Nasopharyngeal colonisation with *Streptococcus pneumoniae* in malnourished children: a systematic review and meta-analysis of prevalence.

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Abstract word count: 216

Paper word count (excluding abstract and references): 2005

Abstract

Background: *Streptococcus pneumoniae* is an intermittent commensal organism in the nasopharynx. Colonisation is a prerequisite for disease and malnourished children are especially susceptible to severe infection. This systematic review examines published prevalence rates of pneumococcal colonisation in the upper respiratory tract of chronically malnourished children under the age of five years.

Methods: A systematic literature search was performed using Medline, PubMed, Web of Science and Scopus. After screening, relevant studies were assessed for quality using STROBE criteria. Colonisation data were extracted and a random-effects model used to pool prevalence estimates.

Findings: Nine studies were included. The prevalence rate of *S. pneumoniae* colonisation in malnourished children during the first month of life ranged from 1.0-2.0% increasing at 2 months to 53.9-80.0%. Carriage remained similar from 3 months to 60 months at 64.1-88.0%. Meta-analysis showed a pooled prevalence of 67.2% in 0-3 months infants (95% CI, 55.6-78.7%), 77.9% in 3-6 months infants (95% CI, 68.1-87.7%) and 77.8% in 6-60 months infants (95% CI, 73.9-81.6%).

Conclusion: In malnourished children, it is plausible that rates of pneumococcal colonisation are higher than in healthy, well-nourished children. Knowledge of colonisation rates can inform policies on vaccination and ancillary interventions during treatment of malnutrition. Future studies should assess the impact of reducing colonisation on disease rates or transmission in these “at risk” individuals.

Keywords: CARRIER STATE, COLONISATION, MALNUTRITION, STREPTOCOCCUS PNEUMONIAE, UPPER RESPIRATORY TRACT

Introduction

Streptococcus pneumoniae (S. pneumoniae) is a leading global cause of respiratory-tract infections and invasive disease in young children, the elderly and immunocompromised patients. In 2015, pneumonia accounted for 16% of all deaths of children less than five years of age.[1] Immunisation programmes using pneumococcal conjugate vaccines (PCV) and polysaccharide vaccines (PPV) reduce colonisation rates and mortality from invasive disease in a serotype-specific manner.[2] Their utility in Low and Middle Income Countries (LMIC) can be limited by cost, variable coverage of locally circulating serotypes,[3] and the high frequency of individuals with medical conditions that alter their immune responses (such as malnutrition or HIV[4]).

The upper respiratory tract (URT) provides an ecological niche for diverse bacterial populations and *S. pneumoniae* frequently but asymptomatically colonises the URT, especially in young children.[5] Progression to mucosal disease (such as sinusitis, otitis media and pneumonia) and invasive pneumococcal disease (IPD) can occur if immunological and mechanical defences are breached and such events are more likely amongst individuals with higher colonisation rates.[6] Nasopharyngeal aerosolization of *S. pneumoniae* is considered the primary mode of population transmission.[7] The high rates of *S. pneumoniae* colonisation seen in the under 5s partly explain the more frequent disease in this age group and why children are thought to be the main reservoir and vector of spread.[8]

Young children also have incomplete protection against disease due to immature adaptive and innate immunity.[9] These systems actively sense colonising mucosal bacteria and maintain a balanced regulatory environment.[10] Higher disease prevalence in LMICs reflects a variety of environmental challenges such as over-crowding, particulate exposure and undernutrition, which can adversely affect mucosal immunity.[11] Pneumonia, for example, is more frequent and more severe in malnourished children in whom immunological studies have shown reduced cellular immunity, phagocyte function, complement and immunoglobulin and cytokine production.[12,13]

Malnutrition, defined as the insufficient, excessive or imbalanced consumption of nutrients, is attributable to 53% of deaths associated with infectious diseases among children less than five years of age in impoverished countries.[6] Malnutrition is

multifactorial, frequently resulting from inadequate breastfeeding, insufficient food availability and recurrent enteric infections.[6,14] Protein-energy malnutrition manifests as kwashiorkor (predominantly protein deficiency) and marasmus (protein and energy deficiency). Micronutrient deficiency due to lack of a specific vitamin or mineral may coexist with these or occur in isolation.[15] Acute malnutrition usually presents as “wasting” (a weight-to-height Z score <-2), compared with “stunting” (a height-to-age Z score <-2), which suggests chronic malnutrition.[16]

Pneumococcal prevalence data could predict the potential of public health interventions targeting nutrition to prevent *S. pneumoniae* disease and/or transmission. The rates of colonisation in malnourished children is therefore relevant to interventions at the individual and population level. This systematic review and meta-analysis describes these rates in malnourished children under the age of 5 years.

Methods

A search of the existing literature was conducted in November 2016 to identify reports of URT pneumococcal colonisation rates in chronically malnourished children aged from 0 to 5 years. We searched PubMed (1955-2016), Medline (1950-2016), Scopus (1960-2016) and Web of Science (1900-2016) using combinations encompassing broad search strings for *Streptococcus pneumoniae*, nasopharyngeal colonisation (including carriage and carrier states) and chronic malnutrition (including kwashiorkor, marasmus and micronutrient deficiencies determined as per the WHO guidelines for the treatment of severely malnourished children).[17] Specific search terms and their combinations are shown in Supplementary materials.

Inclusion criteria were: (i) experimental or observational studies; (ii) English language; (iii) reporting data on URT colonisation of *S. pneumoniae*; (iv) subjects included chronically malnourished children; (v) aged 0-5 years. Studies were excluded which: (i) did not report rates of colonisation; (ii) were case series, case reports, letters or editorials. Retrieved papers were first screened by title and abstract then by full text. Further potential publications were identified from the reference list of included articles.

Data were captured by pre-designed proforma. Quality and risk of bias were assessed using the STROBE tool.[18] Prism (version 7.02, GraphPad, California USA) was used for statistical analysis. Studies were included in meta-analysis if they reported data which allowed calculation of *S. pneumoniae* point prevalence rates and if they included at least 100 participants. Pooled prevalence was calculated using a model which incorporated the study as a random effect and was weighted by the number of participants contributing data.[19]

Results

Sixty-eight citations were identified, of which thirty-three were unique. Nine papers met the inclusion criteria (Figure 1). STROBE quality assessment data are given in Appendix 1.

