Supplemental Appendixes 1-8 for:

Smith TD, Watt H, Gunn L, Car J, Boyle RJ. Recommending oral probiotics to reduce winter antibiotic prescriptions in people with asthma: a pragmatic randomized controlled trial. *Ann Fam Med*. 2016;14(5):422-430.

Supplemental Appendix 1: Study intervention development

Information on the standard information leaflet sent to the control group was gleaned from a number of sources including information leaflets aimed at members of the public, information leaflets directed at health professionals and professional papers [9-15]. This included advice on receiving influenza vaccination, covering the mouth when coughing, hand hygiene, and having an asthma inhaler check. The intervention and control group information leaflets were developed by 3 authors (TDHS, RJB, JC) with input from the trial site research team until it was felt that all the information – both the standard advice and advice about probiotics – would be understood by most people with asthma. Both information leaflets consisted of 2 pages sent on a single sheet of double-sided A4 paper.

Help Protect Yourself Against Winter Infections

We are writing to you because our records show that you or your child has asthma and are therefore at risk of serious chest infections. Please find below some advice about several measures you can take to help you to stay healthy this winter.

Flu jabs

On average 10-15% of people catch influenza (real flu) each year[1] and if you have asthma you are at a higher risk of serious complications[2]. This is why we invite everyone with asthma to have a flu jab each autumn to protect you from most strains of flu for the forthcoming winter period. It does not protect you against other coughs, colds, sore throats etc. that are not caused by the flu virus. Please find enclosed your personalised letter with more information about the vaccine, details of your allocated appointment time and how to change your appointment if needed.

Catch it, bin it, kill it

When someone who has an infection sneezes or coughs, the air around them can be filled with droplets that carry their germs. Anyone who breathes in these droplets could develop the same infection later on. It is good practice for people with infections to cover their mouth or nose when they cough or sneeze and then to wash their hands afterwards[3]. See http://www.nhs.uk/Video/Pages/catch-it-bin-it-kill-it.aspx for a demonstration of how viruses can be passed from person to person[4].

Hand hygiene

Touching somewhere where someone has left their germs and then rubbing our hands on our lips, mouth or eyes is a common method by which we pick up infections[3]. For example, we can pick up germs from a door handle, till checkout, handrail or a seat that someone else has used in the previous 24 hours. Washing hands after we may have been exposed to germs is sensible, especially before eating[3]. If we do not have access to hand washing facilities then making an effort not to touch our faces until we can, or making use of alcohol gel in the meanwhile can be effective[3].

Inhaler technique and asthma control

Evidence shows that if you have a good inhaler technique and asthma control you are likely to have fewer asthma exacerbations each year[5]. If you are not sure whether you are using your inhaler(s) correctly or think that you may need to change what you are using to treat your asthma, why not book in to see one of our respiratory nurses to check? We recommend that everyone with asthma should see one of our respiratory nurses at least once a year. Contact one of the reception team on 01270 275050 to make an appointment.

As part of research into best patient care, we are looking at the effectiveness of advice leaflets such as this and so may wish to check your medical records after this winter, to see whether you had an infection. Anyone who does this already has access to your medical records through their role at the surgery and will keep any personal details confidential. If you would rather we do not look at your records for this purpose, or wish to discuss any content of this information leaflet please let us know.

Ashfields' Research Team
Tel: 01270 275053 Text message: 07825 781066
Email: SCCCG.AshfieldsAsthmaLeaflet@nhs.net
Post: Research Nurses, Ashfields Primary Care Centre, Middlewich Road, Sandbach. CW11 1EQ.

References
1. Clark et al. Influenza: epidemiology, clinical features, therapy, and prevention. *Seminars Resp. & Crit. Care Medicine* 2011;32(4):373-392.
2. Bueving et al. Is influenza vaccination in asthma helpful? *Curr. Opin. Allergy & Clin. Immunol.* 2005;5(1):65-70.
3. Cook GW. This winter make the common cold less common. *Asthma Magazine* Nov/Dec 2004.
4. http://www.nhs.uk/Video/Pages/catch-it-bin-it-kill-it.aspx
5. Melani et al. Inhaler mishandling remains common in real life and is associated with reduced disease control. *Resp. medicine* 2011;105(6):930-938.

Version 1, 27th April 2013

NHS Ashfields Primary Care Centre

The control leaflet is shown above and the intervention leaflet below.

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Probiotics

Evidence has shown that consuming a probiotic a day can prevent just under half of all respiratory infections like colds, sinusitis, sore throats and ear infections[3]. In order to help reduce your chance of getting a chest infection this winter, we have obtained funding so that you can obtain probiotic capsules, using the tokens enclosed with this leaflet, to take throughout the colder months of the year (October to March). The probiotic capsules are called Lab4 and more details about them can be viewed at http://www.lab4probiotics.co.uk/. In order to receive delivery of enough capsules to take the recommended quantity of 1 capsule a day for free you will need to contact the company who make them, Cultech, on 01639 814309 or via their website http://www.provenprobiotics.co.uk/ with your tokens to hand.

Catch it, bin it, kill it

When someone who has an infection sneezes or coughs, the air around them can be filled with droplets that carry their germs. Anyone who breathes in these droplets could develop the same infection later on. It is good practice for people with infections to cover their mouth or nose when they cough or sneeze and then to wash their hands afterwards[4]. See http://www.nhs.uk/Video/Pages/catch-it-bin-it-kill-it.aspx for a demonstration of how viruses can be passed from person to person[5].

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2. Bueving et al. Is influenza vaccination in asthma helpful? *Curr. Opin. Allergy & Clin. Immunol.* 2005;5(1) 65-70.
3. Hao et al. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database 2011(9) 006895.*
4. Cook GW. This winter make the common cold less common. *Asthma Magazine* Nov/Dec 2004.
5. http://www.nhs.uk/Video/Pages/catch-it-bin-it-kill-it.aspx
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While there is good evidence that leaflets can be used in a productive way during a face-to-face consultation, for example in reinforcing information about progression of common infections which do not need antibiotics [31], it is less clear whether postal leaflets used without any personal contact can be similarly effective in influencing action of patients. However, the potential benefit of probiotics in reducing infection rates is thought to be in terms of prevention rather than cure [1] so it was not practical to recruit all eligible patients attending a face-to-face consultation.

As this study involved all patients with asthma within the surgery (with the exception of under 5s and those living in the same household as others involved) it was not

practical to pilot the study leaflets locally without unblinding future participants or preventing those in the pilot study from taking part. There was no evidence from the instances where participants discussed probiotics with the clinical staff, the trial team or the supplier Cultech that the information leaflets were misunderstood with the only recurring aspect that seemed to confuse participants was why the probiotic capsules would be provided for free. It is unclear whether this put any participants off applying for probiotics who would otherwise have wished to take them.

