Antibiotic Use in Relation to the Risk of Breast Cancer

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Context Use of antibiotics may be associated with risk of breast cancer through effects on immune function, inflammation, and metabolism of estrogen and phytochemicals; however, clinical data on the association between antibiotic use and risk of breast cancer are sparse.

Objective To examine the association between use of antibiotics and risk of breast cancer.

Design, Setting, and Participants Case-control study among 2266 women older than 19 years with primary, invasive breast cancer (cases) enrolled in a large, nonprofit health plan for at least 1 year between January 1, 1993, and June 30, 2001, and 7953 randomly selected female health plan members (controls), frequency-matched to cases on age and length of enrollment. Cases were ascertained from the Surveillance, Epidemiology, and End Results cancer registry. Antibiotic use was ascertained from computerized pharmacy records.

Main Outcome Measure Association between extent of antibiotic use and risk of breast cancer.

Results Increasing cumulative days of antibiotic use were associated with increased risk of incident breast cancer, adjusted for age and length of enrollment. For categories of increasing use (0, 1-50, 51-100, 101-500, 501-1000, and ≥1001 days), odds ratios (95% confidence intervals) for breast cancer were 1.00 (reference), 1.45 (1.24-1.69), 1.53 (1.28-1.83), 1.68 (1.42-2.00), 2.14 (1.60-2.88), and 2.07 (1.48-2.89) ($P<.001$ for trend). Increased risk was observed in all antibiotic classes studied and in a subanalysis having breast cancer fatality as the outcome. Among women with the highest levels of tetracycline or macrolide use, risk of breast cancer was not elevated in those using these antibiotics exclusively for acne or rosacea (indications that could be risk factors for breast cancer due to altered hormone levels), compared with those using them exclusively for respiratory tract infections, adjusted for age and length of enrollment (odds ratio, 0.91; 95% confidence interval, 0.44-1.87).

Conclusions Use of antibiotics is associated with increased risk of incident and fatal breast cancer. It cannot be determined from this study whether antibiotic use is causally related to breast cancer, or whether indication for use, overall weakened immune function, or other factors are pertinent underlying exposures. Although further studies are needed, these findings reinforce the need for prudent long-term use of antibiotics.

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THE HYPOTHESIS THAT USE OF antibiotics may increase risk of cancer was first proposed several decades ago.[1] Biological and epidemiologic studies of this association are limited, and the various mechanisms of action of antibiotics may actually have opposite effects on cancer risk.[2] For example, use of antibiotics reduces the capacity of intestinal microflora to metabolize phytochemicals into compounds that may protect against cancer.[1,3,4] However, antibiotic use also disrupts the intestinal microfloral metabolism of estrogens.[5,6] This results in lower levels of circulating estrogens, which might decrease the risk of some hormonal cancers.[2,5,6] Additionally, use of antibiotics may be associated with cancer risk through effects on immune function and inflammation, although little is known about these mechanisms.[2,7]

The only epidemiologic study of the association between antibiotic use and cancer risk is a cohort study of incident breast cancer in Finland. The investigators found that women younger than 50 years who self-reported previous and/or present antibiotic use for urinary tract infections had an elevated risk of breast cancer, compared with women without such usage (relative risk, 1.74; 95% confidence interval [CI], 1.13-2.68).

For editorial comment see p 880.

Baseline bacteriuria was not associated with subsequent incidence of breast cancer, providing some assurance that antibiotic use, not the underlying infection, was the actual risk factor.[8] Nonetheless, antibiotic exposure was measured only as a binary variable without consideration for antibiotic class, length of use, or use for conditions other than urinary tract infections.

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Table 1. Selected Characteristics of Controls and Cases*

Characteristic	No. (%) Controls (n = 7953)	No. (%) Cases (n = 2266)	*P* Value
Patient Characteristics			
Age at reference date, y			
≤40	471 (5.9)	140 (6.2)	.61
41-50	1647 (20.7)	435 (19.2)	
51-60	1830 (23.0)	516 (22.8)	
61-70	1671 (21.0)	477 (21.1)	
71-80	1599 (20.1)	473 (20.9)	
≥81	735 (9.2)	225 (9.9)	
Education beyond high school	1713 (62.3)	800 (68.6)	<.001
White race	2779 (91.3)	1133 (91.6)	.75
Health Care/Pharmacy Characteristics			
Length of enrollment, y			
1-10	1725 (21.7)	497 (21.9)	.39
11-15	1299 (16.3)	364 (16.1)	
16-20	1421 (17.9)	438 (19.3)	
≥21	3508 (44.1)	967 (42.7)	
No. of annual health care visits†			
0-5	2387 (48.7)	606 (42.5)	<.001
6-10	1808 (36.9)	587 (41.2)	
≥11	709 (14.5)	232 (16.3)	
Pharmacy co-payment status			
Fully covered	3054 (38.7)	812 (35.9)	.11
$1-$5 co-payment	3846 (48.7)	1150 (50.8)	
$6-$15 co-payment	967 (12.3)	289 (12.8)	
No pharmacy coverage	30 (0.4)	11 (0.5)	
Clinical Characteristics			
Age at menarche, y			
<11	285 (4.7)	107 (5.9)	.01
11-14	5056 (83.1)	1513 (83.7)	
≥15	744 (12.2)	187 (10.4)	
Parous	5305 (85.6)	1618 (84.5)	.23
Age at first birth >30 y	296 (12.9)	149 (15.5)	.05
Body mass index‡			
<18.5	103 (1.7)	24 (1.3)	.004
18.5-24.9	2815 (46.7)	797 (42.4)	
25.0-29.9	1796 (29.8)	612 (32.6)	
≥30.0	1311 (21.8)	445 (23.7)	
First-degree family history of breast cancer	900 (14.5)	371 (19.5)	<.001
High mammographic breast density§	1214 (42.8)	733 (61.9)	<.001
Hysterectomy	1942 (31.4)	555 (29.9)	.24
Postmenopausal	5963 (85.0)	1645 (78.7)	<.001
Age at menopause ≥50 y	1993 (40.1)	604 (42.3)	.13
Oral contraceptive use‖	2867 (46.7)	1012 (54.6)	<.001
Postmenopausal hormone use‖	4539 (65.9)	1297 (64.7)	.32

