Title: Estimating the proportion of bystander selection for antibiotic resistance in the US

Authors: Christine Tedijanto[1], Scott Olesen[2], Yonatan Grad[2,3], Marc Lipsitch[1,2]

Author Affiliation: [1] Center for Communicable Disease Dynamics, Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; [2] Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; [3] Division of Infectious Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Corresponding Author: Christine Tedijanto, ctedijanto@g.harvard.edu

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Abstract

Bystander selection -- the selective pressures exerted by antibiotics on microbial flora that are not the target pathogen of treatment -- is critical to understanding the total impact of broad-spectrum antibiotic use; however, to our knowledge, this effect has never been quantified. Using the 2010-2011 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey (NAMCS/NHAMCS), the Human Microbiome Project, and additional carriage and etiological data from existing literature, we estimate the magnitude of bystander selection for a range of clinically relevant antibiotic-species pairs as the proportion of all exposures of an antibiotic experienced by a species for conditions in which that species was not the causative pathogen ("proportion of bystander exposures"). For outpatient prescribing in the United States, we find that this proportion over all included antibiotics is over 80% for 8 out of 9 organisms of interest. Low proportions of bystander exposure are often associated with infrequent bacterial carriage or a high proportion of antibiotic prescribing focused on conditions caused by the species of interest. Using the proportion of bystander exposures, we roughly estimate that *S. aureus* and *E. coli* may benefit from 90.7% and 99.7%, respectively, of the estimated reduction in antibiotic use due to pneumococcal conjugate vaccination, despite not being the pathogen targeted by the vaccine. These results underscore the importance of considering antibiotic exposures to bystanders, in addition to the targeted pathogen, in measuring the impact of antibiotic resistance interventions.

Significance Statement

The forces that contribute to changing population prevalence of antibiotic resistance are not well understood. Bystander selection -- the inadvertent pressures imposed by antibiotics on the microbial flora other than the pathogen targeted by treatment -- is hypothesized to be a major factor in the propagation of antibiotic resistance, but its extent has not been characterized. We estimate the proportion of bystander exposures across a range of antibiotics and organisms and describe factors driving variability of these

proportions. Impact estimates for antibiotic resistance interventions, including vaccination, are often limited to effects on a target pathogen. However, the reduction of antibiotic treatment for illnesses caused by the target pathogen may have the broader potential to decrease bystander selection pressures for resistance on many other organisms.

Introduction

Antibiotic use creates a selective pressure favoring resistant microbes. Because the majority of antibiotics are not targeted to a specific bacterial species or body site, the bacteria that comprise the human microbiome are subject to the selective pressures applied by most antibiotic consumption. Therefore, antibiotic exposures, while designed to control the pathogenic bacterium causing an infection (we use the term "target pathogen"), also promote the emergence of *de novo* resistance, the selection for resistant strains that are already present, and the clearance of susceptible strains among both the target species and other organisms in the microbiome (1). These selective pressures experienced by microbial flora exposed to antibiotics due to a condition caused by another bacterial species (or by no bacterium at all, in which case treatment is often inappropriate (2)) can be called "bystander selection". These off-target effects (3) are a concern with appropriate prescribing and are at the core of the concern about inappropriate prescribing, for which every exposed bacterium is a bystander. Recent studies have reported that 30% of all outpatient, oral antibiotic prescriptions in the United States (2) and 8.8-23.1% of systemic antibiotic prescriptions in primary care in England (4) are inappropriate.

Quantifying these bystander effects is of importance for evaluating the potential and actual impact of interventions designed to reduce the need for appropriate antibiotic treatments (e.g., infection control and vaccines). Each of these measures can reduce selective pressure for resistance on the target pathogen: vaccination by reducing the incidence of disease from, say, *Streptococcus pneumoniae* and thus the need

for antibiotic treatment (5, 6), and infection control by reducing the incidence of hospital-acquired infections that will require treatment. Often overlooked is the impact that averted treatment in each case may have beyond the pathogens whose transmission these interventions block, because each treatment averted would have exerted selection on bystanders as well. For stewardship interventions, which aim to avert inappropriate treatment of conditions that are never or seldom caused by bacteria, the primary goal of the intervention is to avert bystander selection of the patient's normal flora.

Given the importance of bystander selection in the rationale for each of these kinds of interventions, it is surprising that its extent has not, to our knowledge, previously been quantified. Bystander selection is driven by the incidence and etiology of bacterial infections, antibiotic prescribing practices, and composition of the microbial flora. All of these factors are highly heterogeneous, varying over time and by age, gender, and geographic location. In addition, antibiotic prescribing depends upon safety and toxicity profiles in certain populations. Microbiome diversity varies between and within individuals, depending on demographic characteristics, diet, and disease. Characterizing the impact of bystander selection at the population level therefore requires understanding which antibiotics are prescribed when and which microbes are likely to experience those pressures.

This work aims to quantify bystander selection due to outpatient prescribing in the US at the population level for a range of clinically relevant species and antibiotic combinations, using prescriptions as a measured proxy for exposures and, ultimately, for selection, and using data from the Human Microbiome Project and from studies of bacterial carriage to estimate the microbial communities subject to selection. We estimate the extent of bystander selection as the proportion of total exposures of a species to an antibiotic for which that species was not the target pathogen of the antibiotic, and will refer to this measure as the "proportion of bystander exposures". Understanding the contribution of bystander

exposures to the landscape of selective pressures for antibiotic resistance will help to inform interventions including vaccines and antibiotic stewardship. Given the special section of the current issue of *PNAS* on vaccines and antimicrobial resistance, we spell out how such estimates can contribute to estimating the impact of vaccines, in particular pneumococcal conjugate vaccines, whose impact on antimicrobial resistance has received arguably the most attention of any vaccine (7, 8).

Results

Data source characteristics. After applying exclusion criteria to the National Ambulatory Medical Care Survey (NAMCS) and National Hospital Ambulatory Medical Care Survey (NHAMCS) from 2010-2011 and weighting with nationally representative sampling weights, 97.9% of total visits remained in the analysis, with 13.7% of these visits resulting in at least one antibiotic prescription. Visits with one or more of the diagnoses included in bystander calculations (see Methods) accounted for 60.7% of prescriptions of our antibiotics of interest. Of included visits with one of these diagnoses, 5.8% were clinical encounters with patients less than one year old and 19.2% with patients between the ages of 1 and 5 years old.