A further offer with a repeated leaflet may have been useful but in order to fit in with the pragmatic nature of the trial, it was felt that it was better only to contact participants with a leaflet at a time when they would usually have received correspondence from the surgery anyway (their annual invite letter for an influenza vaccination). Furthermore, this study aimed to see whether a low cost intervention – including an extra leaflet in post that would have been sent anyway – was effective, and subsequent methods of communication would have significantly increased the cost although probably improved uptake of the probiotic capsule.

Supplemental Appendix 2: Full details of outcome measures

Any deviations in outcome measures from the trial protocol are noted below. All outcome measures came from review of participants' medical notes to obtain history of face-to-face, telephone or third party consultations and acute antibiotic prescriptions during the six-month trial period. Only a minority of respiratory infection episodes result in GP consultation [32], although it is likely that there will be a correlation between severity and likelihood of consulting. However, diary-keeping of symptoms can be confusing as symptoms of allergic rhinitis, common in people with asthma [33], are often indistinguishable from those of upper respiratory tract infections (URTIs) [34].

Primary outcome measure

The primary endpoint was the percentage of participants who within the six month period for which probiotics were recommended, were prescribed at least one acute course of one of the following antibiotics:

- Amoxicillin
- Azithromycin
- Cefaclor
- Cefalexin
- Ciprofloxacin
- Clarithromycin
- Co-amoxiclav
- Doxycycline
- Erythromycin
- Phenoxymethylpenicillin

These were selected based on guidelines from the local health authority for treatment of respiratory infections, and are not recommended in local guidelines as first-line treatment for other common infections such as cellulitis or urinary tract infections.

Secondary outcome measures

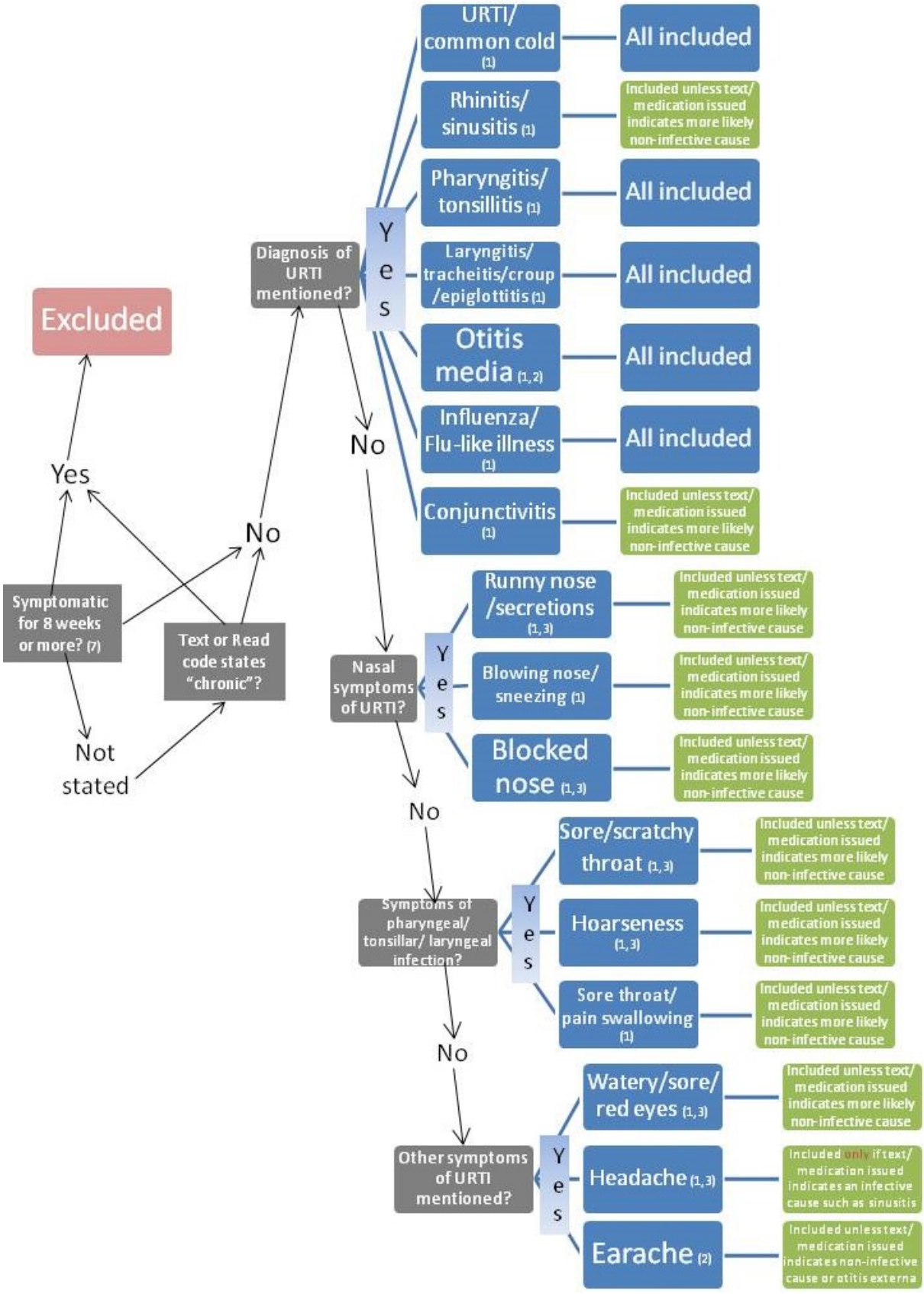
1. Mean number of antibiotic prescriptions for any of the above antibiotics per participant. *This is another way of measuring effects on antibiotic prescribing.*

The following eight secondary outcome measures looked at antibiotic prescribing more generally:

2. Total cost of all antibiotic prescriptions listed above during the six-month study period per participant. *This was based on the NHS drug tariff for England and Wales at the time the prescription was issued. This was a minor change in how antibiotics are costed compared to that planned in the study protocol, since these more accurate data were available. The protocol mentioned cost of antibiotics as a single outcome measure but did not specify whether this was to be the ones selected for use in respiratory infections or all antibiotics, which have therefore been separately reported as two secondary outcome measures (this one and outcome 5).*
3. Percentage of study group prescribed at least one course of any type of oral antibiotics.
4. Mean number of any type of oral antibiotic prescriptions per participant during the six months.
5. Total cost of all types of oral antibiotic prescriptions per participant *(determined as per 2)*.