(continued)

Understanding whether an association between antibiotic use and breast cancer exists is particularly important given the high incidence of breast cancer and widespread antibiotic use in many countries. Breast cancer is the most frequently diagnosed nonskin malignancy and the second leading cause of cancer mortality in US women.[9] It is also the most common cancer in women worldwide, with more than 1 million cases diagnosed each year.[10] Antibiotics are used extensively and overused in many countries, though efforts are under way to curb overuse.[11] In the United States, more than 22.6 million antibiotic prescriptions for nonbacterial acute respiratory infections were filled in 1995 alone.[12]

The objective of this case-control study was to determine if an association exists between antibiotic use and risk of breast cancer in a sample of 10219 women enrolled at Group Health Cooperative (GHC), a large, nonprofit health plan in western Washington State. Although the biological mechanisms through which antibiotics might alter cancer risk may also be relevant to other cancers, we selected breast cancer for this study because it is the topic of the only other epidemiologic study of antibiotic use and cancer risk, and it is an important cancer in women.

METHODS

Selection of Study Participants

In this case-control study, all participants were selected from women enrolled in GHC continuously for at least 1 year before their reference date and for whom computerized pharmacy records were available. The reference date for each case was the date of breast cancer diagnosis. The reference date for each control was a randomly selected date from the calendar years in which the cases were diagnosed. Cases were all women older than 19 years who were newly diagnosed with primary, invasive breast cancer between January 1, 1993, and June 30, 2001, identified through the Seattle-Puget Sound Surveillance, Epidemiology, and End Results cancer registry. Control participants were randomly selected from GHC enrollment files during the years the cases were diagnosed and were frequency matched to cases at a ratio of 3:1 on birth year (5-year intervals) and duration of GHC enrollment with computerized pharmacy records available (≤10 years, 11-15 years, 16-20 years, and ≥21 years of enrollment).

We identified 2266 cases of primary, invasive breast cancer and 385 cases of in situ breast cancer. After exclusion of the in situ breast cancers, the control: case matching ratio was slightly greater

than 3:1, for a total of 7953 controls (TABLE 1). The project was approved by the GHC human subjects review committee. Each study participant was assigned a unique study code number; no personal identifiers were used in the analysis.

Table 1. Selected Characteristics of Controls and Cases* (cont)

Characteristic	No. (%) Controls (n = 7953)	No. (%) Cases (n = 2266)	*P* Value
Clinical Characteristics (cont)			
No. of postmenopausal hormone prescriptions¶			
0	3798 (47.8)	1059 (46.7)	<.001
1-25	2352 (29.6)	593 (26.2)	
26-50	868 (10.9)	286 (12.6)	
≥51	935 (11.8)	328 (14.5)	

*Data were 100% complete for age, length of enrollment, co-payment status, and number of health care visits. The percentages of missing data for other variables for controls and cases, respectively, were: education (65%, 49%), race (62%, 45%), age at menarche (23%, 20%), parity (22%, 15%), age at first birth among parous women (57%, 41%), body mass index (24%, 17%), family history (22%, 16%), breast density (64%, 48%), hysterectomy (22%, 18%), menopausal status (12%, 8%), age at menopause (17%, 13%), oral contraceptive use (22%, 18%), ever use of postmenopausal hormones (13%, 12%); number of postmenopausal hormone prescriptions (100% in cases and controls: 4857 women with no postmenopausal hormone prescriptions were assumed to be nonusers).
†Average annual number of primary care and specialty health care visits over a 5-year period prior to the reference date, among the 6329 women continuously enrolled for at least 5 years between 1990 and 2001.
‡Calculated as weight in kilograms divided by the square of height in meters.
§In upper 2 Breast Imaging Reporting and Data System categories.
‖Ever use, from self-report questionnaire and pharmacy database.
¶From pharmacy database only.

Antibiotic Use

Data on antibiotic use were obtained from the GHC pharmacy database, which began in 1977. In this database, each prescription fill is a separate record including the patient identifier, drug name and strength, dosage form, date dispensed, quantity dispensed, and dosing instructions (eg, "take 2 tablets 4 times a day"). Studies of samples of GHC members indicate that 97% to 98% of participants fill all or almost all (90%-100%) their prescriptions at GHC pharmacies, suggesting a highly complete database.[13] We limited our study to antimicrobial anti-infective agents, excluding antiviral, antifungal, antimalarial, and antituberculin agents. We included all dosage forms, except mouth rinses and topical forms, because we were interested in intestinal effects and systemic absorption of antibiotics.

We selected 2 measures of antibiotic exposure: the cumulative number of days of antibiotic use and the total number of antibiotic prescriptions for each study participant, as ascertained from the computerized pharmacy database over all years of enrollment prior to each participant's reference date, as far back as 1977. After ascertaining that results were not sensitive to reasonable variations in the categorization of antibiotic use, such as tertiles, quartiles, or quintiles, we chose categories of antibiotic use that roughly corresponded to what clinicians might consider "lower use," "moderate use," and "higher use."

