Recommending oral probiotics to reduce winter antibiotic prescriptions in people with asthma: a pragmatic randomized controlled trial

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ABSTRACT

Purpose: Evidence from studies mainly in children has shown that orally administered probiotics may prevent respiratory tract infections and associated antibiotic use [1]. We evaluated whether advice to take daily probiotics can reduce antibiotic prescribing for winter respiratory infections in people with asthma.

Methods: This was a randomized controlled, parallel-group pragmatic study for participants aged ≥5 years with asthma in a UK primary care setting. The intervention was a postal leaflet with advice to take daily probiotics (Lab4, Cultech, Swansea) from October 2013 to March 2014, compared to a standard winter advice leaflet. Primary outcome was the proportion of participants prescribed antibiotics for respiratory infections.

Results: 1302 participants were randomly assigned to control (n=650) or intervention (n=652). There was no significant difference in the primary outcome measure with 177/638 (27.7%) receiving antibiotics in the intervention group compared to 170/632 (26.9%) controls (OR 1.04, 95% CI 0.82, 1.34). Uptake of probiotics was low, but outcomes were similar in those who accessed probiotics (aOR 1.08, 95% CI 0.69, 1.69, compared to controls). We also found no evidence of an effect on respiratory infections or asthma exacerbations.

Conclusions: In this pragmatic community-based trial in people with asthma, we found no evidence that advising use of winter probiotics reduces antibiotic prescribing.

Trial Registration: ISRCTN 61742917
KEY WORDS

Infectious disease: respiratory infections
Respiratory system: asthma
Health promotion/disease prevention
Probiotics
Antibiotic prescribing

ABBREVIATIONS

ITT – intention to treat
LRTI – lower respiratory tract infection
PP – per protocol
RCT – randomized controlled trial
SAE – serious adverse event
URTI – upper respiratory tract infection
Acute respiratory infection is the commonest reason for attending primary care appointments and accounts for 80% of antibiotic prescriptions [2]. A Cochrane review in 2011 found that, in randomized controlled trials (RCTs) of specific patient populations, probiotic prophylaxis significantly reduces both upper respiratory tract infections (URTIs), and antibiotic prescribing rates for these infections [1]. We wanted to determine whether giving advice to take regular probiotics is an effective strategy for reducing antibiotic prescribing rates for respiratory infections in people with asthma, most of whom are older than the predominantly young child populations analyzed in the Cochrane review. People with asthma are especially vulnerable to viral URTIs, which are the commonest trigger of acute asthma exacerbations [3] and contribute substantially to the burden of unnecessary antibiotic prescriptions. Only one previous pilot study assessed effects of probiotics (combined with acupuncture) on respiratory infection rates in people with asthma. In this trial, reduced infection rates were found with the intervention, but the study was underpowered (n=17) and the findings were not statistically significant (P=0.18) [4]. Probiotics alone for preventing antibiotic use in asthmatics have not, to our knowledge, been evaluated in a prospective controlled trial [4-7].

We undertook a pragmatic trial to assess whether advice to take probiotic treatment, implemented as part of routine winter infection advice, could reduce antibiotic prescription rates and respiratory infections in older children and adults with asthma in a primary care setting.
METHODS

Study Design

This was a parallel-group prospective RCT of a pragmatic community-based intervention – advice leaflets including recommendations to take a probiotic supplement daily through the winter months – for reducing antibiotic prescription in participants with asthma. Trial registry number ISRCTN 61742917.

Participants

All participants were registered patients at Ashfields Primary Care Centre, a semi-urban practice in the UK caring for 23,000 patients with a nationally representative socioeconomic and racial mix. Inclusion criteria were ≥5 years (due to unreliability of asthma diagnosis in preschool children); a current diagnosis of asthma [8]; random selection of one person per household only. All patients in the practice population registered with a current diagnosis of asthma and fulfilling the inclusion criteria were enrolled in the study.