6. Percentage of group having at least one new URTI episode for which antibiotics were prescribed. *Of note, this outcome measure and the following 3 secondary outcome measures were included in the statistical analysis plan after clinical trial registration, and are therefore post-hoc analyses. They were added because the information was available from the practice electronic records, and were considered to be relevant additional measures of antibiotic use for specific respiratory indications.*
7. Mean number of new URTI episodes for which antibiotics were issued per participant.
8. Percentage of group having at least one respiratory episode for which antibiotics were prescribed.
9. Mean number of all respiratory episodes for which antibiotics were issued per participant.

People were considered to be suffering from URTIs if they fulfilled the criteria in the flow chart shown below [1,35,36]. A new URTI episode was defined as one where there was at least one day completely free of symptoms since the previous respiratory episode, in line with two studies included in the Cochrane review [1,37,38]. Where this information was not available, it was assumed that any infection presenting four weeks or more after the earliest known date of symptoms of a previous respiratory episode was a new infection. This allows a week longer for recovery than the mean length for acute bronchitis according to the NICE guidelines “Respiratory tract infections – antibiotic prescribing” [39].



Flow chart to determine participants diagnosed with URTIs according to standard definitions [1,35,36]. To stop duplication of respiratory episodes, URTIs that were not recorded as resolving and were within 4 weeks of onset were not included if they developed into LRTI or asthma exacerbation during this time.

Any antibiotic prescribed during a consultation when someone was seen for a respiratory episode was considered to be prescribed for the respiratory illness unless an alternative reason was recorded. If there was no reason recorded on the day of issue but a respiratory infection was assumed to be ongoing according to the definition in the previous paragraph, any of the ten antibiotics listed in the primary outcome measure were assumed to be for the respiratory illness, whereas alternative antibiotics were not.

The following eight secondary outcome measures looked at the effects of probiotics on respiratory infection rates more generally:

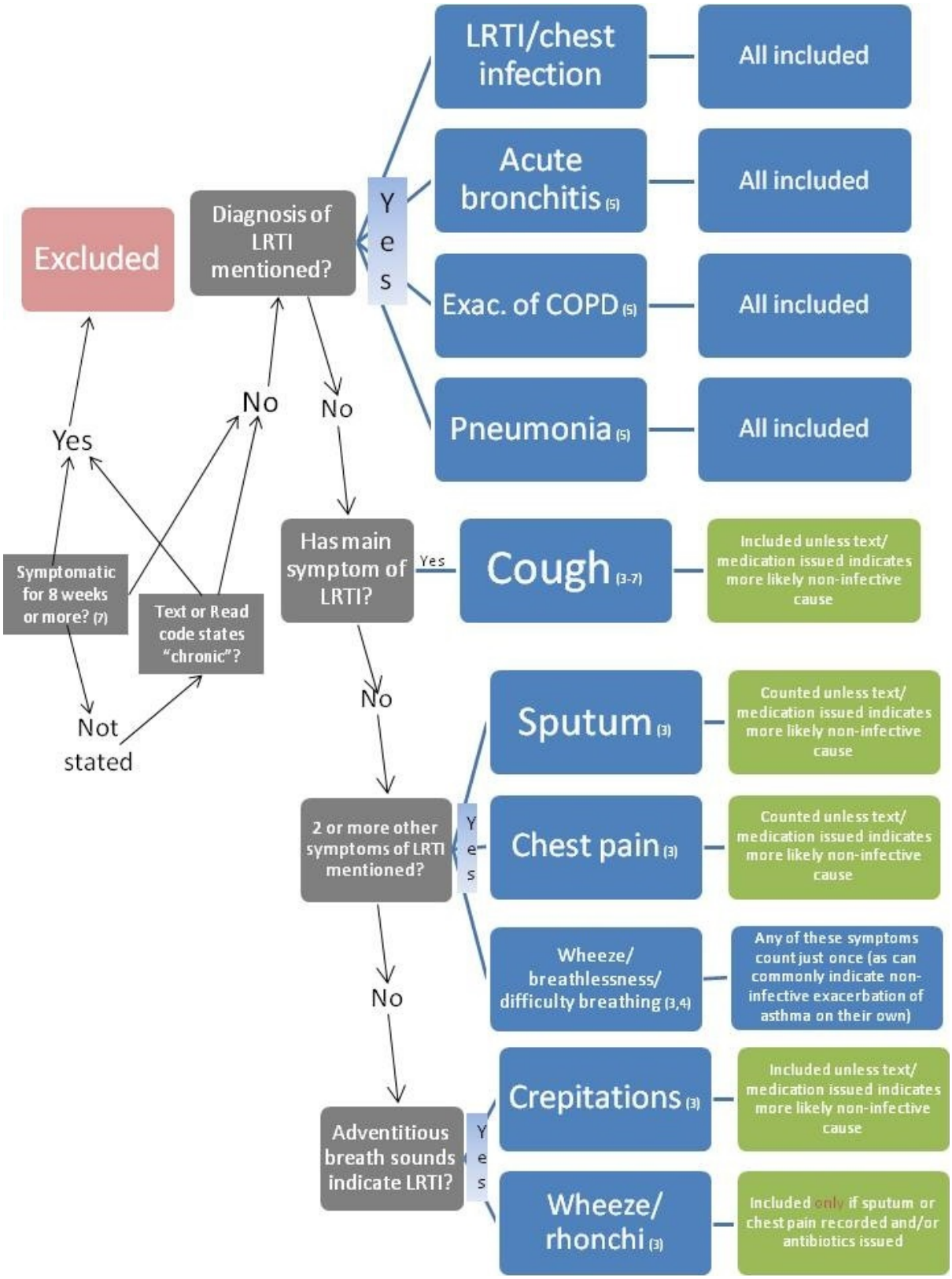
10. Percentage of participants who consulted at least once for URTI during the six-month study period.
11. Mean number of URTI episodes per participant during the six months.
12. Percentage of participants who consulted at least once for lower respiratory tract infection (LRTI) during this six-month period.
13. Mean number of LRTI episodes per participant during the six-month study period.
14. Percentage of participants consulting at least once for an acute exacerbation of asthma during the six months. *This outcome and outcome 15 were added to the statistical analysis plan after registration of the trial protocol, so are post-hoc analyses. They were included because the information was available from the practice electronic records, and LRTIs are an important complication of URTIs.*
15. Mean number of acute asthma exacerbation episodes per participant during the six months.
16. Percentage of participants consulting at least once with acute respiratory symptoms during this time. *This might be due to URTI, LRTI, or an exacerbation*

of asthma. This outcome measure and outcome 17 were added to the statistical analysis plan after registration of the trial protocol, so are post-hoc analyses. They were added because the information was available from the practice electronic records, and asthma exacerbations are an important complication of URTIs.