To estimate the days of use for each prescription, we divided the quantity of antibiotic prescribed by the quantity intended to be taken per day. We summed the days of use across the 8 most common classes of antibiotic prescriptions used by study participants (representing 97% of all antibiotic prescriptions) to obtain the total cumulative days of use for each participant. We also separately estimated each participant's total number of days of use for each of the 6 most common antibiotic classes, which represented the classes with sufficient use to separately estimate risk.

Dosing instructions were available for 48 948 (46%) of 106 663 unique antibiotic prescriptions representing the 8 most common antibiotic classes filled by study participants. The remaining 57 715 prescriptions with missing or incomplete dosing instructions represented 1820 unique combinations of drug name, strength, quantity of pills, and, when available, dosing instructions. An experienced GHC pharmacist (C.A.R.), blinded to case-control status, imputed days of use for these 1820 unique combinations, using the drug name, strength, quantity prescribed, and her extensive knowledge of prescription practices. The imputed days of use were then added into the total days of use variables for each participant.

The proportion of cases and controls with prescriptions requiring imputation was similar. Imputations were required for all antibiotic classes, ranging from a low of 39% for cephalosporins to a high of 80% for nitrofurantoins. The proportion of antibiotic prescriptions requiring imputation increased somewhat across increasing levels of antibiotic use; among women using antibiotics for 1 to 50 days, 51% of prescriptions required imputation, while among those using antibiotics for more than 1000 days, 57% of prescriptions required imputation. For each antibiotic class, the modal number of days of use was 10 for both the imputed days and the days calculated from the pharmacy database.

Other Factors

The known and suspected risk factors for breast cancer, and on other factors relevant to this analysis, included age, level of education, race, length of enrollment at GHC, number of primary and specialty health care visits, pharmacy co-payment status, age at menarche, parity, age at first birth, body mass index, first-degree family history of breast cancer, mammographic breast density, prior hysterectomy, menopausal status, age at menopause, and use of oral contraceptives and postmenopausal hormones. Information on these factors was obtained from the GHC Breast Cancer Surveillance program questionnaire, enrollment files, cost and utilization files (available from 1990 on), and the pharmacy database.

Since 1986, the Breast Cancer Surveillance Program questionnaire has been sent to all female members of GHC when they turn 40 years old and to all new enrollees older than 40 years. Approximately 85% of GHC members who

are at least 40 years old complete the questionnaire, and it is updated at each mammography visit.[14,15] In addition, beginning in 1996, any woman younger than 40 years who has a mammogram also completes the questionnaire. For this study, data on risk factors were obtained from the most recent questionnaire completed prior to each participant's reference date. When menopausal status was missing from the questionnaire, we classified women older than 55 years at the reference date as postmenopausal.

To determine "ever use" of oral contraceptives and postmenopausal hormones, we accepted either self-reported use of oral contraceptives or postmenopausal hormones (from the surveillance questionnaire) or at least 1 oral contraceptive or postmenopausal hormone prescription in the pharmacy database. Information on duration of postmenopausal hormone use was not available; therefore, the total number of postmenopausal hormone prescriptions was evaluated as a separate risk factor to approximate length of use.

Medical Record Reviews

It is possible that indication for antibiotic use, rather than antibiotic use itself, is the pertinent underlying exposure. To assess the evidence that indication for antibiotic use was associated with risk of breast cancer, we conducted a substudy of women in our data set with more than 100 cumulative days of tetracycline use and/or more than 50 cumulative days of macrolide use. We selected tetracyclines and macrolides because they are used for a variety of chronic conditions. Of 330 study participants (90 cases and 240 controls) fitting the antibiotic use criteria, 308 medical records were reviewed to identify indication for antibiotic prescription; 7 records were unavailable and 15 had no information pertaining to antibiotic use, which can occur if a patient uses another provider for health care but GHC pharmacies for prescription fills, or if a telephoned prescription request is not followed up with a note in the medical records, as in some dental prescriptions.

If women with high use of antibiotics for acne or rosacea had elevated risk of breast cancer in comparison to women with high use of antibiotics for respiratory tract infections, then we would have some evidence that underlying medical conditions associated with hormonal imbalances might be pertinent underlying risk factors. We compared women with at least 50 days of tetracycline or macrolide use exclusively for acne or rosacea with women with at least 50 days of use exclusively for respiratory tract infections. These were the only indications occurring with sufficient frequency for a quantitative analysis.

Statistical Analyses

χ^2 Tests were used for categorical variables and analysis of variance for continuous variables. Pearson correlation coefficients were used to evaluate concordance between the total number of antibiotic prescriptions and cumulative days of antibiotic use. We used unconditional logistic regression to estimate the relative risk of breast cancer associated with antibiotic use and to perform tests for trend. All logistic regression analyses were adjusted for the matching variables (ie, age and length of enrollment), which were modeled as continuous variables. For all analyses, we estimated odds ratios (ORs) separately for premenopausal and postmenopausal women but found no appreciable differences in results and therefore present only the combined analyses.

Death due to breast cancer was examined separately as an outcome. The rationale was that women who obtain mammograms may be more likely to have their breast cancer diagnosed earlier than women who do not obtain mammograms. Women obtaining mammograms may be less likely to die from breast cancer due to the advantages of early detection and treatment. It also is possible that these women may utilize more health care in general and may be more likely to fill antibiotic prescriptions than women who do not obtain mammograms. If antibiotic use is only a proxy for health care–seeking or detection by mammography, we would expect an attenuated association between antibiotic use and fatal breast cancer. Using *International Classification of Diseases, Ninth Revision* codes from GHC death file databases, which are downloaded from Washington State vital statistics databases, we identified breast cancer as the underlying cause of death in 177 cases between 1993 and December 31, 2001.

