Effect of antimicrobial treatment for acute otitis media on carriage of *Streptococcus pneumoniae* with reduced susceptibility to penicillin in a randomized, double-blind, placebo-controlled trial

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ABSTRACT

Background: Concerns that antimicrobial treatment for acute otitis media (AOM) may select for resistant bacterial lineages have led to conflicting guidelines for clinical management of AOM.

Methods: We measured effects of amoxicillin-clavulanate on carriage of penicillin-susceptible *Streptococcus pneumoniae* (PSSP) strains and on carriage of strains with reduced penicillin susceptibility (RSSP) at end-of-treatment and 15d, 30d, and 60d after treatment in a previously-conducted randomized, double-blind, placebo-controlled trial in Finland. The trial enrolled 322 children 6-35 months of age with stringently-defined AOM. Children who did not show clinical improvement received open-label antimicrobial rescue treatment, irrespective of the blinded treatment assignment. The intention-to-treat populations of the trial arms thus received care resembling immediate antimicrobial therapy and watchful waiting.

Results: We analyzed 1358 nasopharyngeal specimens. Immediate amoxicillin-clavulanate reduced PSSP carriage prevalence by 88% (95%CI: 76-96%) at end-of-treatment and 27% (–3-49%) after 60d, but did not measurably alter RSSP carriage prevalence. By end-of-treatment, 7% of children who carried PSSP at enrollment remained colonized in the amoxicillin-clavulanate arm, compared to 61% of PSSP carriers who received placebo; efficacy persisted 60d after treatment among children who carried PSSP at enrollment. Among children not carrying pneumococci, amoxicillin-clavulanate reduced PSSP acquisition by >80% over 15d. No effect was detected among children who carried RSSP at enrollment. Thus, although amoxicillin-clavulanate had a selective impact favoring continued carriage of RSSP, absolute risk of carrying non-susceptible lineages was unaffected by treatment.

Conclusions: Antimicrobial treatment for AOM reduced penicillin-susceptible pneumococcal colonization but did not alter children's risk of carrying resistant pneumococcal lineages.

INTRODUCTION

Acute otitis media (AOM) is the leading cause of healthcare visits and antimicrobial prescribing among children in high-income countries.[1] Although many infections are self-limiting, antimicrobial therapy accelerates the resolution of otoscopic signs and symptoms.[2,3] Because AOM is prevalent among children, routinely treating infections with antimicrobial therapy may exert considerable selective pressure in favor of drug resistance. This concern has led to conflicting views and guidelines about the optimal clinical management of AOM,[4] with certain countries recommending "watchful waiting" as a strategy to limit unnecessary antimicrobial prescribing.[5,6]

Limited evidence of how treating AOM with antimicrobial drugs impacts selection of resistant bacteria has contributed to uncertainty about the extent to which resistance risks may offset clinical benefits of treatment.[4] Associations between previous antimicrobial treatment and carriage of resistant bacteria have been reported in observational studies.[7,8] Endpoints of drug-susceptible and drug-resistant bacterial carriage have been measured in few trials, and have been reported incompatibly when measured,[3,9,10] making outcomes difficult to interpret.[11] Individual-level effects of antimicrobial treatment on the clearance, replacement, or acquisition of bacterial strains, and the persistence of these effects over time, have not been assessed. To improve the evidence base for treatment guidelines, we assessed the impact of immediate antimicrobial therapy for AOM on nasopharyngeal carriage dynamics of *Streptococcus pneumoniae* with varying degrees of susceptibility to penicillin up to two months after treatment in a clinical trial.

METHODS

Study design and patients

We assessed nasopharyngeal carriage of *S. pneumoniae* as a secondary outcome in a randomized, double-blind, placebo-controlled trial of amoxicillin-clavulanate for the treatment of stringently-defined AOM at Turku University Hospital in Turku, Finland. Trial design, primary clinical endpoints, adverse events, and the full study protocol have been reported previously.[2] Briefly, children 6 to 35 months old seeking care were screened for AOM diagnosis, defined as fulfilling the following three criteria: presence of middle ear fluid, acute inflammatory signs in the tympanic membrane, and acute symptoms such as fever, ear pain, or respiratory symptoms. Analyses reported in this manuscript were not specified in the original study protocol.

Upon enrollment, children were randomized to immediate antimicrobial therapy or placebo, and attended scheduled follow-up visits with study physicians. Rescue therapy with open-label antimicrobial drugs was initiated if children's condition did not improve or worsened during treatment, based on parental report and clinical assessment of symptoms and otoscopic signs. In this sense, the intention-to-treat populations of the treatment and placebo arms were blinded to prescribing strategies that resembled immediate antimicrobial therapy and watchful waiting, respectively.

We performed nasopharyngeal swabbing in-clinic at enrollment, at scheduled visits, and when children sought care for new episodes, to determine pneumococcal carriage. We analyzed carriage outcomes for scheduled visits occurring at end-of-treatment (day 8) and 15, 30, and 60 days after treatment. To compare effects among children according to time since end-of-treatment, we numbered days from enrollment or, where applicable, from initiation of rescue treatment. Susceptibility of isolates to penicillin was determined using standard breakpoints, as detailed below.[12]

Ethics

Parents provided written informed consent on behalf of children. The ethics committee of the Hospital District of Southwest Finland Ethical approved the original study. Analyses of secondary carriage endpoints were exempted by the institutional review board at Harvard TH Chan School of Public Health.

Randomization and masking

Patients were enrolled by study physicians and randomly assigned (1:1) amoxicillin-clavulanate or placebo via computer-generated allocation numbers, which were provided over the telephone by nurses in the Pediatric Infectious Disease Ward of Turku University Hospital. Patients, study physicians, and laboratory personnel were blinded to the randomization codes for the duration of the trial. The allocation list was generated as a block of ten and kept by the hospital pharmacy. Pharmacists concealed assignments by labeling identical study drug containers with allocation numbers. Placebo drug powders and capsules were identical to the active treatment in appearance, taste, and smell. The biostatistician performing analyses presented in this manuscript was not blinded to treatment assignments.

Procedures

Eligible patients were randomized to amoxicillin-clavulanate (40mg of amoxicillin plus 5.7mg of clavulanate per kg of body weight daily, divided into two doses) or placebo for 7 days. Study physicians encouraged the use of analgesic and antipyretic agents, and allowed the use of decongestant nose drops and sprays. To enable comparisons of immediate versus delayed antimicrobial treatment, the main rescue treatment was open-label amoxicillin-clavulanate 40/5.7mg per kg/day, divided into two doses, for 7 days. However, study physicians could also prescribe high-dose amoxicillin-clavulanate or intramuscular ceftriaxone as rescue treatment based on clinical assessment, consistent with Finnish treatment guidelines.