17. Mean number of acute respiratory episodes for each participant.

    A participant was defined as having LRTI according to the flow chart shown below [35,40-43]. A new episode of LRTI was defined as per URTI episodes above, and if someone with URTI subsequently developed LRTI before URTI had resolved, only LRTI was included to stop a continuation of the same respiratory episode being counted twice.

    An asthma exacerbation was defined according to the documented presence of reported or auscultated wheeze, or auscultated expiratory rhonchus, or according to a documented temporary need for additional asthma treatment or hospitalization. A new episode of asthma exacerbation was determined according to the European Respiratory Society definition of a preceding period of at least one week on usual treatment and out of hospital [44]. In anyone in whom URTI or LRTI progressed into an acute asthma exacerbation without becoming symptom free or within four weeks of onset (as per the definition for a new episode), only the acute asthma exacerbation was counted to stop a continuation of a single respiratory episode being counted more than once.



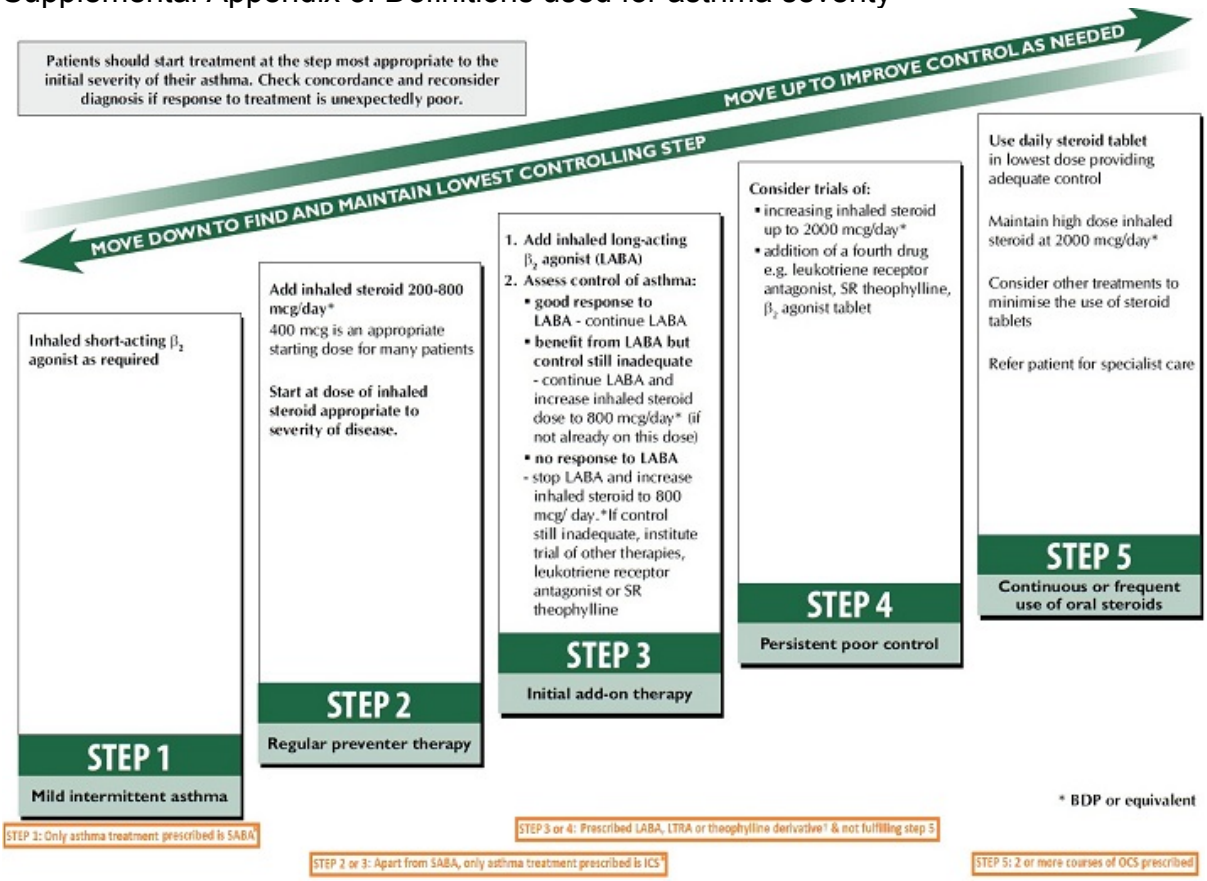
Flow chart to determine participants diagnosed with LRTIs according to standard definitions [1,40-43]. To stop duplication of respiratory episodes, LRTIs that were not recorded as resolving and were within 4 weeks of onset were not included if they developed into an asthma exacerbation during this time.

Per protocol (PP) analyses were undertaken using two different datasets:

1. Participants who used their voucher to order Lab4 probiotic capsules (Cultech) at least once during the six-month study period.
2. Participants who used their voucher to order Lab4 probiotic capsules at least twice during the six-month study period. *This was a deviation from the registered trial protocol, as data were not available for the protocol-defined group of participants who took probiotic for at least half of the trial period. However, ordering the probiotic for a second time was felt to be an appropriate surrogate measure.*

Outcome data were extracted from participants' medical records for the six-month period when probiotic consumption was recommended, 1st October 2013 to 31st March 2014, by a single investigator (TDHS) blind to treatment allocation. Once the data had been checked by another investigator (RJB) blind to treatment allocation, and the database locked and statistical analysis plan approved, the locked database was sent to the statistician (HW) for analysis.

Supplemental Appendix 3: Definitions used for asthma severity



Definitions used for asthma severity using the 5 step chart taken from the British Thoracic Society (BTS) and the Scottish Intercollegiate Guidelines Network (SIGN)'s revised 2011 guidelines: British guideline on the management of asthma [45]. The orange boxes have been added to show how the measure of asthma severity used in this trial relates to the guidelines, when looking at prescriptions issued in the 12 month period preceding the trial. *One course of oral corticosteroids for asthma is allowed during that 12 months as a rescue medication but two or more courses would take the participant into step 5 for the purposes of this trial. †No participant received any oral β_2 agonist. SABA: short-acting β_2 agonist; ICS: inhaled corticosteroids; LABA: long-acting β_2 agonist; LTRA: leukotriene receptor antagonist; OCS: oral corticosteroids.