We examined the sensitivity of the results to various assumptions about the "days of use" antibiotic exposure variable in 8 separate subanalyses: (1) we restricted the analysis to the 5302 women who had at least 15 years of pharmacy data; (2) we restricted the analysis to the 8409 women who filled at least 1 antibiotic prescription during enrollment; (3) we assumed that participants used only 75% of their prescription; (4) we assumed that participants used all of their prescription, but took an average of 75% of the daily dose (thus extending their days of use by 33%); (5) we excluded all antibiotic use within the 2-year period prior to the reference date in an attempt to address the possibility that antibiotic use was associated with prediagnosable breast cancer; (6) similarly, we excluded all antibiotic use within the 4-year period prior to the reference date; (7) we examined risk of breast cancer in relation to only the calculated days of antibiotic use; and (8) we examined risk of breast cancer in relation to only the imputed days of antibiotic use.

Data retrieval was performed using SAS version 8.0 (SAS Institute Inc, Cary, NC), and analyses were performed using Intercooled Stata version 6.0 (Stata Corp, College Station, Tex); $P<.05$ was used to determine statistical significance.

RESULTS

Cases and controls were similar with respect to age, race, length of GHC enrollment with computerized pharmacy data available, pharmacy co-payment status, parity, hysterectomy status, age at menopause, and ever use of postmenopausal hormones (Table 1). A larger propor-

tion of cases than controls were educated beyond high school, had a higher number of health care visits, were premenopausal, had ever used oral contraceptives, and had filled more than 25 postmenopausal hormone prescriptions. Cases were also more likely than controls to have menarche before age 11, first birth after age 30 years, higher body mass index, a first-degree family history of breast cancer, and higher mammographic breast density. As shown in

Table 2. Characteristics of Control Participants and Breast Cancer Cases, by Cumulative Number of Antibiotic Prescriptions*

Characteristic	Total No. of Antibiotic Prescriptions†					*P* Value
	0	1-10	11-25	26-50	≥51	
Control Participants						
Total	1478	4042	1726	522	185	
Age at reference date, mean (SD), y	58.9 (14.4)	61.0 (14.3)	62.3 (14.3)	62.4 (13.9)	62.3 (13.6)	<.001
Education >high school, No. (%)	78 (53.4)	894 (62.1)	498 (63.5)	173 (62.7)	70 (66.7)	.18
White race, No. (%)	217 (84.8)	1423 (90.2)	764 (93.3)	270 (95.1)	105 (98.1)	<.001
Years of enrollment, mean (SD)	9.6 (6.3)	17.9 (6.7)	20.9 (5.1)	21.4 (4.3)	22.8 (3.3)	<.001
No. of health care visits, mean (SD)‡	3.6 (3.1)	5.2 (3.5)	7.6 (4.4)	10.3 (5.9)	12.4 (7.4)	<.001
Pharmacy co-payment >$5, No. (%)	195 (13.6)	458 (11.4)	245 (14.2)	71 (13.6)	28 (15.1)	.02
Age at menarche <11 y, No. (%)	19 (4.1)	145 (4.3)	71 (4.5)	32 (6.6)	18 (10.4)	.001
Parous, No. (%)	395 (83.2)	2945 (85.1)	1390 (86.8)	421 (85.7)	154 (87.0)	.29
Age at first birth>30 y, No. (%)	12 (10.1)	159 (13.2)	95 (14.4)	22 (9.8)	8 (8.8)	.24
Body mass index >24.9, No. (%)§	218 (47.8)	1679 (50.2)	819 (52.4)	290 (59.9)	101 (57.1)	<.001
First-degree family history of breast cancer, No. (%)	57 (12.1)	485 (14.0)	237 (14.8)	81 (16.5)	40 (22.6)	.007
High mammographic breast density, No. (%)‖	71 (48.0)	635 (42.6)	334 (41.4)	124 (44.0)	50 (45.5)	.60
Hysterectomy, No. (%)	133 (27.8)	984 (28.5)	533 (33.4)	209 (42.7)	83 (47.2)	<.001
Postmenopausal, No. (%)	928 (92.2)	3062 (83.2)	1383 (83.9)	432 (86.8)	158 (87.8)	<.001
Age at menopause ≥50 y, No. (%)	152 (39.4)	1128 (41.2)	526 (40.9)	135 (32.9)	52 (35.4)	.02
Oral contraceptive use, No. (%)¶	210 (45.3)	1566 (45.7)	768 (48.5)	238 (48.9)	85 (48.3)	.32
Postmenopausal hormone use, No. (%)¶	281 (31.0)	2422 (66.4)	1255 (76.3)	423 (84.1)	158 (87.8)	<.001
No. of postmenopausal hormone prescriptions, mean (SD)#	2.9 (12.0)	14.9 (25.3)	22.6 (30.7)	28.2 (33.1)	35.9 (37.8)	<.001
Breast Cancer Cases						
Total	332	1164	498	196	76	
Age at reference date, mean (SD), y	61.1 (13.9)	60.7 (14.1)	64.5 (14.2)	62.6 (14.1)	64.5 (13.0)	.001
Education >high school, No. (%)	60 (74.1)	430 (70.4)	186 (64.1)	91 (70.0)	33 (61.1)	.18
White race, No. (%)	91 (87.5)	596 (91.6)	270 (91.2)	124 (93.9)	52 (96.3)	.31
Years of enrollment, mean (SD)	9.5 (6.4)	17.1 (7.0)	20.2 (5.0)	21.4 (4.4)	21.8 (4.1)	<.001
No. of health care visits, mean (SD)‡	5.1 (3.7)	5.4 (3.3)	8.0 (4.3)	10.4 (5.5)	11.3 (7.7)	<.001
Pharmacy co-payment >$5, No. (%)	49 (14.9)	148 (12.7)	69 (13.9)	24 (12.2)	10 (13.2)	.85
Age at menarche <11 y, No. (%)	9 (6.6)	57 (5.9)	27 (6.1)	11 (6.0)	3 (4.3)	.97
Parous, No. (%)	122 (79.2)	851 (82.3)	418 (89.7)	161 (85.2)	66 (90.4)	.001
Age at first birth>30 y, No. (%)	7 (11.9)	73 (15.0)	39 (15.0)	25 (22.9)	5 (10.6)	.19
Body mass index >24.9, No. (%)§	83 (55.3)	558 (55.0)	248 (54.4)	123 (65.4)	45 (64.3)	.05
First-degree family history of breast cancer, No. (%)	26 (17.2)	203 (19.8)	99 (21.4)	27 (14.4)	16 (21.9)	.29
High mammographic breast density, No. (%)‖	53 (69.7)	376 (60.7)	184 (60.9)	87 (65.4)	33 (62.3)	.52
Hysterectomy, No. (%)	34 (23.9)	287 (28.6)	126 (27.8)	73 (39.3)	35 (48.0)	<.001
Postmenopausal, No. (%)	223 (81.1)	826 (76.6)	379 (80.0)	152 (80.4)	65 (87.8)	.09
Age at menopause ≥50 y, No. (%)	48 (48.5)	323 (42.6)	150 (41.6)	61 (41.2)	22 (36.1)	.61
Oral contraceptive use, No. (%)¶	73 (52.5)	551 (55.0)	236 (51.9)	108 (57.1)	44 (64.7)	.29
Postmenopausal hormone use, No. (%)¶	95 (38.3)	638 (61.4)	349 (75.9)	151 (82.1)	64 (87.7)	<.001
No. of postmenopausal hormone prescriptions, mean (SD)#	5.0 (14.6)	15.8 (26.0)	27.0 (34.4)	26.8 (31.7)	37.0 (34.0)	<.001