All studies used conventional microbiology to detect colonisation and followed recommendations made by the WHO for measuring nasopharyngeal colonisation.[20] Rayon-tipped swabs were the most frequently used swabs (4/9 studies, 44.4%). A single study from Venezuela obtained additional oropharyngeal samples.[21]

Five studies derived from vitamin A supplementation random controlled trials (RCTs): two in India,[22,23] one in Bangladesh,[24] and two in The Gambia.[25,26] One study was a zinc supplementation RCT in Nepal.[27] Two case-control studies - one in Venezuela[21], one in The Gambia[12] - compared colonisation in malnourished children with and without *S. pneumoniae* infection. One paper was a clinical trial investigating a urinary pneumococcal diagnostic tool in Ecuador.[28]

Reported prevalence of *S. pneumoniae* colonisation in malnourished children aged 0 to 5 years ranged from 1.0% to 88.0% (Table 1). The prevalence rate of colonisation ranged from 1.0 to 2.0% at birth, increasing within the first two months of life to 53.9% to 80.0%. Colonisation rates in the period between 3 to 60 months of life ranged from 64.1% and 88.0%.

The pooled prevalence estimate of *S. pneumoniae* colonisation was 67.2% (95% CI, 55.6%-78.7%) in children aged from 0 to ≤ 3 months, 77.9% (95% CI, 68.1%-87.7%) aged greater than 3 months to ≤ 6 months and 77.8% (95% CI, 73.9%-81.6%) aged greater than 6 months to ≤ 60 months (Figure 2).

All nine papers were graded as good quality and design (Appendix 1). Control groups were used and appropriate in eight studies. Intervention trials used malnourished controls without pneumococcal infection, supplemented with placebos. The control group in the Gambian RCT study were provided with a lower-dose vitamin A supplement rather than a placebo.[26] In Venezuela, matched controls of nearest-age siblings or cousins living in the same household were used.[21]

Discussion

We found nasopharyngeal colonisation to be frequent in children with malnutrition under five years in low- and middle-income countries (53.9% to 88.0% from 2 months to 5 years). Pooled prevalence rates increased greatly from 1.0-2.0% at birth to 53.9-80.0% at 2 months, reflecting the point of first acquisition of *S. pneumoniae*. From 3 months up to 5 years of age, colonisation remains high between 64.1 and 88.0%.

Nasopharyngeal colonisation rates amongst healthy, well-nourished children less than 5 years of age before the introduction of PCV have been previously reported as 64.8% in low-income and 47.8% in lower-middle income countries.[29] The prevalence rates found in these healthy children are lower than those found in our review of malnourished children. This difference may reflect clinical and methodological differences but suggests that prevalence rates of *S. pneumoniae* are higher in malnourished states. Our results are supported by a study in Venezuela that reported colonisation rates of 73% in children under 5.[30]

Immune changes associated with protein-energy malnutrition are many: atrophic changes to the thymus, leading to poorly developed peripheral lymphoid organs and decreased T cell function and number.[14] Malnutrition also reduces immunoglobulin A secretion, impairs complement activity and immunoglobulin responses to encapsulated bacteria and, indirectly, decreases phagocytic activity.[31,32] In a persuasive case-control study, children with stunting in Venezuela were more likely to present with pneumococcal colonisation and acute respiratory tract infection compared to age-matched relatives from the same household.[21] A recent study, not included in our review due to search time limits, found a strong association between stunting and *S. pneumoniae* colonisation.[30]

The role of micronutrient deficiency and supplementation in acute bacterial infections remains controversial but zinc supplementation is effective in preventing pneumonia, and is recommended by the WHO.[33] Suboptimal zinc status damages epithelial function and impairs antibody-mediated responses.[27] A case-control study included in our systematic review found a strong interaction between zinc status and *S. pneumoniae* colonisation.[27] In mice, zinc-deficiency has been associated with higher nasopharyngeal colonisation density of *S. pneumoniae* following pneumococcal challenge and reduced responses to PspA immunisation.[33]

Vitamin A deficiency promotes rapid bacterial colonisation in infants,[34] potentially permitting greater bacterial adherence, colonisation, and infection.[23] It is known to play a role in immune homeostasis as well as B- and T-cell homing to the intestinal mucosa.[35] It is plausible that it performs a similar function at the nasal mucosa. Supplementation can reduce infant mortality in areas of endemic deficiency,[36] although studies examining the specific effects on bacterial colonisation are inconsistent. New-borns in south India dosed with vitamin A had a lower rate of pneumococcal colonisation at 3 months compared to those receiving placebo, but this was not apparent in a similar trial in Bangladesh.[23,24] A third trial comparing high and low dose vitamin A supplementation found no difference in colonisation between groups.[26]

Baseline measurements confirming micronutrient malnutrition were not systematically carried out within the supplementation trials[22-25,26,27] and deficiency within the population is assumed. However, evidence of deficiency in those geographies was good: 17-37% of young children within the geographic area studied in south India had low serum retinol levels;[22,23,37] the Gambian population had significantly lower levels than expected in the UK [38]; the Bangladesh study reported a high prevalence of maternal night blindness, a clinical sign of vitamin A deficiency;[24] in the Nepal zinc trial 42% of children within a previous trial in the same area had low serum zinc concentration.[27,39]

Our review encompassed multiple forms of macro and micronutrient malnutrition which, with limited data, might make pooled rates of colonisation difficult to interpret. The studies reported here represent a relatively limited geographic sample which may not represent the worldwide malnourished population. Additionally, inclusion of studies of malnourished children only does not allow for a detailed appraisal of the association between malnutrition and pneumococcal colonisation. Two studies combined anthropometric measurements of wasting and stunting to define their study population.[12,21] These represent acute and chronic malnutrition respectively, and each may differently impact mucosal immunity and *S. pneumoniae* colonisation rates.

There is not enough uniformity of reporting of vaccination within the included studies to comment on pneumococcal vaccination within study populations. Vaccines can have a profound effect on serotype prevalence,[40,41] if not always overall prevalence, and vaccination status could be a confounding variable.

Other limitations of this study include bias arising from selective publication and methodological limitations. For example, two studies compared colonisation in malnourished children with and without pneumococcal infection. As colonisation is a prerequisite to infection, this may artificially inflate colonisation rate estimates.[12,21] Studies embedded within trials were not originally designed to deliver a cross-sectional representation of the wider population and sampling frames may not have fully accounted for seasonal variation. For example, colonisation rates are higher in the Gambia during the dry season, perhaps due to a combination of atmospheric conditions which promote colonisation and increased transmission due to social factors such as higher school attendance (and therefore intermixing) outside of harvesting seasons.[12,42]

Studies designed specifically to investigate pneumococcal colonisation in a malnourished population are essential for the identification of high-risk groups and to understand their responses to pneumococcal vaccines. There is evidence that both protein-energy malnutrition and micronutrient deficiencies can affect responses to vaccination.[43,44] The drivers of colonisation rates are complex but well-designed studies could be used to detect other risk factors or confounders for increased *S. pneumoniae* prevalence in the nasopharynx, including the effect on somatic growth.[21] Importantly, we do not know if colonisation produces antibody-mediated protection in this population as it does in healthy children.[45]

The combination of pneumonia and malnutrition has an enormous impact on child mortality in developing countries. This systematic review found high prevalence of *S. pneumoniae* colonisation in malnourished children under five years of age. Although the available data are limited, *S. pneumoniae* colonisation appears more prevalent in this population compared to healthy children without malnutrition. Current evidence is insufficient to judge if correction of nutritional deficits would lead to lower colonisation rates.