Supplemental Appendix 4: Blinding and contamination

While participants were not blinded to treatment allocation, every attempt was made to keep clinical staff and statistical analyzers blinded as to allocation of the two different information leaflets. One of the authors of this study (TDHS) worked at Ashfields Primary Care Centre throughout the trial period at 0.75 full-time equivalent hours. In the course of his clinical work, he became unblinded on just one occasion when a participant disclosed he was taking probiotic in the course of an otherwise normal consultation. Two other GPs surveyed who were not part of the trial team reported one and two participants respectively who had asked whether or not they should take the probiotics recommended in the leaflet or otherwise disclosed that they were already taking them. No other unblinding events were reported.

TDHS also became intentionally unblinded acting as an investigator in eight other cases due to queries from clinical colleagues about other participants' suitability to take probiotics, and from Cultech due to queries about applicants for free Lab4 probiotics. The outcome assessor (TDHS) was otherwise blinded until after the data collection had been entered, cleaned and locked.

It is difficult to state the actual contamination rate in the study but we can assume it was low. Only one person per household was randomized as two people receiving different leaflets would obviously unblind participants to the other branch of the study (see inclusion criteria). In accordance with advice from the Research Ethics Committee, participants were not actively aware that there was more than one version of the information leaflet. However, participants were informed on both the control and intervention advice leaflets (see Figures S1 and S2) that the

effectiveness of the leaflets was being studied by the research team and given ways to contact the research team if they had any queries. Only five participants contacted the research team but in each case it was to discuss whether to start the probiotic or not rather than to query any of the additional information on either leaflet. No one contacted the research team or Cultech Ltd. to protest that they had heard there was another leaflet or to request that they received probiotics despite receiving the control advice leaflet. There was one case where a study participant with asthma, randomised to probiotic advice, contacted the surgery to request that she receive additional probiotics to give to her daughter who was not on the asthma register and she was advised that the probiotics were only being provided free for people with asthma.

There was a low level of unblinding of the participants' physicians as to which group they had been randomized to. There was a low loss of outcome data and contamination rates were kept low. In hindsight, 20% loss of outcome data was a conservative estimate for loss of outcome data. Our rationale for using such a conservative measure was based around the unknown. Having given participants the option of contacting the trial team to withdraw consent for staff to access their medical records to obtain the study data (in line with recommendations by the Research Ethics Committee), we could not find any figures for opt outs or indeed any previous trials taking this approach. In our trial, not one single person contacted the research team to withdraw consent, and so the only known participants where there was loss of outcome data was the 2.5% who deregistered from the surgery. This was presumably due to moving away from the area as Ashfields Primary Care Centre is

the sole supplier of primary care services to more than 95% patients living in its catchment area.

Supplemental Appendix 5: Details of SAEs and losses to follow up amongst the PP groups

Two participants who left the study (due to death or moving out of area) obtained Lab4 probiotics (Cultech). They were both in the 60 and over age group. One obtained three probiotic packages and moved out of area during the trial, deregistering with the surgery in February. The other was one of the two participants who died, a participant in the 60 and over group, who received two packages of probiotics. They were at step 5 of BTS asthma treatment (indicating severe asthma) [45] and were already known to have terminal adenocarcinoma of the lung before the trial began. The death was expected. Prior to this, they were involved in another SAE when they were admitted with a discomfort and shuffling gait in order to exclude spinal cord compression successfully.

The difference in “other adverse events” became non-significant when Hochberg’s procedure was used to correct for multiple testing [16] and cases were heterogeneous with only hospital admission for chest pain occurring more than once (see table below). In these two cases, admission was made to exclude a different diagnosis in each case, with myocardial infarction and pulmonary embolism excluded successfully, and observation of the participants’ notes for a further five months showed no recurring or persisting symptoms.

Study number	No. probiotic packages	Category of SAE	All recorded SAEs
XY0262	1	Other	Emergency Caesarean section due to failure to progress
XY0281	3	Other	Emergency admission for acute urinary retention
		Other	Subsequent elective admission for TURP
XY0283	3	Other	Planned elective surgery for CABG (cancelled by hospital)
		Other	Elective surgery for CABG (proceeded)
XY0349	2	Other	Admission to successfully exclude spinal cord compression
		Respiratory	Expected death - had adenocarcinoma lung since before trial
XY0407	3	Other	Admission excluded PE, diagnosed with musculoskeletal pain
XY0601	1	Other	Diagnosed and treated for testicular torsion
XY0728	1	Other	Elective admission for anterior vaginal repair
XY0769	1	Infection	Admitted with leg cellulitis
		Other	Was given alcohol detoxification on same admission
XY1250	3	Other	Admitted to successfully exclude MI, diagnosed atypical chest pain
XY1392	3	Other	Elective admission for total knee replacement
		Infection	Readmitted for postoperative infection

Only leg infections and chest pain occurred in more than one participant and these were thought to have different causes and so not bear any repeated relationship to Lab4 probiotic (Cultech). TURP – transurethral resection of the prostate, CABG – coronary artery bypass graft, PE – pulmonary embolism, MI – myocardial infarction.

Supplemental Appendix 6: Accounting for differences in PP groups compared to randomized control group

The participants who accessed probiotics in the intervention group – used for PP analyses – show some differences from those who did not access probiotics (not shown directly) and from the control group (shown in Table 1). They were generally older ($p<0.0001$) and had been given a first diagnosis in later life ($p<0.0001$). However, when age of first diagnosis was adjusted for age and sex it became non-significant. Those obtaining probiotics were also more likely to have had an asthma review in the last 12 months ($p=0.014$), and to have received an influenza vaccine the previous vaccination season ($p=0.0007$). These differences were also largely due to differences in age distributions as there was a smaller significance ($p=0.03$ for asthma review and $p=0.01$ for influenza vaccination) when analyses adjusted for age and sex. Additional adjusted analysis was performed for those who received influenza vaccination during the study period which is likely to correlate with a history of previous vaccination and attending for asthma reviews [46].

There was a higher proportion of participants who had been prescribed antibiotics in the previous 12 months amongst the PP groups. Unadjusted analysis just touched significance only amongst those who received 2 to 3 probiotic packages ($p=0.04$) but there was no significant difference in either group who obtained probiotics when analysis adjusted for age and sex. Analyses of outcome measures included adjustment for past antibiotic use as agreed before analysis in the statistical plan.

Other differences included history of chronic diseases with some diseases more and some less common amongst those who requested probiotics but only those with a history of cancer had a significant difference ($p=0.01$). Again, this was non-significant

using analysis adjusted for age and sex and the overall numbers are small with only around 5% of those in the study having any history of cancer. Those requesting probiotics tended to have slightly more severe asthma (according to the definition shown in Appendix 5 [45]) than those in the control group, and although this was not significant in adjusted or unadjusted analyses, outcome measures were adjusted for this in accordance with the statistical plan.