*See Table 1 footnote regarding completeness of data.
†As of 1977 or start of enrollment, whichever was later.
‡Average annual number of primary care and specialty health care visits over a 5-year period prior to the reference date, for the 6329 women continuously enrolled for at least 5 years between 1990 and 2001. Numbers of women with data across the 5 prescription categories, for controls and cases, respectively: 342 and 98 (for 0 prescriptions); 2606 and 718 (1-10 prescriptions); 1366 and 388 (11-25 prescriptions); 433 and 154 (26-50 prescriptions); 157 and 67 (≥51 prescriptions).
§Body mass index calculated as weight in kilograms divided by the square of height in meters.
‖Upper 2 Breast Imaging Reporting and Data System categories.
¶Ever use, from self-report questionnaire and pharmacy database.
#From pharmacy database only.

TABLE 2, in control participants, increasing cumulative number of prescriptions was associated with older age, white race, longer length of enrollment at GHC, increasing number of health care visits, pharmacy co-payment greater than $5, age at menarche younger than 11 years, higher body mass index, family history of breast cancer, prior hysterectomy, generally younger age at menopause, ever use of postmenopausal hormones, and higher number of postmenopausal hormone prescriptions. In control participants, both never users and high users of antibiotics were more likely to be postmenopausal than were women with low levels of use. Among breast cancer cases, increasing cumulative number of prescriptions was associated with older age, longer length of enrollment at GHC, increasing number of health care visits, parity, higher body mass index, prior hysterectomy, ever use of postmenopausal hormones, and higher number of postmenopausal hormone prescriptions (Table 2).

A total of 110 191 antibiotic prescriptions were dispensed to study participants. Eight classes represented 97% of these prescriptions: macrolides (13.2% of all antibiotic prescriptions), tetracyclines (12.7%), penicillins (31.9%), cephalosporins (11.1%), sulfonamides (18.0%), nitrofurantoins (4.6%), metronidazole (3.5%), and quinolones (1.8%). The most frequently prescribed generic drugs were erythromycin, tetracycline, amoxicillin, penicillin VK, cephalexin, trimethoprim-sulfamethoxazole (included in "sulfonamide" class), and nitrofurantoin.

Cumulative duration of antibiotic use ranged from 0 to 7600 days, and cumulative number of antibiotic prescriptions ranged from 0 to 194. There was excellent correlation between the number of antibiotic prescriptions and the number of days of antibiotic use (Pearson correlation coefficients between 0.82 and 0.91 for each of the 6 most commonly used classes of antibiotics).

For all antibiotic classes considered together, increasing cumulative days of use was associated with increased risk of incident breast cancer after adjustment for age and length of GHC enrollment (TABLE 3). For categories of increasing days of use (0, 1-50, 51-100, 101-500, 501-1000, and ≥1001 days), the ORs (95% CIs) for breast cancer were 1.00 (reference), 1.45 (1.24-1.69), 1.53 (1.28-1.83), 1.68 (1.42-2.00), 2.14 (1.60-2.88), and 2.07 (1.48-2.89) ($P<.001$ for trend). Results were similar when the cumulative number of antibiotic prescriptions was used as the measure of antibiotic exposure (TABLE 4). The increased risk of incident breast cancer was noted for all antibiotic classes. Further adjustment for other variables shown in Table 1 did not materially affect the results. Specifically, additional adjustment for each of these variables typically did not change the risk estimates by more than 2% and in no case changed the risk estimates by more than 5%.

