Association between Antibiotic Resistance and Community Prescribing: A Critical Review of Bias and Confounding in Published Studies

Douglas Steinke* and Peter Davey
Medicines Monitoring Unit (MEMO), Department of Clinical Pharmacology and Therapeutics, University of Dundee, Dundee, Scotland

The reported association between antibiotic prescribing and resistance may be subject to bias or confounding. Bias describes any effect at any stage of investigation or inference tending to produce results that depart systematically from the true value. A confounding variable is one that is associated independently with both exposure and outcome. Confounding variables may create an apparent association or mask a real association. Pharmacoepidemiology is the study of the use and the effects of drugs in large numbers of people. We have used standard pharmacoepidemiological methods to investigate sources of bias and confounding in the association between prescribing and resistance. We conclude that the association is statistically valid and that the consistency of evidence supports a cause-effect relationship. Nonetheless, several important sources of bias and confounding must be taken into account in future studies that analyze the impact of prescribing policies on resistance.

In the United Kingdom, guidance to medical practitioners stresses the importance of prudent prescribing of antimicrobial agents in hospitals and in the community [1]. In hospitals, studies of the relationship between antibiotic prescribing and resistance have been hindered by difficulties in defining terms, selection biases, artifacts produced by study methods, and failure to control for confounding variables [2]. Pharmacoepidemiology has been defined as “the study of the use of and the effects of drugs in large numbers of people” [3]. The validity of such a study is crucially dependent on the definition of exposure to drugs, of the potential outcomes of drug treatment, and of biases or confounding variables that may influence the relationship [4]. We have identified potential sources of bias and confounding in studies that investigate the association between community antibiotic prescribing and used these criteria to critique the published evidence.

THE LIKELY RELATIONSHIP BETWEEN EXPOSURE TO ANTIBIOTICS AND COLONIZATION OR INFECTION WITH DRUG-RESISTANT BACTERIA

Antibiotic treatment is likely to influence colonization with resistant bacteria in 2 ways [5]. The first is by promoting mutation of bacteria, and the second is by facilitating the persistence of drug-resistant strains that are already present in the normal flora (figure 1). However, colonization with drug-resistant bacteria may occur independently of antibiotic exposure either by acquisition of drug-resistant bacteria or dissemination of genetic determinants through contact with other individuals, or by
Figure 1. The influence of antibiotic treatment on colonization of the normal flora with drug-resistant bacteria, adapted from Austin et al. [5]. The 2 main factors determining colonization that are independent of antibiotic treatment are the following: A, direct colonization or cross-infection with drug-resistant bacteria; B, spontaneous mutation of drug-sensitive bacteria to drug-resistant bacteria, or dissemination of genetic determinants of resistance from other bacteria. Both of these events can happen either before or after antibiotic treatment. Antibiotic treatment may influence colonization with resistant bacteria in 2 ways: (1) by promoting mutation from sensitive to resistant; and (2) by facilitating the persistence of drug-resistant strains that are already present in the normal flora.

DEFINITIONS OF BIAS AND CONFOUNDING

Bias is defined as any effect at any stage of investigation or inference tending to produce results that depart systematically from the true value [17]. Bias in epidemiological studies is usually divided into 2 broad types, information bias and selection bias (table 1) [4].

Misclassification may either be of exposure to drugs [18, 19], or of the outcome of drug treatment, which in this case is the accurate ascertainment of colonization or infection with drug-resistant bacteria [20–25]. Classification of exposure to drugs in the community is complex and particularly prone to error (figure 2). In North America and most of the countries of Western Europe, antibiotics are only available to people in the community by obtaining a doctor’s prescription. However, it would be naïve to assume that prescription of an antibiotic is either necessary or sufficient for exposure to an antibiotic (figure 2).

Misclassification of exposure or outcome may be either nondifferential or differential. Nondifferential misclassification occurs when the degree of misclassification is similar for all patients and independent of both exposure and outcome. However, differential misclassification can occur when the outcome influences the classification of exposure. For example,
isolation of a resistant organism may lead doctors to record information about prior exposure to antibiotics more accurately or completely than they would normally do.