Ethics and Consent

The study was approved by London-Bloomsbury Research Ethics Committee (reference 13/LO/0783). Informed consent was not obtained from study participants although they were given the option to request that their data were not included in study analyses.

Randomization, treatment allocation and blinding

Where there was more than one eligible participant per household, the participant was randomly selected. For included participants, the randomization sequence was
computer-generated with 1:1 intervention/control ratio in random block sizes of 4, 6 and 8, stratified by age (5 to 18, 19 to 34, 35 to 59, and ≥60 years). Clinical staff and the outcome assessor were blind to treatment allocation until all study data had been entered, cleaned, and locked in the study database to be sent to the study statistician.

**Study intervention**

In the UK, all patients with asthma are invited to receive annual influenza vaccination. In this study participants were sent information leaflets (see Appendix 1) over a two week period in late September 2013 together with their routine invitation for annual influenza vaccination. Participants randomized to the control group received a leaflet with standard advice about measures that have been reported to help reduce infections or asthma exacerbations [9-15]. Participants randomized to the intervention group received an information leaflet with an additional section recommending taking one Lab4 probiotic capsule (Cultech) daily from October to the end of March. In addition, the intervention group received three tokens with which they could request supplies 2 months at a time from the manufacturer via telephone or internet, which are the methods by which Lab4 is currently sold. Lab4 is a patented blend of four probiotic bacteria comprising two strains of *Lactobacillus acidophilus* CUL60 (NCIMB 30157) and CUL21 (NCIMB 30156), *Bifidobacterium bifidum* CUL20 (NCIMB 30153) and *Bifidobacterium animalis* (var. lactis) CUL34 (NCIMB 30172) at a total $2.5 \times 10^{10}$ colony forming units per capsule.

**Outcome measures**
All outcome measures were pre-specified in the statistical analysis plan, which was finalised prior to database lock. The primary outcome measure was the proportion of participants who, within the six-month period for which probiotics were recommended, were prescribed at least one new course of one of the following antibiotics locally used for respiratory infections: amoxicillin, azithromycin, cefaclor, cefalexin, ciprofloxacin, clarithromycin, co-amoxiclav, doxycycline, erythromycin, phenoxymethylpenicillin. Secondary outcomes were based on consultations for URTIs, lower respiratory tract infections (LRTIs), asthma exacerbation or any respiratory infection, and the number and cost of antibiotic courses prescribed during the six-month intervention period. Full outcome measures along with methods used to define different forms of respiratory infection, asthma exacerbations and new episodes of illness are described in Appendix 2.

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 blind to treatment allocation.

**Statistical analysis**

In the study primary care practice, 28.4% of all patients with a current asthma diagnosis had received ≥1 of the specified antibiotics during the winter prior to this study (October 2012 to March 2013). The Cochrane systematic review found probiotics are associated with reduced antibiotic prescribing for acute URTI compared with placebo (RR 0.67, 95% CI 0.45, 0.98) [1]. For 80% power to detect a smaller effect size (RR 0.77) in this pragmatic study, with anticipated 20% loss of
outcome data and a 5% contamination rate in the control group, we planned to randomize 1258 participants.

Intention to treat (ITT) analysis included all participants with data available for the relevant outcome, in the groups to which they were originally assigned, whether or not they took the intervention. Per protocol (PP) analyses were used to compare two subgroups – participants who ordered probiotics ≥1 or ≥2 times – with the control group. Adjusted analyses used logistic regression for binary outcomes, linear regression for continuous outcome variables and t-tests for cost data with bootstrapping to provide robust confidence intervals on these estimates. Variables adjusted for were age group, sex, asthma severity (determined as per Appendix 3) and numbers of courses of any antibiotics prescribed in the 12 months before entry into the trial. Sensitivity analyses further adjusted for receiving influenza vaccine during the trial. Multiple comparisons were assessed using Hochberg’s procedure to control for false discovery [16].
RESULTS

Recruitment and flow of participants is shown in Figure 1. Losses to follow-up were low – 14 (2.1%) participants in the intervention group; 18 (2.8%) in the control group. Primary analyses included the remaining 1270 participants (638 intervention; 632 control), with the 2 fatalities also included in adverse events analysis. The recommended probiotic was accessed by 121 (19.0%) participants in the intervention group at least once (≥2 months’ supply), and 86 (13.5%) at least twice (≥4 months). Blinding and contamination is discussed in Appendix 4.