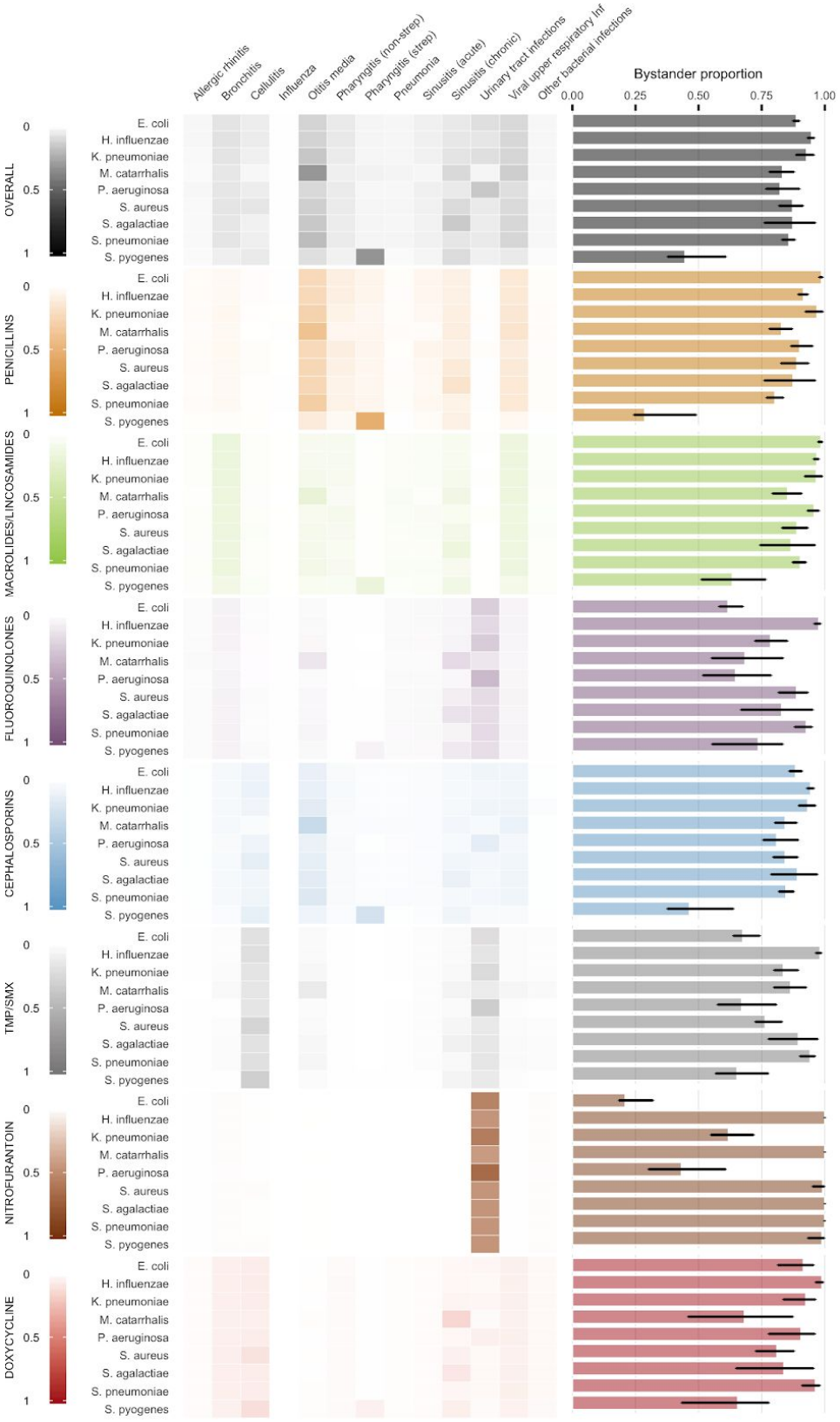

Figure 1. Proportion of total antibiotic exposures by drug class and species associated with diagnoses, and proportion of bystander exposures over all conditions by antibiotic class and species with 95%

confidence intervals. Penicillins include penicillin, amoxicillin, and amoxicillin-clavulanate; macrolides/lincosamides include azithromycin, clarithromycin, and clindamycin; fluoroquinolones include ciprofloxacin, levofloxacin, and moxifloxacin; cephalosporins include ceftriaxone, cephalexin, and cefdinir. Doxycycline, nitrofurantoin, and trimethoprim-sulfamethoxazole results are for the individual antibiotics. "Overall" estimates reflect exposures to any of the drugs listed above.

Estimating the proportion of bystander selection, by species and antibiotic or class. The proportion of total exposures of a given species to an antibiotic (or class) that were associated with a given diagnosis ranged from 67.2% for exposures of nitrofurantoin to *P. aeruginosa* due to UTI to 0% for cases in which the antibiotic was never prescribed for the condition (e.g. doxycycline and influenza, nitrofurantoin and sinusitis) (Figure 1, heatmap). Within a specific antibiotic class, the proportions tended to be elevated across all organisms for particular conditions, indicating that incidence of the condition and corresponding volume of antibiotic use were the key drivers of this value. For example, results were higher across all organisms for penicillins and suppurative otitis media, a common condition leading to frequent use of that antibiotic class. While a high proportion of pneumonia cases also lead to antibiotic prescriptions, low incidence in the outpatient setting led to a low proportion of total exposures due to this condition. Nitrofurantoin presents an extreme case where use is targeted towards a single, common condition; thus, the majority of exposures across all organisms were associated with UTI. This proportion is also a function of etiology. In the case of fluoroquinolone use, the proportion of total exposures associated with UTI was especially high for the causative agents -- *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. This effect is more distinct among organisms with low carriage prevalence in the general population, such as *P. aeruginosa* in the previous example and *S. pyogenes* and strep throat.

The proportion of bystander exposures was variable across species and antibiotic classes, ranging from 21% for *E. coli* exposures to nitrofurantoin to 100% for *M. catarrhalis* and *S. agalactiae* exposures to nitrofurantoin (Figure 1, bar chart). The proportion of bystander exposures exceeded 80% for 8 out of 9

organisms (all except *S. pyogenes*) when considering exposures to any of our antibiotics of interest, and above 80% for 96 out of 135 (71.1%) antibiotic-species pairs (Supplemental Figure 1). These results indicate that for the majority of antibiotic and species combinations, fewer than 20% of the exposures of that species to the antibiotic in question occur in the context of treating a disease caused by that species. The proportion of bystander exposures is inversely related to the proportion of total exposures associated with each condition. Across each row in Figure 1, if the species of interest was an etiological agent for the conditions contributing the highest proportion of total exposures, we would expect the bystander proportion to be low, and vice versa. For example, the majority of exposures of *E. coli* to cephalosporins are due to cellulitis and suppurative otitis media, neither of which are caused by *E. coli*, leading to almost all exposures being bystander. However, the majority of exposures of *E. coli* to fluoroquinolones are due to UTIs, which are often caused by *E. coli*; thus the proportion of bystander exposures is much lower for this pairing. Supplemental Figure 1 also displays the high variability of bystander results within drug classes, reflecting the preferred use of these drugs for different conditions.