Selection bias is likely to be a particular problem when it is difficult to be precise about the date of onset of the outcome. It is relatively easy to be precise about the date of onset of symptoms of an infection caused by drug-resistant bacteria, but symptomatic infection is likely to be preceded by a period of asymptomatic colonization that is harder to define. Selection bias is always more likely to occur in studies that rely on identification of prevalent cases rather than incident cases [4]. The problem for the design of studies of antibiotic resistance is that an incident case of infection (e.g., no infection during the previous 6 months) could still be a prevalent case of colonization (e.g., colonized for more than 6 months before the onset of symptomatic infection). The only way to be sure that antibiotic exposure preceded colonization is to obtain microbiological samples before treatment.

There is some evidence to show that some drug-resistant bacterial strains are less virulent than drug-sensitive strains [26]; this could lead to selection bias in studies of the relationship between drug resistance and clinical outcome.

Publication bias is an additional problem that needs to be considered in any literature review [33]. Techniques have been devised for statistical analysis of publication bias that can be applied when there is a reasonable number of homogeneous studies [33]. For example, a recent review identified 20 case-control studies that examined the association between vancomycin treatment and vancomycin-resistant enterococci (VRE), of which 15 were sufficiently homogeneous for meta-analysis. The regression asymmetry test ($P < .01$) and the adjusted rank correlation test ($P = .13$) both suggested that there was bias against publication of studies with negative results.

A confounding variable is a variable other than the risk factor under study that is associated independently with both exposure and outcome. It may create an apparent association or mask a real association [17]. Confounding is usually divided into confounding by association and confounding by indication [30] (table 2). Examples of confounding variables in studies of antibiotic resistance include comorbidities [27], hospitalization...
Figure 2. Factors influencing drug exposure (adapted from Collet et al. [4]). Note that even when a drug is bought and used at the time of prescription, personal use may mean that incomplete doses and courses are taken, and personal variation in absorption or drug clearance will further influence exposure.

[28, 29], and the indication for prescription of the antibiotic [31, 32].

In general, information and selection bias will tend to be towards the null—that is, these forms of bias will usually mask a true association between exposure and outcome. In our experience, if preliminary studies show an association, this association is likely to be stronger in subsequent studies that use more rigorous designs to minimize sources of information or selection bias [34]. In contrast, confounding may create an apparent association between exposure and outcome. For example, exposure to ulcer-healing drugs is strongly associated with risk of lung cancer. However, this association is entirely a result of the fact that both exposure to ulcer-healing drugs and risk of lung cancer are independently associated with smoking. Similarly, exposure to the oral contraceptive pill is associated with risk of cervical cancer because both are independently associated with sexual activity. An example of confounding in a study of antibiotic resistance is provided by a meta-analysis of reports that have investigated the association between antecedent vancomycin treatment and hospital-acquired VRE [35]. These authors found that the strength of association was much greater in 10 studies that did not adjust for duration of hospitalization (OR, 3.1; 95% CI, 1.8–5.3) compared with 5 studies that did adjust for duration of hospitalization (OR, 1.4; 95% CI, .74–2.60). In this example, duration of hospitalization was a confounding variable that distorted
Confounding by association
- Age: Increasing age is associated both with increasing use of antibiotics and with increasing risk of infection with drug-resistant bacteria.
- Comorbidities: All 3 of these confounders increase the overall risk of infection and hence the risk of exposure to antibiotics. However, these conditions are independently associated with an increased proportion of infections that are caused by inherently drug-resistant bacteria. For example, cystic fibrosis increases the risk of chest infection in general and the proportion of infections caused by inherently drug-resistant bacteria, such as *Pseudomonas aeruginosa* or *Pseudomonas cepacia*.
- Urinary tract abnormalities: Urinary tract abnormalities and urinary catheters influence both the probability of exposure to antibiotics in general, or to specific antibiotics such as quinolones, and the probability of infection with drug-resistant bacteria [27].
- Catheters and other devices: Prosthetic devices are particularly prone to infection with drug-resistant, coagulase-negative staphylococci.
- Hospitalization: Patients who are hospitalized are at increased risk of colonization with drug-resistant bacteria from the hospital environment. However, hospitalization is independently associated with comorbidities or more severe disease, and hence exposure to antibiotics. For example, in comparison with patients with chest infections caused by amoxicillin-sensitive *Haemophilus influenzae*, those infected with amoxicillin-resistant strains were both more likely to have been previously hospitalized and to have received antibiotics in the community [28].
- Being the child of a health care worker: Children of health care workers have an increased risk of colonization with penicillin-resistant *Streptococcus pneumoniae* [29]. It is plausible that this occurs because of independent increases in the risk of exposure to antibiotics and of colonization with penicillin-resistant *S. pneumoniae* (PRSP).