Declarations

Funding: None
Conflicts of interest: None declared
Ethical approval: Not required as data are anonymised and openly available.

Author contributions: All authors contributed to the design, analysis, and writing of the manuscript.

References

1. World Health Organization. Pneumonia: Fact sheet 2016. http://www.who.int/mediacentre/factsheets/fs331/en/ (accessed 29/11/2017).
2. Whitney CG, Farley MM, Hadler J et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *N Engl J Med* 2003; 348(18): 1737-46.
3. Gordon SB, Kanyanda S, Walsh AL et al. Poor Potential Coverage for 7-Valent Pneumococcal Conjugate Vaccine, Malawi. *Emerg Inf Dis* 2003; 9(6):747-749
4. Jallow S & Mahdi SA. Pneumococcal conjugate vaccine in HIV-infected and HIV-exposed, uninfected children. *Expert Review of Vaccines* 2017; 16(5):453-465
5. Mehr S, Wood N. Streptococcus pneumoniae--a review of carriage, infection, serotype replacement and vaccination. *Paediatric respiratory reviews* 2012; 13(4): 258-64.
6. Glennie SJ, Williams NA, Heyderman RS. Mucosal immunity in resource-limited setting: is the battle ground different? *Trends in microbiology* 2010; 18(11): 487-93.
7. Musher DM. How contagious are common respiratory tract infections? *The New England journal of medicine* 2003;348(13):1256-66. doi: 10.1056/NEJMra021771 [published Online First: 2003/03/28]
8. Althouse BM, Hammitt LL, Grant L et al. Identifying transmission routes of Streptococcus pneumoniae and sources of acquisitions in high transmission communities. *Epidemiol Infect* 2017; 145:2750-2758.
9. Bogaert D, De Groot R, Hermans PW. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. *Lancet Infect Dis* 2004; 4(3): 144-54.
10. Paton JC, Andrew PW, Boulnois GJ, Mitchell TJ. Molecular analysis of the pathogenicity of Streptococcus pneumoniae: the role of pneumococcal proteins. *Annu Rev Microbiol* 1993; 47: 89-115.
11. Mills RO, Twum-Danso K, Owusu-Agyei S, Donkor ES. Epidemiology of pneumococcal carriage in children under five years of age in Accra, Ghana. *Infect Dis (Lond)* 2015; 47(5): 326-31.
12. Adegbola RA, Falade AG, Sam BE, et al. The etiology of pneumonia in malnourished and well-nourished Gambian children. *Pediatr Infect Dis J* 1994; 13(11): 975-82.
13. Zelaya H, Laino J, Villena J, Alvarez S, Aguero G. Lactobacillus rhamnosus CRL1505 beneficially modulates the immuno-coagulative response after pneumococcal infection in immunocompromised malnourished mice. *Can J Microbiol* 2013; 59(10): 684-93.
14. Schaible UE, Kaufmann SH. Malnutrition and infection: complex mechanisms and global impacts. *PLoS Med* 2007; 4(5): e115.
15. Waterlow JC. Classification and definition of protein-energy malnutrition. *Monogr Ser World Health Organ* 1976; (62): 530-55.
16. World Health Organization. WHO child growth standards: length/height for age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age, methods and development: World Health Organization; 2006.
17. World Health Organization. Management of severe malnutrition: a manual for physicians and other senior health workers. Geneva; 1999.
18. Vandenbroucke JP, von Elm E, Altman DG, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and Elaboration. *PLOS Medicine* 2007; 4(10): e297.
19. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. *Res Synth Methods* 2010; 1(2): 97-111.

20. Satzke C, Turner P, Virolainen-Julkunen A, et al. Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. *Vaccine* 2013; 32(1): 165-79.
21. Verhagen LM, Gomez-Castellano K, Snelders E, et al. Respiratory infections in Enepa Amerindians are related to malnutrition and Streptococcus pneumoniae carriage. *The Journal of infection* 2013; 67(4): 273-81.
22. Coles CL, Kanungo R, Rahmathullah L, et al. Pneumococcal nasopharyngeal colonization in young South Indian infants. *Pediatr Infect Dis J* 2001; 20(3): 289-95.
23. Coles CL, Rahmathullah L, Kanungo R, et al. Vitamin A supplementation at birth delays pneumococcal colonization in South Indian infants. *J Nutr* 2001; 131(2): 255-61.
24. Coles CL, Labrique A, Saha SK, et al. Newborn vitamin A supplementation does not affect nasopharyngeal carriage of Streptococcus pneumoniae in Bangladeshi infants at age 3 months. *J Nutr* 2011; 141(10): 1907-11.
25. Darboe MK, Fulford AJ, Secka O, Prentice AM. The dynamics of nasopharyngeal streptococcus pneumoniae carriage among rural Gambian mother-infant pairs. *BMC Infect Dis* 2010; 10: 195.
26. Darboe MK, Thurnham DI, Morgan G, et al. Effectiveness of an early supplementation scheme of high-dose vitamin A versus standard WHO protocol in Gambian mothers and infants: a randomised controlled trial. *Lancet* 2007; 369(9579): 2088-96.
27. Coles CL, Sherchand JB, Khatry SK, et al. Zinc modifies the association between nasopharyngeal Streptococcus pneumoniae carriage and risk of acute lower respiratory infection among young children in rural Nepal. *J Nutr* 2008; 138(12): 2462-7.
28. Hamer DH, Egas J, Estrella B, MacLeod WB, Griffiths JK, Sempertegui F. Assessment of the Binax NOW Streptococcus pneumoniae urinary antigen test in children with nasopharyngeal pneumococcal carriage. *Clin Infect Dis* 2002; 34(7): 1025-8.
29. Adegbola RA, DeAntonio R, Hill PC, et al. Carriage of Streptococcus pneumoniae and other respiratory bacterial pathogens in low and lower-middle income countries: a systematic review and meta-analysis. *PLoS One* 2014; 9(8): e103293.
30. Verhagen LM,Hermsen M, Rivera-Olivero IA, et al. Nasopharyngeal carriage of repiratory pathogens in Warao Amerindians: significant relationship with stunting. *Tropical Medicine and International Health* 2017; 22(4): 407-414.
31. Caulfield LE, de Onis M, Blossner M, Black RE. Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles. *Am J Clin Nutr* 2004; 80(1): 193-8.
32. Savino W. The thymus gland is a target in malnutrition. *Eur J Clin Nutr* 2002; 56 Suppl 3: S46-9.
33. Strand TA, Hollingshead SK, Julshamn K, Briles DE, Blomberg B, Sommerfelt H. Effects of zinc deficiency and pneumococcal surface protein a immunization on zinc status and the risk of severe infection in mice. *Infect Immun* 2003; 71(4): 2009-13.
34. Chandra RK. Increased bacterial binding to respiratory epithelial cells in vitamin A deficiency. *BMJ* 1988; 297(6652): 834-5.
35. Oliveira LM, Teixeira FME, Sato MN. Impact of Retinoic Acid on Immune Cells and Inflammatory Diseases. *Mediators of inflammation* 2018; 2018: 3067126.
36. Klemm RD, Labrique AB, Christian P, et al. Newborn vitamin A supplementation reduced infant mortality in rural Bangladesh. *Pediatrics* 2008; 122(1): e242-50.
37. Rahi JS, Sripathi S, Gilbert CE, Foster A. Childhood blindness due to vitamin A deficiency in India: regional variations. *Arch Dis Child* 1995; 72(4): 330-3.