Outcome of randomization

Table 1 shows the baseline characteristics of both randomization groups with few significant differences between randomized groups. The participants who accessed probiotics in the intervention group – used for PP analyses – show some differences from the control group. They were generally older and diagnosed with asthma later in life, and more likely to have had an asthma review and influenza vaccine in the last 12 months. There was no significant difference between groups in the proportion prescribed antibiotics in the last 12 months, or in other chronic disease rates, when adjusted for age and sex.

Effect of the study intervention on antibiotic use

Table 2 shows the study outcomes relating to antibiotic use, for the ITT and PP analyses. We found no evidence for difference between treatment groups in our primary outcome measure, prescription of a specified antibiotic during the study period, in ITT (OR 1.04, 95% CI 0.82, 1.34); or PP analyses – ≥1 probiotic token used (aOR 1.08, 95% CI 0.69, 1.69), ≥2 probiotic tokens (aOR 1.04, 95% CI 0.62,
We also found no evidence for a difference in secondary outcomes: use of any antibiotic, any antibiotic given for URTI/any respiratory illness, number of antibiotic courses or total cost of antibiotics – in unadjusted or adjusted analyses. We found weak evidence for a higher mean number of respiratory episodes for which antibiotics were given in the intervention (ITT) group compared to the control group (i.e. a detrimental effect), but when adjusted for multiple testing this was not found to be significant [16]. For the 10 separate antibiotic outcomes evaluated the threshold is \( P=0.005 \), using the false discovery method of Hochberg.

**Effect of the study intervention on respiratory health**

Table 3 shows outcomes related to respiratory health, for the ITT and PP analyses. We found no evidence for difference between treatment groups in respiratory outcomes: any URTI, any asthma exacerbation, any respiratory infection or total episodes of each of these categories in ITT or PP analyses, using unadjusted or adjusted analyses. We found weak evidence for detrimental effects in the intervention group in the number of people with an LRTI and mean number of LRTIs per person, but these were not significant when adjusted for multiple testing (threshold for respiratory outcomes \( P=0.0125 \)) [16].

**Adverse effects of the intervention**

There was no significant difference in serious adverse events (SAEs) between treatment groups (Table 4) when corrected for multiple testing [16]. This is further discussed in Appendix 5.
DISCUSSION

In this pragmatic RCT of a recommendation for adults and older children with asthma to take daily probiotics over a single winter, we found no evidence that the intervention of advice leaflets recommending probiotic supplementation, with free access to such supplements, can reduce antibiotic prescriptions or promote improved respiratory health. Although only around 20% of these participants followed the intervention leaflet advice and took probiotics (PP groups), we found no evidence that probiotics influenced study outcomes in the PP groups. It is possible that those who accessed the probiotic would have had more respiratory infections and resulting antibiotics without the probiotic but adjustment for likely confounding factors failed to show any evidence to support this (see also Appendix 6). These results differ from previous findings in controlled trials mainly involving younger children that probiotics reduce respiratory infections and resulting antibiotic prescription rates, and cast doubt on the reproducibility of those findings in older children and adults with asthma using information leaflets. Our data suggest that real world use of probiotics to prevent winter infections and reduce antibiotic use, cannot yet be recommended despite positive findings in a Cochrane review – at least not in older children and adults with asthma. Winter infections are not necessarily all captured, since we only analyse those reported to the doctor. However, there is little reason to believe that there would be differential reporting of infections between treatment arms. Antibiotic prescriptions are generally captured, since they would appear in GP records, so this more important outcome is collected robustly.