Confounding by indication
- Definition: The indication for drug treatment is the confounding variable. "In clinical practice, one would expect treated patients to differ from untreated patients, as the former have an indication for the treatment. To the extent that the indication is related to the outcome variable as well, the indication can function as a confounding variable" [30].
- Example: Central-line associated bloodstream infection is an indication for vancomycin treatment because of the high probability that these infections are caused by β-lactam-resistant, coagulase-negative staphylococci [31]. However, patients with central lines are likely to be located in intensive care or high dependency units, which increases the risk of colonization with vancomycin-resistant enterococci (VRE) [32]. So, the clinical indication "line infection" could be a confounder of the association between vancomycin treatment and colonization with VRE.

(i.e., magnified) the association between vancomycin treatment and colonization with VRE. It is plausible that increasing duration of hospitalization could independently increase both the risk of exposure to vancomycin and the risk of colonization with VRE, and that this would confound any real association between exposure and outcome.

### STUDY DESIGNS AND THEIR IMPLICATIONS FOR BIAS AND CONFounding

Studies can be conducted at the population level (relating global use of antibiotics to prevalence of antibiotic resistance) or at the individual level. At the individual level are 3 methods that can be used to identify subjects as case patients or control patients [35]:

1. **Clinical cultures**: subjects are identified through clinical cultures that are ordered as part of normal patient care.
2. **Cross-sectional surveillance**: subjects are identified through systematic sampling (single cultures are obtained from all members of a random sample of people from the general population).
3. **Surveillance of acquisition**: serial cultures are obtained to identify incident cases (those who have a negative culture result for drug-resistant bacteria, followed by a positive culture result).

**Studies based on clinical cultures done at the population level.** Studies based on clinical isolates are open to bias arising from the factors that determine whether or not a patient consults a doctor, whether the doctor obtains a bacteriological sample, and if so, the method that the doctor uses for taking and processing samples (table 3). In addition, studies conducted at the population level are particularly subject to information bias for both exposure and outcome. It is impossible to distinguish between high drug exposure a rising from single treatments of a large number of individual patients versus multiple treatments of a small number of individual patients. Similarly,
classification of outcome may be biased by multiple sampling from single individuals with drug-resistant bacteria [25]. The only negative study at the population level was unable to eliminate multiple samples from the analysis and found no relationship between prescribing of trimethoprim and resistance to trimethoprim in 3 regions of Finland (table 3) [41]. In contrast, a recent study from Wales that did eliminate multiple urine samples from analysis showed a statistically significant relationship between trimethoprim prescribing and resistance [37] (table 3). This study was also able to investigate another source of bias, which is the potential for an association between prescribing and use of microbiological diagnostic tests by primary care doctors [42, 43]. They found no relationship between the number of trimethoprim prescriptions per 1000 practice population and the number of urinary samples submitted to the laboratory.

Investigation of confounding in studies at the population level is limited by the lack of detail regarding the characteristics of the study population. For example, the study by Magee et al. [37] used data from 190 different primary care practices. This allows some crude adjustment for the age or sex distribution of each practice population, or other variables, such as social deprivation.