38. Bates CJ, Villard L, Prentice AM, Paul AA, Whitehead RG. Seasonal variations in plasma retinol and carotenoid levels in rural Gambian women. *Trans R Soc Trop Med Hyg* 1984; 78(6): 814-7.
39. Tielsch JM, Khatry SK, Stoltzfus RJ, et al. Effect of daily zinc supplementation on child mortality in southern Nepal: a community-based, cluster randomised, placebo-controlled trial. *Lancet* 2007; 370(9594): 1230-9.
40. Devine VT, Cleary DW, Jefferies JMC et al. The rise and fall of pneumococcal serotypes carried in the PCV era. *Vaccine* 2017; 35(9):1293-1298.
41. Usuf E, Bottomley C, Bojang E et al. Persistence of nasopharyngeal pneumococcal vaccine serotypes and increase of non-vaccine serotypes among vaccinated infants and their mothers five years after PCV13 introduction in The Gambia. Clin Infect Dis 2018; ISSN 1058-4838 DOI:https://doi.org/10.1093/cid/ciy726
42. Bojang A, Jafali J, Egere UE, et al. Seasonality of Pneumococcal Nasopharyngeal Carriage in Rural Gambia Determined within the Context of a Cluster Randomized Pneumococcal Vaccine Trial. *PLoS One* 2015; 10(7): e0129649.
43. Rho S, Kim H, Shim SH et al. Protein energy malnutrition alters mucosal IgA responses and reduces mucosal vaccine efficacy in mice. *Immunology letters* 2017; 190:247-256.
44. Penkert RR, Iverson A, Rosch JW & Hurwitz JL. Prevnar-13 vaccine failure in a mouse model for vitamin A deficiency. *Vaccine* 2017; 35(46):6264-6268.
45. Zhang Q, Bernatoniene J, Bagrade L, et al. Serum and mucosal antibody responses to pneumococcal protein antigens in children: relationships with carriage status. *Eur J Immunol* 2006; 36:46-57.
46. Riboldi BP, Quevedo A, Fuchs S et al. Respiratory Infections in Enepa Amerindians; interpret with caution. Journal of Infection 2013; 67(4)
47. Verhagen LM, Gomez-Castellano K, Snelders E et al. Post hoc power calculations and statistical analysis of case control studies: reply to Riboldi et al. Journal of Infection 2014; 68(2):194-195.