Application to vaccine impact on antimicrobial exposures in bystanders. We provide here some preliminary estimates of the bystander impact of vaccines to illustrate how such calculations might be performed. Because the requisite quantities have not all been estimated in the same population, we combine estimates from different populations for the purposes of illustration, but we note the need for additional data to improve the level of confidence in such calculations by comparing quantities within a single population.We take the example of the pneumococcal conjugate vaccine (PCV), which reduces bacteremia, meningitis, pneumonia and otitis media caused by 7, 10 or 13 serotypes of pneumococci, depending on the formulation.

Considering only immediate direct effects, we made a conservative estimation of the effect of PCV on antimicrobial exposures of bystander organisms. A randomized controlled trial of the seven-valent pneumococcal conjugate vaccine found a 7.8% reduction in otitis media among vaccinated vs. control children, and a 5.4% in all-cause antimicrobial prescribing, mainly attributed to reduced otitis media (5). Restricting attention to the 0-1 and 1-5 year old age groups in our study, this would translate to a 4.9% reduction in exposure of *S. aureus* to antibiotics, and a 5.38% reduction in exposure of *E. coli* to antibiotics in these age groups. See Materials and Methods for the way this figure is calculated.

Much larger estimates of impact on otitis media are obtained in studies that account for herd immunity effects and for the possibility that PCVs can indirectly prevent some non-pneumococcal otitis media (9). An Israeli study found a 57-71% reduction in all-cause otitis media associated with the rollout of PCV13 in various age groups up to the third birthday (10), while a study in the UK found a 36% reduction in all-ages otitis media comparing the post-PCV13 period to the pre-PCV7 period, and a 29% reduction in otitis media-associated antimicrobial prescribing for the same comparison (6). Impact on total antimicrobial prescribing was not reported by Lau et al. (6) If we assume that the ratio of 0.69 percentage points reduction in total prescribing per percentage point reduction in otitis media can be extrapolated from the RCT in California (5), this 36% reduction in otitis media would correspond to a 25% reduction in all-cause antibiotic prescribing. Using our estimates of prevalence and bystander proportion, this would yield a 24.9% and 22.7% reduction in outpatient exposure of bystanders *E.coli* and *S. aureus* to antibiotics. While these calculations require a number of assumptions, they underscore the potentially substantial impact of vaccines on bystander selection and the need for improved data on the impact of vaccination on use of specific antimicrobials in specific populations.

Discussion

For most bacterial species, the majority of their antibiotic exposures were the result of a condition that they did not cause. This held true across a range of different organisms and antibiotics. Carriage prevalence was the key predictive factor of the differences in proportion of bystander exposures between organisms, with species that were commonly carried asymptomatically (Supplemental Table 2), such as *E. coli*, *H. influenzae* and *S. pneumoniae*, having consistently high bystander proportions, and more rarely carried species such as *S. pyogenes*, which is frequently associated with antibiotic-treated disease, having lower ones. Among drugs/drug classes, nitrofurantoin, used almost exclusively for urinary tract infections, had low bystander proportions for common urinary tract pathogens, which frequently would be the cause of nitrofurantoin treatment. In contrast, broad-spectrum drug classes such as beta-lactams, cephalosporins and fluoroquinolones typically have high bystander proportions for most or all species considered, because they are used for a wide variety of conditions caused by a wide variety of species, as well as for treatment of conditions that are often nonbacterial.

Quantifying the bystander effect for different antibiotic-species combinations has several potential applications. Mathematical transmission models of antibiotic prescribing and resistance commonly assume that treatment incidence is unrelated to whether a person is colonized with the bacterium of interest -- effectively assuming that bystander selection is the rule rather than the exception (11–13), and these findings confirm this has been a sensible assumption, at least for outpatient antibiotic use.

For policy discussions, the high bystander proportions obtained here suggest that interventions to reduce antimicrobial use may have broad effects in reducing the strength of selection across a number of bacterial species, not only the ones involved in the pathogenesis of the disease targeted by such efforts. For example, improved adherence to guidelines on unnecessary antimicrobial prescribing might mainly

affect prescribing for respiratory infections, yet might reduce selection for resistance on potential pathogens that reside on the skin (e.g. *S. aureus*) or in the gut (e.g. *E. coli* and *Klebsiella* species), as well as on respiratory bacteria. In the area of antimicrobial stewardship, these findings suggest that each reduction in inappropriate antibiotic prescribing for a particular indication may have broad impacts across many species but may not dramatically reduce the exposures to antibiotics of any one species, as long as prescribing for other indications remains unchanged.

As discussed, another example of an intervention that can reduce antimicrobial prescribing is vaccination. Vaccines can reduce the incidence of resistant infections directly (by preventing disease from their target pathogens) and indirectly (by preventing the need for antibiotic prescribing, thereby protecting bystander bacteria from exposure to antibiotics that can promote resistance). High bystander proportions are seen here for many organism-drug combinations, particularly for broad-spectrum antibiotic classes that are frequently prescribed for respiratory infections, and respiratory infections (including otitis media) account for a large fraction of total antimicrobial use. These considerations suggest that vaccines against pathogens that cause respiratory infections, such as *Bordetella pertussis, Streptococcus pneumoniae,* influenza virus, and respiratory syncytial virus, may substantially reduce the exposure of a broad range of pathogenic bacterial species to antibiotics, via prevention of bystander selection. Notably, this includes vaccines that prevent viral respiratory infections, which are often inappropriately treated with antibiotics (14) and perhaps prevent bacterial secondary infections that might be appropriately treated if they occurred (15). We have described an approach for using estimates of bystander exposures to estimate how vaccines could reduce exposure across various non-target pathogens. However, quantifying the impact of vaccines on antimicrobial resistance is a complex task, and many components of such calculations will depend on the population, vaccine, and timescale considered, among other variables.

It is informative to consider the antimicrobial agents not included in our analysis. Most antimycobacterial agents have little effect on other bacterial species, while most broad-spectrum antibacterial classes are of little use against *Mycobacterium tuberculosis*. The exception to both rules is the fluoroquinolones, for which bystander selection has been documented both in treatment of what was thought to be pneumonia but was actually tuberculosis (16) and in treatment with fluoroquinolones in a tuberculosis ward promoting the spread of fluoroquinolone-resistant *S. pneumoniae. (17)*. With this exception, bystander selection by antimycobacterial drugs is expected to be limited, and bystander selection on M. tuberculosis is also expected to be limited. This is reflected in an appropriate focus for tuberculosis resistance management in ensuring adequate treatment to prevent emergence of resistance and prevent transmission, rather than on bystander-focused interventions. Similarly, we note that antiviral agents, such as the neuraminidase inhibitor oseltamivir for influenza, have no substantial known activity against other components of the (bacterial) microbiome, so the rationale for prudent use of oseltamivir would include avoiding side effects and costs, but not avoiding selection for resistance. Extending the scope beyond anti-infectives, a recent *in vitro* study found that 24% of 835 therapeutic compounds with molecular targets in human cells inhibited the growth of at least one bacterial species commonly found in the human gut microbiome (18). This work suggests that bystander selection may not be limited to antimicrobials, and further research is needed to elucidate which drug-species combinations may be prone to such effects.