**Studies based on linking data about clinical cultures and antibiotic prescribing from individual patients.** This study design is still subject to bias arising from the factors that influence diagnostic sampling and is subject to prevalence study bias because it is impossible to be sure that isolation of resistant bacteria from a clinical sample represents the true onset of the disease (table 4). However, the ability to link data about exposure and outcome considerably reduces the risk of information bias and increases the ability to investigate confounding. Investigation of confounding is limited by the size of the study sample, by the richness of the information available for each study subject, and by prior information about the nature of confounding variables. For example, in a study of 412 patients with respiratory infection, we were only able to adjust for age, sex, and prior hospitalization [28]. Data about other potential confounders or risk factors were available, but the sample size was too small to include more than these key variables. In contrast, in a study of 13,765 patients with urinary infections, we were able to adjust for age, sex, hospitalization, diabetes, and prior exposure to hormone replacement therapy, oral contraceptive pills, or steroids [47]. The choice of these variables was limited by the fact that the database only contained information about prescribing. Furthermore, the drugs were selected on the basis that it was plausible that they might confound an association between antibiotic prescribing and resistance. The study could still be confounded if there was an unexpected association between prescribing of another class of drugs (e.g., β-blockers), antibiotic prescribing, and antibiotic resistance.

The only negative study based on individual patient data found that patients with gonococcal urethritis caused by ciprofloxacin-resistant Neisseria gonorrhoeae were less likely to have received ciprofloxacin than were control patients [49]. However, they were less likely to have received other antibiotics as well, suggesting that they were less likely to be seeking medical attention. Infection with drug-resistant strains of N. gonorrhoeae is more likely to be influenced by transmission of resistant strains between individuals than by exposure to antibiotics (figure 1).

**Studies that ascertained outcome by surveillance.** The major advantage of this study design is that it minimizes selection bias (table 5). This is because bacteriological samples can be obtained with standardized methods from every member of a sample that has been randomly drawn from the population at large. It is also possible to eliminate prevalence study bias by obtaining samples before exposure to antibiotics. However, the major disadvantage of studies of colonization is that they do not provide direct evidence about the relationship between antibiotic prescribing and the risk of infection with drug-resistant bacteria.

Many studies have shown an association between carriage of penicillin-resistant Streptococcus pneumoniae (PRSP) and prior exposure to penicillins or to other antibiotics [52]. The studies that are cited in table 5 are merely examples. One study is particularly interesting because it suggests that carriage of PRSP is associated with exposure to β-lactams at low daily dose for prolonged periods of time [51].

The only negative study of colonization found no association in children who were attending day care between prior antibiotic exposure and fecal colonization with drug-resistant E. coli (Reves et al., table 5). Significant risk factors were an age of <12 months and attendance at a center with an enrollment of >40 diapered children, suggesting that, as with that of gonorrhea, [49] acquisition of drug-resistant strains was more likely to be influenced by cross infection than by prior antibiotic exposure.