Figure 1

Flow chart showing selection of articles

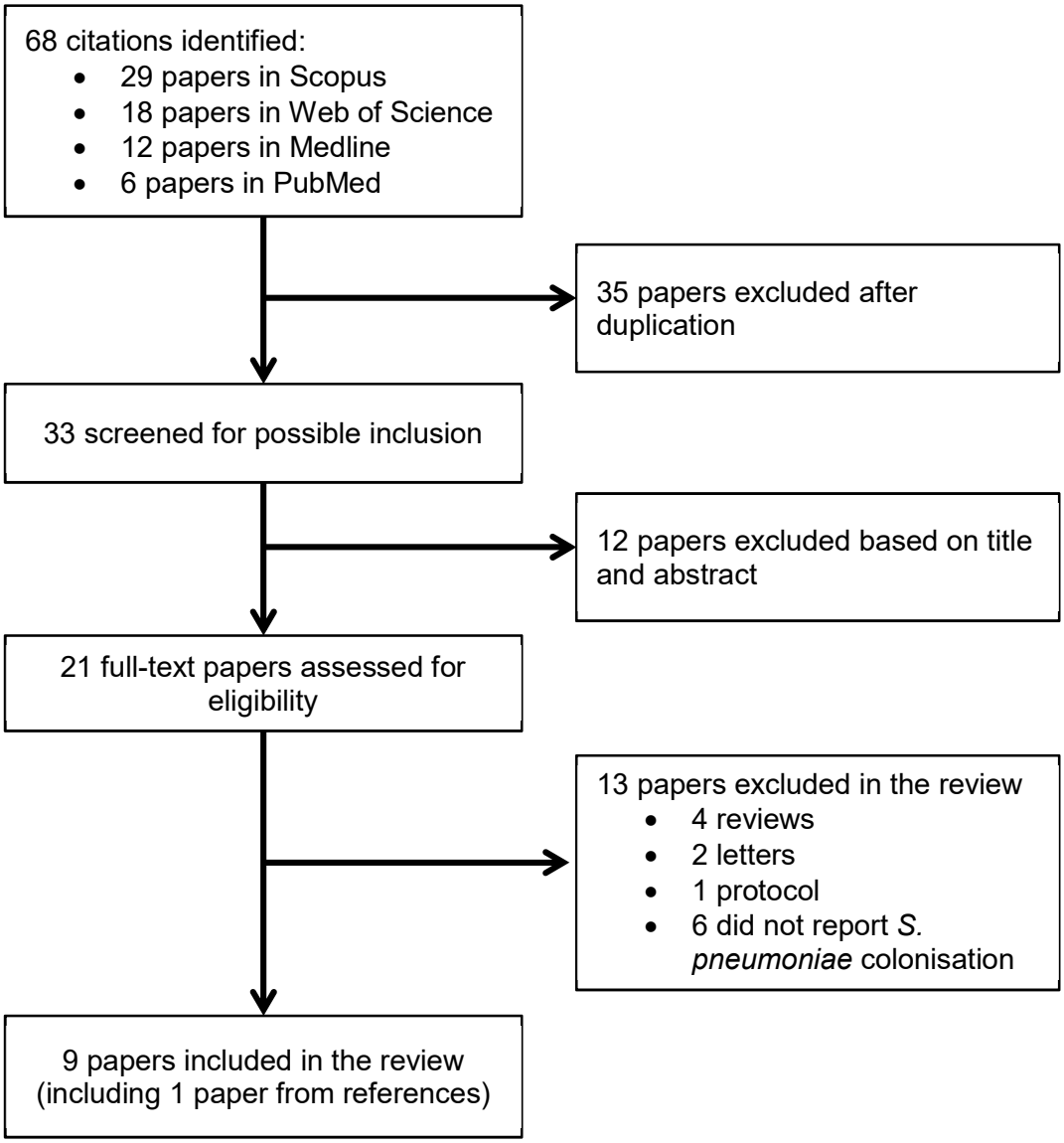

Figure 2

Meta-analysis of S. pneumoniae carriage in malnourished children under 5 years of age. Children aged: (A) 0 to ≤ 3 months, (B) <3 to ≤ 6 months, (C) <6 to ≤ 60 months. The prevalence carriage rate for each study is represented by a square. An unfilled diamond represents the pooled prevalence estimate. The 95% confidence interval (CI) for each study is denoted by a horizontal line crossing the symbol. Weight for the random effects analysis for each study is given (%).

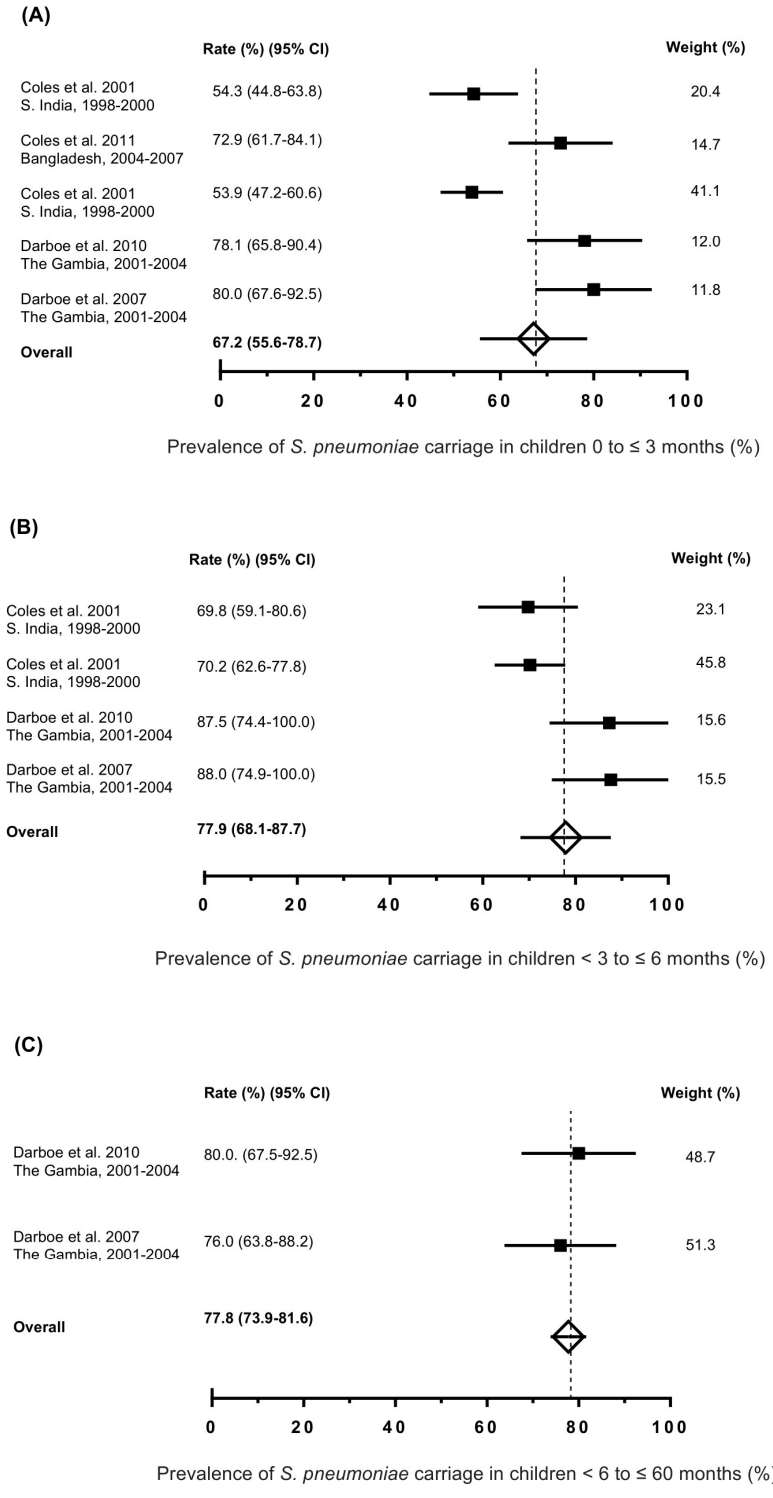

Table 1

Summary table of studies reporting colonisation rates of *Streptococcus pneumoniae* in chronically malnourished children.

Study	Study period	Country	Condition	Population	n	*Streptococcus pneumoniae* colonisation rate (%)							
						Birth	2m	3m	4m	5m	6m	12m	Overall
Coles et al. 2001[22]	Oct 1998 – Jan 1999	South India	Vit A def	New-borns given placebo	232		54.3		67.9		69.8		
Coles et al. 2011[24]	Jan 2004 – Jan 2007	Bangladesh	Vit A def	New-borns given placebo	225			72.9					
Coles et al. 2001[23]	Oct 1998 – Jun 1999	South India	Vit A def	Child living in area of endemic Vit A def, of which unknown proportion received VA dose	464		53.9		64.1		70.2		
Darboe et al. 2010[25]	Sept 2001 – Oct 2004	The Gambia	Vit A def	Child living in area of endemic Vit A def, of which unknown proportion received VA dose	197	1.5	78.1			87.5		78.6	
Darboe et al. 2007[26]	Sept 2001-Oct 2004	The Gambia	Vit A def	0-12m infant receiving standard WHO Vit A dose 0-12m infant receiving high-dose Vit A	197	2.0 1.0	76.0 80.0			88.0 88.0		81.0 76.0	
Verhagen et al. 2013[21]	Aug 2011	Venezuela	WHZ or HAZ <-2	Controls aged 0-10y of which 58% are acutely or chronically malnourished	40								70.0 (0-5y)
Adegbola et al. 1994[12]	Nov 1990-Oct 1992	The Gambia	WFA<70% NCHS mean or oedema	Malnourished children aged 3m-5y without pneumonia	100								72.0
Coles et al. 2008[27]	Dec 2003 – July 2004	Nepal	Zn def	Control groups aged 1-35m given placebo	604								78.7
Hamer et al. 2002[28]	Unknown	Ecuador	WAZ	Healthy 2-60m who were: Malnourished Mildly malnourished	210								60.0 72.0