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
≥1 URTI (OR 0.55 95% CI 0.35, 0.86) [1]. Our data challenge this finding – our 95% CI do not overlap with the point estimate of the OR/RRs of the Cochrane review. Differences between our study and those in the Cochrane review include the age group studied, our focus on people with asthma, the probiotic strain(s) used and our pragmatic trial design – the use of information leaflets and the offer of free probiotics. The developing immune system at younger ages may be more sensitive to immunological changes in the gastrointestinal tract triggered by taking probiotics [17] and 95% of subjects included in the Cochrane review were under 8 years old compared to only 3% in our study. Three recent RCTs published subsequent to the Cochrane review, found positive effects of probiotics in pediatric populations when used to prevent respiratory infections [18-20]. The studies in the Cochrane review [1] also included very few people with asthma and it is possible that the immunopathology of asthma leads to differential response to probiotics [21,22]. One small pilot study suggested probiotics may have positive effects in asthma, although the findings were inconclusive due to low statistical power [4]. All three studies included in the meta-analysis on antibiotic prescribing in the Cochrane review, and all four studies in another meta-analysis showing similar evidence for a benefit, evaluated Lactobacillus rhamnosus GG, in some instances in conjunction with a second probiotic, Bifidobacterium lactis Bb-12 [23]. Immune effects of probiotics may be species- or strain- rather than genus-specific [24,25]. It is also possible that publication bias, which is difficult to assess in meta-analyses of small numbers of studies, or time-lag bias may have contributed to the Cochrane review’s findings [26]. We identified a data-entry error in the 2011 Cochrane review,
and an issue of differential loss to follow-up – an assumption that the 18.3% in the probiotic group and 4.6% in the control group who were lost to follow-up would all have had no URTIs – both relating to the included studies of Cobo Sanz et al. (See Appendix 7) [1,27]. Together with our negative trial findings, these issues support a need to update the Cochrane systematic review of probiotics for URTI. –

One final explanation for the difference between our findings and those of previous studies may be the use of intact probiotic capsules or tablets rather than a liquid formulation. Only one of two RCTs in the Cochrane review using a non-liquid probiotic formulation for prevention of respiratory infections showed a benefit in reducing URTIs [28,29]. Since then, one other RCT showed reduced symptoms of URTIs using probiotic capsules [20], although it is unclear whether the young participants dissolved the capsules’ powder contents in liquid as other pediatric studies have done [30]. If probiotics prevent URTI through local effects on the upper respiratory mucosa, then direct contact between probiotic and mucosa may be important for efficacy.