Nasopharyngeal specimens were collected with dacron swabs (Copan diagnostics, Corona, CA USA), and plated on selective agar immediately after the study visit to determine pneumococcal carriage. Plates were incubated in 5% CO_2 at ≥35ºC and examined at 18-24h and 36-48h. Penicillin susceptibility was determined via a two-step approach. Samples were first screened for reduced susceptibility using oxacillin disks. For samples with a decreased (<20mm) zone of inhibition, minimal inhibitory concentrations (MICs) were determined using penicillin G E-tests (bioMérieux, Cambridge, MA, USA). All samples with a zone of exclusion ≥20mm, or a zone of exclusion <20mm and MIC ≤0.064μg/mL, were classed as penicillin-susceptible. Intermediate resistance was defined as a zone of exclusion <20mm and MIC >0.064μg/mL or ≤2μg/mL, and resistance was defined as a zone of exclusion <20mm and MIC >2μg/mL. A susceptible control strain (ATCC 49619) was tested together with clinical isolates for validation.

Outcomes

The endpoints of interest were carriage of penicillin-susceptible, intermediately-resistant, or resistant *S. pneumoniae* at scheduled visits 8, 15, 30, and 60 days after the start of treatment with the study drug or rescue therapy. We conducted comparisons of pneumococcal carriage prevalence, prevalence of penicillin-susceptible *S. pneumoniae* (PSSP), and prevalence of *S. pneumoniae* with reduced susceptibility to penicillin (RSSP), as defined by intermediately-resistant or resistant phenotype. Because otopathogens carried in the nasopharynx provide a reservoir for recurrent or persisting infection following treatment for AOM,[13,14] we defined treatment efficacy as the reduction in prevalence of nasopharyngeal carriage.

Statistical analysis

Power considerations

Enrollment was guided by statistical power considerations for the primary clinical endpoint of the study (treatment failure in the amoxicillin-clavulanate arm as compared to the placebo arm).[2] Assuming 50% carriage prevalence in the placebo arm at end-of-treatment, the study had over 90% power at two-sided type II error threshold $p<0.05$ for detecting a reduction in carriage prevalence of 50% or more in the treatment arm.

Definition of treatment effects

Children were analyzed according to the study arms to which they were randomized in an intention-to-treat framework. We calculated the relative risk (RR) of carriage endpoints in the two arms via the prevalence ratio of any pneumococcal carriage, of PSSP carriage, and of RSSP carriage. We measured efficacy of immediate antimicrobial prescribing against each colonization endpoint at follow-up visits as $(1-RR)\times 100\%$. To understand impacts on acquisition and clearance of *S. pneumoniae*, we also performed subgroup analyses estimating treatment efficacy against any pneumococcal carriage among children who were not colonized, or carried PSSP or RSSP, at enrollment.

To determine the selective impact of antimicrobial treatment on pneumococci carried by children treated for AOM, we next assessed the susceptibility of strains carried by children who had been assigned to immediate amoxicillin-clavulanate or placebo.[11] We measured the conditional relative risk (cRR) for strains carried at follow-up visits to show reduced susceptibility as the ratio, between the study arms, of the proportions of pneumococcal isolates classed as RSSP, out of all pneumococcal isolates tested.

Last, we calculated risk differences to measure the prevalence of carriage that could be attributed to, or averted by, immediate antimicrobial prescribing. For each scheduled visit, we calculated differences in pneumococcal carriage prevalence—including all pneumococci, PSSP, and RSSP—within the two study arms. We again performed subgroup analyses according to carriage status at enrollment.

Measurement of prevalence and statistical testing

We conducted statistical inference over 100,000 bootstrap-resampled datasets to measure medians and 95% confidence intervals (CIs) around prevalence, relative risk, treatment efficacy, and risk difference estimates. As detailed in the supplemental text (**Text S1**) this bootstrap approach allowed us to generate unbiased estimates of PSSP and RSSP carriage prevalence in the presence of missing data.

Role of the funding source

The funder had no role in study design, collection, analysis, or interpretation of data, writing of the report, or the decision to submit for publication. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

RESULTS

Enrollment and sample processing

Of 162 patients initially randomized to amoxicillin-clavulanate and 160 initially randomized to placebo, two children in the treatment arm and three children in the placebo arm left the study prior to the end-of-treatment visit (**Figure 1**). Rescue therapy was initiated in 11 (7%) children randomized to amoxicillin-clavulanate, compared to 53 (33%) children randomized to placebo.

At enrollment, 193 of 318 (61%) children carried *S. pneumoniae* (**Table 1**). In total, pneumococcal carriage was determined at 675 of 810 (83%) scheduled visits among children assigned amoxicillin-clavulanate, and at 683 of 800 (85%) scheduled visits among children assigned placebo (**Figure 1**). Resistance determinations were available for 662 (98%) and 640 (94%) pneumococcal isolates in these arms, respectively. Baseline characteristics of children with complete follow-up data to day 60 did not differ from baseline characteristics of children missing data from any scheduled visit (**Table S1**).

Efficacy of amoxicillin-clavulanate therapy for AOM against pneumococcal colonization

Among children from whom we obtained swabs as scheduled for the end-of-treatment visit, we detected pneumococcal carriage in 18 of 146 (12%) randomized to amoxicillin-clavulanate and 63 of 141 (45%) randomized to placebo (**Figure 2**), indicating 73% (95%CI: 58% to 84%) efficacy against pneumococcal colonization at end-of-treatment. Only 4% of children in the amoxicillin-clavulanate arm, compared to 34% in the placebo arm, carried PSSP at end-of-treatment. In contrast, 8% and 11% of children in the treatment and placebo arms, respectively, carried RSSP at end-of-treatment. These findings translated to

estimates of 88% (76% to 96%) and 25% (–54% to 65%) efficacy, respectively, against PSSP and RSSP carriage at end-of-treatment. In total, 32% (23% to 42%) prevalence of pneumococcal carriage among children treated for AOM could be averted by immediate amoxicillin-clavulanate therapy, almost entirely due to the prevention of PSSP carriage in 30% (21% to 38%) of treated children.

Estimated efficacy against any pneumococcal carriage and PSSP carriage declined over successive visits to 21% (–6% to 42%) and 27% (–3% to 49%), respectively (**Figure 2**). We did not detect strong statistical evidence (defined by a 95%CI entirely above zero) of efficacy against RSSP carriage at any visit. Only 8% to 11% of children in the two arms carried RSSP at any follow-up visit.

Variation in outcomes according to carriage at enrollment

Estimates of treatment efficacy against pneumococcal colonization varied according to carriage at the enrollment visit (**Figure 3** and **Table 2**). Among children carrying PSSP at enrollment, between 61% and 67% of those assigned placebo carried pneumococcus at follow-up visits, whereas prevalence among children assigned amoxicillin-clavulanate dropped to 7% by end-of-treatment and reached only 35% after 60d. This signified 89% efficacy (77% to 98%) among PSSP carriers at end-of-treatment, with 45% efficacy (17% to 66%) persisting up to 60d after treatment. No children who carried PSSP at enrollment were observed to carry RSSP at any follow-up visit (**Table 2**). Immediate amoxicillin-clavulanate prevented 54% (40% to 68%) of children who carried PSSP at baseline from carrying PSSP at end-of-treatment, an effect persisting among 28% (9% to 48%) of these children 60d after treatment.