Analyses of the effects on antibiotic use and on respiratory health in the PP groups compared to the randomized control groups looked at adjusted and unadjusted analyses. Adjusted analyses as published in Tables 2 and 3, adjusted for age group, sex, asthma severity, and use of any antibiotics in the 12 months prior to the study. In measures where the P value approached significance (P<0.05), further adjustment was made for participants receiving influenza vaccination in the same season as the trial. This further adjustment made little difference to the resulting P values (see table below).

Outcome measure	Unadjusted	Adjusted for age, sex, asthma severity & 12 month previous use of antibiotics	Further adjusted for receiving flu vaccination during trial
Combined all respiratory episodes given antibiotics*	0.025	0.016	0.020
Had any LRTI without wheeze*	0.038	0.045	0.036
Number of LRTIs without wheeze*	0.024	0.021	0.027

The effect of adjustments carried out as per the pre-stipulated statistical analysis plan on for outcome measures with significant p values (<0.05) comparing the ITT intervention group to the control group. When multiple comparisons were accounted for using the method of Benjamini and Hochberg [16], there were no significant findings in either PP intervention group compared to the control group using unadjusted or adjusted data.

As those in the intervention group who elected to take Lab4 probiotics (Cultech) (the PP groups) were more likely to have obtained antibiotics for respiratory infections the previous winter, they may have been more likely to otherwise obtain them during the study period. The baseline characteristics suggest they may have more severe asthma (measured by what drugs they have been prescribed) although this may also reflect healthcare-seeking behaviour rather than disease severity. Like many UK sites, both the official asthma review and the influenza vaccination recorded on baseline characteristics are annual events at Ashfields Primary Care Centre when patients with current asthma are invited to attend, regardless of the severity of their disease. The higher attendance rate for influenza vaccination amongst those who accessed probiotics suggests a difference in healthcare-seeking behaviour.

In summary, those who accessed the probiotic intervention may have had more severe asthma, but their increased attendance for influenza vaccination suggests they may have different healthcare seeking behaviour compared with those who did not access the intervention, and this could explain their increased uptake of the probiotic intervention. We attempted to adjust all analyses for these possible differences, by including age group, sex, asthma severity and use of antibiotics in the past 12 months in the model; and by additionally adjusting for influenza vaccination during the trial period in a post-hoc analysis. This additional adjustment had no significant impact on the study outcomes.

It is not always possible to fully adjust statistically for differences in baseline measures in self-selecting groups, and the relatively low uptake of probiotic amongst those given the intervention advice leaflet meant that this study may be underpowered to pick up significant differences in the PP groups. However, the outcome data generally showed no sign of positive effects from probiotics. Of the 18 outcome measures assessed, only three point estimates showed effect estimates in a beneficial direction for the randomized intervention group – number of patients having any asthma exacerbations/wheeze during the trial, total number of asthma exacerbations/wheeze and cost per person of all antibiotics regardless of whether they were for respiratory or non-respiratory causes. For the two PP groups, only one out of the 18 outcome measures showed an effect estimate in a beneficial direction – number of any antibiotic courses for any condition including non-respiratory as well as respiratory causes; and a further two outcome measures for one but not both PP groups – number of patients taking an antibiotic for any condition and total number of antibiotic courses from the specified list for respiratory conditions.

An additional notable difference amongst those in the PP groups who followed the advice of the intervention leaflet to take probiotics, is that they had received their diagnosis at a significantly later age, although this probably reflects the generally older age group of people wishing to take the probiotic as the difference was non-significant when adjusted for age. In older participants, the earliest known age of diagnosis is likely to be less reliable as UK patient records have generally only been electronic for ten to twenty years and the data of this study came entirely from electronic records. Dates of earlier diagnoses which were made in the days of paper

notes are often not transferred successfully. Outcome measures were adjusted for age of participants which would be likely to nullify any differences between the PP groups and the randomized control group with regard to age of diagnosis. There have been different phenotypes of asthma described partly based on age of onset [47] so we cannot exclude the possibility that probiotics have differential effects in different asthma phenotypes.

Supplemental Appendix 7: Details of data-entry error and effects of differential loss to follow-up recorded in the Cochrane review relating to Cobo Sanz *et al.*

The 2011 Cochrane systematic review found participants treated with probiotics had a reduced risk of antibiotic use for acute URTIs (RR 0.67 95% CI 0.45, 0.98) and for having ≥1 URTI (RR 0.55 95% CI 0.35, 0.86) [1]. The latter is a corrected figure which we recalculated using the original data from Cobo Sanz *et al.* [27] due to a data-entry error in the Cochrane review. However, this figure does make the same assumptions about how to handle the differential loss to follow up between intervention and control groups as the Cochrane review did for ≥3 URTI episodes. Those lost to follow up – 18.3% in the probiotic group, and 4.6% in the control group – are all assumed in the ITT analysis to have had no URTI during the trial period whereas there is no reason to suppose this was the reason for their loss to follow-up. This gives an impression of fewer URTI episodes in the probiotic group in this study which is entirely created by the differential loss to follow-up. If the data entry in the Cochrane review is corrected, with imputation of missing data from Cobo Sanz *et al.* assuming that the same proportion of dropouts within a group had URTIs as those for whom there were available data, then the pooled analysis for number of people experiencing ≥1 URTI becomes non-significant (OR 0.64, 95% CI 0.36, 1.12). The meta-analysis of antibiotic prescribing for URTIs does not include data from this paper and so is unaffected.

Supplemental Appendix 8: Discussion of LRTI and asthma exacerbation outcome measures

Studies of probiotics for preventing LRTI alone are scarce and our finding of no effect on LRTI is consistent with previous literature [19,29,48-50]. Our finding that probiotics do not prevent asthma exacerbations is also consistent with the small amount of prior work in this area [6].

REFERENCES

1. Hao Q, Lu Z, Dong BR, Huang CQ, Wu T. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst Rev.* 2011;(9):006895. http://dx.doi.org/10.1002/14651858.CD006895.pub2. Published Sep 7, 2011. Accessed Sep 8, 2011.

2. Cosby JL, Francis N, Butler CC. The role of evidence in the decline of antibiotic use for common respiratory infections in primary care. *Lancet Infect Dis*. 2007;7(11):749-756.

3. Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet. http://www.sciencedirect.com/science/article/pii/S0140673610613803. Published Sep 2010. Accessed Nov 5, 2014.