Our broader analysis has several limitations. Firstly, the analysis combines three different data sources to calculate a population-level, average estimate of the quantities of interest. For simplicity, we only consider age group stratification in our analysis; in reality, the factors contributing to bystander exposures may vary across sex, socioeconomic status, and many other characteristics. In addition, carriage prevalences and etiologies are uniformly applied across visits. This may bias our estimates depending on the extent of microbial ecological or etiological relationships. For example, presence (or lack) of

organism A in the microbiome contributes to the pathogenicity of organism B. Organism A would be more (or less) prone to bystander exposure of antibiotics used to treat the condition caused by organism B than we calculate. While we estimate the impact on the organism at the species level, selection pressures may be more relevant at the strain level, particularly if virulence and resistance are associated. We also do not consider the impact of antibiotic pharmacodynamics and pharmacokinetics and how these vary by indication and by body site.

Secondly, the limitations of the datasets used in our analysis also apply to our results. For example, the HMP was conducted in a restricted study population and prevalence estimates may not be generalizable to the US population. Similarly, etiologic studies are burdensome and thus often conducted among very small populations. Additionally, though NAMCS/NHAMCS are unique in providing a large sample of outpatient visits with corresponding diagnoses and prescriptions, a direct link between diagnosis and prescription is unavailable - therefore, exposures may be incorrectly counted as “bystander”, when the prescription was in fact written for a second diagnosis caused by the species of interest. Outpatient prescribing constitutes approximately 90% of total antimicrobial volume for human health in developed countries (19, 20), but certainly further work is needed to consider the inpatient context as it affects nosocomial pathogens.

Finally, in this analysis, prescriptions are used as a proxy for exposure, which is itself a proxy for selective pressure. NAMCS/NHAMCS do not contain information on whether or not the prescriptions were filled; even after being filled, we have no information on compliance to the listed medications. Furthermore, little is known about how exposures of a particular antibiotic correspond to selection pressures. This may differ widely by antibiotic, dose, organism, body site, and context (e.g. microbiome composition) (21). The classes of antibiotics considered here may be assumed to exert selection on the

normal flora of the gut because they are taken orally; direct evidence of this effect has been reported (22). Likewise, selection on the flora of the upper respiratory tract is likely the rule for many of these classes, including macrolides, penicillins, cephalosporins, and trimethoprim/sulfamethoxazole because they are routinely prescribed for upper respiratory infections and are documented to affect bacterial carriage at in the nasopharynx (23–25). Antibiotics in major classes including penicillins and cephalosporins (26), macrolides (27) and fluoroquinolones (28) have been detected in sweat, indicating that they can exert selection on skin flora. The concentrations in these body compartments will vary, and subinhibitory concentrations likely play an important role in selection for resistance (29). The metric used in the present study, counting prescriptions as exposures, could be refined in studies of individual drugs, classes, microbes or body sites to incorporate more biological detail.

The bystander proportions quantified in this analysis are a step toward better characterizing the dynamics of antibiotic resistance and should be considered in the development and prioritization of interventions. Studies of the effects of antibiotic use on the microbiome are greatly needed to further understand the impact of bystander, and total, exposures.

Materials and methods

Data sources. All estimates were based on three main data sources - the National Ambulatory Medical Care Survey/National Hospital Ambulatory Medical Care Survey (NAMCS/NHAMCS) collected by the National Center for Health Statistics, the Human Microbiome Project and other studies of carriage prevalence, and etiological studies.

The NAMCS and NHAMCS are cross-sectional national surveys designed to collect data on ambulatory care services provided at office-based physician practices, emergency, and hospital outpatient

departments throughout the United States. At each sampled visit, patient characteristics (e.g. age), visit characteristics (e.g. reason for visit, diagnosis, prescriptions), and physician characteristics are recorded, including up to 3 diagnoses and up to 8 prescribed medications. Sampling is based on a probability multi-stage sampling scheme. The most recent 2 years of data available for both NAMCS and NHAMCS were used (2010-2011) for this analysis. As the focus of our analysis was outpatient antibiotic use, visits that resulted in hospital or observation unit admission were excluded.

The first phase of the Human Microbiome Project (HMP) consisted of collecting microbiome samples from 300 healthy individuals between the ages of 18 and 40 at multiple timepoints and across five major body sites, including nasal passages, oral cavity, skin, gastrointestinal tract, and urogenital tract. Microbial composition was characterized using MetaPhlAn2 (30), a method for taxonomic profiling of whole-metagenomic shotgun samples. Prevalence estimates from HMP data were based on presence of the species at any body site. For children under 5 years old, carriage prevalences were compiled from primary sources in the literature (Supplemental Table 1). As individual carriage studies tended to collect samples from only one body site, carriage prevalences at each body site were estimated as an average across studies weighted by sample size, and overall prevalence was calculated assuming independence at each body site. This process was also used to estimate carriage prevalences of *S. pyogenes* and *S. pneumoniae* in the >5 age group, as MetaPhlAn2 did not distinguish between these and closely related species (e.g. *S. mitis*, *S. oralis*). Etiologies for conditions of interest were based on etiologic studies cited in the medical resource UpToDate (Supplemental Table 3).

