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Antibiotic Usage in Relation to Resistant Bacterial Infections and Liver and Kidney Cancer Outcomes in South Carolina

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ANTIBIOTIC USAGE IN RELATION TO RESISTANT BACTERIAL INFECTIONS AND LIVER AND KIDNEY CANCER OUTCOME IN SOUTH CAROLINA

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DEDICATION

I would like to dedicate this dissertation and doctoral degree to my family, those near and far, old and new.

For Muffin, who set me on this path and Milo, who kept me company on it.

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ABSTRACT

Antibiotics are one of the most beneficial discoveries in medicine and public health. However, the use, overuse, and misuse of these drugs have led to increases in antibiotic resistant bacterial infections (ARI). Furthermore, previous epidemiological studies have linked antibiotic use to breast cancer, but these studies have not included effects on metabolic organs, such as the liver and kidneys. This dissertation investigates the role of antibiotic use in infections and liver and kidney cancers in the state of South Carolina. Using ecological study methods and Poisson regression to determine relative risk ratios, it was found that antibiotic use is a risk factor for the development of ARIs and kidney cancer, but not liver cancer. Census tracts with higher percentages of black populations were found to be more at risk for these outcomes, including liver cancer. Case-control methodology was used to investigate individual risk for liver and kidney cancer outcomes, and demographic and geographic variables were examined as confounders or effect modifiers between these relationships. Using conditional logistic regression to calculate odds ratios (OR), it was determined that antibiotic usage is not a risk factor for liver cancer, with ORs of 1.07 (0.77-1.49) for 5 to 36 total prescriptions, 1.33 (0.72-1.46) for 64 to 204 total prescriptions, and 1.39 (0.98-1.98) for 205 to 4374 total prescriptions per participant. No association was found between liver cancer and days of use of antibiotics, nor was their increased risk by specific antibiotic classes. Despite these findings, antibiotic

usage was associated with higher odds of kidney cancer outcomes, with ORs of 1.50 (1.27-1.78) for 18 to 131 total prescriptions and 1.43 (1.20-1.69) for 132 to 12362 total prescriptions per participant. For days of use of antibiotics, ORs were 1.41 (1.20-1.67) for 116 to 950 total days of use, and 1.46 (1.23-1.73) for 951 to 123588 total days of use per participant. Also, increased kidney cancers risks were associated with certain classes of antibiotics for some or all levels of exposure by total prescription number and days of use. Overall, these findings suggest that antibiotics must be used in a more judicious manner in medical settings

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CHAPTER 1

INTRODUCTION

Statement of the Problem

Antibiotic resistant bacterial infections are responsible for approximately one third of intensive care unit and hospital acquired infections (1-4). Due to the widespread and conventional use of antibiotics, it has become increasingly common to isolate resistant and multi-drug resistant organisms from patients who would have normally hosted susceptible bacteria. While this trend increases, the utility and effectiveness of our present stock of antibiotics dwindles (5), allowing for an unchecked emergence of these infections. Prudent use of antibiotics can help curb this trend, but in order to do so, patterns of antibiotic usage must be determined. These patterns can be studied in relation to resistant infections, but also spatially, allowing for a bigger picture of antibiotic prescribing practices. This research will provide a basis for understanding traditional antibiotic usage against a backdrop of various demographic and geographic factors, and may lead to strategies for a more judicious and mindful use of antimicrobial drugs.

Furthermore, a paucity of literature currently exists examining the association between antibiotic usage and cancers. To date, only two studies have been published investigating this link, and both studies focused on antibiotic utilization and breast cancer (6;7). While both studies demonstrated that

antibiotics may indeed be a risk for breast cancer, no other cancer has been studied in relation to antibiotics. Physiologically, the liver and kidneys experience the most exposure to drugs in the bloodstream, and therefore would presumably carry a substantial amount of risk for antibiotic induced cancers. Additionally, in respect to liver and kidney cancer, the American Cancer Society predicts rising numbers of cases and deaths attributable to each of these cancers from 2011 to 2012 (8;9). It is feasible that this upward trend may result from the inappropriate or excessive intake of antibiotics by these individuals. This study aims to fill the gaps in the research regarding antibiotic usage and its relationship to cancer, as well as to examine antibiotic usage as a further risk factor for liver and kidney cancer.

Background

The overuse of antibiotics as chemotherapeutic treatments for bacterial infections poses serious public health care concerns. It has been shown, many times over, that antibiotics are being prescribed inappropriately and excessively in the medical fields (10-14). The reasons for this include physician ignorance, patient pressures, or inadequate examination times (15-18). Overuse of antibiotics is the main driving force behind the recent emergence of antibiotic resistant bacterial infections (19;20). Bacteria have the ability to rapidly evolve, acquiring mutations and drug resistant genes from other bacterial populations, allowing them to quickly overcome pharmaceuticals we depend on for therapies. To compound the situation, new antibiotic development has stalled, decreasing the stock of effective antibiotics medicine has to offer to fight these resistant

infections (5). Furthermore, the use of antibiotics in the veterinary and agriculture fields continue to expose humans to these drugs (21-24). Antibiotic usage and prescribing patterns vary geographically, as it has been shown that antibiotics are prescribed more often in urban areas and communities with higher population densities (25-27). They can also be isolated more readily from farming, agriculture, aquaculture, and coastal ecosystems (28;29). These spatial differences in exposures to antibiotics must be described and understand to prevent potential adverse outcomes to public health.

In addition, antibiotics are very powerful drugs and have many known adverse side affects. These range from relatively mild, even asymptomatic, reactions to serious and life threatening conditions. Some of the most serious include toxicities of the body's organs or organ systems resulting in use of antibiotics (30;31). Among the organs within the body, the liver and kidneys play the lead role in metabolizing and excreting chemotherapeutics, and are therefore prone to developing toxicities (32-36). Although toxicities can be induced by antibiotics, it is feasible that even more serious complications can arise. Studies by Knekt (7) and Velicer (6) have shown associations between treatment of women with antibiotics and breast cancer, showing that these chemotherapeutics may have a much more serious role in disease development. Associations between bacterial infections and various cancers have also been uncovered. For example, infections with *Streptococcus bovis* have been associated with colon cancer, *Helicobacter pylori* with gastric cancer, and *Salmonella typhi* with gallbladder. However, the studies by Knekt and Velicer have ruled out initial

bacterial infection as risk factors for cancer, but rather that the subsequent consumption of antibiotics confers the actual risk.

The American Cancer Society reported that in 2011, an estimated 26,190 cases of liver and intrahepatic bile duct cancer occurred resulting in 6,330 deaths in the Unites States (9). It is expected in 2012 that there will be a total number of 28,720 cases and 6,570 deaths (8). Additionally, in 2011, 60,920 cases of kidney and renal pelvis cancer were reported, leading to 13,120 deaths (9). It is estimated that in 2012, 64,770 cases will be reported and will result in 13,570 deaths (8). Both of these types of cancers display increasing trends in cases reported and resulting deaths. Moreover, the literature points to elevated risks of liver and kidney cancers in certain ethnicities and genders, the highest risks found in African Americans and men. Determining and understanding the etiologies behind these trends, the role of antibiotic usage in relation to liver and kidney cancers, and cancer disparities among populations may provide insight into more cautious prescribing of antibiotics.