There is a need for new ways to prevent URTI and reduce antibiotic prescribing in asthmatics and non-asthmatics that are cost-effective, safe and acceptable to patients. In a population of people with asthma, we found that probiotics were not effective in preventing antibiotic prescription, in contrast to the findings of the Cochrane review. We also found no effect on URTI, LRTI or asthma exacerbation rates. Our data suggest that probiotics may not be effective for prophylaxis against URTIs, and cast doubt on the reproducibility of earlier positive trials. Therefore, there is not currently enough evidence to recommend their use for preventing infections
and antibiotic use in at risk populations such as asthmatics. Further work is needed to evaluate whether specific probiotic formulations modulate systemic immune responses or mucosal defences, before developing new interventions to reduce the burden of respiratory infection.
<table>
<thead>
<tr>
<th></th>
<th>Randomized control group (n=650)</th>
<th>Randomized intervention group (n=652)</th>
<th>Obtaining any probiotic package (n=123)</th>
<th>Obtaining 2-3 probiotic packages (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-18 years</td>
<td>80 (12)</td>
<td>80 (12)</td>
<td>10 (8)</td>
<td>7 (8)</td>
</tr>
<tr>
<td>19-34 years</td>
<td>95 (15)</td>
<td>101 (15)</td>
<td>7 (6)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>35-59 years</td>
<td>254 (39)</td>
<td>251 (38)</td>
<td>34 (28)</td>
<td>21 (24)</td>
</tr>
<tr>
<td>60 years and over</td>
<td>221 (34)</td>
<td>220 (34)</td>
<td>72 (59)</td>
<td>58 (66)</td>
</tr>
<tr>
<td><strong>Age:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-18 years</td>
<td>48 (32, 66)</td>
<td>49 (32, 65)</td>
<td>64*** (46, 70)</td>
<td>65*** (54, 74)</td>
</tr>
<tr>
<td>19-34 years</td>
<td>95 (43)</td>
<td>101 (49)</td>
<td>52 (42)</td>
<td>42 (48)</td>
</tr>
<tr>
<td>35-59 years</td>
<td>26.8 (23.5, 30.7)</td>
<td>27.2 (24.2, 30.7)</td>
<td>28.2 (25.0, 31.2)</td>
<td>27.4 (24.9, 30.9)</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>277 (89)</td>
<td>316* (91)</td>
<td>109 (89)</td>
<td>82 (93)</td>
</tr>
<tr>
<td><strong>Body mass index:</strong></td>
<td>48 (32, 66)</td>
<td>49 (32, 65)</td>
<td>64*** (46, 70)</td>
<td>65*** (54, 74)</td>
</tr>
<tr>
<td><strong>Ethnic Group:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>577 (89)</td>
<td>595 (91)</td>
<td>109 (89)</td>
<td>82 (93)</td>
</tr>
<tr>
<td>Black</td>
<td>2 (0.3)</td>
<td>1 (0.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Asian</td>
<td>4 (0.6)</td>
<td>1 (0.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mixed</td>
<td>45 (7)</td>
<td>39 (6)</td>
<td>12 (10)</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Not Specified</td>
<td>22 (3)</td>
<td>16 (3)</td>
<td>2 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Smoking status:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>385 (60)</td>
<td>368 (57)</td>
<td>76 (62)</td>
<td>54 (61)</td>
</tr>
<tr>
<td>Ex smoker</td>
<td>188 (29)</td>
<td>200 (31)</td>
<td>37 (30)</td>
<td>29 (33)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>66 (10)</td>
<td>67 (10)</td>
<td>7 (6)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Not specified</td>
<td>11 (2)</td>
<td>17 (3)</td>
<td>3 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td><strong>Asthma Severity:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 1</td>
<td>103 (16)</td>
<td>116 (18)</td>
<td>15 (12)</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Step 2 or 3</td>
<td>283 (44)</td>
<td>272 (42)</td>
<td>51 (42)</td>
<td>41 (47)</td>
</tr>
<tr>
<td>Step 3 or 4</td>
<td>202 (31)</td>
<td>207 (32)</td>
<td>40 (33)</td>
<td>27 (31)</td>
</tr>
<tr>
<td>Step 5</td>
<td>62 (9)</td>
<td>57 (9)</td>
<td>16 (13)</td>
<td>13 (15)</td>
</tr>
<tr>
<td><strong>Other disease registers:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>56 (9)</td>
<td>37* (6)</td>
<td>5 (4)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>36 (6)</td>
<td>32 (5)</td>
<td>10 (8)</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Stroke or TIA</td>
<td>11 (2)</td>
<td>17 (3)</td>
<td>2 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>COPD</td>
<td>43 (7)</td>
<td>46 (7)</td>
<td>9 (7)</td>
<td>8 (9)</td>
</tr>
<tr>
<td>Cancer</td>
<td>31 (5)</td>
<td>37 (6)</td>
<td>13* (11)</td>
<td>9* (10)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>5 (0.8)</td>
<td>4 (0.6)</td>
<td>1 (0.8)</td>
<td>1 (1)</td>
</tr>
<tr>
<td><strong>In last 12 months:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any antibiotics</td>
<td>330 (51)</td>
<td>327 (50)</td>
<td>71 (58)</td>
<td>55* (63)</td>
</tr>
<tr>
<td>Oral corticosteroids</td>
<td>132 (20)</td>
<td>123 (19)</td>
<td>26 (21)</td>
<td>21 (24)</td>
</tr>
<tr>
<td>Asthma review</td>
<td>377 (58)</td>
<td>373 (57)</td>
<td>86* (70)</td>
<td>64** (73)</td>
</tr>
<tr>
<td>Influenza vaccine</td>
<td>416 (64)</td>
<td>409 (63)</td>
<td>98*** (80)</td>
<td>72*** (82)</td>
</tr>
</tbody>
</table>