Calculations of bystander proportions. A bystander exposure was defined as a prescription of antibiotic *a* received by an individual carrying species *s* for a diagnosis of condition *c* that was not caused by *s*. Exposures were estimated on average at the population-level. Let B_{as} be the proportion of bystander

exposures of antibiotic *a* received by species *s*, equivalent to one minus the ratio of N_{as}, the number of exposures of antibiotic *a* received by species *s* for a case of condition *c* caused by species *s*, and T_{as}, the total number of exposures of antibiotic *a* received by species *s*. Additionally, let d_{acg} be the number of prescriptions of antibiotic *a* written for condition *c* in age group *g*, let p_{sg} be the proportion of the population colonized with species *s* in age group *g*, and let e_{scg} be the proportion of cases of condition *c* caused by species *s* in age group *g*. Since p_{sg} and e_{scg} were collected from different data sources, p_{scg} was modified to $e_{scg} + (1 - e_{scg})p_{sg}$ so that $e_{scg} \leq p_{scg}$ for every condition and species of interest. Since the inputs d_{acg}, e_{scg}, and p_{sg} may be highly variable by age, estimates were summed over three age strata *g* (<1 year old, 1-5 years old, and over 5 years old). The proportion of bystander exposures for antibiotic *a*, species *s*, and condition *c* were calculated as follows:

$$B_{as} = 1 - \frac{N_{as}}{T_{as}}$$

$$T_{as} = \sum_{g=1}^{G} \sum_{c=1}^{C} d_{acg} \times p_{scg}$$

$$N_{as} = \sum_{g=1}^{G} \sum_{c=1}^{C} d_{acg} \times e_{scg}$$

Conditions were based on diagnostic categories by Fleming-Dutra et al. (2) with the following exceptions: 1) “Other bacterial infections” includes “miscellaneous bacterial infections” and other intestinal infectious diseases (ICD-9CM codes: 001-008), but excludes a subset of infectious diseases (041, 130-139), mastoiditis (383), and peritonsillar abscess (475); 2) we include only cellulitis (681-682) instead of the category “Skin, cutaneous and mucosal infections”; 3) we include viral pneumonia (480) with “Pneumonia”. Bystander exposure calculations included the set of conditions, *C*, for which antibiotic use

was relatively high (>2% of weighted prescriptions; viral upper respiratory tract infection contributed the most, at 10% of weighted prescriptions) and reasonable estimates of e_{scg} were available. When diagnoses were excluded, this was most often due to one of these two limitations. Influenza was also included due to clear etiology and vaccination-related interest.

The proportion of total exposures of antibiotic *a* received by species *s* and associated with a given condition $c^{\star}$ was calculated as:

$$\frac{\Sigma_{g=1}^{G} d_{ac^{\star}g} \times p_{sc^{\star}g}}{\Sigma_{g=1}^{G}\Sigma_{c=1}^{C} d_{acg} \times p_{scg} + \Sigma_{g=1}^{G} d_{a\bar{c}g} \times p_{sg}} = \frac{\Sigma_{g=1}^{G} d_{ac^{\star}g} \times p_{sc^{\star}g}}{T_{as} + \Sigma_{g=1}^{G} d_{a\bar{c}g} \times p_{sg}}$$

The second term in the denominator was added to account for exposures of antibiotic *a* that were not associated with any of our conditions of interest, where $d_{a\bar{c}g}$ represents prescriptions of antibiotic *a* that occur at visits unassociated with any of our conditions of interest. The use of p_s in this term implies that our species of interest are rarely, if ever, causative agents for conditions that are not included in our analysis.

Confidence intervals were estimated by simulation. Variances were estimated for d_{acg} using the `survey` package in R (31). For HMP prevalence estimates, with A presences and B absences, random draws were simulated from a beta distribution with parameters (A+0.5, B+0.5), the posterior distribution using Jeffreys prior. Resampling was done similarly for etiological fractions. The proportion of bystander exposures was calculated for 1000 iterations of random draws of d_{acg}, p_{scg}, and e_{scg}. The 2.5th and 97.5th percentiles were utilized as the bounds of the 95% confidence interval.

Impact of vaccine. To approximate the impact of a vaccine in reducing antimicrobial exposure of nontargeted species (e.g. *E. coli* for a pneumococcal vaccine) we initially assume as an input the observed reduction r in all-cause antimicrobial use in a particular age group, such as the 5.4% reduction in all-cause antibiotic use in a randomized pneumococcal conjugate vaccine trial in 0-2 year-olds (which we approximate with the average values from 0-1 and 1-5). We reason as follows:

Table 1 shows the possible combinations of presence/absence of E. coli in a treated patient, and E. coli as cause or not cause of the treatment. One cell (absent, but causal) is empty because by assumption the species must be present to cause treatment. Let A, B, and D represent proportions of all treatments so $A + B + D = 1$. In our example, the total treatment reduction is r=0.054 of all treatments. But this is unequally apportioned.

All of the reduction is in categories A and B, because we assume that PCV would have no effect on the rate of treatment for a disease that was caused by *E. coli*.

Define p_{Ec} as the prevalence of E. coli in the microbiome data for the relevant age group. Then by our modeling assumptions, $p_{Ec} = \frac{B}{A+B}$. Thus, the amount of treatment reduction in category B is $r\frac{B}{A+B} = rp_{Ec}$.

We seek the proportional reduction in $B + D$, the exposure of *E. coli* to treatment. D is unchanged, so the reduction is $\frac{rp_{Ec}}{B+D} = \frac{rp_{Ec}}{1-A}$. Defining the proportion of bystander exposures for *E. coli* to all antibiotics as $B_{all,Ec} = \frac{B}{B+D} = \frac{B}{1-A}$, some algebra yields the quantity we seek, the reduction in *E. coli*'s total (causal plus bystander) exposure to antibiotics attributable to a reduction r in all-cause antibiotic treatment from a vaccine that prevents no disease caused by *E. coli*:

$$\frac{rp_{Ec}}{1-A} = r(p_{Ec} + B_{\text{all},Ec} - p_{Ec}B_{\text{all},Ec}).$$

Analogous calculations can be made for any other bacterial species for which disease is not reduced by the vaccine. For pathogens (e.g. *H. influenzae*) in which vaccination may cause a reduction in the amount of disease they cause (e.g. through indirectly preventing non-pneumococcal otitis media (9, 10)), this estimate would be a lower bound.

Table 1. Classifying all-cause antibiotic treatments with respect to a potential bystander species, *E. coli*, as present or not, and cause of treatment or not.

	E. coli present in treated patient		
E.coli is the cause of treatment		-	+
	-	A	B
	+		D

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Conflicts of Interest

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References

1. Lipsitch M, Samore MH (2002) Antimicrobial Use and Antimicrobial Resistance: A Population Perspective. *Emerg Infect Dis* 8(4):347–354.

2. Fleming-Dutra KE, et al. (2016) Prevalence of Inappropriate Antibiotic Prescriptions Among US Ambulatory Care Visits, 2010-2011. *JAMA* 315(17):1864–1873.

3. Blaser MJ (2016) Antibiotic use and its consequences for the normal microbiome. *Science* 352(6285):544–545.

4. Smieszek T, et al. (2018) Potential for reducing inappropriate antibiotic prescribing in English primary care. *J Antimicrob Chemother* 73(suppl_2):ii36–ii43.

5. Fireman B, et al. (2003) Impact of the pneumococcal conjugate vaccine on otitis media. *Pediatr Infect Dis J* 22(1):10–16.