Table 3. Association of Incident Breast Cancer With Cumulative Days of Antibiotic Use*

No. of Days of Antibiotic Use	No. (%) Controls (n = 7948)	No. (%) Cases (n = 2266)	OR (95% CI)†	*P* Value for Trend
Cumulative‡				
0	1478 (18.6)	332 (14.7)	Reference	<.001
1-50	2365 (29.8)	675 (29.8)	1.45 (1.24-1.69)	
51-100	1515 (19.1)	435 (19.2)	1.53 (1.28-1.83)	
101-500	2215 (27.9)	682 (30.1)	1.68 (1.42-2.00)	
501-1000	214 (2.7)	83 (3.7)	2.14 (1.60-2.88)	
≥1001	161 (2.0)	59 (2.6)	2.07 (1.48-2.89)	
Macrolides				
0	1478 (29.8)	332 (23.4)	Reference	<.001
1-50	337 (61.3)	924 (65.1)	1.49 (1.24-1.78)	
51-100	285 (5.8)	96 (6.8)	1.68 (1.25-2.24)	
≥101	153 (3.1)	68 (4.8)	2.26 (1.61-3.18)	
Tetracyclines				
0	1478 (32.4)	332 (25.9)	Reference	<.001
1-50	2381 (52.2)	681 (53.0)	1.55 (1.28-1.87)	
51-100	327 (7.2)	130 (10.1)	2.20 (1.68-2.88)	
≥101	378 (8.3)	141 (11.0)	2.12 (1.63-2.76)	
Penicillins				
0	1478 (22.9)	332 (18.2)	Reference	<.001
1-50	3327 (51.5)	966 (52.8)	1.52 (1.29-1.79)	
51-100	1044 (16.2)	308 (16.8)	1.60 (1.31-1.95)	
≥101	608 (9.4)	223 (12.2)	2.02 (1.62-2.53)	
Cephalosporins				
0	1478 (32.6)	332 (25.9)	Reference	<.001
1-50	2723 (60.0)	824 (64.4)	1.55 (1.30-1.85)	
51-100	232 (5.1)	77 (6.0)	1.70 (1.25-2.32)	
≥101	108 (2.4)	47 (3.7)	2.30 (1.57-3.38)	
Sulfonamides				
0	1478 (27.0)	332 (21.5)	Reference	<.001
1-50	3174 (57.9)	946 (61.3)	1.50 (1.27-1.77)	
51-100	478 (8.7)	147 (9.5)	1.57 (1.23-2.01)	
≥101	349 (6.4)	118 (7.7)	1.72 (1.32-2.24)	
Nitrofurantoins				
0	1478 (57.2)	332 (49.5)	Reference	.006
1-50	901 (34.9)	270 (40.2)	1.44 (1.13-1.84)	
51-100	77 (3.0)	24 (3.6)	1.45 (0.88-2.40)	
≥101	129 (5.0)	45 (6.7)	1.66 (1.11-2.48)	

Abbreviations: CI, confidence interval; OR, odds ratio.
*The reference group for all analyses in this table is made up of all cases and controls in the study population who had no antibiotic prescription fills. A participant appearing as a user of any specific antibiotic class will appear as a user of another specific class only if she had at least 1 prescription fill for that other class.
†ORs adjusted for age and for length of Group Health Cooperative enrollment.
‡Representing the 8 most commonly used antibiotic classes (97% of all antibiotic prescriptions).

When analyses of the association between days of antibiotic use and incident breast cancer were restricted to women with 15 to 24 years of pharmacy data prior to their reference date, an increased risk of incident breast cancer with increasing days of use was still present, though ORs were slightly attenuated and CIs for nitrofurantoins included 1. When the analysis shown in Table 3 was repeated restricted to women who had filled at least 1 antibiotic prescription, an increased risk of incident breast cancer was still present, though the ORs were attenuated for all antibiotic classes (typically by about 30%) and CIs for sulfonamides and nitrofurantoins included 1. The results in Table 3 were essentially unchanged in analyses that assumed either that 75% of the prescription was taken or that 75% of the daily dose was taken but the entire prescription was used, in analyses in which antibiotic use within 2 and 4 years before reference date was excluded, and in analyses in which imputed days of antibiotic use were evaluated separately from calculated days of use.

The association between cumulative days of antibiotic use and death due to breast cancer was strong for all antibiotic classes, controlling for age, length of enrollment, and ever use of postmenopausal hormones (TABLE 5). Further adjustment for other variables shown in Table 1 did not materially affect the results. When these analyses were repeated excluding antibiotic use within 2 and 4 years before the reference date, results were similar.

In a substudy of 308 women with high use of macrolides and tetracyclines, the most common indications for prescriptions were acne and/or rosacea (41%) and respiratory tract infections (20%). Other indications occurred infrequently; the third most common indication was skin infection, which occurred in only 9% of this subset of participants. Risk of incident breast cancer was not elevated in women with at least 50 days of tetracycline or macrolide use exclusively for acne or rosacea (n=136) compared with women with at least 50 days of use exclusively for respiratory tract infections (n=65), after adjusting for age and length of enrollment (OR, 0.91; 95% CI, 0.44-1.87).

COMMENT

In this population-based case-control study, we found that increasing cumulative days of antibiotic use and increasing cumulative number of antibiotic prescriptions were associated with increased risk of incident breast cancer, after controlling for age and length of enrollment. Increasing cumulative days of antibiotic use was also associated with death due to breast cancer, controlling for age, length of enrollment, and ever use of postmenopausal hormones. All classes of antibiotics were associated with increased risk.