Proposal and Specific Aims

Using both ecological and case control studies, I propose to study antibiotic usage and its effects on antibiotic resistant bacterial infections, as well as its relationship with liver and kidney cancers in South Carolina. First, antibiotic usage or antibiotic prescription rates will be determined for various demographic aspects and geographic variables in the state. This will yield information regarding state wide antibiotic usage practices and provide the foundation for the associations between resistant infections and cancer. Next,

the frequencies antibiotic resistant bacterial infections will be examined in relation to antibiotic utilization rates. Cases of liver and kidney cancers in South Carolina can be linked to both rates of antibiotic usage and resistant bacterial infections in this way. These investigations will be accomplished by appending Medicaid administrative claims data this State Health Plan (SHP) claims data and linking this to data from the South Carolina Central Cancer Registry (SCCCR). This data linkage between Medicaid and SHP to SCCCR databases is unique to this study, as this type of linkage is not possible in other states.

Additionally, based on analyses by Velicer et al, separate case control studies will be performed to determine the association between antibiotic usage and liver and kidney cancers, respectively. Velicer's study was focused on breast cancer, and therefore women over age 19 years were the focus of the study (n = 10,219). Cases (n = 2,266) presented with primary, invasive breast cancer, and were enrolled at Group Health Cooperative (GHC) for at least one year. Controls (n = 7,953) were randomly selected and frequency matched to cases based on age and length of enrollment in GHC (6). For the liver and kidney cancer aims in this exploration, my study population will again focus on male and female Medicaid and SHP recipients and cases of cancer for both genders will be ascertained from SCCCR. South Carolina provides a distinctive and ideal location in which to perform these studies due to its large rural population and geographically diverse regions. Of the two studies that have examined the relationship between cancer and antibiotic usage, neither has

addressed disparities among gender or different population types, which is a goal of this study.

Therefore, the specific aims of this investigation follow:

- To describe antibiotic usage and patterns, antibiotic resistant bacterial infections, and cases of liver and kidney cancers in South Carolina using ecological study methodologies and Medicaid and SHP administrative claims data linked with SCCCR data. These associations will be investigated against the background of various demographic and geographic factors.
- To evaluate the association, overall, by gender, and by population type, between antibiotic usage and the risk of liver cancer among Medicaid and SHP recipients and individuals registered in SCCCR. Due to the inherent toxicity of antibiotics and the role of the liver during drug metabolism, this analysis may provide outcome based perspectives and strategies for the use of antibiotics.
- To evaluate the association, overall, by gender, and by population type between antibiotic usage and the risk of kidney cancer among Medicaid and SHP recipients and individuals registered in SCCCR. Due to the inherent toxicity of antibiotics and the role of the kidneys during drug excretion, this analysis may provide outcome based perspectives and strategies for the use of antibiotics.

These analyses strive to not only understand the effects of antibiotic usage on resistant infections and cancers, but also to uncover the antibiotic

prescribing patterns and practices for the state of South Carolina. When these rates are further described in combination with infections and cancer cases, it can be determined if antibiotics are risk factors for or driving forces behind these diseases.

Significance of Research

The overuse of antibiotics has become problematic around the world (12;13;19;37;38). These irresponsible and immoderate practices have ushered in an era of increasing frequencies of antibiotic resistant infections and may lead to more serious risks including cancer. It is important to understand the effects of antibiotic overuse in response to bacterial infections, as the use of these drugs act as an impetus for the selection of antibiotic resistant bacteria. Infections caused by these types of bacteria require much more intensive and complicated treatments, placing patients in even more reduced states of health and resulting in economic losses (13;14). This investigation seeks to temper the antibiotic overuse-bacterial resistance cycle by elucidating aspects of the primary cause, rates of antibiotic usage and prescribing in South Carolina.

Additionally, only two studies have examined the association between the utilization of antibiotics and its role in the development of cancers to date. Yet neither study examines the risk of other cancers besides breast cancer. This investigation aims to bridge the gap in this knowledge by expanding previous methodologies to liver and kidney cancers. As these organs are responsible for the majority of drug metabolism and excretion, it is biologically plausible that they would be targeted for negative effects from circulating antibiotics in the

bloodstream. Case control studies which examine the association between antibiotic utilization and liver and kidney cancers will clarify the role of antibiotics as risk factors for cancer, and will provide medical and clinical insight into a more appropriate and moderate use of these drugs.

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CHAPTER 2

MATERIALS AND METHODS

Overall Study

Data Sources:

This study will use existing demographic and pharmacy data and diagnostic codes from South Carolina Medicaid administrative claims merged with State Health Plan (SHP) claims data. Specifically, pharmacy or drug files from Medicaid and SHP will be used to ascertain antibiotic prescribing data. These appended datasets will also be integrated with liver and kidney cancer incidences from the South Carolina Central Cancer Registry (SCCCR). The study period includes dates from January 1, 2000 to December 31, 2009. These data will be linked by unique patient identification numbers, but personal data will not be used in this analysis, with the exception of geocoding. Geocoding will be performed at the South Carolina Budget and Control's Office of Research and Statistics. Researchers and investigators at the University of South Carolina will not have access to protected health information used for geocoding. Geocoding of the patient records will provide data for antibiotic usage, antibiotic resistant bacterial infections, and cancer incidences. United States Census Bureau data will be used to determine geographical aspects of the state of South Carolina, including numbers and locations of rural and urban areas, metropolitan

city centers, and population densities. Coastal areas will be defined by the U.S. Census fixed distance inland boundary of 50 miles from the coast to inland. Interior areas will range from 51 miles from the coast to the inland border of the state.

Inclusion Criteria:

Study participants will represent both males and females aged 18 years or older. This restricts the dataset to adults only. All participants will be selected from patients enrolled in Medicaid or SHP continuously for at least 1 year before the diagnosis of liver or kidney cancer. Furthermore, all participants in this study must have an existing address to allow for geocoding. In some cases, if individuals have two or more different addresses during the study period, the address of the longest residence will be utilized for this study.