Among children carrying RSSP at enrollment, 71% and 83% carried pneumococcus at end-of-treatment after randomization to amoxicillin-clavulanate and placebo, respectively, indicating 15% (–22% to 44%) efficacy against any colonization (**Figure 3**). Efficacy estimates at subsequent follow-up visits remained statistically indistinguishable from zero. By 60d after treatment, 69% and 60% of children assigned amoxicillin-clavulanate and placebo, respectively, carried *S. pneumoniae*. Of the children carrying RSSP at enrollment and assigned amoxicillin-clavulanate, none carried PSSP until 60d after treatment (**Table 2**), whereas 5% to 8% prevalence of PSSP carriage was detected at follow-up visits by children assigned placebo.

We estimated 82% (–6% to 100%) and 86% (24% to 100%) efficacy against pneumococcal colonization at end-of-treatment and 15d after treatment among children who were not colonized at enrollment (**Figure 3**). However, prevalence of colonization among such children was low; the averted prevalence 15d after treatment amounted to only 10% (1% to 19%). We did not identify clear reductions in prevalence of carriage at subsequent follow-up visits. The majority of pneumococcal isolates collected after treatment from children who were not colonized at enrollment were penicillin-susceptible (**Table 2**). Among children who were not colonized at enrollment, no RSSP carriage was detected among those assigned placebo, whereas RSSP prevalence reached 6% (0% to 15%) by 60d after treatment among those assigned amoxicillin-clavulanate. However, this reflected acquisition of RSSP by only three children assigned amoxicillin-clavulanate, with zero to two children simultaneously carrying RSSP at any scheduled visit.

Selective impact of amoxicillin-clavulanate therapy for AOM

Owing to the differential treatment efficacy against PSSP and RSSP, the proportion of carried strains showing reduced susceptibility to penicillin at end-of-treatment was 2.74 (95%CI: 1.62 to 4.88) times higher among children assigned amoxicillin-clavulanate (12 of 18, 67%) than among children assigned placebo (13 of 53, 25%; **Table 3**). This effect persisted at least 30d after treatment, at which point the proportion of carried strains showing reduced susceptibility to penicillin was 2.28 (1.18 to 5.53) times higher among children assigned amoxicillin-clavulanate (35%) than among those assigned placebo (15%). By 60d after treatment, the proportion of pneumococcal isolates with reduced susceptibility to penicillin remained higher among children assigned amoxicillin-clavulanate (cRR 1.40 (0.64 to 3.20)), although this difference no longer reached conventional thresholds for statistical significance..

Penicillin-resistant pneumococcal carriage

Three children carried *S. pneumoniae* strains that were classed as fully resistant to penicillin (defined by an oxacillin disk reading <20mm and MIC>2μg/mL) (**Figure 4**). Two of these children carried strains registering an intermediate MIC at enrollment, and were found to carry penicillin-resistant isolates of the same serotype at least once after treatment with amoxicillin-clavulanate as the study drug or rescue therapy. A third child who received amoxicillin-clavulanate carried a strain registering a resistant MIC at all study visits, with the exception of the end-of-treatment visit, at which an intermediate MIC was detected. We detail the clinical course of these children in the supplemental text (**Text S2**).

DISCUSSION

Our findings demonstrate a sustained, selective impact of antimicrobial treatment for AOM on pneumococcal carriage among young children in a high-income country. In our trial, children randomized to immediate amoxicillin-clavulanate therapy were less likely than those randomized to placebo to carry PSSP up to 60d after treatment. By end-of-treatment, RSSP constituted 67% of carried strains among children assigned amoxicillin-clavulanate, compared to 25% of carried strains among children assigned placebo; this difference in susceptibility of carried strains persisted up to 60d after treatment. However, immediate amoxicillin-clavulanate did not impact absolute prevalence of RSSP carriage. These findings clarify the magnitude and duration of the impact of immediate antimicrobial therapy, as compared to watchful waiting, on carriage of *S. pneumoniae* with varying susceptibility to penicillin.

Our analysis also illustrates how antimicrobial effects on bacterial acquisition and clearance mediate differences between study arms in carriage prevalence. In our trial, 39% of PSSP carriers assigned placebo cleared colonization by end-of-treatment under circumstances resembling watchful waiting, either naturally or due to receipt of antimicrobial drugs as rescue therapy, compared to 18% of RSSP carriers. Immediate amoxicillin-clavulanate cleared PSSP colonization by end-of-treatment in an additional 54% of children, but had no measurable impact on colonization among RSSP carriers. Among children who were not colonized at enrollment, immediate amoxicillin-clavulanate lowered the risk of acquiring PSSP by >80% over the first two weeks after treatment. These measurements provide a basis for understanding treatment effects in settings with differing PSSP and RSSP prevalence, and thus modeling transmission of susceptible and resistant lineages under differing treatment protocols.

Among placebo-controlled trials of antimicrobial treatment for AOM, our study enrolled the largest sample of children and included the longest continuous monitoring of carriage.[15] By stratifying according to carriage status at enrollment, our analysis is the first to assess effects of antimicrobial treatment for AOM on clearance and acquisition of PSSP and RSSP. Several other trials of antimicrobial therapy for AOM in high-income countries provide complementary insight into effects on pneumococcal carriage. Consistent with our findings, no difference in absolute prevalence of RSSP was reported at end-of-treatment or 21-25d after treatment among children assigned 10d of amoxicillin-clavulanate compared to those assigned placebo,[3] or at the end of a 10d versus 5d course of amoxicillin-clavulanate,[9] in two trials in the United States. Thus, the proportions of pneumococcal isolates showing reduced susceptibility to penicillin at end-of-treatment were higher among children assigned 10d amoxicillin-clavulanate than among children assigned either placebo or 5d amoxicillin-clavulanate, owing to reductions in PSSP carriage—evidence of a selective impact that was not assessed in primary analyses.[3,9] Similar changes in the proportion of isolates showing reduced susceptibility were evident after treatment in a trial comparing immediate antimicrobial prescribing and watchful waiting,[10] although effects on absolute prevalence of PSSP and RSSP were not reported, and carriage was assessed only 12d after treatment. Unified reporting of absolute and conditional effects on bacterial carriage and susceptibility will facilitate direct comparison of results from future trials.[11] Our finding that reductions in PSSP carriage persist up to 60d after treatment further suggests that future studies should monitor carriage over longer follow-up windows than previous trials have included.