Appendix 1

Table of STROBE assessment of papers included in systematic review

Reporting Item	Coles et al. 2001[22]	Coles et al. 2011[24]	Coles et al. 2001[23]
Title and Abstract (1)	(a) Study design in abstract (b) Informative, clear abstract	(a) Study design in abstract (b) Clear, balanced summary	(a) Study design outline in abstract (b) Clear abstract
Background/rationale (2)	*Spn* leading cause of child mortality, vaccines unavailable. Endemic VA def in areas of high *Spn* infection. VA may reduce carriage rates	Data suggests correlation between endemic VA def and *Spn* colonisation in early infancy	Region-specific data required to form effective vaccines as serotype prevalence varies temporally, geographically and by age. Little data in S. India
Objectives (3)	To evaluate the impact of VA on *Spn* NP colonisation in young children of endemic VA def	To evaluate efficacy of new-born VA supplementation in preventing NP carriage among 3mo old infants in rural Bangladesh	To study the epidemiology of NP colonisation, risk factors and distribution of serotypes in S. Indian infants to determine the effectiveness of conjugate vaccines
Study design (4)	Double-blinded, placebo case-controlled VA supplementation trial	Double-blinded, placebo case-controlled VA supplementation trial	Double-blinded, placebo case-controlled VA supplementation trial
Settings (5)	South India. Oct 1998-Jan 1999. Area of endemic VA def	Bangladesh. Jan 2004- Jan 2007. Endemic VA def	South India, Oct 1998-June 1999. Endemic VA def
Participants (6)	Carriage study from InPACT study embedded in VASIN trial. Eligibility criteria, sources, randomisation method and controls given	Carriage study from JiVitA-2 embedded in JiVitA1 trial. Criteria given. No randomisation method given but refers to JiVitA-2 paper	InPACT study embedded in VASIN trial. Have to refer VASIN trial for eligibility criteria and participant details
Variables (7)	All variables apart from effect modifiers given	Exposures, confounding variables given but not clearly stated. No mention of effect modifiers	Primary and additional outcomes not clear, exposures, confounders and effect modifiers not directly specified

Data sources/measurements (8)	Specimen and lab procedures given	Specimen and lab procedures given	Specimen and lab procedures given
Bias (9)	Minimised. Use of control (selection), re-interviewed participants at random, reviewed data collection forms on weekly basis to avoid missing data, high quality data collection method	Use of controls and randomisation. Imbalance in sample size (VA 275 vs. placebo 225) reflects survival bias (survival until 12w old)	No details of reducing bias
Study size (10)	Study size calculated for intervention data (464 required to detect 17.5% reduction in NP colonisation with 80% power). Placebo group = 232	Used data from S. India study (Coles 2000) – 500 infants to detect difference of 17%. Placebo group = 225	3000 embedded in VASIN trial to detect minimum reduction in infant mortality by 30%. No study sample calculation for InPACT.
Quantitative variables (11)	Grouped according to treatment and placebo group	Grouped according to treatment and placebo group	Age groups split
Statistical methods (12)	(a) t test for continuous, (b) two-tailed Chi-squared or Fisher's for bivariate associations, (c) database analysed for missed data (d) OR used to assess association between risk factors and colonisation, (e) OR used rather than relative risk due to sample collection at 2mo intervals	(a) Chi-squared and t test for baseline (b) Bivariate logistic regression models (c) No mention of missed data (d) OR and 95%CI used to measure associations	Associations tested by two-tailed chi square test or Fisher's exact test. (b) Regression models include receipt of VA as a covariate (c) OR used instead of relative risk as prevalence rates for each age group are underestimate due to dynamics of colonisation

Participants (13)	Number reported at each stage, non-participation given, flow diagram given	Number given at each stage, including non-participants. No flow diagram	Number at each stage given, flow chart given
Descriptive data (14)	(a) Table 1 for baseline data (b) number of participants with missing data given	Baseline characteristics given. No indication of missed data	Demographics in table 1
Outcome data (15)	Table 2 shows number in each exposure category	No table given	Carriage rates in text given in results section
Main results (16)	Unadjusted and adjusted estimates (OR) with p-values. Baseline, effect of VA on colonisation, effect of VA delaying colonisation, VA on invasive serotype colonisation, on colonisation with Ab-resistant pneumococci	Unadjusted and adjusted OR given. Multivariate models to control for effects of baseline and other covariates	Carriage rates of unknown proportion receiving VA given. Adjusted OR given for risk factors of NP carriage adjusted for effect of VA in table 3
Other analyses (17)	None	None	None
Key results (18)	Summarises results, refers to objectives	Summarises	Summarised simply
Limitations (19)	Serum retinol levels at each interval not measures so VA def is assumed but good evidence of endemic VA def from previous studies in same area. Low power to detect differences between 2 groups	Carriage only measured at 3mo	Specimens collected at 2mo intervals so may underestimate true colonisation. States mothers suffer from night blindness but does not investigate if infants are VA def

Interpretation (20)	Interprets with reference to objective, limitations, compares to previous similar studies and results	Refers to S. Indian paper and states data was not significant and VA does not decrease colonisation. S. Indian paper data for 4mo. *Spn* colonisation dynamic process so may not be valid comparison. Considers objectives, limitations and current evidence	Comparable data to other regions in developing world. Few risk factors shows to modify carriage
Generalisability (21)	Discusses how results may vary with different populations, settings and malnutrition setting	When pooled with S. Indian data, new-born VA supplementation unlikely to decrease colonisation	Cannot apply data from study population to a macroscopic level due to large socioeconomic differences
Other information – funding (22)	USAID and Institute for Sight and Life, John Hopkins School of Hygiene and Public Health	Specimen and lab procedures given	USAID and Institute for Sight and Life, John Hopkins School of Hygiene and Public Health