Antibiotic Usage:

Antibiotic usage will be assessed by using Medicaid and SHP data. This study will be limited to antimicrobial agents effective against bacterial infections and will exclude antiviral, antifungal, and antiparasitic agents. Variables in the Medicaid Pharmacy file include: date dispensed, class of drug, national drug code (NDC), quantity, number of refills, days supplied, and therapeutic class. Variables in the SHP drug file include: date written, days of therapy, date dispensed, NDC, and drug strength. Two measures of antibiotic usage can be used, but both will have to be adjusted for the time the participant was enrolled in Medicaid or SHP. The first measure will be indicated primarily by the cumulative

days of antibiotic usage during the study period. This will be indicated by the days supplied variable in Medicaid and the days of therapy variable in SHP. These values will then be summed up for each participant to generate the cumulative days of antibiotic use. Alternatively, this measure can be calculated by dividing the quantity of antibiotic prescribed by the quantity intended to be taken per day. In addition to cumulatively summing up days of use per participant, they can be summed up separately for each of the most common classes of antibiotics prescribed for study participants (for example, macrolides, tetracyclines, penicillins, cephalosporins, sulfonamides, and nitrofurantoins). Days of cumulative use categories will include: 0 days, 1 to 50 days, 51 to 100 days, 101 to 500 days, 501 to 1000 days, and more than 1001 days. The other measure of antibiotic usage is the total number of antibiotic prescriptions per participant during the study period. Total number of prescription categories will include: 0 prescriptions, 1 to 5 prescriptions, 6 to 10 prescriptions, 11 to 15 prescriptions, 16 to 20 prescriptions, and more than 20 prescriptions.

Ascertainment of Antibiotic Resistant Bacterial Infections: The cases of antibiotic resistant bacterial infections will be ascertained from ICD-9 codes for resistant infections from Medicaid administrative claims and SHP administrative claims data. These codes include: V09.8 infection with microorganisms resistant to other specified drugs, 041.12 methicillin resistant *Staphylococcus aureus*, V09.0 infection with microorganisms resistant to penicillins, V09.2 infection with microorganisms resistant to macrolides, V09.3 infection with microorganisms to tetracyclines, V09.4 infection with microorganisms resistant to aminoglycosides,

V09.6 infection with microorganisms resistant to sulfonamides, 038.12 methicillin resistant *Staphylococcus aureus* septicemia, and 482.42 methicillin resistant pneumonia due to *Staphylococcus aureus*.

Selection of Liver and Kidney Cancer Cases and Controls:

Cases of liver and kidney cancer will be ascertained from SCCCR. Primary liver cancer cases, including hepatocellular carcinoma and bile duct carcinomas will be included. Kidney cancers will include renal cell carcinomas and renal pelvis cancers. Incidence density sampling will be used as controls will be randomly selected from Medicaid and SHP enrollees during the same years the cases were diagnosed. Controls will be frequency matched to cases at a ratio of 3:1 on birth year, by 2 year intervals, and duration of enrollment in Medicaid or SHP with pharmacy records availability (≤ 2 years, 3 – 6 years, 7 – 10 years, and ≥ 11 years of enrollment). Frequency matching will ensure that the characteristics of the population of controls are similar to the characteristics of the cases.

Assessment of Other Risk Factors:

Known and suspected risk factors for liver and kidney cancer include age, sex, race, smoking, liver cirrhosis, co-infections, and alcohol consumption. Information on these factors will be obtained from Medicaid and SHP administrative claims data or from SCCCR data, if possible.

Specific Aim #1: Antibiotic Usage Patterns in Relation to Resistant Bacterial Infections and Liver and Kidney Cancer Cases

Objective:

The objective of this study is to describe antibiotic usage in the state of South Carolina in respect to a variety of geographical and population based factors. Antibiotic usage will be reflected by either the number of antibiotic prescriptions or the cumulative days of antibiotic usage during the study period. Antibiotic resistant bacterial infections and cancer outcomes can then be described against antibiotic usage patterns.

Methodology:

Exposure data will be ascertained from Medicaid and SHP pharmacy and drug files and outcome data will be ascertained from SCCCR data. Antibiotic usage will be summed for each census tract, using either number of cumulative prescriptions or days of use for the exposure variable. The exposure variable will be divided into tertiles of exposure, low, medium, and high. Infections, liver cancer, and kidney cancer cases will also be summed by census tract for the outcomes. Census tracts will be classified based on demographic and geographic criteria from United States Census Bureau data from the year 2000 and Department of Natural Resources data. Multivariate Poisson regression with standard errors will be used to ascertain incidence rate ratios, which will be used as estimates for relative rate ratios. Only significant variables will be included in the multivariate model. This will allow for the determination of important

demographic and geographic factors in relation to prescribing patterns of antibiotics in the state of South Carolina. Data retrieval, management, and analyses will be performed using SAS version 9.2 (SAS Institute Inc., Cary, NC) and $P < 0.05$ will be used to determine statistical significance.

Specific Aims #2 and #3: Liver and Kidney Cancer Case-control Studies

Objective:

The objectives of these studies are to describe and assess the association between the use of antibiotics and liver and kidney cancer outcomes. This will be assessed overall, by class of antibiotic, sex, and varying population type. The data will be stratified by the most commonly used classes of antibiotics in the dataset. Associations will be described for males and females separately, for urban and rural populations, as well as by counties in relation to low or high minority populations within the county itself. Further analyses may be carried out based on the stratification of other variables in the dataset.

Statistical Analyses:

Conditional logistic regression will be used to estimate the odds ratios of liver and kidney cancer associated with antibiotic use overall, by antibiotic class, sex, and population type. Calculating odds ratios by class of antibiotic will allow for ascertainment of cancer risk by type of antibiotic, as one class may be associated more strongly than another with a cancer outcome. Furthermore, odds ratios will be calculated separately by sex and population to examine any gender or geographic disparities that may exist between the association between

antibiotic usage and cancer outcomes. The cancer outcome variable will be categorical, either yes (1) or no (0), while the predictor variable of days of antibiotic use or number of prescriptions will be continuous. All logistic regression analyses will be adjusted for the matching variables (age, length of enrollment in Medicaid and SHP, etc.), which will be modeled as continuous variables. Data retrieval, management, and analyses will be performed using SAS version 9.2 (SAS Institute Inc., Cary, NC) and $P < 0.05$ will be used to determine statistical significance.

CHAPTER 3

LITERATURE REVIEW

Part I. Antibiotic Toxicity and Metabolism

Antibiotics are traditionally defined as naturally occurring agents that have microbicidal or microbiostatic activity, but also include semi-synthetic, or synthetic agents with these actions (1;2). In general, antibiotics, also referred to as antimicrobials, provide positive outcomes for the majority of patients, offering relatively quick and easy treatments for a myriad of infectious diseases. However, despite the success of these drugs, they also pose risks to patients because of their many associated side effects and adverse reactions. Some of these effects can be relatively mild and even unknown to the patient, whereas other effects can be life threatening. These include anaphylaxis, organ toxicities, including those of the ears, liver, and kidneys, cardiac arrhythmias, and seizures (3;4). In fact, approximately 25% of adverse reactions seen in hospitalized patients arise from the use of antibiotics. Antibiotic related side effects also have economic impacts to patients, as nephrotoxicities due to vancomycins and aminoglycosides can add up to $2,500 per patient with this toxicity (5). These costs can continue to climb if patients must stay longer in the hospital or receive more advanced and specialized therapies to treat antibiotic induced adverse reactions (6).