Whereas our study did not detect increases in RSSP carriage prevalence due to antimicrobial treatment, this observation has been reported in several studies undertaken in lower-income countries with higher RSSP prevalence. In the Dominican Republic, children with respiratory tract infections randomized to a low-dose, 10d amoxicillin regimen had higher prevalence of RSSP carriage after treatment than children randomized to a high-dose, 5d regimen.[16] In Malawi, increases in RSSP carriage prevalence were

reported after treatment of malaria with sulfadoxine-pyrimethamine and treatment of suspected bacterial infections with cotrimoxazole.[17] Whereas most studies of antimicrobial therapy for AOM have been conducted in the United States and Europe,[15] these findings suggest trials are warranted in low- and middle-income countries to guide appropriate treatment practices for such settings, as higher RSSP prevalence may alter the relationship between antimicrobial treatment and carriage dynamics.

It is important to note that our study measured only the direct effect of antimicrobial therapy.[18] Large-scale changes in treatment practices may spur indirect effects on carriage of PSSP and RSSP among children not treated for AOM. Widespread serotype replacement after introduction of pneumococcal conjugate vaccines illustrates that interventions targeting young children may confer such indirect, population-level effects on the composition of circulating lineages.[19–21] Several lines of evidence demonstrate similar indirect effects of antimicrobial treatment interventions on pneumococcal carriage and susceptibility. Mass azithromycin administration for the prevention of trachoma caused community-wide increases in prevalence of macrolide-resistant *S. pneumoniae* carriage in a cluster-randomized trial in Ethiopia.[22] In ecological studies, outpatient antibiotic prescribing predicts the proportion of pneumococcal isolates showing reduced penicillin susceptibility at geographic scales ranging from municipalities[23] to countries.[24]

Our study has several limitations. Because our trial was designed to assess clinical failure as a primary endpoint, stratified analyses addressing carriage of RSSP, and outcomes among children who carried RSSP at enrollment, had reduced statistical power. Although data from four time points up to 60d after treatment provided a more detailed view of pneumococcal carriage dynamics than other trials have afforded,[3,10,9] we did not obtain swabs for 22% and 32% of children from follow-up visits 30d and 60d after treatment. Last, our analysis does not address the association of carriage endpoints with antimicrobial prescribing for secondary AOM episodes or other causes during follow-up. An advantage of the modified intention-to-treat framework we employ is that this circumstance does not hinder our interpretation of carriage outcomes as causal effects of the initial randomized assignment.

We conducted this study before the introduction of pneumococcal conjugate vaccination in the Finnish national immunization program. Whereas a higher proportion of vaccine-targeted serotypes than non-vaccine serotypes tended to be resistant to antimicrobial drugs in the pre-vaccine era, increases in the proportion of resistant non-vaccine serotypes have been reported amid serotype replacement, generally mitigating any net change in the prevalence of resistance.[25–27] Thus, our findings remain relevant for settings with routine immunization.

In conclusion, our study demonstrates that immediate amoxicillin-clavulanate therapy for AOM reduces prevalence of PSSP carriage, indirectly increasing the proportion of carried strains showing reduced susceptibility to penicillin. We detected no increase in absolute prevalence of RSSP among children assigned amoxicillin-clavulanate therapy as compared to children assigned placebo. These findings suggest antimicrobial treatment for AOM may not increase risk for individual children to carry resistant strains of *S. pneumoniae*, although selective impacts of widespread treatment of AOM remain of concern at the population level. Quantifying these effects of different AOM treatment strategies on carriage of PSSP and RSSP provides a basis for evaluating antimicrobial resistance risks against the clinical benefits of antimicrobial therapy for AOM.[4]

RESEARCH IN CONTEXT

Evidence before the study: Antimicrobial therapy hastens the resolution of signs and symptoms of acute otitis media (AOM). However, fears that this treatment approach may select for resistant lineages of pathogens such as *Streptococcus pneumoniae* have led to conflicting guidelines for immediate antimicrobial therapy or watchful waiting as an AOM management strategy. To understand the effect of antimicrobial treatment of AOM on carriage of *S. pneumoniae* with varying susceptibility to penicillin, we searched for trials in ClinicalTrials.gov, PubMed, and Google Scholar, and the Cochrane Review database. Besides our trial, no others have assessed long-term effects (≥30d) of antimicrobial treatment on bacterial carriage and susceptibility. All other trials to date have reported carriage endpoints ≤12d after treatment; one study also evaluated carriage 21-25d after treatment. Studies have been inconsistent in reporting either absolute effects of treatment on the prevalence of susceptible and resistant lineages, or conditional effects on the susceptibility of lineages carried after treatment, leading to conflicting conclusions. Studies have not assessed effects of treatment on the dynamics of acquisition or loss of strains with varying susceptibility.

Added value of this study: This analysis, the first of its kind to monitor individual-level changes in carriage at scheduled visits over long-term follow-up, shows that antimicrobial therapy clears colonization of penicillin-susceptible *S. pneumoniae*, leading to reduced prevalence of such strains up to 60 days after treatment, without impacting carriage dynamics of lineages with reduced susceptibility. Antimicrobial therapy also delays pneumococcal carriage acquisition by two weeks among children not carrying pneumococcus.

Implications of all the available evidence: As compared to watchful waiting, immediate antimicrobial treatment of AOM confers a long-term (≥60d) selective effect on the composition of pneumococcal strains carried by children due to selective clearance of penicillin-susceptible pneumococcal strains, and delayed acquisition of penicillin-susceptible strains among children not already colonized.