Comparisons are made between randomized groups and for those within the intervention group who obtained probiotic packages at least once, and two to three times. Data are number (percent) or median (25th, 75th centile). TIA: transient ischemic attack; COPD: chronic obstructive pulmonary disease. *p<0.05, **p<0.01 and ***p<0.001 comparing intervention group or PP groups obtaining probiotics to the control group (unadjusted analysis). No other such comparisons were significant.
### TABLE 2 Effects of advice to take probiotics on antibiotic prescribing outcomes

<table>
<thead>
<tr>
<th></th>
<th>Randomized control group (n=632)</th>
<th>Randomized intervention group† (n=638)</th>
<th>Obtaining any probiotic package‡ (n=121)</th>
<th>Obtaining 2-3 probiotic packages‡ (n=86)</th>
<th>Odds Ratios (95% CI) for specified group compared to randomized control group:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taken any selected antibiotic</td>
<td>170 (26.9)</td>
<td>177 (27.7)</td>
<td>38 (31.4)</td>
<td>28 (32.6)</td>
<td>1.04 (0.82, 1.34)</td>
</tr>
<tr>
<td>Taken any antibiotic</td>
<td>212 (33.5)</td>
<td>216 (33.9)</td>
<td>46 (38)</td>
<td>32 (37.2)</td>
<td>1.01 (0.80, 1.28)</td>
</tr>
<tr>
<td>Had URTI without wheeze, treated by antibiotics</td>
<td>27 (4.3)</td>
<td>30 (4.7)</td>
<td>5 (4.1)</td>
<td>4 (4.7)</td>
<td>1.11 (0.65, 1.88)</td>
</tr>
<tr>
<td>Had respiratory episode, treated by antibiotics</td>
<td>129 (20.4)</td>
<td>143 (22.4)</td>
<td>31 (25.6)</td>
<td>24 (27.9)</td>
<td>1.13 (0.86, 1.47)</td>
</tr>
<tr>
<td>Number of selected antibiotic courses</td>
<td>0.42 (17.4, 9.5)</td>
<td>0.47 (16.3, 11.4)</td>
<td>0.50 (9.8, 11.6)</td>
<td>0.51 (19.8, 12.8)</td>
<td>1.11 (0.88, 1.38)</td>
</tr>
<tr>
<td>Number of any antibiotic courses</td>
<td>0.59 (19.6, 13.9)</td>
<td>0.61 (19.0, 14.9)</td>
<td>0.64 (24.0, 14.0)</td>
<td>0.63 (20.9, 16.3)</td>
<td>1.03 (0.84, 1.26)</td>
</tr>
<tr>
<td>No. URTIs without wheeze given antibiotics</td>
<td>0.04 (4.1, 0.2)</td>
<td>0.05 (4.1, 0.6)</td>
<td>0.04 (4.1, 0.0)</td>
<td>0.05 (4.7, 0)</td>
<td>1.24 (0.72, 2.12)</td>
</tr>
<tr>
<td>Combined all respiratory episodes given antibiotics</td>
<td>0.23 (4.2, 2.2)</td>
<td>0.30* (16.1, 6.3)</td>
<td>0.32 (19.0, 6.6)</td>
<td>0.35 (20.9, 7.0)</td>
<td>1.31 (1.03, 1.66)</td>
</tr>
<tr>
<td>Mean (percentage with one, percentage with two or more):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (standard deviation):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cost per person of selected antibiotics (£)</td>
<td>1.49 (SD=10.9)</td>
<td>1.75 (SD=16.58)</td>
<td>4.41 (SD=37.18)</td>
<td>5.74 (SD=44.08)</td>
<td>£0.25 (-1.29, 1.79)</td>
</tr>
<tr>
<td>Total cost per person of all antibiotics (£)</td>
<td>2.41 (SD=11.68)</td>
<td>2.38 (SD=16.96)</td>
<td>4.74 (SD=37.18)</td>
<td>5.98 (SD=44.07)</td>
<td>£-0.04 (-1.62, 1.54)</td>
</tr>
</tbody>
</table>