Five primary mechanisms of antibiotic toxicity can be described. Direct effects on tissues can occur due to interactions between the drug and or the drug's metabolites on a particular tissue or organ within the body (4). These are in part due to the chemical and molecular structures of the antibiotics, the body's own immune responses to and formation of toxic anti-metabolites from antibiotics (3). Examples of direct effects include anemias caused by chloramphenicols and nerve toxicities, resulting in deafness and vertigo, due to aminoglycosides. Hypersensitivities, and specifically type I hypersensitivity, are responsible for anaphylaxis due to antibiotics, and result from individuals' own genetic predispositions to these agents (3). These can include certain types of anemias, serum sickness, and other allergic syndromes. Changes in the patient's microbial flora may predispose them to adverse reactions, such as fungal infections by *Candida* or *Aspergillus*. When using antibiotics, and especially broad spectrum antibiotics, much of the host's normal microflora is eliminated. This allows opportunistic species of fungus, as well as bacteria, to colonize and infect susceptible individuals. In many cases, drug interactions can be the cause of toxicities. When two or more drugs are taken in combination, regardless of their classes or purposes, interactions are always possible. Sometimes, the interactions can be relatively harmless to the patient and merely result in the inactivation of one or both of the drugs. In other cases, severe toxicities can occur from combinations of pharmaceuticals. For example, the use of furosemide, used to treat high blood pressure, in conjunction with cephaloridine can lead to kidney toxicity. Lastly, upon treatment with antibiotics, microbial cells

may lyse and release toxic products and components from the cell. These toxins are capable of producing secondary conditions, including erythema nodosum leprosum, seen in about 50% of lepromatous leprosy patients treated with dapsone, and Jarisch-Herxheimer reactions, which result from treatment of neurosyphilis with intravenous penicillin (4). It can be noted that many of these side affects can be attributed to a particular class of antibiotics, or even a specific antibiotic. To illustrate, trovafloxacin and temafloxin were found to be responsible for hepatotoxicity and hemolysis, respectively. Due to the severity and high incidence of these toxicities, deaths from trovafloxacin and an incidence of toxicity in 1 of every 3500 patients treated with temafloxacin, these antibiotics were removed from the market. Despite the withdrawal of the most toxic antibiotics from pharmaceutical circulation, many antibiotics remaining on the market or in development and clinical trail phases do possess certain, deemed appropriate, levels of toxicities (3).

Antibiotic Metabolism and Effects on the Liver and Kidneys

The liver and kidneys play vital roles in the metabolism and excretion of pharmaceuticals, including antibiotics. Due to these roles, these organs are usually associated with antibiotic induced toxicities. The liver is the most metabolically active tissue per unit of weight, and is therefore responsible for the bulk of drug metabolism. In addition to its metabolic activities, the liver is also the largest organ in the body, is perfused by blood containing drugs from the digestive tract, and houses the majority of drug metabolizing enzymes found in organs of the body, further cementing its vast role in drug metabolism. The liver

accomplishes two phases of drug metabolism, and depending on the drug, both of these phases will be completed in sequence, or only one of the two phases will be performed. Phase 1 reactions include oxidation, reduction, and hydrolysis of the drug which prepares it for the second phase to produce conjugation products. Phase 2 conjugation reactions, which include glucoronidation, sulphation, and acetylation, increase the water solubility of the drug permitting its excretion in bile or urine. Phase 2 reactions can also inactivate the drug or its active metabolites produced in phase 1 (7;8). Various factors influence the rate of drug metabolism by the liver. Age, sex, host microflora, nutrition, circulation pathways, genetic predispositions, and drug interactions have all been shown to affect drug metabolism (7).

Drug induced liver injury (DILI) causes most cases of liver failure seen in the United States, composing 13% of these cases (9), but severe antibiotic caused hepatotoxicities are relatively rare, occurring in less than 5 people per 100,000 per year (10). Usually, antibiotic induced hepatotoxicity is asymptomatic, causing mild liver impairment, and resolves on its own. In a few cases, it can be more serious, requiring the need for a liver transplant or can lead to death resulting from liver failure (11;12). The majority of antibiotic induced hepatotoxicity cases can be classified as idiosyncratic, meaning that the condition is host specific, occurs in a small population of those treated, and cannot be predicted from the antibiotic's pharmacology or pre-clinical toxicology testing (13). Most of the time, causes of DILI are poorly understood and rather vague, but studies have shown that 45.5% of cases are caused by antibiotics

and that this is the largest class of therapeutic agents associated with DILI (9) and liver biopsies (14). However, predisposing factors, such as age, pre-existing liver disease, use of other drugs, bacterial infections, and alcohol consumption can influence a patient's susceptibility to DILI (15). Furthermore, dosage of the antibiotic is related to development of DILI. Drugs administered in greater than 50 mg/day resulted in 77% of cases of DILI in a Swedish study (16).

The kidneys not only mediate drug excretion but also play a role in the metabolism of drugs. Studies have shown that the kidneys carry out certain metabolic functions at a faster rate than the liver (17-19), and its metabolic repertoire has thus expanded in the past few decades. The kidneys receive a large amount of blood during circulation, about 25% of resting cardiac output (20) and are therefore routinely and directly exposed to drugs and compounds in the bloodstream (19). Biochemical reactions in the kidneys, which include the same types of phase 1 and phase 2 reactions that occur in the liver, are capable of activating or inactivating drug compounds and may produce metabolic byproducts which are toxic to the organ. Blood flow through the kidneys, the pH of urine, and urine filtration pathways provide increased concentrations of drugs in various sections of the kidney, which may subsequently lead to damage of the organ (21).

Drug induced nephrotoxicities are moderately common sources of kidney injury, with the incidence of drug induced nephrotoxicity as high as 66% in the elderly population. Different classes of antibiotics can cause various effects on the structure and function of kidneys which can lead to injury. For example,

aminoglycosides are accountable for tubular cell toxicities. This class of antibiotics are toxic to renal tubular cells because of their continuous role in concentrating and absorbing filtrate, thus exposing them to higher concentrations of the antibiotic. Many classes of antibiotics, including vancomycins, quinolones, beta lactams, and sulfonamides, are responsible for acute interstitial nephritis. These drugs bind antigens, or can even act as antigens, and are then deposited in the interstitium, the extravascular space surrounding the tubules (22), of the kidney causing an immune reaction. Exposure to some antibiotics, such as ciprofloxacins and sulfonamides, can result in crystal nephropathies, which is the production of urine insoluble crystals in the kidneys leading to impairment of function (20;23). While certain antibiotics are inherently nephrotoxic, some produce damage because of prolonged treatment with the drug or in a dose dependent manner (24). Patient risk factors for drug induced nephrotoxicities include age over 60 years, underlying kidney conditions, diabetes, and heart failure. Certain types of bacterial infections are also associated with antibiotic induced nephrotoxicity, as Gram negative bacterial sepsis treated with aminoglycosides have been shown to result in nephrotoxicity in 10% to 20% of cases (25).