Our findings were robust. We estimated similar, though slightly attenuated, risks of incident breast cancer when we restricted our analyses to women with at least 15 years of pharmacy data. When we restricted analyses to women with at least 1 antibiotic prescription, risks of incident breast cancer were still elevated, though attenuated by about 30% in comparison to models with nonusers as the reference group. Varying the assumptions about how antibiotics were actually taken or restricting the analyses to only

Table 4. Association of Incident Breast Cancer With Number of Antibiotic Prescriptions*

No. of Antibiotic Prescriptions	No. (%) Controls (n = 7953)	No. (%) Cases (n = 2266)	OR (95% CI)†	*P* Value for Trend
Total‡				
0	1478 (18.6)	332 (14.7)	Reference	
1-10	4042 (50.8)	1164 (51.4)	1.49 (1.28-1.73)	
11-25	1726 (21.7)	498 (22.0)	1.57 (1.31-1.87)	<.001
26-50	522 (6.6)	196 (8.7)	2.07 (1.66-2.59)	
≥51	185 (2.3)	76 (3.4)	2.31 (1.69-3.15)	
Macrolides				
0	1478 (29.8)	332 (23.4)	Reference	
1-10	3373 (68.1)	1034 (72.8)	1.50 (1.25-1.79)	<.001
≥11	102 (2.1)	54 (3.8)	2.66 (1.83-3.88)	
Tetracyclines				
0	1478 (32.4)	332 (25.9)	Reference	
1-10	2944 (64.5)	898 (69.9)	1.64 (1.36-1.98)	<.001
≥11	142 (3.1)	54 (4.2)	2.14 (1.49-3.07)	
Penicillins				
0	1478 (22.9)	332 (18.2)	Reference	
1-10	4433 (68.7)	1288 (70.4)	1.53 (1.31-1.80)	<.001
≥11	546 (8.5)	209 (11.4)	2.11 (1.68-2.65)	
Cephalosporins				
0	1478 (32.6)	332 (25.9)	Reference	
1-10	2964 (65.3)	912 (71.3)	1.57 (1.31-1.87)	<.001
≥11	99 (2.2)	36 (2.8)	1.90 (1.25-2.89)	
Sulfonamides				
0	1478 (27.0)	332 (21.5)	Reference	
1-10	3781 (69.0)	1143 (74.1)	1.51 (1.28-1.79)	<.001
≥11	220 (4.0)	68 (4.4)	1.56 (1.14-2.15)	
Nitrofurantoins				
0	1478 (57.2)	332 (49.5)	Reference	
1-10	1050 (40.6)	316 (47.1)	1.45 (1.14-1.83)	.001
≥11	57 (2.2)	23 (3.4)	1.90 (1.12-3.24)	

Abbreviations: CI, confidence interval; OR, odds ratio.
*The reference group for all analyses in this table is made up of all cases and controls in the study population who have no antibiotic prescription fills in the pharmacy database. A participant appearing as a user of any specific antibiotic class will appear as a user of another specific class only if she had at least 1 prescription fill for that other class.
†ORs adjusted for age and for length of Group Health Cooperative enrollment.
‡Representing all antibiotic classes.

calculated or only imputed days of antibiotic use did not materially affect the estimates of incident breast cancer risk. Additionally, incorporation of 2- and 4-year latency periods between antibiotic exposure and reference dates did not materially change risk estimates for incident or fatal breast cancers.

Our findings are consistent with those of Knekt et al,[8] but we observed an increased risk of incident breast cancer for both premenopausal and postmenopausal women among women who used antibiotics for any indication, whereas the risk of incident breast cancer in the study by Knekt et al was increased only in women younger than 50 years and the study addressed only antibiotic use for urinary tract infections.

In a subset of study participants with heavy use of macrolide and tetracycline antibiotics, we found no difference in risk of incident breast cancer among women using these antibiotics for acne and/or rosacea compared with women using these drugs for respiratory tract infections. Because the severity of acne and rosacea can be related to levels of estrogen, unlike most respiratory tract infections, we reasoned that this indication for long-term antibiotic use might be associated with an increased risk of breast cancer. Our results did not support this hypothesis, but our study had limited power; the number of women included in the subset was small and the CI was wide. Additional studies are needed to further clarify whether indication for antibiotic use is associated with risk of breast cancer.

The hypothesis that some classes of antibiotics may increase risk of breast cancer is plausible; antibiotics have effects on intestinal microflora and on immune and inflammatory responses.[2] For example, antibiotic use may increase risk of breast cancer by decreasing phytochemical metabolism by intestinal microflora.[4,16] Phytochemicals are hypothesized to play an inhibitory role at several points in the carcinogenesis pathway by modulating enzymes involved in carcinogen and steroid hormone metabolism.[16-19] Also, use of tetracycline may be associated with increased production of prostaglandin E2, a hallmark of the inflammatory response, catalyzed by cyclooxygenase 1 and 2.[20] Overexpression of cyclooxygenase 2 is associated with mammary carcinogenesis, while inhibition of prostaglandins and other inflammatory responses by nonsteroidal anti-inflammatory drugs is associated with a 20% to 40% decreased risk of breast cancer.[21-24] Although this evidence suggests that antibiotics may be associated with breast cancer, it is also possible that a weakened immune system (either alone or in conjunction with use of antibiotics) is the biologically relevant basis of this association.