4. Stockert K, Schneider B, Porenta G, Rath R, Nissel H, Eichler I. Laser acupuncture and probiotics in school age children with asthma: a randomized, placebo-controlled pilot study of therapy guided by principles of Traditional Chinese Medicine. *Pediatr Allergy Immunol*. 2007;18(2):160-166.

5. Chen YS, Jan RL, Lin YL, Chen HH, Wang JY. Randomized placebo-controlled trial of lactobacillus on asthmatic children with allergic rhinitis. *Pediatr Pulmonol* 2010;45:(11) 1111-1120. http://dx.doi.org/10.1002/ppul.21296. Published Oct 26, 2010. Accessed Oct 27, 2010.

6. Giovannini M, Agostoni C, Riva E, et al.; Felicita Study Group. A randomized prospective double blind controlled trial on effects of long-term consumption of fermented milk containing Lactobacillus casei in pre-school children with allergic asthma and/or rhinitis. *Pediatr Res*. 2007;62(2):215-220.

7. Wheeler JG, Shema SJ, Bogle ML, et al. Immune and clinical impact of Lactobacillus acidophilus on asthma. *Ann Allergy Asthma Immunol*. 1997;79(3):229-233.

8. NHS Commissioning Board, BMA, NHS Employers. Asthma (AST). In: *Quality and Outcomes Framework Guidance for GMS Contract 2013/14.* London, England: NHS Employers; 2013:88-94.

9. Clark NM, Lynch JP, 3rd. Influenza: epidemiology, clinical features, therapy, and prevention. *Semin Respir Crit Care Med*. 2011;32:(4) 373-392. http://dx.doi.org/10.1055/s-0031-1283278. Published Aug 19, 2011. Accessed Aug 22, 2011.

10. Bueving HJ, Thomas S, Wouden JC. Is influenza vaccination in asthma helpful? *Curr Opin Allergy Clin Immunol*. 2005;5(1):65-70.

11. Cook GW. This winter make the common cold less common: Advice to help you stay healthy. *Asthma Mag*. 2004;9(6):9-11.

12. Health Protection Agency. Infection control precautions to minimise transmission of Respiratory Tract Infections (RTIs) in the healthcare setting. http://www.hpa.org.uk/webc/hpawebfile/hpaweb_c/1317131892566. Published Jan 12, 2012. Accessed Jan 25, 2013.

13. Jefferson T, Del Mar CB, Dooley L, Ferroni E, Al-Ansary LA, Bawazeer GA, *et al*. Physical interventions to interrupt or reduce the spread of respiratory viruses. *Cochrane Database of Syst Rev.* 2011;(7):006207. http://dx.doi.org/10.1002/14651858.CD006207.pub4. Published Jul 6, 2011. Accessed Jul 7, 2011.

14. Yardley L, Miller S, Schlotz W, Little P. Evaluation of a Web-based intervention to promote hand hygiene: exploratory randomized controlled trial. *J Med Internet Res*. 2011;13(4):e107.

15. Melani AS, Bonavia M, Cilenti V, et al; Gruppo Educazionale Associazione Italiana Pneumologi Ospedalieri. Inhaler mishandling remains common in real life and is associated with reduced disease control [published correction appears in Respir Med. 2012;106(5):757]. *Respir Med*. 2011;105(6):930-938.

16. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. *Behav Brain Res*. 2001;125(1-2):279-284.

17. Singh M, Ranjan Das R. Probiotics for allergic respiratory diseases—putting it into perspective.. *Pediatr Allergy Immunol*. 2010;21(2 Pt 2):e368-e376.

18. Kumpu M, Kekkonen RA, Kautiainen H, Järvenpää S, Kristo A, Huovinen P, *et al*. Milk containing probiotic Lactobacillus rhamnosus GG and respiratory illness in children: a randomized, double-blind, placebo-controlled trial. *European Journal of Clinical Nutrition*. 2012;66:(9) 1020-1023. http://dx.doi.org/10.1038/ejcn.2012.62. Published Sep 2012. Accessed Sep 7, 2012.

19. Maldonado J, Cañabate F, Sempere L, Vela F, Sánchez AR, Narbona E, et al. Human milk probiotic Lactobacillus fermentum CECT5716 reduces the incidence of gastrointestinal and upper respiratory tract infections in infants. [published correction appears in J Ped Gastr Nit. 2012;54(4):571(5):757]. *JPed Gastr Nit*. 2012;54:(1) 55-61. http://dx.doi.org/10.1097/MPG.0b013e3182333f18. Published Jan 2012. Accessed Dec 28, 2011.

20. Rerksuppaphol S, Rerksuppaphol L. Randomized controlled trial of probiotics to reduce common cold in schoolchildren. *Pediatrics International* [Online] 2012;54:(5) 682-687. http://onlinelibrary.wiley.com/doi/10.1111/j.1442-200X.2012.03647.x/abstract Published Jul 10, 2012. Accessed Sep 25, 2012.

21. Das RR, Naik SS, Singh M. Probiotics as additives on therapy in allergic airway diseases: a systematic review of benefits and risks. *BioMed Research International* [Online] 2013;2013: 231979. Available from: doi: http://dx.doi.org/10.1155/2013/231979. Published 2013. Accessed Aug 19, 2013.

22. Jacquet A. Probiotic-derived factors: efficient treatment for allergic asthma?. *Clinical & Experimental Allergy* [Online] 2013;43:(3) 268-270. http://dx.doi.org/10.1111/cea.12078. Published Feb 17, 2013. Accessed Feb 18, 2013.

23. Liu S, Hu P, Du X, Zhou T, Pei X. Lactobacillus rhamnosus GG supplementation for preventing respiratory infections in children: a meta-analysis of randomized, placebo-controlled trials. *Indian Pediatr*. 2013;50(4):377-381.

24. Popova M, Molimard P, Courau S, Crociani J, Dufour C, Le Vacon F, *et al*. Beneficial effects of probiotics in upper respiratory tract infections and their mechanical

actions to antagonize pathogens. *Journal of Applied Microbiology*. 2012;113(6):1305-1318. http://dx.doi.org/10.1111/j.1365-2672.2012.05394.x Published Aug 3, 2012. Accessed Nov 19, 2012.

25. Villena J, Oliveira ML, Ferreira PC, Salva S, Alvarez S. Lactic acid bacteria in the prevention of pneumococcal respiratory infection: future opportunities and challenges. *Int Immunopharmacol*. 2011;11(11):1633-1645. http://dx.doi.org/10.1016/j.intimp.2011.06.004. Published Jun 25, 2011. Accessed Oct 28, 2011.