Part II. Antibiotic Usage and Trends

The past few decades have shown a marked increase in the global use of antibiotics in hospital and outpatient settings in both public and private sectors (26-29). Data from the National Ambulatory Medical Care Survey and the National Hospital Ambulatory Medical Care Survey show that prescriptions of

some classes of antibiotics have increased by shocking rates. In just the 1990's, the use of azithromycin and clarithromycin increased by 388%, quinolones by 78%, and amoxicillin and clavulanate by 72% (30). This increase stems from a number of reasons, the most bothersome being that physicians will prescribe antibiotics to patients merely because they feel the patient wants or expects them or that it will reduce return visits (31). For example, antibiotics are prescribed to approximately 75% of adults with acute pharyngitis, or sore throat (32). In these cases, physicians report that they do so because they believe the patient expects them, the patient will be unsatisfied or come back if not given a prescription, or that it is easier to write a prescription than to explain why the patient's condition does not require an antibiotic (33-35). Further studies have shown that prescriptions of antibiotics lend a heightened level of credibility to the patient's illness and that they will subsequently be more likely to return in the future with a similar illness and expect another prescription (36-38).

In the United States, otitis media is the most common complaint for the administration of antibiotics in children. This includes a second prescription of antibiotics after the initial treatment. Differences in rates of repeat prescriptions were observed depending on the cost of the initial antibiotic used. Pediatric Medicaid populations showed that a repeat treatment occurred in 11.6% of children when a less expensive antibiotic was used compared to 13.2% when a more expensive antibiotic was used (39). Parents are often driving forces when it comes to antibiotic prescriptions, as physicians are more likely to prescribe antibiotics to a child if that is their parents' wish (40). Yet, by educating parents

about the judicious use of antibiotics, numbers of prescriptions can decrease. A two year randomized trial in Massachusetts and northern Washington showed that antibiotic prescribing rates dropped by 15% in groups of children aged 36 months to less than 72 months when their parents were educated about appropriate antibiotic use opposed to 9.8% in the control group. Prescribing rates for children between 3 months to less than 36 months were reduced by 18.6% in the treatment group and 11.5% in the control group (41).

Canadian and American studies have determined that up to 50% of antibiotic prescriptions are unnecessary or inappropriate for which they are prescribed (42;43). In the United States, about 75% of antibiotics prescribed on an outpatient basis are for five different respiratory infections, including otitis media, sinusitis, pharyngitis, bronchitis, and generic upper respiratory tract infections (44). Moreover, the majority of these prescriptions are viewed as unnecessary, as most of these infections are viral in nature or the use of antibiotics would not provide any clinical effects (32;45). An alarming 55% of the antibiotics prescribed for the respiratory infections described above are deemed excessive, costing an excess of $726 million a year (44).

Globally, antibiotic prescribing is also on the rise, as antibiotic usage in India remains high due to the improper use of these drugs and a lack of knowledge about the primary benefits and outcomes due to antibiotics (46). High rates of broad spectrum antibiotics and newer classes of antibiotics are being prescribed in India, especially by public clinics and pharmacies. It was shown that 39% of patients at a public clinic or retail pharmacy were prescribed at least

one antibiotic, and 43% of patients attending private clinics left with at lease one antibiotic prescription (26). Antibiotic use in Dutch hospitals was shown to rise because of an increased duration in hospital stay and the use of more intensive treatments to discharge patients earlier. It was noted in these hospitals that prescriptions of co-amoxiclav increased the most and that there were aberrations to the usual prescribing patterns of cephalosporins (47). European studies have showed variable rates of antibiotic prescriptions in 26 different study countries. While France had the highest rate of prescriptions, a shift was also noticed from the older broad spectrum antibiotics to new broad spectrum drugs. Furthermore, seasonal fluctuations caused marked increases in antibiotics in countries which already had high rates of antibiotic usage (48).

It is also important to note the usage of antibiotics in the dental and veterinary fields. Roughly 7% to 10% of antibiotic prescriptions occur due to dental treatments (49). In a 2001 British study, researchers found that 75% of patients attending emergency dental clinics were prescribed antibiotics inappropriately. This was due to poor understandings of the pathologies of dental infections or the lack of knowledge about the indications of the use of antibiotics by the prescribing dentists. In addition, these clinics saw large number of patients, which lead to shorter appointment times, perhaps hindering a proper diagnosis and treatment strategy by the dentist (50).

Veterinary antibiotics are used for the same purposes in animals as in humans, to prevent and treat infectious diseases. In fact, the same classes of antibiotics used to treat humans can also be used to treat dogs, cats, pigs, and

horses (1). However, 30% to 90% of these antibiotics are excreted by these animals in their active forms, ending up in soils, groundwater, and freshwater ecosystems, and eventually run off into the oceans (51). While antibiotic treatment strategies for animals are beyond the scope of this study, its important to recognize that overuse of veterinary antibiotics exposes humans to these drugs in their natural environments.

Antibiotic Usage and Bacterial Resistance

A major reason for the increase in antibiotic prescribing rates, and also a primary cause for this increase, is the emergence of antibiotic resistant bacterial infections. As an inherent biological process, antibacterial resistance has always occurred in nature, but due to the selective pressure placed upon pathogenic strains of bacteria by the use of antibiotics, resistance has increased in both speed and scope in the medical field (31). Antibiotic resistance emerged as a problem shortly after the introduction of penicillin in the 1940's (52). This was brought to attention due to the many outbreaks of resistant infections in pediatric nurseries and maternity wards in hospitals and infections of surgical wounds shortly thereafter (53). Bacteria possess a genetic arsenal with which they are able to overcome the detrimental effects of antibiotics. Faced with selective pressures, they have evolved methods to render an antibiotic useless in a number of ways. Bacteria can inactivate a drug by use of detoxifying enzymes, reduce the transport of the drug into the cell or expel the drug from the cell's interior, acquire genetic mutations to cope with the drug, or gain resistance genes from other bacteria via horizontal transfer methods (52). In addition to

bacterial tactics, antibiotic development in general has come to a halt, resulting in fewer antibiotics that are available as those which bacteria can overcome can no longer be used (54). As bacteria rapidly make existing antibiotics ineffective, an increase in resistant infections can be observed. Moreover, bacteria are capable of becoming resistant to more than one antibiotic, leading to multi-drug resistant bacterial strains, such as *Streptococcus pneumonia* (55). These multi-drug resistant bacterial infections are responsible for an increase in morbidity and mortality in intensive care unit patients in hospitals (56).