6. Lau WCY, et al. (2015) Impact of pneumococcal conjugate vaccines on childhood otitis media in the United Kingdom. *Vaccine* 33(39):5072–5079.

7. Atkins KE, et al. (2017) Use of mathematical modelling to assess the impact of vaccines on antibiotic resistance. *Lancet Infect Dis*. doi:10.1016/S1473-3099(17)30478-4.

8. Lipsitch M, Siber GR (2016) How Can Vaccines Contribute to Solving the Antimicrobial Resistance Problem? *MBio* 7(3). doi:10.1128/mBio.00428-16.

9. Dagan R, Pelton S, Bakaletz L, Cohen R (2016) Prevention of early episodes of otitis media by pneumococcal vaccines might reduce progression to complex disease. *Lancet Infect Dis* 16(4):480–492.

10. Ben-Shimol S, et al. (2016) Impact of Widespread Introduction of Pneumococcal Conjugate Vaccines on Pneumococcal and Nonpneumococcal Otitis Media. *Clin Infect Dis* 63(5):611–618.

11. Cobey S, et al. (2017) Host population structure and treatment frequency maintain balancing selection on drug resistance. *J R Soc Interface* 14(133). doi:10.1098/rsif.2017.0295.

12. Bonhoeffer S, Lipsitch M, Levin BR (1997) Evaluating treatment protocols to prevent antibiotic resistance. *Proc Natl Acad Sci U S A* 94(22):12106–12111.

13. Davies NG, Flasche S, Jit M, Atkins KE (2017) Within-host dynamics explain the coexistence of antibiotic-sensitive and resistant bacteria. *bioRxiv*. Available at: https://www.biorxiv.org/content/early/2017/11/10/217232.abstract.

14. Goyal MK, et al. (2017) Racial and Ethnic Differences in Antibiotic Use for Viral Illness in Emergency Departments. *Pediatrics* 140(4). doi:10.1542/peds.2017-0203.

15. Loeb M (2010) Community-acquired pneumonia. *BMJ Clin Evid* 2010. Available at: https://www.ncbi.nlm.nih.gov/pubmed/21418681.

16. Shen G-H, et al. (2012) Does empirical treatment of community-acquired pneumonia with fluoroquinolones delay tuberculosis treatment and result in fluoroquinolone resistance in Mycobacterium tuberculosis? Controversies and solutions. *Int J Antimicrob Agents* 39(3):201–205.

17. von Gottberg A, et al. (2008) Emergence of levofloxacin-non-susceptible Streptococcus pneumoniae and treatment for multidrug-resistant tuberculosis in children in South Africa: a cohort observational surveillance study. *Lancet* 371(9618):1108–1113.

18. Maier L, et al. (2018) Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*. doi:10.1038/nature25979.

19. Swedres-Svarm (2014) Consumption of antibiotics and occurrence of antibiotic resistance in

Sweden. Solna/Uppsala ISSN 1650-6332.

20. Public Health Agency of Canada (2017) Canadian Antimicrobial Resistance Surveillance System 2017 Report.

21. Ferrer M, Méndez-García C, Rojo D, Barbas C, Moya A (2017) Antibiotic use and microbiome function. *Biochem Pharmacol* 134:114–126.

22. Lange K, Buerger M, Stallmach A, Bruns T (2016) Effects of Antibiotics on Gut Microbiota. *Dig Dis* 34(3):260–268.

23. Dagan R, Barkai G, Leibovitz E, Dreifuss E, Greenberg D (2006) Will reduction of antibiotic use reduce antibiotic resistance?: The pneumococcus paradigm. *Pediatr Infect Dis J* 25(10):981–986.

24. Samore MH, et al. (2006) Mechanisms by which antibiotics promote dissemination of resistant pneumococci in human populations. *Am J Epidemiol* 163(2):160–170.

25. Feikin DR, et al. (2000) Increased carriage of trimethoprim/sulfamethoxazole-resistant Streptococcus pneumoniae in Malawian children after treatment for malaria with sulfadoxine/pyrimethamine. *J Infect Dis* 181(4):1501–1505.

26. Høiby N, Pers C, Johansen HK, Hansen H, The Copenhagen Study Group on Antibiotics in Sweat (2000) Excretion of β-Lactam Antibiotics in Sweat—a Neglected Mechanism for Development of Antibiotic Resistance? *Antimicrob Agents Chemother* 44(10):2855–2857.

27. Strausbaugh LJ, Bolton WK, Dilworth JA, Guerrant RL, Sande MA (1976) Comparative pharmacology of josamycin and erythromycin stearate. *Antimicrob Agents Chemother* 10(3):450–456.

28. Høiby N, et al. (1997) Excretion of ciprofloxacin in sweat and multiresistant Staphylococcus epidermidis. *Lancet* 349(9046):167–169.