**Randomized controlled trials.** Random allocation of drug exposure should equalize the distribution of all potential confounders, even unknown ones, across the different levels of drug exposure [4]. In addition, random allocation of drug exposure minimizes selection bias and prevalence study bias (provided that subjects are sampled before drug exposure to identify prevalent cases of carriage of drug-resistant bacteria). This study design has been used to compare selection of drug-resistant bacteria from the fecal flora by trimethoprim alone versus trimethoprim-sulfamethoxazole (table 6). Collectively, these studies do not provide convincing evidence that trimethoprim alone is more likely to select resistant bacteria than is the combination [53–56]. However, 3 of the 4 studies showed selection of drug-
<table>
<thead>
<tr>
<th>Setting</th>
<th>Bacteria; source; antibiotic resistance</th>
<th>Main findings</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Studies at the population level showing an association between prescribing and resistance</td>
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<td></td>
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<tr>
<td>Two regions of Israel</td>
<td>Gram-negative bacteria; urinary isolates; trimethoprim resistance</td>
<td>Prescribing rates of ampicillin, cotrimoxazole, cephalxin, and nitrofurantoin were significantly higher in the region that had the highest resistance rates.</td>
<td>Raz et al. [36]</td>
</tr>
<tr>
<td>All primary care practices in Wales</td>
<td>Gram-negative bacteria; urinary isolates; trimethoprim resistance</td>
<td>The prevalence of trimethoprim-resistant bacteria was positively associated with the rate of prescribing of trimethoprim and of all other antibiotics.</td>
<td>Magee et al. [37]</td>
</tr>
<tr>
<td>Children &gt;5 years old in Sweden</td>
<td><em>Haemophilus influenzae</em>; any source; erythromycin resistance</td>
<td>Seasonal variations were seen in both the prevalence of resistant <em>H. influenzae</em> and prescribing of erythromycin.</td>
<td>Ringertz et al. [38]</td>
</tr>
<tr>
<td>A total of 206 health authority areas in Finland</td>
<td><em>S. pyogenes</em>; from any source from out-patients; erythromycin resistance</td>
<td>The proportion of isolates resistant to erythromycin clearly increased with increasing local erythromycin use (P = .006; logistic regression analysis).</td>
<td>Seppala et al. [39]</td>
</tr>
<tr>
<td>Finland</td>
<td><em>S. pyogenes</em>; from throat swabs, pus samples, and blood cultures; erythromycin resistance</td>
<td>Use of macrolide antibiotics decreased from 2.40 defined daily doses per 1000 inhabitants per day in 1991 to 1.38 in 1992 (P = .007) and remained near the lower level during the study period. The change in use was followed by a steady decrease in the frequency of erythromycin resistance, from 16.5% in 1992 to 8.6% in 1996 (OR, 0.5; 95% CI, 0.4–0.5).</td>
<td>Seppala et al. [40]</td>
</tr>
<tr>
<td>Studies at the population level not showing an association between prescribing and resistance</td>
<td></td>
<td>No clear correlation between the use of trimethoprim and the level of resistance was found.</td>
<td>Huovinen et al. [41]</td>
</tr>
</tbody>
</table>
Table 4. Studies of the association between antibiotic prescribing and infection with drug-resistant bacteria based on linking data about clinical cultures and antibiotic prescribing from individual patients.

<table>
<thead>
<tr>
<th>Study population</th>
<th>Bacteria; source; antibiotic resistance</th>
<th>Main findings</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Studies showing an association between prescribing and resistance</strong></td>
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<tr>
<td>68 patients with lower respiratory tract isolates</td>
<td><em>Haemophilus influenzae</em>; sputum isolates; β-lactamase production</td>
<td>A recent course of antibiotics, especially ampicillin/amoxicillin, was significantly ($P &lt; .05$) more common in the β+ group ($β+17/34$, $β−3/34$).</td>
<td>Johnson et al. [44]</td>
</tr>
<tr>
<td>1717 cases of community acquired bacteremia</td>
<td><em>Escherichia coli</em>, other coliforms, and <em>Staphylococcus aureus</em>; blood culture isolates; resistance to ampicillin, sulphonamides and trimethoprim.</td>
<td>Previous antibiotic prescriptions were strongly associated with resistance to ampicillin, sulphonamides, and trimethoprim in <em>E. coli</em>. The association was less pronounced for <em>S. aureus</em> and enteric rods other than <em>E. coli</em>.</td>
<td>Pedersen et al. [45]</td>
</tr>
<tr>
<td>412 patients with respiratory isolates from community or hospital</td>
<td><em>H. influenzae</em>; sputum isolates; amoxicillin resistance</td>
<td>Hospitalization (irrespective of use of any community-prescribed antibiotic) was associated with amoxicillin resistance (RR 4.5 [1.7–12.5]) and the prescription of β-lactam antibiotics in the community (irrespective of prior hospitalization) was also associated with amoxicillin resistance (RR 3.9 [1.6–9.8]).</td>
<td>Seaton et al. [30]</td>
</tr>
<tr>
<td>13,765 men and women who submitted urine samples from the community</td>
<td><em>E. coli</em> and other coliforms; urine isolates; trimethoprim resistance</td>
<td>With adjustment for significant risk factors and confounding variables, logistic regression analysis showed that exposure to trimethoprim (OR, 4.36; 95% CI, 2.9–5.0) or any other antibiotic (OR, 1.32; 95% CI, 1.1–1.6) were significantly associated with resistance to trimethoprim.</td>
<td>Steinke et al. [46, 47]</td>
</tr>
<tr>
<td>116 children</td>
<td><em>Streptococcus pneumoniae</em>; middle ear fluid, blood, CSF, and nasopharyngeal isolates; penicillin resistance</td>
<td>Frequent antibiotic use, prior hospitalization, and duration of hospital stay ($P &lt; .001$ for all 3) were associated with infection with resistant strains.</td>
<td>Reichler et al. [48]</td>
</tr>
<tr>
<td><strong>Studies not showing an association between prescribing and resistance</strong></td>
<td></td>
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<tr>
<td>157 patients with gonococcal urethritis</td>
<td><em>Neisseria gonorrhoeae</em>; urethral swabs; ciprofloxacin resistance</td>
<td>Case patients were less likely to be treated with ciprofloxacin or other antibiotics for gonococcal urethritis than were controls ($P ≤ .001$).</td>
<td>Gordon et al. [49]</td>
</tr>
</tbody>
</table>