FIGURES

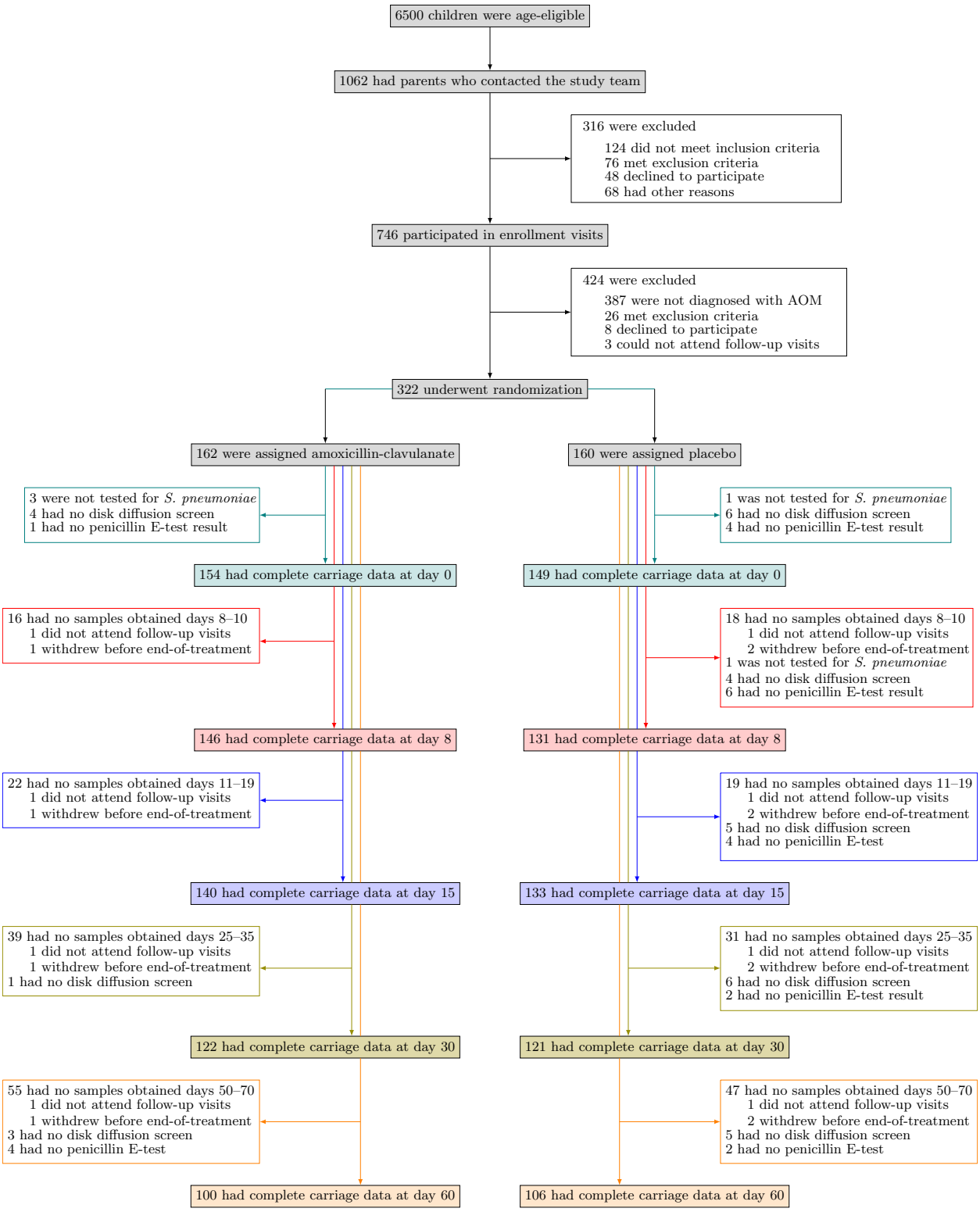

***Figure 1*: Trial outline.** We illustrate the recruitment, enrollment, and randomization of patients together with the completion of follow-up visits as scheduled and processing of nasopharyngeal specimens. Samples were not obtained if children did not attend a

visit within the specified time windows of scheduled visits at days 8, 15, 30, and 60, or if children or parents refused the nasopharyngeal swab.

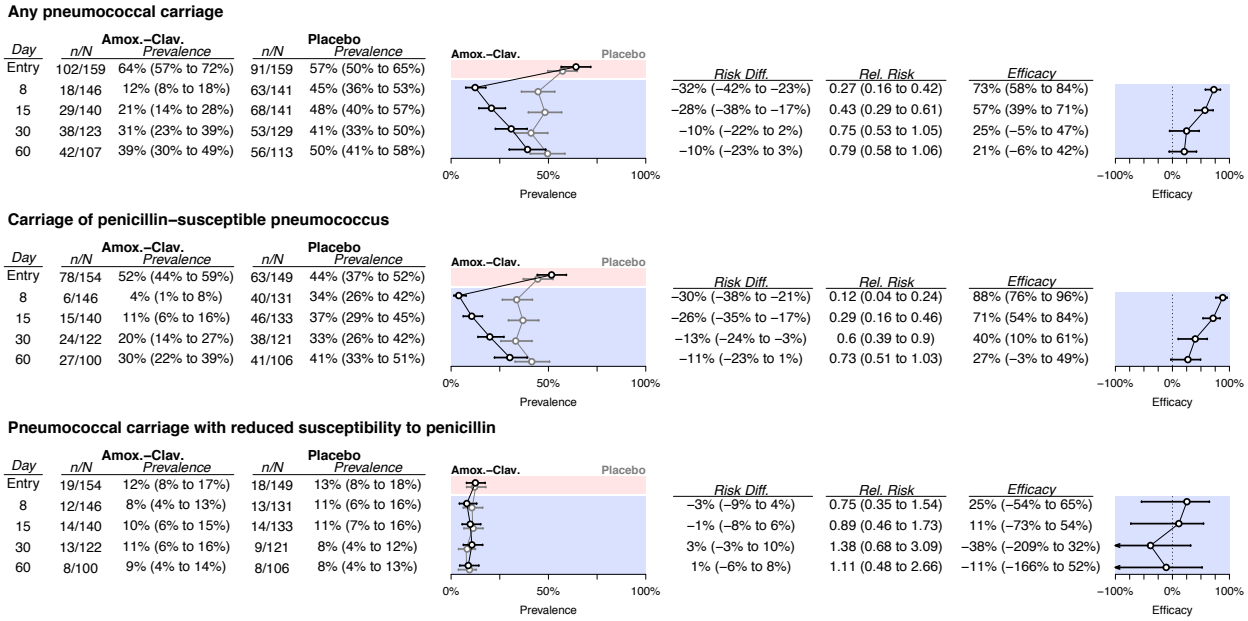

Figure 2: **Efficacy of immediate amoxicillin-clavulanate against carriage of *S. pneumoniae* with varying susceptibility to penicillin.** Tables and plots indicate the number carrying *S. pneumoniae*, PSSP, and RSSP, measured out of all swabs with microbiological determinations available (carriage presence/absence, or carriage presence/absence and penicillin susceptibility, given carriage). Prevalence estimates are presented as median (95% confidence interval) based on the bootstrap approach (see **Methods** and **Text S1**) taken to correct for bias from incomplete testing of specimens; consequently, median reported estimates may not equal *n*/*N*.