Comparison is made between randomized groups comparing the intervention group to the control group, and between the PP groups of participants following additional advice and obtaining probiotics during the study, comparing them to the randomized control group. †From unadjusted analyses. ‡From analyses adjusted for age group, sex, asthma severity and use of any antibiotics in 12 months prior to study. *p<0.05 compared to control group.
<table>
<thead>
<tr>
<th>Outcome Description</th>
<th>Randomized control group (n=632)</th>
<th>Randomized intervention group† (n=638)</th>
<th>Obtaining any probiotic package‡ (n=121)</th>
<th>Obtaining 2-3 probiotic packages‡ (n=86)</th>
<th>Odds Ratios (95% CI) for specified group compared to randomized control group:</th>
<th>Incidence Rate Ratio (95% CI) for specified group compared to randomized control group:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Had any URTI without wheeze</td>
<td>43 (6.8)</td>
<td>48 (7.5)</td>
<td>11 (9.1)</td>
<td>7 (8.1)</td>
<td>1.11 (0.73, 1.71)</td>
<td>1.56 (0.76, 3.20)</td>
</tr>
<tr>
<td>Had any LRTI without wheeze</td>
<td>63 (10.0)</td>
<td>87* (13.6)</td>
<td>17 (14.0)</td>
<td>11 (12.8)</td>
<td>1.43 (1.01, 2.01)</td>
<td>1.29 (0.71, 2.33)</td>
</tr>
<tr>
<td>Had any asthma exacerbations/wheeze</td>
<td>85 (13.4)</td>
<td>84 (13.2)</td>
<td>19 (15.7)</td>
<td>15 (17.4)</td>
<td>0.98 (0.71, 1.35)</td>
<td>1.12 (0.64, 1.99)</td>
</tr>
<tr>
<td>Had any respiratory infection</td>
<td>177 (28.0)</td>
<td>188 (29.5)</td>
<td>40 (33.1)</td>
<td>29 (33.7)</td>
<td>1.07 (0.84, 1.37)</td>
<td>1.19 (0.77, 1.84)</td>
</tr>
<tr>
<td>Received influenza vaccine during trial</td>
<td>430 (68.0)</td>
<td>454 (71.2)</td>
<td>111*** (91.7)</td>
<td>78** (90.7)</td>
<td>1.16 (0.91, 1.47)</td>
<td>4.34 (2.12, 8.91)</td>
</tr>
<tr>
<td>Received asthma review during trial</td>
<td>221 (35.0)</td>
<td>207 (32.4)</td>
<td>47 (38.8)</td>
<td>36 (41.9)</td>
<td>0.89 (0.71, 1.13)</td>
<td>0.94 (0.62, 1.43)</td>
</tr>
</tbody>
</table>