**NOTE.** $+$, Positive; $-$, negative.
### Table 5. Examples of studies using surveillance to examine the association between antibiotic prescribing and colonization with drug-resistant bacteria.

<table>
<thead>
<tr>
<th>Setting and study population</th>
<th>Bacteria; source; antibiotic resistance</th>
<th>Main findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studies showing an association between prescribing and resistance</td>
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<tr>
<td>919 children from 5 different communities in Iceland</td>
<td><em>Streptococcus pneumoniae</em>; nasopharyngeal swabs; penicillin resistance</td>
<td>By multivariate analysis age (&lt;2 years), area (highest antimicrobial consumption), and individual use of antimicrobial agents significantly influenced the odds of carrying penicillin-resistant pneumococci. By univariate analysis, recent antimicrobial use (2–7 weeks) and use of cotrimoxazole were also significantly associated with carriage of penicillin-resistant pneumococci.</td>
<td>Arason et al. [50]</td>
</tr>
<tr>
<td>941 children, 3 to 6 years old, attending 20 randomly sampled schools in France</td>
<td><em>S. pneumoniae</em>; nasopharyngeal swabs; penicillin resistance</td>
<td>Children treated with low daily doses of an oral β-lactam (defined as lower than clinical recommendations) had an increased risk of PRSP carriage, compared with children who did not (OR, 5.9; 95% CI, 2.1–16.7; <em>P</em> = .002). A treatment of long duration (&gt;5 days) with a β-lactam was associated with an increased risk of penicillin-resistant <em>S. pneumoniae</em> (PRSP) carriage (OR, 3.5; 95% CI, 1.3–9.8; <em>P</em> = .02).</td>
<td>Guillemot et al. [51]</td>
</tr>
<tr>
<td>179 children from a military population in the United States</td>
<td><em>S. pneumoniae</em>; nasopharyngeal swabs; penicillin resistance</td>
<td>Frequent courses of antimicrobial treatment correlated both with carriage of pneumococci (<em>P</em> &lt; .009) and with carriage of PRP (<em>P</em> &lt; .0001). However, long-term antimicrobial prophylaxis was protective against carriage of pneumococci (<em>P</em> &lt; .002).</td>
<td>Fairchok et al. [29]</td>
</tr>
<tr>
<td>Studies not showing an association between prescribing and resistance</td>
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<tr>
<td>203 children attending 12 day-care centers, 51 children attending a well-child clinic (controls), and 64 medical students</td>
<td><em>Escherichia coli</em>; feces; trimethoprim resistance and multiple antibiotic resistance.</td>
<td>In a case-control study among the day-care center children, significant risk factors were an age of &lt;12 months and attendance at a center with an enrolment of &gt;40 diapered children (ORs of 2.2 and 3.5, respectively); ethnicity, duration of attendance, and prior antibiotic administration were not associated with colonization.</td>
<td>Reves et al. [10]</td>
</tr>
</tbody>
</table>

**NOTE.** Case and control patients were identified by systematic surveillance cultures. All of these studies used a cross-sectional study design.
ment is associated with an increase in the prevalence of subjects with *E. coli* isolates that are resistant to the drug that they received [58, 60, 61]. Moreover, one study showed that treatment with amoxicillin-clavulanate was associated with an increase in the proportion of symptomatic urinary tract infections caused by bacteria resistant to amoxicillin, amoxicillin-clavulanate, and cefaclor [58]. These studies provide convincing evidence that treatment with a variety of antibiotics causes a transient increase both in the proportion of *E. coli* from the normal flora that are drug resistant, and in the proportion of subjects who carry drug-resistant *E. coli*.