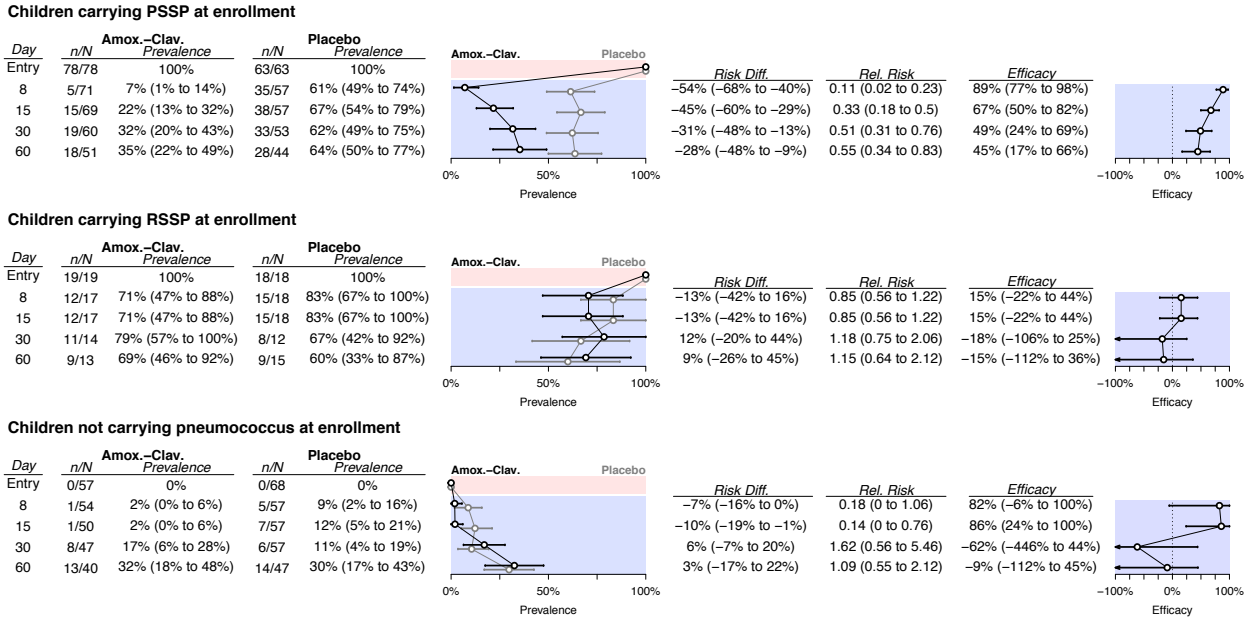

Figure 3: **Efficacy of immediate amoxicillin-clavulanate against carriage of *S. pneumoniae*, according to carriage at enrollment.** Tables and plots indicate the number carrying *S. pneumoniae* at follow-up visits, according to carriage at enrollment, measured out of all swabs with microbiological determinations available (carriage presence/absence, or carriage presence/absence and penicillin susceptibility, given carriage); prevalence estimates are presented as median (95% confidence interval) based on the bootstrap approach (see **Methods** and **Text S1**) taken to correct for bias from incomplete testing of specimens. We expand on results presented in this Figure—detailing susceptibility of isolates carried at follow-up—in **Table 2**.

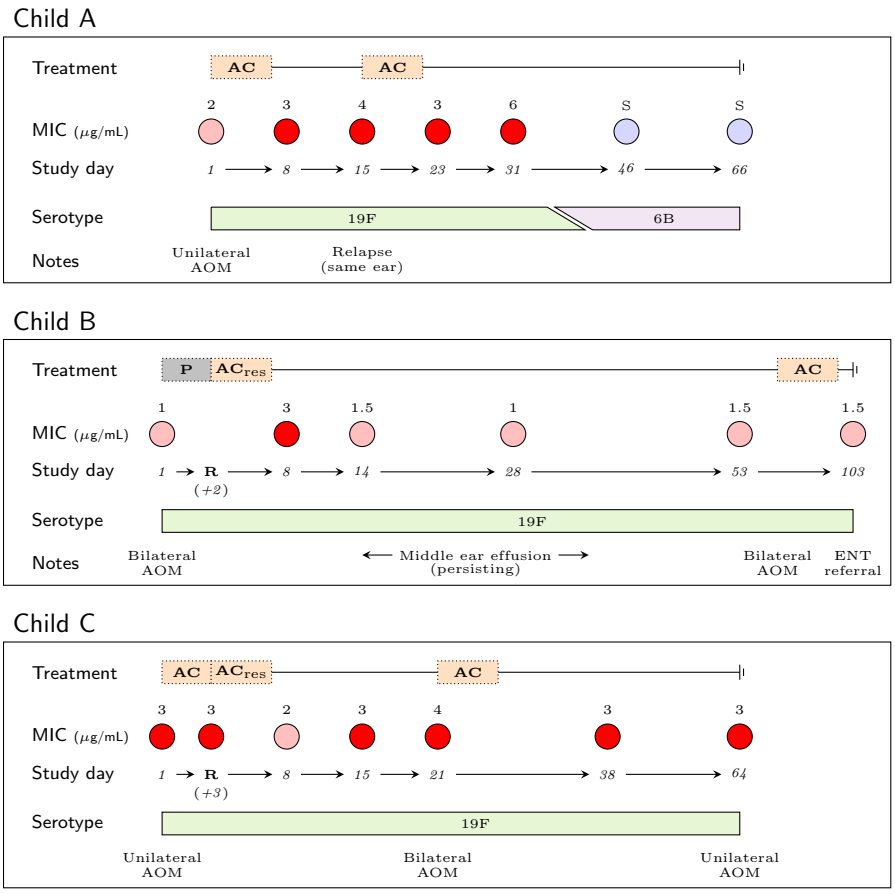

***Figure 4*: Observations among children who carried resistant isolates during the study.** Study drug and rescue therapy assignments (P: placebo; AC: amoxicillin-clavulanate) are illustrated together with isolates (with MIC values) taken from children from the enrollment visit to the last visit performed. Study days are numbered from the first day of treatment with study drug or rescue therapy. Colors indicate penicillin sensitivity of the carried strain: blue, penicillin sensitive; pink, intermediate penicillin resistance; red, penicillin resistance.

TABLES

Table 1: **Baseline characteristics of patients.**

Characteristic	All children		Children carrying *S. pneumoniae* at enrollment		Children not colonized by *S. pneumoniae* at enrollment	
	Amox.-Clav. (*N*=162)	Placebo (*N*=160)	Amox.-Clav. (*N*=102)	Placebo (*N*=91)	Amox.-Clav. (*N*=57)	Placebo (*N*=68)
Age (mo.)						
Mean (±SD)	15.6 (±7.3)	16.0 (±7.9)	15.7 (±7.3)	16.4 (±8.2)	15.5 (±7.4)	15.5 (±7.5)
Male child						
N (%)	92 (57)	91 (57)	59 (58)	46 (51)	31 (54)	45 (66)
Received antimicrobial drugs within preceding month						
N (%)	37 (23)	34 (21)	21 (21)	14 (15)	15 (26)	20 (29)
Number of siblings						
0, N (%)	73 (45)	66 (41)	46 (45)	34 (37)	24 (42)	31 (46)
1–2, N (%)	79 (49)	89 (56)	51 (50)	53 (58)	28 (49)	36 (53)
3 or more, N (%)	10 (6)	5 (3)	5 (5)	4 (4)	5 (9)	1 (1)
Daycare attendance						
N (%)	87 (54)	87 (54)	63 (47)	49 (34)	23 (30)	37 (43)
Parental smoking[1]						
N (%)	57 (35)	48 (30)	33 (32)	24 (26)	23 (40)	24 (35)
Uses pacifier						
N (%)	80 (49)	86 (54)	50 (49)	42 (46)	30 (53)	43 (63)
Duration of breastfeeding (mo.)[2]						
Mean (±SD)	7.5 (±4.7)	7.1 (±4.5)	7.4 (±4.4)	6.8 (±4.1)	7.7 (±5.3)	7.5 (±4.9)
Received ≥1 dose of pneumococcal conjugate vaccine						
N (%)	3 (2)	4 (3)	2 (2)	4 (4)	1 (2)	0 (0)
Severe bulging						
N (%)	45 (28)	41 (26)	33 (32)	30 (33)	12 (21)	10 (15)
Bilateral AOM						
N (%)	61/160 (38)	68/158 (43)	41/100 (41)	34/90 (38)	19/57 (33)	34/67 (51)
Fever ≥39ºC in previous 24 hours						
N (%)	24 (15)	18 (11)	16 (16)	13 (14)	8 (14)	5 (7)
Pneumococcal carriage						
Any, N (%)	102/159 (64)	91/159 (57)				
PSSP, N (%)	78/154 (52)[3]	63/149 (45)[3]	78/97 (81)[3]	63/81 (78)[3]		
RSSP, N (%)	19/154 (13)[3]	18/149 (13)[3]	19/97 (19)[3]	18/81 (22)[3]		