Trends in Antibiotic Resistant Bacterial Infections

The overuse of antibiotics has been linked to an increase in rates of bacterial resistance (57-60). These bacteria have consequently been associated with an increase in the incidences of resistant bacterial infections. Antibiotic resistant Gram positive organisms are increasingly causing more infections and they are now responsible for one third of all nosocomial infections (61). In the early 2000's, concern was placed on methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus* (VRE) species (62), and from 1990 to 2005 there was a substantial increase in MRSA infection in the united States. MRSA infections increased from 2% to 39.7% in the United States in the past two decades (63). It is estimated that 79% of all nosocomially acquired coagulase negative *Staphylococcus* species are methicillin resistant (64), and about 43% of *Staphylococcus aureus* isolates from patients are methicillin resistant. Most of these cases occurred in patients aged 18 to less than 50 years old. These infections occurred more often in African American and Hispanic

patients. Hospital onset of *Staphylococcus aureus* cases were more likely caused by MRSA, with an odds ratio of 1.58 and a confidence interval of 1.46 to 1.70 (65). Even in 2012, MRSA can still be viewed as a public health threat as the incidence of these cases continues to rise (66). VRE infections comprise a large number of hospital acquired infections in the United States, causing more morbidity, mortality, and costs than vancomycin susceptible *Enterococcus* infections (67). A twenty fold increase in *Enterococcus* isolates which were vancomycin resistant was seen from 1993 to 1989. From 1990 to 1997, an additional 17% increase in VRE isolates was reported. This is a sobering trend because prior to these increases, vancomycin was seen as the last therapeutic resort when all other antibiotics had failed (63).

In addition, infections caused by antibiotic resistant Gram negative microorganisms, such as members of the *Enterobacteriaceae* and the *Klebsiella*, *Escherichia*, and *Pseudomonas* genuses, are cause for concern as they account for a substantial proportion of nosocomial and bloodstream infections (7;68). To illustrate this trend, approximately one-third, or 36.6%, of isolates from Ghent University Hospital in Ghent, Belgium were proven to be antibiotic resistant bacterial strains (7). A susceptibility study performed using data from 1994 to 2000 showed increasing resistance to commonly used antibiotics by Gram negative organisms. The study looked at Gram negative isolates from 43 states in the United States including the District of Columbia, and found that the activity of most antibiotic therapeutics decreased by 6% at most over the study period. Furthermore, the overall susceptibility to ciprofloxacin decreased from 86% to

76% percent during the time of the study (69). This trend shows that bacteria are becoming more adept at overcoming the bactericidal and bacteriostatic effects of traditional antibiotics, allowing for eventual increases in antibiotic resistant pathogenic bacterial populations and infections. Much of this resistance is due to the exposure to previous antimicrobial drugs, either the inappropriate initial use of antimicrobials or the length of exposure to antibiotics prior to infection (68;70). Inappropriate use of antibiotics includes the utilization of an antibiotic to which the infecting organism is already resistant to or the use of no antibiotics at the inception of treatment (68). Furthermore, an odds ratio of 1.07, with a confidence interval of 1.01 to 1.12, was associated with the isolation of multiply resistant *Pseudomonas aeruginosa* and total days of antibiotic treatment, whereas *Enterobacteriaceae* infections were associated with prior exposure to ampicillin (70). Moreover, the recent emergence of resistant *Campylobacter jejuni* infections has led to elevated rates of global morbidity and mortality. This is due to the use of antibiotics in food animals, as these agents have allowed for these bacteria to develop resistance to common medical antibiotics. High rates of *Campylobacter* resistance have been seen to a number of antibiotics, including tetracycline, amoxicillin, ampicillin, metronidazole, and cephalosporins. Resistance to fluoroquinolones by *Campylobacter* has also emerged in the United States, Asia, and many European countries (71).

While antibiotics are used to treat infections in humans, they are also widely used in agriculture and farming. Half of all antibiotics utilized in the Unites States are used for these purposes, as they are given to livestock, poultry, and

fish to aid in their growth and health (72;73). The animals treated by antibiotics and the humans administering them are then more likely to select and harbor resistant pathogenic bacteria within their bodies. Studies of environmental and intestinal microbial communities, as well as stable soil bacterial communities, show the presence of large amounts of antimicrobial resistant gene elements, even after the antibiotics have been removed (74;75). While bacteria in these communities may not come into direct contact with humans, horizontal or lateral transfer of genes from these bacteria to human pathogens poses a real threat to public health (76). In this way, antibiotic use in the agricultural domain can directly impact the incidence of human antibiotic resistant bacterial infections.

Geographic Trends in Antibiotic Prescribing and Exposure

Many studies have pointed to various prescribing patterns for antibiotics based on geographic locations. One such study in 2004 attempted to determine the prevalence of antibiotic resistant *Escherichia coli* in urban and non-urban areas. Mexico, Kenya, Peru, and the Philippines were used for urban samples, while non-urban areas were sampled from Ghana, Zimbabwe, Venezuela, Curacao, Mexico, and the Philippines. The population of the area was used as classification for urban and non-urban regions, as a population of at least 150,000 inhabitants was considered urban. It was discovered that antibiotic resistant infections were most common in urban populations, as resistance ranged anywhere from 1% to 63% to various antibiotics. This was explained by a higher exposure to antibiotics in these urban areas, from both agricultural and medical fields (77). Furthermore, a study in China also found that antibiotics

were prescribed more often in urban settings rather than rural settings. In this study, researchers were sent to various locations as simulated patients to determine antibiotic prescribing patterns. While none of these "patients" were actually sick, none described having symptoms which required antibiotics and none asked for antibiotics, 65% of "patients" in urban areas received a prescription while 55% received prescriptions on rural areas. However, this study showed that more expensive and powerful antibiotics were being prescribed in rural areas (78). In addition, population density is a common way of measuring crowding or overcrowding of an area, and studies have shown that areas with greater densities harbor more antibiotic resistant organisms and have a higher overall consumption of antibiotics than less populated areas (79).

Exposure to antibiotics can also vary geographically based on practices in the veterinary and agriculture industry. For example, areas devoted to farming have much higher exposures to antibiotics. More tetracycline is consumed by farm animals rather than humans on an annual basis in most developed countries in the world. These antibiotics are given to animals in sub-therapeutic concentrations to aid in their growth, but the presence of these antibiotics in the ecosystem often leads to the emergence of resistant bacterial strains. Additionally, fruit orchards are sprayed with tetracycline and oxytetracycline to prevent infections with *Erwinia amylovora*, the cause of fire blight. Coastal areas also have higher exposures to antibiotics such as tetracycline as it is used to treat infections in lobster, catfish, and salmon (80). Shrimp farming operations in coastal areas use a variety of antibiotics including quinolones, sulfonamides, and

tetracyclines to prevent infection in shrimp populations (81). Exposure to antibiotics in these areas is often higher than usual as soil and water can serve as reservoirs for these antimicrobial agents as well as resistant bacteria.

Part III. Antibiotic Usage and Cancer

The hypothesis that the use of antibiotics may increase the risk of cancer in humans was first suggested in 1981 by Setchell et al (82). To date, only two studies in which the association between the usage of antibiotics and the development of cancer was examined exist, and both studies examined the risk of only breast cancer in relation to antibiotic usage. The first study, conducted in 2000 by Knekt et al in Finland, determined that women below the age of 50 years who were treated with antibiotics for urinary tract infections had an elevated risk of developing breast cancer. The relative risk reported was 1.74 with a 95% confidence interval of 1.13 to 2.68. In this study, the presence of bacteria in the urine, or the actual urinary infection, was not associated with the increased risk, pointing to the use of antibiotics as the real risk factor (83). Limitations of this study included the classification of antibiotic usage as only a binary variable and not accounting for the different classes of antibiotics or the length of treatment (84).