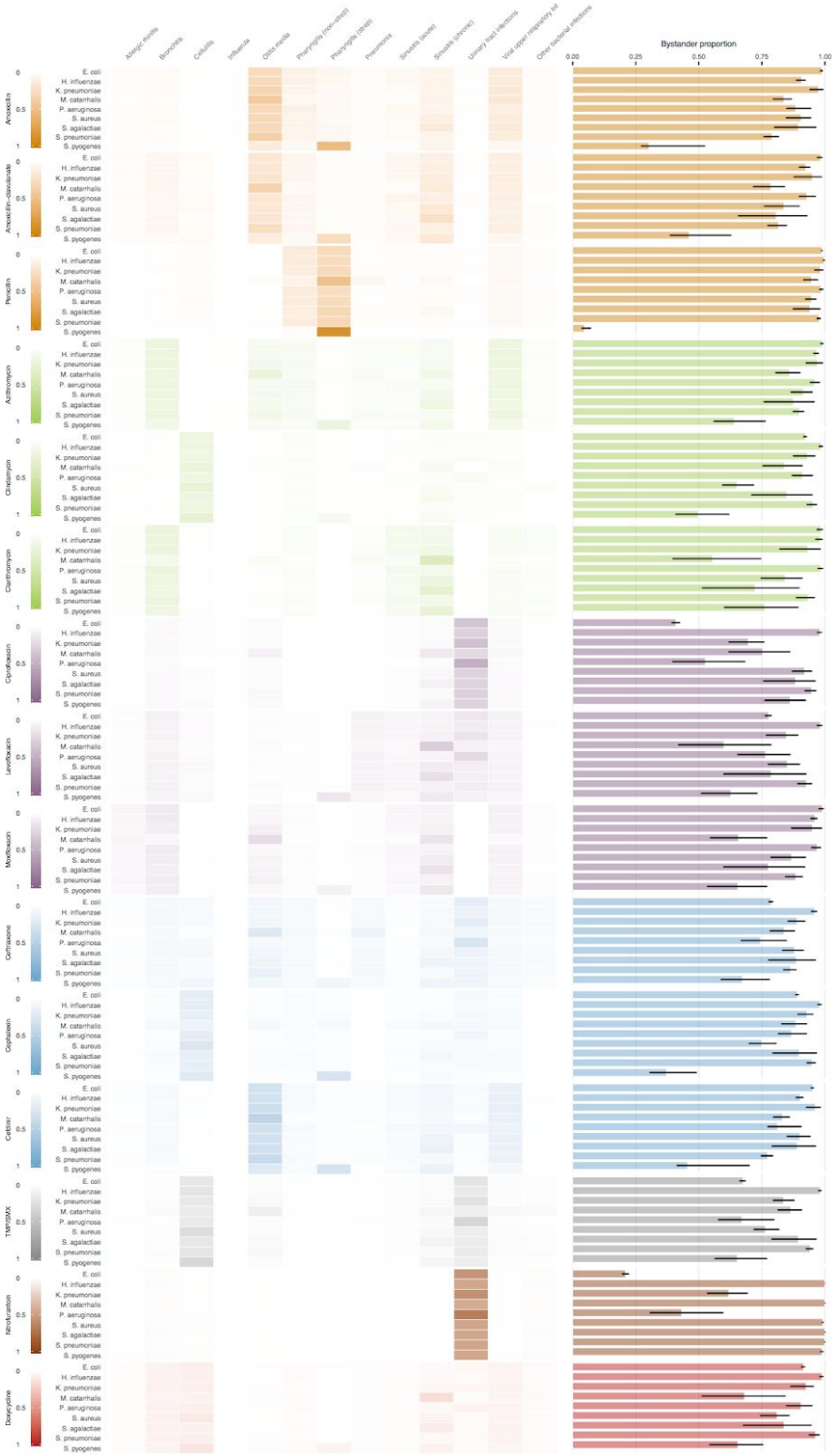
29. Andersson DI, Hughes D (2014) Microbiological effects of sublethal levels of antibiotics. *Nat Rev Microbiol* 12(7):465–478.

30. Truong DT, et al. (2015) MetaPhlAn2 for enhanced metagenomic taxonomic profiling. *Nat Methods* 12(10):902–903.

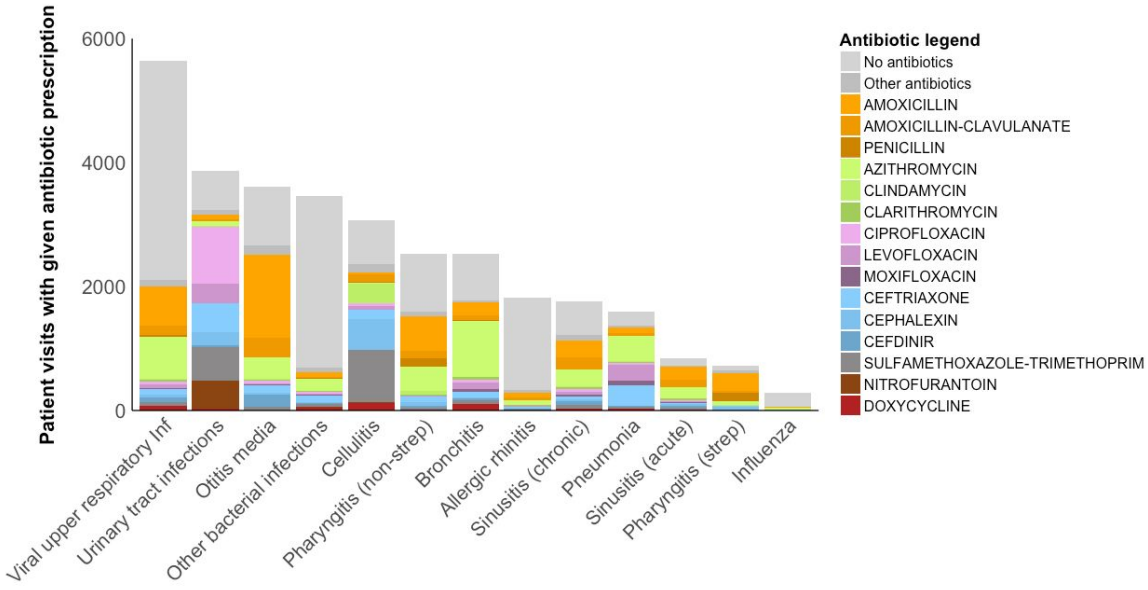
31. Lumley T (2016) survey: analysis of complex survey samples. R package version 3.32.

Supporting information

Supplemental Figure 1. Total exposures by antibiotic, species, and condition, and proportion of bystander exposures over all conditions by antibiotic and species.



Supplemental Figure 2. Number of sampled outpatient visits (unweighted) from NAMCS/NHAMCS 2010-2011 with given diagnosis and antibiotic prescription.



Supplemental Table 1. Carriage studies used to characterize microbial prevalences for which HMP data was unavailable. In addition to prevalences among children <5 years old, additional carriage studies were also used for *S. pyogenes* and *S. pneumoniae* in the >5-year-old age group as taxonomic profiling of HMP data via MetaPhlAn2 does not distinguish between these and similar species. Specific studies were not identified for *P. aeruginosa* and *S. agalactiae* for children from 1 to 5 years old; the prevalences among children under 1 year old were imputed in these cases.

Article	Age group	Body site	Organisms
Bäckhed et al. 2015 (1)	<1 year old	Gastrointestinal	*P. aeruginosa* *S. agalactiae*
Bogaert et al. 2011 (2)	1-5 years old	Nasopharyngeal	*H. influenzae*
Mainous et al. 2006 (3)	1-5 years old	Nasopharyngeal	*S. aureus*
Regev-Yochay et al. 2004 (4)	<1 year old 1-5 years old	Nasopharyngeal	*S. aureus* *S. pneumoniae*
Verhaegh et al. 2010 (5)	<1 year old 1-5 years old	Nasopharyngeal	*M. catarrhalis*
Pettigrew et al. 2012 (6)	<1 year old 1-5 years old	Upper respiratory tract	*H. influenzae* *M. catarrhalis* *S. pneumoniae*

Holgerson et al. 2015 (7)	<1 year old 1-5 years old	Oral	*E. coli* *H. influenzae* *K. pneumoniae* *S. aureus* *S. pyogenes*
Yassour et al. 2016 (8) (DIABIMMUNE cohort)	<1 year old 1-5 years old	Gastrointestinal	*E. coli* *H. influenzae* *K. pneumoniae* *S. aureus*
Ginsburg et al. 1985 (9)	All	Throat	*S. pyogenes*
Gunnarsson et al. 1997 (10)	All	Throat	*S. pyogenes*
Hammitt et al. 2006 (11)	All	Nasopharyngeal	*S. pneumoniae*
Huang et al. 2009 (12)	All	Nasopharyngeal	*S. pneumoniae*

Supplemental Table 2. Carriage prevalence estimates by age group and species from HMP and sources shown in Supplemental Table 1.