The strengths of this observational study include the use of population-based cases and controls, the identification and validation of case diagnoses through the Surveillance, Epidemiol-

Table 5. Association of Fatal Breast Cancer With Cumulative Days of Antibiotic Use*

No. of Days of Antibiotic Use	No. (%) Controls (n = 7948)	No. (%) Cases (n = 177)	OR (95% CI)†	*P* Value for Trend
Cumulative‡				
0	1478 (18.6)	24 (13.6)	Reference	<.001
1-50	2365 (29.8)	57 (32.2)	2.00 (1.09-3.66)	
51-100	1515 (19.1)	32 (18.1)	2.55 (1.29-5.03)	
101-500	2215 (27.9)	54 (30.5)	3.42 (1.77-6.60)	
≥501	375 (4.7)	10 (5.7)	4.37 (1.82-10.51)	
Macrolides				
0	1478 (29.8)	24 (21.6)	Reference	<.001
1-50	3037 (61.3)	71 (64.0)	2.85 (1.44-5.67)	
51-100	285 (5.8)	10 (9.0)	5.72 (2.23-14.67)	
≥101	153 (3.1)	6 (5.4)	5.75 (1.81-18.24)	
Tetracyclines				
0	1478 (32.4)	24 (23.1)	Reference	.001
1-50	2381 (52.2)	59 (56.7)	3.44 (1.70-6.96)	
51-100	327 (7.2)	10 (9.6)	5.02 (1.91-13.19)	
≥101	378 (8.3)	11 (10.6)	5.21 (2.09-13.02)	
Penicillins				
0	1478 (22.9)	24 (17.9)	Reference	.002
1-50	3327 (51.5)	75 (56.0)	2.41 (1.29-4.50)	
51-100	1044 (16.2)	18 (13.4)	2.38 (1.09-5.19)	
≥101	608 (9.4)	17 (12.7)	4.43 (1.97-9.98)	
Cephalosporins				
0	1478 (32.6)	24 (22.6)	Reference	.008
1-50	2723 (60.0)	71 (67.0)	2.48 (1.27-4.86)	
51-100	232 (5.1)	9 (8.5)	4.43 (1.74-11.27)	
≥101	108 (2.4)	2 (1.9)	2.39 (0.50-11.39)	
Sulfonamides				
0	1478 (27.0)	24 (20.9)	Reference	.14
1-50	3174 (57.9)	76 (66.1)	2.96 (1.57-5.58)	
51-100	478 (8.7)	10 (8.7)	3.04 (1.24-7.45)	
≥101	349 (6.4)	5 (4.4)	2.04 (0.69-6.08)	
Nitrofurantoins				
0	1478 (57.2)	24 (41.4)	Reference	.21
1-50	901 (34.9)	30 (51.7)	3.15 (1.30-7.64)	
51-100	77 (3.0)	0	NA	
≥101	129 (5.0)	4 (6.9)	3.27 (0.89-12.06)	

Abbreviations: CI, confidence interval; NA, not available; OR, odds ratio.
*The reference group for all analyses in this table is made up of all cases who died from breast cancer and who had no antibiotic prescriptions in the pharmacy database, and of all controls in the study population who had no antibiotic prescription fills in the pharmacy database. A participant appearing as a user of any specific antibiotic class will appear as a user of another specific class only if she had at least 1 prescription fill for that other class.
†ORs adjusted for age, length of Group Health Cooperative enrollment, and ever use of postmenopausal hormones.
‡Representing the 8 most commonly used antibiotic classes (97% of all antibiotic prescriptions).

ogy, and End Results registry, the ability to include all participants because no direct participant involvement was required, and the use of the GHC pharmacy database to assess antibiotic use in an unbiased manner for cases and controls. Our method of measuring antibiotic use did not capture data for inpatient antibiotic use or antibiotics purchased outside GHC, and could not determine whether prescriptions dispensed were actually used. However, we have no reason to suspect differences between cases and controls, and therefore if any bias exists because of misclassification of antibiotic exposure, we would expect that the current results are biased toward the null.

We had missing data for some of the known or suspected risk factors for breast cancer shown in Table 1, and no information for other potential risk factors, such as alcohol use and lactation. Whether this missing information is problematic is difficult to determine; it may have limited our ability to detect confounding. Also, at the highest levels of antibiotic exposure, sample sizes were small and CIs were wide; however, the confidence limits consistently excluded 1. We have some assurance that antibiotic use is not simply a proxy for health care–seeking or mammography-detection bias; the association between cumulative days of antibiotic use and fatal breast cancer was similar to that for the association with incident breast cancer, and number of health care visits did not confound the association between use of antibiotics and incident breast cancer.

Given that we found an association using relatively straightforward measures of antibiotic use, more detailed analyses including timing of exposure and considering various antibiotic doses might further clarify this association. For example, the amount of antibiotic use at particularly sensitive times in breast development, such as adolescence, pregnancy, or during menopause, may be pertinent. We were unable to conduct such analyses because there were relatively few women with pharmacy records covering the time span from adolescence or childbearing years through postmenopause. Additionally, it is possible that risks of breast cancer differ between women with long-term use of low-dose antibiotics and those with intermittent use of higher-dose antibiotics.

In summary, we found that increased use of antibiotics was associated with increased risk of incident and fatal breast cancer for a variety of antibiotic classes. It cannot be determined from this study whether the use of antibiotics is causally related to breast cancer, or whether the indication for antibiotic use, overall weakened immune function, or other factors are the pertinent underlying exposures. While the implications for clinical practice will not be clear until additional studies are conducted, the results of this study support the continued need for prudent long-term use of antibiotics and the need for further studies of the association between antibiotic use and cancer risk.

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Study concept and design: Velicer, Heckbert, Lampe, Potter, Taplin.
Acquisition of data; administrative, technical, or material support: Velicer, Robertson, Taplin.
Analysis and interpretation of data: Velicer, Heckbert, Lampe, Potter.
Drafting of the manuscript: Velicer.
Critical revision of the manuscript for important intellectual content: Heckbert, Lampe, Potter, Robertson, Taplin.
Statistical expertise: Heckbert.
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