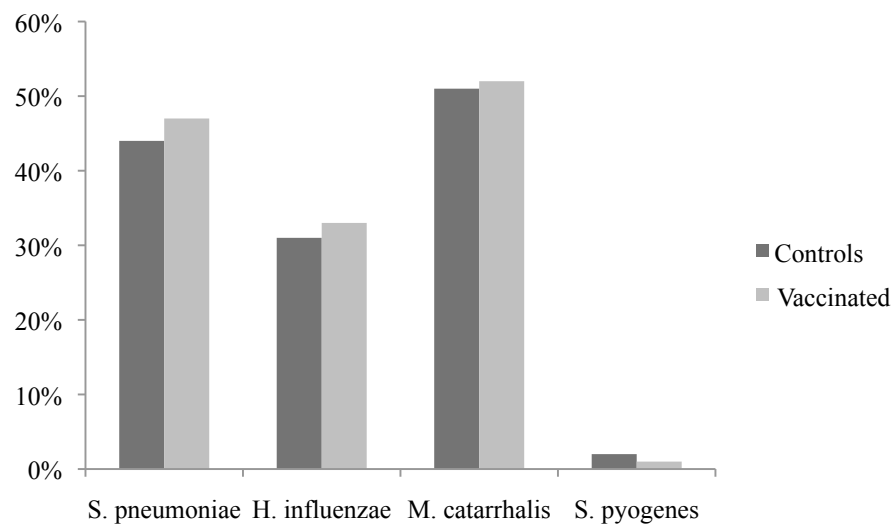


Figure 2

Figure legends

Figure 1 Study flow chart

Figure 2 Bacterial carriage in vaccinated children and controls (% of all cultures)