26. Dwan K, Gamble C, Williamson PR, Kirkham JJ, Reporting Bias G. Systematic review of the empirical evidence of study publication bias and outcome reporting bias - an updated review. *PLoS ONE*. 2013;8:(7) e66844. http://dx.doi.org/10.1371/journal.pone.0066844. Published Jul 5, 2013. Accessed Jul 17, 2013.

27. Cobo Sanz JM, Mateos JA, Muñoz Conejo A. [Effect of Lactobacillus casei on the incidence of infectious conditions in children]. *Nutr Hosp*. 2006;21(4):547-551.

28. de Vrese M, Winkler P, Rautenberg P, et al. Effect of Lactobacillus gasseri PA 16/8, Bifidobacterium longum SP 07/3, B. bifidum MF 20/5 on common cold episodes: a double blind, randomized, controlled trial. *Clin Nutr*. 2005;24(4):481-491.

29. West NP, Pyne DB, Cripps AW, et al. Lactobacillus fermentum (PCC®) supplementation and gastrointestinal and respiratory-tract illness symptoms: a randomised control trial in athletes. *Nutr J*. 2011;10(1):30.

30. Hatakka K, Blomgren K, Pohjavuori S, et al. Treatment of acute otitis media with probiotics in otitis-prone children-a double-blind, placebo-controlled randomised study. *Clin Nutr*. 2007;26(3):314-321.

31. de Bont EGPM, Alink M, Falkenberg FCJ, Dinant GJ, Cals JWL. Patient information leaflets to reduce antibiotic use and reconsultation rates in general practice: A systematic review. *BMJ Open* [Online]. 2015;5: e007612. http://bmjopen.bmj.com/content/5/6/e007612.short?g=w_open_current_tab [Accessed 20160119].

32. McNulty CAM, Nichols T, French DP, Joshi P, Butler CC. Expectations for consultations and antibiotics for respiratory tract infection in primary care: the RTI clinical iceberg. *Br J Gen Pract*. 2013;63(612):e429-e436.

33. Bousquet J, Khaltaev N, Cruz AA, et al.; World Health Organization; GA(2)LEN; AllerGen. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*. 2008;63(Suppl 86):8-160.

34. Hellgren J, Cervin A, Nordling S, Bergman A, Cardell LO. Allergic rhinitis and the common cold—high cost to society. *Allergy*. 2010;65(6):776-783.

35. Knutson D, Braun C. Diagnosis and management of acute bronchitis. *Am Fam Physician*. 2002;65(10):2039-2044.

36. Venekamp RP, Sanders S, Glasziou PP, Del Mar CB, Rovers MM. Antibiotics for acute otitis media in children. *Cochrane Database of Systematic Reviews* [Online] 2013;1: 000219. Available from: doi: http://dx.doi.org/10.1002/14651858.CD000219.pub3 [Accessed 20130226; 3/19/2014 1:44:29 PM].

37. Berggren A, Lazou Ahrén I, Larsson N, Önning G. Randomised, double-blind and placebo-controlled study using new probiotic lactobacilli for strengthening the body immune defence against viral infections. *European journal of nutrition* [Online] 2011;50:(3) 203-210. Available from: doi: http://dx.doi.org/10.1007/s00394-010-0127-6 [Accessed 22/3/2014].

38. Leyer GJ, Li S, Mubasher ME, Reifer C, Ouwehand AC. Probiotic effects on cold and influenza-like symptom incidence and duration in children. *Pediatrics*. 2009;124(2):e172-e179.

39. NICE. *Respiratory tract infections – antibiotic prescribing. Prescribing of antibiotics for self-limiting respiratory tract infections in adults and children in primary care. CG69.* London: National Institute for Health and Clinical Excellence; 2008.

40. van Elden LJ, Sachs AP, van Loon AM, et al. Enhanced severity of virus associated lower respiratory tract disease in asthma patients may not be associated with delayed viral clearance and increased viral load in the upper respiratory tract. *J Clin Virol*. 2008;41(2):116-121.

41. Panpanich R, Lerttrakarnnon P, Laopaiboon M. Azithromycin for acute lower respiratory tract infections. [Online]. *Cochrane Database Syst Rev*. 2004;(4):CD001954.

42. Becker LA, Hom J, Villasis-Keever M, van der Wouden JC. Beta2-agonists for acute bronchitis. *Cochrane Database of Systematic Reviews* [Online] 2011(7) 001726. Available from: doi: http://dx.doi.org/10.1002/14651858.CD001726.pub4 [Accessed 20110707; 3/17/2014 7:21:46 AM].

43. El-Gohary M, Hay AD, Coventry P, Moore M, Stuart B, Little P. Corticosteroids for acute and subacute cough following respiratory tract infection: a systematic review. *Family practice* [Online] 2013;30:(5) 492-500. Available from: doi: http://dx.doi.org/10.1093/fampra/cmt034 [Accessed 20130925; 3/17/2014 6:03:12 AM].

44. Reddel HK, Taylor DR, Bateman ED, et al.; American Thoracic Society/European Respiratory Society Task Force on Asthma Control and Exacerbations. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med*. 2009;180(1):59-99.

45. Scottish Intercollegiate Guidelines Network, The British Thoracic Society. *British Guideline on the Management of Asthma. A national clinical guideline.* London/Edinburgh, United Kingdom; 2008, revised January 2012.

46. Lu PJ, Euler GL, Callahan DB. Influenza vaccination among adults with asthma findings from the 2007 BRFSS survey. *Am J Prev Med*. 2009;37(2):109-115.

47. Haldar P, Pavord ID, Shaw DE, et al. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med*. 2008;178(3):218-224.

48. Hojsak I, Abdović S, Szajewska H, Milosević M, Krznarić Z, Kolaček S. Lactobacillus GG in the prevention of nosocomial gastrointestinal and respiratory tract infections. *Pediatrics*. 2010;125(5):e1171-e1177.

49. Cohen R, Martin E, de La Rocque F, Thollot F, Pecquet S, Werner A, *et al*. Probiotics and prebiotics in preventing episodes of acute otitis media in high-risk children: a randomized, double-blind, placebo-controlled study. *Pediatric Infectious Disease Journal* [Online] 2013;32:(8) 810-814. Available from: doi: http://dx.doi.org/10.1097/INF.0b013e31828df4f3 [Accessed 20130716; 11/5/2013 11:27:38 AM].

50. Merenstein D, Murphy M, Fokar A, et al. Use of a fermented dairy probiotic drink containing Lactobacillus casei (DN-114 001) to decrease the rate of illness in kids: the DRINK study. A patient-oriented, double-blind, cluster-randomized, placebo-controlled, clinical trial. *Eur J Clin Nutr*. 2010;64(7):669-677.