[1]No parents reported smoking inside the household.
[2]Duration of any breastfeeding at the time of the enrollment visit.
[3]Prevalence estimates are presented as median (95% confidence interval) based on the bootstrap approach (see **Methods** and **Text S1**) taken to correct for bias from incomplete testing of specimens.

Table 2: **Prevalence of pneumococcal carriage averted by immediate antimicrobial therapy, according to carriage status at enrollment.**

Carriage status at enrollment	Endpoint	Study day	Amox.-Clav.		Placebo		Risk difference, % (bootstrap)[1]
			n/N	Prevalence, % (bootstrap)[1]	*n/N*	Prevalence, % (bootstrap)[1]	
PSSP carriers	Any carriage						
		8	5/71	7 (1, 14)	35/57	61 (49, 74)	-54 (-68, -40)
		15	15/69	22 (13, 32)	38/57	67 (54, 79)	-45 (-60, -29)
		30	19/60	32 (20, 43)	33/53	62 (49, 76)	-31 (-48, -13)
		60	18/51	35 (22, 49)	28/44	64 (50, 77)	-28 (-48, -9)
	PSSP carriage						
		8	5/71	7 (1, 14)	35/57	61 (49, 74)	-54 (-68, -40)
		15	15/69	22 (13, 32)	37/56	67 (54, 79)	-45 (-60, -29)
		30	19/60	32 (20, 43)	31/51	62 (49, 75)	-31 (-48, -13)
		60	17/50	35 (22, 49)	26/42	64 (50, 77)	-28 (-48, -9)
	RSSP carriage						
		8	0/71	0	0/57	0	0
		15	0/69	0	0/56	0	0
		30	0/60	0	0/51	0	0
		60	0/51	0	0/42	0	0
RSSP carriers	Any carriage						
		8	12/17	71 (47, 88)	15/18	83 (67, 100)	-13 (-42, 16)
		15	12/17	71 (47, 88)	15/18	83 (67, 100)	-13 (-42, 16)
		30	11/14	79 (57, 100)	8/12	67 (42, 92)	12 (-20, 44)
		60	9/13	69 (46, 92)	9/15	60 (33, 87)	9 (-26, 45)
	PSSP carriage						
		8	0/17	0	1/17	5 (0, 17)	-5 (-17, 0)
		15	0/17	0	1/17	5 (0, 17)	-5 (-17, 0)
		30	0/14	0	1/11	8 (0, 25)	-8 (-25, 0)
		60	2/11	19 (0, 39)	2/15	12 (0, 28)	6 (-17, 30)
	RSSP carriage						
		8	12/17	71 (47, 88)	13/17	78 (58, 94)	-7 (-36, 21)
		15	12/17	71 (47, 88)	13/17	78 (58, 94)	-7 (-36, 21)
		30	11/14	79 (57, 100)	6/11	58 (32, 83)	20 (-13, 5)
		60	5/11	49 (27, 77)	7/15	47 (26, 73)	3 (-31, 38)
Not carrying pneumococcus	Any carriage						
		8	1/54	2 (0, 6)	5/57	9 (2, 18)	-7 (-16, 0)
		15	1/50	2 (0, 6)	7/57	12 (5, 21)	-10 (-19, -1)
		30	8/47	17 (6, 28)	6/57	11 (4, 19)	7 (-7, 20)
		60	13/40	33 (18, 48)	14/47	30 (17, 43)	3 (-17, 22)
	PSSP carriage						
		8	1/54	2 (0, 6)	4/56	9 (2, 18)	-7 (-16, 0)
		15	0/50	0	7/57	12 (5, 21)	-12 (-21, -5)
		30	5/46	12 (4, 24)	6/57	11 (4, 19)	2 (-10, 15)
		60	8/37	26 (14, 41)	13/46	30 (17, 43)	-4 (-22, 15)
	RSSP carriage						
		8	0/54	0	0/56	0	0
		15	1/50	2 (0, 6)	0/57	0	2 (0, 6)
		30	2/46	4 (0, 13)	0/57	0	4 (0, 13)
		60	2/37	6 (0, 15)	0/46	0	6 (0, 15)

[1]Prevalence estimates are presented as median (95% confidence interval) based on the bootstrap approach (see **Methods** and **Text S1**) taken to correct for bias from incomplete testing of specimens.

***Table 3*: Relative risk for carried *S. pneumoniae* to show reduced susceptibility to penicillin, given immediate antimicrobial therapy.**

Study day	Amox.-Clav.		Placebo		Conditional relative risk
	n/N	Proportion, %	*n/N*	Proportion, %	
8	12/18	67 (45, 87)	13/53	25 (15, 35)	**2.74 (1.62, 4.88)**
15	14/29	48 (32, 65)	14/60	23 (14, 33)	**2.08 (1.22, 3.69)**
30	13/37	35 (22, 48)	7/45	15 (7, 25)	**2.28 (1.18, 5.53)**
60	8/35	23 (12, 34)	8/49	16 (8, 25)	1.40 (0.64, 3.20)

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CONFLICTS OF INTEREST

JAL has received research funds from Pfizer to Harvard University and consulting fees from Pfizer for unrelated studies. ML has received consulting fees/honoraria from Merck, Pfizer, Affinivax, and Antigen Discovery, and grant support from Pfizer and PATH Vaccine Solutions to Harvard University for unrelated studies. All other authors declare no conflicts of interest.