In contrast, *S. pneumoniae*, *α*-hemolytic streptococci, and *Haemophilus influenzae* are not universally present in the nasopharyngeal flora and antibiotic treatment influences both the number of subjects who are carriers of these bacteria, and the proportion of bacteria that are drug resistant [57, 62]. This makes interpretation of study results potentially confusing [52]. For example, before treatment with amoxicillin-clavulanate or cefaclor, 224 (53%) of 426 children were carriers of *S. pneumoniae*, of whom 87 (39%) had isolates that were resistant to penicillin [62]. At the end of treatment only 116 children (27%) were carriers, but 65 *S. pneumoniae* isolates (56%) were resistant to penicillin. One month after treatment the proportion of carriers remained lower than before treatment (32% vs. 53%), but the proportion of penicillin-resistant strains was greater than before treatment (50% vs. 39%). There were 27 new carriers of *S. pneumoniae* after antibiotic treatment and 24 (89%) of these strains were resistant to penicillin. In this example, antibiotic treatment was associated with 2 opposing effects: reduction in the prevalence of carriage of *S. pneumoniae* and an increase in the proportion of penicillin-resistant strains. The net effect of drug treatment was a reduction in the prevalence of carriage of penicillin-resistant *S. pneumoniae*, from 87 children (20%) to 69 children (16%). Similar results with respect to carriage of *S. pneumoniae* were found in a second study [57]. Treatment with amoxicillin-clavulanate reduced the prevalence of carriage of *S. pneumoniae* (from 49% to 26% 1 month after treatment), increased the proportion of penicillin-resistant strains of *S. pneumoniae* (from 28% to 43%) and resulted in a net decrease in the prevalence of children carrying penicillin-resistant *S. pneumoniae* (from 17% to 11%). In contrast, treatment with either amoxicillin-clavulanate or azithromycin increased both the proportion of carriage of *α*-hemolytic streptococci (from 14% to 42%) and the proportion of penicillin-resistant strains (from 27% to 49%). This resulted in a 5-fold net increase in the prevalence of children carrying penicillin-resistant *α*-hemolytic streptococci (from 4% to 21%) [57].

### Table 6. Randomized trials comparing changes in the resistance of the fecal flora of community subjects after treatment with trimethoprim (TMP) or trimethoprim-sulfamethoxazole (TMP-SMZ).

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Indication</th>
<th>Duration, days</th>
<th>Baseline TMP resistance</th>
<th>Acquired TMP-resistance on TMP</th>
<th>Acquired TMP-resistance on TMP-SMZ</th>
<th>Difference, % (95% CI)a</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980</td>
<td>UK</td>
<td>UTI</td>
<td>5</td>
<td>0/42 (0)</td>
<td>0/22 (0)</td>
<td>0/20 (0)</td>
<td>0 (NA)</td>
<td>Lacey et al. [53]</td>
</tr>
<tr>
<td>1981</td>
<td>USA</td>
<td>Recurrent UTI</td>
<td>28</td>
<td>NRb</td>
<td>3/30 (10)</td>
<td>4/18 (22)</td>
<td>−12 (−34 to +10)</td>
<td>Guerrant et al. [54]</td>
</tr>
<tr>
<td>1982</td>
<td>Mexico</td>
<td>Prophylaxis of TD</td>
<td>14</td>
<td>37/100 (37)</td>
<td>29/33 (88)</td>
<td>46/46 (100)</td>
<td>−12 (−23 to −1)</td>
<td>Murray et al. [55]</td>
</tr>
<tr>
<td>1985</td>
<td>Finland</td>
<td>UTI</td>
<td>10</td>
<td>9/93 (10)</td>
<td>6/43 (14)</td>
<td>7/44 (16)</td>
<td>−2 (−17 to +13)</td>
<td>Huovinen et al. [56]</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no of subjects with *E. coli* or other Enterobacteriaceae resistant to TMP/total no. of subjects (%), unless otherwise indicated. NR, not reported; TD, travellers diarrhea; UK, United Kingdom; USA, United States of America; UTI, urinary tract infection.