Reporting Item	Darboe et al. 2010[25]	Darboe et al. 2007[26]	Verhagen et al. 2013[21]
Title and Abstract (1)	(a) Study design in abstract (b) Informative, clear abstract	(a) Study design in abstract (b) Clear, balanced summary	(a) Study design outline in abstract (b) Clear abstract
Background/rationale (2)	*Spn* carriage highest in infancy, search for new vaccine is essential. Need to understand the distribution and dynamics of carriage of serotype	Nation polices of VA supplementation in young children has reduced all-cause mortality. Higher dose thought to increase body stores in babies born to VA def mothers	Lack of data on clinical presentations and aetiologies of ARTIs in indigenous people in South America
Objectives (3)	To study the longitudinal distribution and dynamics of *Spn* carriage of mother/infant pairs during a high-dose vs low-dose VA study	To compare efficacy of new-born high-dose VA supplementation to standard low-dose suggested by WHO to assess side effects, VA concentration, mucosal integrity, growth and morbidity and immunity	To investigate bacterial NP carriage, viral infections and nutritional status in Enepa Amerindian children 0-10y with and without ARTI and their mothers
Study design (4)	Cohort longitudinal carriage study embedded in double-blinded, placebo case-controlled VA supplementation trial	Double-blinded, placebo case-controlled VA supplementation trial	Matched-age sibling/cousin case-control study with and without ARTI cases and their mothers
Settings (5)	The Gambia. Sept 2001-Oct 2004	Bangladesh. Sept 2001 to Oct 2004	Venezuela August 2011
Participants (6)	All pregnant women in area and their infants unless <2200g, premature (<37w), congenital birth defects, severe peripartum difficulties	All pregnant women in area and their infants unless <2200g, premature (<37w), congenital birth defects, severe peripartum difficulties	145 children 0-10y in 5 isolated Enepa communities diagnosed with ARTI. 0-5y % malnourished given as those both acute (weight-for-height) and chronic (height-for-age) combined

Variables (7)	Outcomes outlines	Primary and secondary outcomes outlined	Primary and additional outcomes not clear, exposures, confounders and effect modifiers not directly specified
Data sources/measurements (8)	Specimen and lab procedures given	Specimen and lab procedures given, breath samples for *H. pylori*, infant urine for gut epithelial integrity, breast milk concentration	Specimen and lab procedures given
Bias (9)	Randomisation in high-dose VA trial. All pregnant women in 6 areas enrolled at 30w	Randomisation in high-dose VA trial. All pregnant women in 6 areas enrolled at 30w	No details of reducing bias
Study size (10)	*Spn* carriage expected at 80% and sample size of 220 calculated to detect 18% improvement. Recruitment target of 110 per group to allow for drop-outs	*Spn* carriage expected at 80% and sample size of 220 calculated to detect 18% improvement. Recruitment target of 110 per group to allow for drop-outs	No study size calculated – 40 controls and 40 cases. Letter[46]: case-controls require calculations, so cases represent cases. Response[47]: "Post hoc power calculations": Incorrect to calculate power calculations after study. CI are accurate reflection of the strength of associations and power
Quantitative variables (11)	Grouped according to treatment and placebo group and split in birth, 2, 5 and 12mo groups	Grouped according to treatment and placebo group and split in birth, 2, 5 and 12mo groups	Age groups split

Statistical methods (12)	VA def and *Spn* carriage compared between group by Pearson's Chi-squared test and ANOVA. Modelling used to test effects of treatment with adjustment for sex and season	VA def and *Spn* carriage compared between group by Pearson's Chi-squared test and ANOVA. Modelling used to test effects of treatment with adjustment for sex and season	Student's t test and non-parametric Wilcoxon signed rank test – univariate analysis. Multivariate logistic regression model for age, sex, nutritional status
Participants (13)	Number reported at each stage, non-participation given, flow diagram given	Number reported at each stage, non-participation given, flow diagram given	Samples taken from 79/80 children (99%). No reason given for missed sample, no flow chart
Descriptive data (14)	No demographics given in this data but summarised in corresponding trial paper in table 3	Baseline characteristics given. No indication of missed data	Characteristics in table 1. 88% cases malnourished, 58% controls malnourished. Malnourished = acute and chronic
Outcome data (15)	Carriage rates for each serotype at birth, 2, 5 and 12mo groups	Carriage rates for each serotype at birth, 2, 5 and 12mo groups	Carriage rates for cases and controls combined in table 2
Main results (16)	Unadjusted and adjusted estimates (OR) with p-values. Serotype distribution, infant age and sex, seasonality, relationship between mother and infant carriage	Unadjusted and adjusted OR given. Multivariate models to control for effects of baseline and other covariates. No difference in primary outcomes for high-dose vs low-dose	Cases of ARTI significantly more malnourished compared to controls. *Spn* carriage significantly higher in malnourished. No statistically significant relationship with carriage and age.
Other analyses (17)	None	No adverse events at dosing	*Spn* carriage associated with mothers with lower BMIs. No association between mothers and children
Key results (18)	Summarises results, refers to objectives	Summarises well	Summarised

Limitations (19)	Assumed VA def within population. Participants had been dosed with VA supplementation but results found no detectable effect on pneumococcal carriage – inclusion in analysis reliable. Infrequent swabbing, no extension to other family members	Mechanism by which mortality is reduced is unknown. Measurement of dose that gives max reduction needs many large-scale trials but risk of adverse effects. Low dose used as control rather than placebo for ethical considerations	Use of sibling/cousin controls may underestimate effect of factors relating to residence and household exposure, e.g. biomass smoke. Larger cross-sectional studies required to find association with carriage and house-hold related factors. Large CI due to small sample size – data collection difficult during rainy season and in rural areas
Interpretation (20)	Equilibrium and loss of colonies reached by 2mo. Age dependence of carriage and relative contribution of mother-infant transmission differ between vaccine and non-vaccine serotypes	No evidence to support use of higher VA dose	*Spn* carriage is high in children up to 10y and chronic malnutrition significantly associated with increased risk of *Spn* carriage – unknown if chronic malnutrition is a risk factor or whether *Spn* carriage affects growth leading to growth deficits and chronic malnutrition
Generalisability (21)	VA shown in other studies to affect carriage rates so data may not truly reflect malnourished population once dosed	Reflects general diet disease in Sub-Saharan Africa. Cannot be extrapolated for more severe VA def and study does not have the power to test possibility of a differential effect on mortality.	Not representative of general population of Enepa Amerindians or other children due to the case-control study design
Other information - funding (22)	Unknown	Summarised	Integrated Microsystems for Biosensing and FUNDIAM