The second study by Velicer et al occurred in 2004 using data from women enrolled at Group Health Cooperative (GHC) and the Surveillance, Epidemiology, and End Results cancer registry (SEER) in Washington state. This case control study focused on the days of treatment with various classes of

antibiotics and their relationships with breast cancer. Pearson correlations showed that the number of antibiotic prescriptions coincided with the number of days of antibiotic use very well, with coefficients ranging from 0.82 to 0.91. Days of antibiotic use were categorized as 0 days, 1 to 50 days, 51 to 100 days, 101 to 500 days, 501 to 1000 days, and greater than or equal to 1000 days. Results showed, for all antibiotic classes combined, elevated risks for the development of incident breast cancer, adjusting for age and length of enrollment in GHC. Using 0 days of treatment as the reference group, the odds ratios for each range of days, and corresponding 95% confidence intervals (CI), were: 1.45 for 1 to 50 days (CI 1.24 to 1.69), 1.53 for 51 to 100 days (CI 1.28 to 1.83), 1.68 for 101 to 500 days (CI 1.42 to 2.00), 2.14 for 501 to 1000 days (CI 1.60 to 2.88), and 2.07 for 1000 days or more (CI 1.48 to 2.89). Increased risks were also associated with incident breast cancer and the number of antibiotic prescriptions, as well as the length of antibiotic use and fatal breast cancers (84).

Velicer et al suggest that antibiotics increase the risk of cancer in a number of ways. First, antibiotics affect the metabolism of the microflora in the gastrointestinal tract, allowing for inefficient or incorrect processing of carcinogens, chemicals, and hormones. Antibiotics can also interfere with immune and inflammatory responses, permitting an increase in production of inflammatory cytokines, prostaglandins, and enzymes (84). These events can trigger mammary cells to proceed down a carcinogenic pathway and eventually produce fatal cancers.

Part IV. Bacterial Infections and Cancer

Bacteria have been linked with cancer since the 1970's. Endogenous bacteria were thought to play a role in the development of cancers because microbial metabolism often produces changes in the body's biochemistry. Bacterial enzymes are capable of hydrolyzing, reducing, and synthesizing a sizeable amount of biochemicals and carcinogens from food sources, hormones, and environmental chemicals. Because the human microflora contains a vast amount of bacteria which interact directly with the body's physiological processes, relationships between colon, breast, and stomach cancer have been examined. It was hypothesized that breast cancer may be influenced by the bacterial production of estrogens and that diet can select for these bacteria and provide them with appropriate substrates. Furthermore, stomach and colon cancer may be attributed to the colonization of these areas with bacteria that produce or metabolize high amounts of nitrates, hormones, and chemical food additives (85).

While the body's innate bacterial population provides a cancer risk, exogenous infection by bacteria account for a substantial amount of cancer cases. By causing chronic infections, producing toxins that can affect the eukaryotic cell cycle, or damaging DNA, bacteria can modify cell growth and promote tumorigenesis. Furthermore, certain bacteria can manipulate the immune system to ignore excessive cell proliferation or to even encourage it (86). Currently, the American Cancer Society estimates that 20% of all cancers globally have origins in bacterial infections (87). Perhaps the most prevalent

association is that between *Helicobacter pylori* infection and subsequent gastric cancer and mucosa associated lymphoid tissue (MALT) lymphoma. However, other associations have been uncovered, including the relationships between *Salmonella typhi* and gallbladder cancer, *Streptococcus bovis* and colon cancer, and *Chlamydophila pneumoniae* and lung cancer (86).

Gastric cancer is one of the most prevalent conditions worldwide and infection with *H. pylori* increased the risk of this type of cancer by 2.2 to 20 fold (88-91). Bacterial-induced increases in inflammation and cell proliferation, abnormal DNA methylation patterns, and cellular mutations are thought to play roles in the formation of cancer, but other risk factors exist, including diets which contain high levels of alcohol and salt (88). The global incidence of gallbladder cancer (GC) is 17 million cases a year, with the highest incidence in Native Americans, Mexican Americans, and populations in the Andes region of South America (86). *Salmonella typhi* and its role in gallbladder cancer was described in an Indian study which proved that patients who were carriers of *Salmonella* had 8.47 times the risk of developing cancer than non-carriers (92). This association mirrors previous findings between *Salmonella* and GC (93;94). However, other risk factors, including gallstones, obesity, environmental chemicals, and chronic infection of the gallbladder can contribute to carcinomas (86).

Colorectal cancer is the third most common cancer in the Unites States and has been linked to a number of bacteria including *Escherichia coli* and several *Streptococci* species, but *Streptococcus bovis* is now viewed as the main

culprit behind this cancer. In 1974, it was first recognized that 25% to 80% of patients with *Streptococcus bovis* infection also had colon carcinomas (95), and the present incidence of *S. bovis* associated colon cancer is between 18% to 62% (96). The exact bacterial mechanism which causes colorectal cancer is poorly understood, but it is thought that an overgrowth of the bacteria or its antigens play a role in tumorigenesis (86;97). Chronic *Chlamydophila pneumoniae* and *Mycoplasma* infection have been associated with lung and prostate cancer, respectively (87;98). Approximately 54% of males and 36% of women with lung carcinomas tested positive for antibodies to *C. pneumoniae*. This data reflected differences between males and females and indicated that males were more likely to be smokers, thus making them more susceptible to infections (98). Persistent *Mycoplasma genitalium* or *hominis* infection has been implicated in prostate cancer as 20.5% to 37.4% of patients with cancer test positive for the presence of *Mycoplasma* in prostate tissue (87;99).

Part V. Cancers of the Liver and Kidneys

Liver Cancer

In 2011, an estimated 26,190 cases of liver and intrahepatic bile duct cancer occurred resulting in 6,330 deaths in the Unites States (100). It is expected in 2012 that there will be a total number of 28,720 cases and 6,570 deaths (101). Primary liver cancer, which does not take into account any secondary tumors, is the sixth most reported cancer in the world and is the third most common cause of cancer fatalities (102). Based on histological typing of

liver tumors, the most common presentation of liver neoplasms is hepatocellular carcinoma (HCC), but other types include childhood hepatoblastoma, adult cholangiocarcinoma which originates from the intrahepatic biliary ducts, and angiosarcoma which originates from the intrahepatic blood vessels. Liver cancer is more common in men than women as men are at least 2.4 times as likely as women to develop this cancer (103).