a Difference is TMP minus TMP-SMZ; a negative value indicates that resistance was more likely to emerge during treatment with TMP-SMZ than with TMP alone.

b In this study, TMP-resistant bacteria emerged or increased during therapy in 15 (50%) of 30 patients who received TMP and 4 (22%) of 18 patients who received TMP-SMZ, but this result was attributable to emergence of *Pseudomonas* species rather than Enterobacteriaceae.

### CONCLUSIONS

Even allowing for publication bias, there is a compelling weight of evidence showing that community prescribing of antibiotics is associated with increased prevalence of both colonization and infection with drug-resistant strains. The studies that we have reviewed (tables 3–6) are too heterogeneous for formal analysis of publication bias. Nonetheless, we have only been able to identify 4 studies reporting no association between antibiotic prescribing and resistance [10, 41, 49, 53].

Each of the study designs reviewed has positive and negative points. Randomized trials with sampling of the normal flora before drug exposure minimize confounding and bias but they are artificial, using selected samples of patients and focusing on carriage of bacteria, rather than infection with drug-resistant strains. At the other extreme, observational studies of clinical isolates are highly vulnerable to bias and confounding, but they provide information about clinical infections in large populations of representative patients. The fact that all study designs (tables 3–6) have demonstrated an association is convincing evidence that the associations is real and has not been produced by chance, bias or confounding [63].
The remaining question is does the strong association between community antibiotic prescribing and resistance indicate cause and effect? Strength of association is just 1 of 5 criteria used to assess whether a valid statistical association can be judged as cause and effect [63]. The other 4 questions that need to be addressed are:

1. Is there biologic credibility to the hypothesis?
2. Is there consistency with other studies?
3. Is the time sequence compatible?
4. Is there evidence of a dose-response relationship?

Clearly there is biologic plausibility to a causal link between community antibiotic prescribing and resistance [5, 64]. Consistency with other studies means that the most persuasive evidence to support a judgment of a cause-effect relationship arises when “a number of studies, conducted by different investigators at various times using alternative methodology in a variety of geographic or cultural settings and among different populations, all show similar results” [63]. The studies that we have reviewed meet these criteria (tables 3–6). Time sequence refers to the evidence that exposure precedes the outcome by a period of time consistent with any proposed biologic mechanism. In the case of antibiotic resistance, this is not as problematic as it is with some other diseases, such as Jakob-Creutzfeld disease, which have prolonged and uncertain periods of latency. Studies of incident cases of colonization with drug-resistant bacteria (tables 5 and 6) provide particularly convincing evidence of a time sequence consistent with selection of drug-resistant bacteria through exposure to antibiotics. A dose-response relationship is perhaps the most problematic piece of evidence to assess, because there is some evidence to show that exposure to low doses of antibiotics is more likely to select drug-resistant bacteria than is exposure to high doses [51, 65]. Nonetheless, duration of antibiotic exposure is consistently positively associated with emergence of resistance [51, 66, 67].

Having established beyond a reasonable doubt that community antibiotic prescribing contributes to the problem of antibiotic resistance, the research agenda needs to move on towards studies that analyze the impact of different prescribing policies on resistance. It should be possible to build information about prescribing and resistance explicitly into models that compare the likely effect of different prescribing policies [68]. The complexity and number of influences in addition to antibiotic prescribing are daunting (figure 1). Nonetheless, we believe that progress can be made provided that researchers heed Box’s warning about mathematical models: “All models are wrong but some are useful” [69].

References


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