Reporting Item	Adegbola et al. 1994[12]	Coles et al. 2008[27]	Hamer et al. 2002[28]
Title and Abstract (1)	(a) Study design in abstract (b) Informative, clear abstract	(a) Study design in abstract (b) Clear, balanced summary	(a) Clinical trial (b) Clear abstract
Background/rationale (2)	Pneumonia and malnutrition cause of mortality in children in the developing world. Unknown aetiology of pneumonia in malnourished children	Zinc def high in South Asia. Zinc def children prone to infections and have higher incidence of infections. Zinc supplementation reduces ALRI risk, unknown if this is due to reducing carriage	Rapid urinary pneumococcal antigen test (Binax NOW) has excellent sensitivity and specificity in adults to diagnose pneumonia but a study has found test to be positive in *Spn* carriage in children
Objectives (3)	To study the bacteriology and virology of pneumonia in malnourished children in the Gambia compared to malnourished without pneumonia and well-nourished with and without pneumonia	To study the effect of zinc supplementation on the association of *Spn* carriage and risk of ALRI	To evaluate the Binax NOW assay test in healthy children aged 2-60mo in Ecuador to determine NP carriage on test results
Study design (4)	Case-control study – 3 control groups	Matched case-control study. Population based, prospective. Embedded within NNIPS4 trial (trial evaluating zinc, iron and folic acid prophylaxis on morbidity, mortality and growth in children 1-35mo old.	Clinical trial of healthy children aged 2-60mo
Settings (5)	The Gambia. Nov 1990-Oct 1992	Rural Nepal, Dec 2003-July 2004	Poor urban neighbourhoods of Quito, Ecuador. Unknown dates

Participants (6)	Presenting cases from outpatient for cases of pneumonia. Malnourished = WAZ<70% of NCHS mean or oedema. Matched-age controls to non-pneumonia cases. Well-nourished with pneumonia from ward during study period and without pneumonia from health centres matched for age	All children <36mo in NNIPS4 trial during study period who met criteria for ALRI. Controls were matched age without ALRI in last 4w and living in the same area	Healthy children aged 2-60mo living in study area. Excluded those with fever, signs of ARTI
Variables (7)	Outcomes: bacteriology and virology for each group. Malnourished with pneumonia tested for HIV. No mentions of predictors or effect modifiers. Malnutrition given but criteria for pneumonia is vague	Outcomes: Colonisation rates in both groups. Exposures: zinc or placebo. Confounders: matched age +/- 3mo	Outcomes: positive NP carriage and positive Binax NOW test result
Data sources/measurements (8)	Lung aspirations, sputum collection. Lab procedures to examine bacteria and viruses given	NP specimen collection	Clean-catch midstream urine collected for Binax NOW test, NP swab 15 minutes within urine collection
Bias (9)	Recruitment bias	Not outlined	2 people read all test results to reduce potential variability in interpretation

Study size (10)	Not calculated, based on availability at the time. 574 children meet malnourished criteria of which 159 had evidence of pneumonia – 119 randomly enrolled. 119 well-nourished with pneumonia controls on ward at the time matched. 52 well-nourished without pneumonia enrolled	Study size calculation. Goal to enrol 440 ALRI cases and 440 controls = 880 children in total. Sample size had power to detect an OR of 1.53 to reject null hypothesis	Sample size calculation – 200 children needed to provide power level of 0.98 (α=0.05) to detect level of increase in the false-positive
Quantitative variables (11)	Summarised	Summarised	Positive carriage determined for whole group and then grouped by age and WAZ score
Statistical methods (12)	Chi-squared or Fisher's exact test. Continuous variables compared using Wilcoxon rank sum test. No mention of handling missed data or sensitivity analysis	Two-tailed McNemar's test and paired student's t test to compare baseline characteristics. Conditional logistic regression model stratified by treatment group and adjusted for covariates	With and without carriage who had false-positive antigen test results compared using Chi-squared test. False-positive test results stratified by age and WAZ score. Logistic regression model used to assess association between carriage and age to see if age or WAZ are predictors of false-positives
Participants (13)	Given but no flow chart given	550 cases, 550 controls	NP swabs from 209 children
Descriptive data (14)	No descriptive data given other than forms of malnutrition (marasmus, kwashiorkor, hypoalbuminemia etc)	Table 1	None
Outcome data (15)	Table 1	Table 1 and 2	Table 1

Main results (16)	Aetiology of pneumonia and serotypes. NP carriage of *Spn* and *H.influenza* high in all groups, isotypes present irrespective of nutritional status	OR for carriage and risk of ALRI. Zinc modifies association between carriage and ALRI. Strong interaction between zinc status and *Spn* carriage	False-positives more common in carriers than non-carriers (21.7%v4.2%). Carriage highest in youngest and decreased with increasing age. No association between mildly malnourished and malnourished and carriage rate. No significance between false-positive results and WAZ score
Other analyses (17)	None	Carriage data in ALRI cases who were symptomatic at the time for NP swab (table 2)	No association between WAZ score and intensity of *Spn* growth in culture
Key results (18)	Summarises results, refers to objectives	Summarises well	Summarised
Limitations (19)	Diagnostic technique undertaken in children with well-defined areas of pulmonary consolidation next to chest wall (more frequent in well-nourished children than malnourished children) – may give differing isolation rate of bacteria	Broad definition of ALRI – not categorised into moderate or severe, no blood cultures to determine aetiology – different pathogens may react differently to zinc. Timing of swabs may not account for changes of *Spn* carriage	Fails to identify children with recent but resolving ARTI. *Spn* antigens shed for weeks post pneumonia so resolved infection may lead to false-positives who did not have carriage. Also false-positives may occur due to test reacting with other streptococci species

Interpretation (20)	Equilibrium and loss of colonies reached by 2mo. Age dependence of carriage and relative contribution of mother-infant transmission differ between vaccine and non-vaccine serotypes	Zinc helps impede infection process rather than stop carriage	Similar results as studies in China and the Gambia
Generalisability (21)	Only studied community acquired pneumonia so cannot be applied to hospital acquired. Can only be applied to developing countries with similar spectrum of malnutrition as the Gambia	Zinc effect restricted to the risk of *Spn*-carriage related ALRI	Binax NOW antigen test should be used with caution for pneumonia diagnosis in young children, especially in developing countries where carriage rates are high
Other information – funding (22)	Unknown	Unknown	Unknown

w=week
mo=month
y=year
Spn=*S.pneumoniae*
NP=nasopharyngeal
ARTI=Acute Respiratory Tract Infection
ALRI=Acute Lower Respiratory Infection
VA=vitamin A
def=deficient/deficiency
WAZ=Weight-for-age Z score
OR=Odds Ratio
CI=Confidence Interval