Comparison is made between randomized groups comparing the intervention group to the control group, and between the PP groups of participants following additional advice and obtaining probiotics during the study, to the randomized control group. †From unadjusted analyses. ‡From analyses adjusted for age group, sex, asthma severity and use of any antibiotics in 12 months prior to study. *p<0.05 compared to control group, **p<0.01 compared to control group, ***p<0.001 compared to control group.
### TABLE 4 Serious adverse events

<table>
<thead>
<tr>
<th>Type of serious adverse event</th>
<th>Randomized control group (n=632)</th>
<th>Randomized intervention group (n=640)</th>
<th>Obtaining any probiotic package (n=122)</th>
<th>Obtaining 2-3 probiotic packages (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Counts, Percentages (95% CI percentages)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>10 (1.6 (0.8-2.9))</td>
<td>6 (0.9 (0.3-2.0))</td>
<td>1 (0.8 (0.02-4.5))</td>
<td>1 (1.1 (0.03-6.2))</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>6 (0.9 (0.3-2.1))</td>
<td>10 (1.6 (0.8-2.9))</td>
<td>0 (0 (0-3.0))</td>
<td>0 (0 (0-4.2))</td>
</tr>
<tr>
<td>Infection (excluding above)</td>
<td>2 (0.3 (0.04-1.1))</td>
<td>4 (0.6 (0.2-1.6))</td>
<td>2 (1.6 (0.2-5.8))</td>
<td>1 (1.1 (0.03-6.2))</td>
</tr>
<tr>
<td>Other</td>
<td>20 (3.2 (1.9-4.8))</td>
<td>31 (4.8 (3.3-6.8))</td>
<td>10* (8.2 (4.0-14.6))</td>
<td>6 (6.9 (2.6-14.4))</td>
</tr>
</tbody>
</table>

This table compares each category of SAE according to the randomized groups and the PP groups of those receiving additional advice who obtained probiotics, counting number of participants affected by each category of SAE. *p<0.05 compared to control group, adjusted for age and sex.
Figure Legends

FIGURE 1 CONSORT flow diagram showing participant recruitment and flow through the study.

* PP1 is ≥1 set of probiotics ordered; PP2 is ≥2 sets of probiotics ordered. Each set included sufficient probiotics for 2 months.

FIGURE 2 Summarised effect size for the study primary outcome.

Data represent the OR (95% CI) for ≥1 antibiotic prescription during the 6-month intervention period, in the intervention compared with control group. OR is unadjusted for the intention to treat (ITT) analysis, and adjusted for age group, sex, asthma severity and use of any antibiotics in the 12 months prior to the study for per protocol (PP) analyses.

* PP1 is ≥1 set of probiotics ordered; PP2 is ≥2 sets of probiotics ordered. Each set included sufficient probiotics for 2 months.
ACKNOWLEDGEMENTS

We are grateful to Jean Pointon from Ashfield Primary Care Centre, NHS South Cheshire CCG, United Kingdom for administrative support especially with treatment allocation. We are grateful to Susan Plummer from Cultech Ltd, Swansea, United Kingdom for agreeing to supply Lab4 probiotics for this study without charge. RJB is supported by a National Institute for Health Research Biomedical Research Centre, and the MRC-Asthma UK Centre for Allergic Mechanisms in Asthma.

CONFLICT OF INTEREST STATEMENT

None of the co-authors has any relevant conflict of interest
REFERENCES


Figure 1:

Assessed for eligibility n=1457

Randomised n=1302

Excluded n=155
Same household n=141
Aged 0-4 years n=14

Probiotic n=652
Accessed probiotic at least once n=123
Accessed probiotic 2-3 times n=88

Followed up n=638
Lost to follow up n=14
Died n=2
Moved out of area n=10
Other reason n=2

Analysed – ITT n=638
PP 1* n=121
PP 2* n=86

Control n=650

Followed up n=632
Lost to follow up n=18
Moved out of area n=17
Other reason n=1

Analysed – ITT n=632
Figure 2:
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.

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

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.
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).

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

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.
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.
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].