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Supporting Information

Effect of antimicrobial treatment for acute otitis media on carriage of *Streptococcus pneumoniae* with reduced susceptibility to penicillin in a randomized, double-blind, placebo-controlled trial

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Text S1 Bootstrap approach to statistical inference and missing-data bias correction

We used the bootstrap to correct for missing-data biases under incomplete testing in prevalence estimates of PSSP and RSSP. Pneumococcal carriage prevalence and prevalence of PSSP and RSSP at each follow-up visit were subject to differential missingness under incomplete testing of specimens for pneumococcus, and under incomplete testing for resistance in specimens that tested positive for carriage. This could lead to bias under the conditions laid out below:

For arm *A*, define N_A as the total number of children, $C_A=p_{C|A}N_A$ as the total number of carriers, and $S_A=p_{S|A}N_A=p_{S|CA}C_A$ as the total number of carriers of pneumococcus with susceptibility *s*. Define $t_{C|A}$ and $t_{S|CA}$, respectively, as the proportions of children tested for pneumococcal carriage and the proportion of carriage-positive specimens for which resistance was characterized. Under an assumption that missingness is unrelated to carriage status within any study arm, excluding untested specimens does not lead to bias in naive measurements of pneumococcal carriage prevalence

$$\hat{p}_{C|A} = \frac{t_{C|A}C_A}{t_{C|A}N_A} = \frac{C_A}{N_A} = p_{C|A}$$

or in measurements of the proportion of carriage-positive specimens with susceptibility *s*:

$$\hat{p}_{S|CA} = \frac{t_{S|CA}t_{C|A}S_A}{t_{S|CA}t_{C|A}C_A} = \frac{S_A}{C_A} = p_{S|CA}$$

. However, measures of the prevalence of pneumococcus with susceptibility *s* were subject to bias when resistance data were missing:

$$\frac{t_{S|CA}t_{C|A}S_A}{t_{C|A}\left(t_{S|CA}C_A + [N_A - C_A]\right)} = \frac{t_{S|CA}S_A}{N_A - C_A\left(1 - t_{S|CA}\right)} \neq \left.\frac{S_A}{N_A}\right|_{t_{S|CA}\neq 1}$$

We therefore measured the prevalence of pneumococci with susceptibility *s* from the unbiased measures $\hat{p}_{S|A} = \hat{p}_{S|CA}\hat{p}_{C|A}$, using the bootstrap-resampled estimates of $\hat{p}_{S|CA}$ and $\hat{p}_{C|A}$.

Text S2 Clinical course of children carrying fully penicillin-resistant *S. pneumoniae*

"Child A" carried an intermediately-resistant (MIC=2µg/mL) strain (serotype 19F) at enrollment and was assigned amoxicillin-clavulanate. The child had unilateral AOM at the enrollment visit, and at the end-of-treatment visit (day 8) his symptoms and otoscopic signs were improving. The child had a relapse of AOM in the same ear as the initial episode on day 15, and was prescribed amoxicillin-clavulanate for 7 days. Both ears were diagnosed healthy at the end of the second course of antimicrobial treatment on day 23. No other episodes of AOM were diagnosed during the follow-up. The strain of *S. pneumoniae* was found to be resistant from end-of-treatment to day 31, when the highest level of resistance was detected (MIC=6µg/mL). The child carried a penicillin-susceptible strain (serotype 6B) at subsequent visits (days 46 and 66).

"Child B" carried an intermediately-resistant (MIC=1µg/mL) strain (serotype 19F) at enrollment and was randomized to placebo. At enrollment, she had bilateral AOM. During follow-up the symtoms and otoscopic signs deteriorated, and rescue treatment was initiated on day 3. After that, the child's overall condition and otoscopic signs improved, but she still had middle ear effusion in both ears until day 97, when she was diagnosed with bilateral AOM and received amoxicillin-clavulanate for 7 days. The carried 19F strain registered a resistant MIC (3µg/mL) at end-of-treatment, but intermediate resistance (MIC 1 to 1.5µg/mL) at subsequent visits to day 103. Eventually, the child was sent for referral to an ear, nose and throat specialist due to persistent middle ear effusion.

"Child C" presented with unilateral AOM and carried a resistant (MIC=3µg/mL) strain (serotype 19F) at enrollment, and received amoxicillin-clavulanate. On day 4 her overall condition had worsened with bulging of the tympanic membrane in both ears. As the study personnel remained blinded to the study group assignment until the completion of the whole trial, she received rescue treatment (amoxicillin-clavulanate) for additional 7 days. On day 15 both ears were healthy. On day 21 she had a bilateral episode of AOM and was prescribed amoxicillin-clavulanate for 7 days. On day 38 both ears were healthy again. On day 64 she had unilateral AOM that was treated with watchful waiting. The carried strain showed resistant MIC readings at all visits except the end-of-treatment visit, when borderline intermediate resistance was detected (MIC=2µg/mL).

Table S1 Baseline attributes of children with missing and complete carriage data

Characteristic	Amox.-Clav.		Placebo	
	Children missing carriage or resistance data at any visit (*N*=80)	Children with no missing data at any visit (*N*=82)	Children missing carriage or resistance data at any visit (*N*=79)	Children with no missing data at any visit (*N*=81)
Age (mo.)				
Mean (±SD)	16.5 (±7.5)	14.6 (±6.9)	16.7 (±8.0)	15.4 (±7.9)
Male child				
N (%)	43 (54)	49 (60)	41 (52)	50 (62)
Received antimicrobial drugs within preceding month				
N (%)	20 (25)	17 (21)	13 (16)	21 (26)
Number of siblings				
0, N (%)	41 (51)	32 (39)	35 (44)	31 (38)
1–2, N (%)	36 (45)	43 (52)	43 (54)	46 (57)
3 or more, N (%)	3 (4)	7 (9)	1 (1)	4 (5)
Daycare attendance				
N (%)	47 (59)	40 (49)	49 (62)	38 (47)
Parental smoking[1]				
N (%)	29 (36)	28 (35)	22 (28)	26 (32)
Uses pacifier				
N (%)	39 (49)	41 (50)	44 (56)	42 (52)
Duration of breastfeeding (mo.)[2]				
Mean (±SD)	7.8 (±4.2)	7.3 (±5.2)	6.5 (±4.2)	7.7 (±4.7)
Received ≥1 dose of pneumococcal conjugate vaccine				
N (%)	1 (1)	2 (2)	1 (1)	3 (4)
Severe bulging				
N (%)	25 (31)	20 (24)	25 (32)	16 (20)
Bilateral AOM				
N (%)	32 (41)	29 (36)	34 (44)	34 (42)
Fever ≥39ºC in previous 24 hours				
N (%)	13 (16)	11 (13)	11 (14)	7 (9)
Pneumococcal carriage				
Any, N (%)	51 (66)	51 (62)	48 (62)	43 (53)
PSSP, N (%)	36 (50)	42 (51)	29 (43)	34 (42)
RSSP, N (%)	10 (14)	9 (11)	9 (13)	9 (11)

[1]No parents reported smoking inside the household.
[2]Duration of any breastfeeding at the time of the enrollment visit.