Species	<1 year old	1-5 years old	>5 years old
E. coli	94.9%	100%	66.3%
H. influenzae	100%	95.9%	68.6%
K. pneumoniae	39.1%	15.0%	7.4%
M. catarrhalis	45.5%	50.8%	2.3%
P. aeruginosa	1.4%	1.4%	1.9%
S. aureus	35.0%	19.1%	12.4%
S. agalactiae	8.2%	8.2%	2.7%
S. pneumoniae	64.3%	64.6%	25.2%
S. pyogenes	1.1%	4.4%	4.7%

Supplemental Table 3. Estimated etiologies by condition. Conditions in which none of our species of interest are causative agents are excluded. If two numbers are shown, the number to the left was applied to children under 5 years old, and the number to the right was applied to individuals over 5. Diagnoses with etiology specified by ICD-9CM code (e.g. 481: pneumococcal pneumonia) were attributed to the appropriate organism.

Species	Cellulitis (13)	Pharyngitis (non-strep) (14)	Pneumonia (15, 16)	Sinusitis (acute) (17)	Sinusitis (chronic) (18)	Strep throat	Otitis media (suppurative) (19, 20)	UTI (21, 22)
E. coli	-	-	-	-	2.9%	-	-	75% | 78.5%
H. influenzae	-	-	- | 0.6%	0.7%	4.4%	-	23% | 26%	-
K. pneumoniae	-	-	-	-	2.9%	-	-	4.7% | 4.8%
M. catarrhalis	-	-	-	0.1%	11.8%	-	14% | 3%	-
P. aeruginosa	-	-	- | 0.4%	-	-	-	-	2.3% | 2.7%
S. aureus	8%	-	- | 1.6%	0.1%	11.8%	-	1% | 3%	-
S. agalactiae	-	-	-	-	5.9%	-	-	-
S. pneumoniae	-	-	27% | 5.1%	0.8%	5.9%	-	35% | 21%	-
S. pyogenes	4.3%	-	- | 0.3%	-	7.4%	100%	3% | 3%	-

References

1. Bäckhed F, et al. (2015) Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe* 17(5):690–703.

2. Bogaert D, et al. (2011) Variability and diversity of nasopharyngeal microbiota in children: a metagenomic analysis. *PLoS One* 6(2):e17035.

3. Mainous AG 3rd, Hueston WJ, Everett CJ, Diaz VA (2006) Nasal carriage of Staphylococcus aureus and methicillin-resistant S aureus in the United States, 2001-2002. *Ann Fam Med* 4(2):132–137.

4. Regev-Yochay G, et al. (2004) Association between carriage of Streptococcus pneumoniae and Staphylococcus aureus in Children. *JAMA* 292(6):716–720.

5. Verhaegh SJC, et al. (2010) Determinants of Moraxella catarrhalis colonization in healthy Dutch children during the first 14 months of life. *Clin Microbiol Infect* 16(7):992–997.

6. Pettigrew MM, et al. (2012) Upper respiratory tract microbial communities, acute otitis media pathogens, and antibiotic use in healthy and sick children. *Appl Environ Microbiol* 78(17):6262–6270.

7. Lif Holgerson P, Holgerson PL, Öhman C, Rönnlund A, Johansson I (2015) Maturation of Oral Microbiota in Children with or without Dental Caries. *PLoS One* 10(5):e0128534.

8. Yassour M, et al. (2016) Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci Transl Med* 8(343):343ra81.

9. Ginsburg CM, et al. (1985) Seroepidemiology of the group-A streptococcal carriage state in a private pediatric practice. *Am J Dis Child* 139(6):614–617.

10. Gunnarsson RK, Holm SE, Söderström M (1997) The prevalence of beta-haemolytic streptococci in throat specimens from healthy children and adults. Implications for the clinical value of throat cultures. *Scand J Prim Health Care* 15(3):149–155.

11. Hammitt LL, et al. (2006) Indirect effect of conjugate vaccine on adult carriage of Streptococcus pneumoniae: an explanation of trends in invasive pneumococcal disease. *J Infect Dis* 193(11):1487–1494.

12. Huang SS, et al. (2009) Continued Impact of Pneumococcal Conjugate Vaccine on Carriage in Young Children. *Pediatrics* 124(1):e1–e11.

13. Chira S, Miller LG (2010) Staphylococcus aureus is the most common identified cause of cellulitis: a systematic review. *Epidemiol Infect* 138(3):313–317.

14. Huovinen P, et al. (1989) Pharyngitis in adults: the presence and coexistence of viruses and bacterial organisms. *Ann Intern Med* 110(8):612–616.

15. Jain, Seema Self, Wesley H. Wunderink, Richard G. Fakhran, Sherene Balk, Robert Bramley, Anna M. Reed, Carrie Grijalva, Carlos G. Anderson, Evan J. Courtney, Mark Chappell, James D. Qi, Chao (2015) Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. *N Engl J Med* 373:415–427.

16. Wubbel L, et al. (1999) Etiology and treatment of community-acquired pneumonia in ambulatory children. *Pediatr Infect Dis J* 18(2):98–104.

17. Gwaltney JM Jr, Scheld WM, Sande MA, Sydnor A (1992) The microbial etiology and antimicrobial therapy of adults with acute community-acquired sinusitis: a fifteen-year experience at the University of Virginia and review of other selected studies. *J Allergy Clin Immunol* 90(3 Pt 2):457–61; discussion 462.

18. Brook I, Thompson DH, Frazier EH (1994) Microbiology and management of chronic maxillary sinusitis. *Arch Otolaryngol Head Neck Surg* 120(12):1317–1320.

19. Celin SE, Bluestone CD, Stephenson J, Yilmaz HM, Collins JJ (1991) Bacteriology of acute otitis media in adults. *JAMA* 266(16):2249–2252.

20. Bluestone CD, Stephenson JS, Martin LM (1992) Ten-year review of otitis media pathogens. *Pediatr Infect Dis J* 11(Supplement):S7–11.

21. Edlin RS, Shapiro DJ, Hersh AL, Copp HL (2013) Antibiotic Resistance Patterns of Outpatient Pediatric Urinary Tract Infections. *J Urol* 190(1):222–227.

22. Gupta K, Scholes D, Stamm WE (1999) Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. *JAMA* 281(8):736–738.