Many risk factors for liver cancer have been elucidated. The most frequent is the presence of liver cirrhosis. Earlier diagnosis and treatment of liver cirrhosis has led to a decrease in mortality from this condition, which in turn has allowed for an increase in incidences of HCC. Danish studies have shown that patients have a 59.9 fold increase in developing HCC if they present with liver cirrhosis as well, and a 10 fold increase in developing cholangiocarcinoma with a cirrhosis co-morbidity (104). Cancer may stem from cirrhotic conditions due to the immense amount of regeneration of hepatic cells that must occur during this illness. When these regenerations progress uncontrolled or unchecked, tumorigenesis may occur (105). Furthermore, changes in hormonal states, ineffective metabolism of carcinogens, and differential immune statuses can interact with cirrhotic physiologies and lead to HCC (104).

Another factor in the development of liver cancer, and cirrhosis, is infection with hepatitis B (HBV) or hepatitis C viruses (HCV) (106). In a global study, Perz et al found that 30% and 27% of liver cirrhosis was attributable to HBV and HCV, respectively. In addition, 53% and 25% of HCC was attributable to HBV and HCV, respectively (105). Other risk factors which may lead to HCC

include alcohol consumption, tobacco smoking, oral contraceptive use, diabetes, and diet and obesity (107). Exposures to aflatoxins, which are toxins produced by fungi in the *Aspergillus* genus, have been associated with causing mutations in the DNA of hepatic cells leading to tumorigenesis, and are also considered a risk factor for HCC (103).

Kidney Cancer

In the United States in 2011, 60,920 cases of kidney and renal pelvis cancer were reported, leading to 13,120 deaths (100). It is estimated that in 2012, 64,770 cases will be reported and will result in 13,570 deaths (101). Incidence rates show that this cancer affects 9 out of 100,000 people per year (108). About 10% of kidney cancers occur in the renal pelvis, the upper most part of the ureter which drains urine from the kidney, and 90% occur in the renal parenchyma, the actual kidney tissue which consists of nephrons. Cancers which originate from the renal parenchyma are referred to as adenocarcinomas or renal cell cancer (RCC) (109). RCC is ranked 10^{th} in cancers of males and 14^{th} in cancers of females. In terms of global urological cancers, RCC is the third most common carcinoma following prostate and bladder cancers, yet it has the highest mortality rate at 40% compared to 20% from prostate and bladder cancers (108). Asian and Pacific Islanders in the United States have the lowest incidences of RCC, which reflect the low incidence of this cancer in their countries of origin, whereas white Hispanics have much higher incidence rates than their counterparts in Latin America (109). African Americans also have a

10% to 20% higher incidence of RCC, but the reason behind this is not yet known (108).

Risk factors for RCC include smoking, exposure to chemical carcinogens such as asbestos, arsenic, organic solvents, and polycyclic aromatic hydrocarbons, as well as exposure to ionizing radiation. Diabetes, high blood pressure, long term dialysis, obesity and diet are additional factors that place individuals at risk for kidney cancers (108;109). Infection with certain viruses can increase the risk for RCC, as those with human immunodeficiency virus (HIV) are 8.5 times more likely to develop this cancer compared to HIV negative individuals. Infections with polyomavirus type 40, adenovirus 7, and herpes viruses may also increase the risk for RCC (108). There is some evidence that the use of diuretic and analgesic drugs can increase the risk, as well as estrogens from oral contraceptives (110).

A strong genetic component is involved with RCC. Von Hippel-Lindau (VHL) disease is caused by mutations in the VHL tumor suppressor gene. It is an autosomal dominant trait which leads to a myriad of carcinomas of the central nervous system, retinas, pancreas, and inner ears. 40% to 60% of patients with VHL develop RCC, and about 30% of those patients advance to metastatic RCC (111). Other types of heredity syndromes are associated with the development of kidney carcinomas, and all stem from chromosomal mutations which lead to neoplasms of the kidneys, other organs, and skin (108).

Conclusion

The overuse and over-prescription of antibiotics is a primary concern for many reasons. The inherent toxicity of these drugs has been shown to produce negative effects in many organs of the body. The liver and kidneys, due to their crucial role in metabolizing and excreting antibiotics, can develop serious toxicities due to treatment with antibiotics. Furthermore, excessive use of antibiotics, in human and animal medicine, generates resistant strains of bacteria, and sometimes multiply drug resistant strains. This is a major public health concern, as the traditional chemotherapeutic treatment of bacterial infections is becoming ineffective. Bacterial infections have also been linked with certain types of cancer, and with the emergence of resistant bacteria, these infections are returning with a higher incidence. The increase in these infections may also correlate with an increase in cancers.

Studies by Knekt and Velicer have shown that antibiotic usage is associated with the development of breast cancer. Many biological and physiological processes may allow for antibiotic drugs to alter cellular growth and proliferation, leading to the formation of carcinomas. It is conceivable that an increase in liver and kidney cancer can also be attributed to antibiotic usage, via the same biological mechanisms which relate to breast cancer. The trends for these types of cancers have shown increases in incidence over the past few decades, and this increasing trend is still predicted in 2012. Dissecting and understanding the associations between antibiotic usage, antibiotic resistant bacterial infections, and liver and kidney cancers may lead to a more judicious

use of antibiotics and improved strategies for antibiotic prescribing in medical and clinical practices.

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CHAPTER 4

An Ecological Study of Antibiotic Usage in South Carolina by Census Tract Type and Racial Composition[1]

Introduction

The past few decades have shown a marked increase in the global use of antibiotics in hospital and outpatient settings in both public and private sectors (1-4). This increase stems from a number of reasons, the most troubling being that physicians will prescribe antibiotics to satisfy patient desires or to reduce return visits (5). Even when the cause of illness is not bacterial, doctors will prescribe antibiotics due to patients' expectations, belief that patients will return for an unnecessary visit if no prescription is written, or because it is easier to write a prescription than to explain why the patient's condition does not require an antibiotic (6-8).

A major reason for the increase in antibiotic prescribing rates, and also a primary cause for this increase, is the emergence of antibiotic resistant bacterial infections. Bacteria are becoming more adept at overcoming the bactericidal and bacteriostatic effects of traditional antibiotics, allowing for eventual increases in antibiotic resistant pathogenic bacterial populations and infections. Much of this

[1] Thathiah, P., S.A. Adams, A. Merchant, R. Moran, K. Bennett, R. S. Norman. 2014. To be submitted to *Cancer Causes and Control*.

resistance is due to the exposure to previous antimicrobial drugs, either the inappropriate initial use of antimicrobials or the length of exposure to antibiotics prior to infection (9;10). As bacteria rapidly make existing antibiotics ineffective, an increase in resistant infections can be observed. Moreover, bacteria are capable of becoming resistant to more than one antibiotic, leading to multi-drug resistant bacterial strains, such as *Streptococcus pneumonia* (11). It has clearly been shown that the overuse and misuse of antibiotics has been linked to increased rates of bacterial resistance in recent medicine (12-15).

Studies have pointed to various prescribing patterns for antibiotics based on geographic locations. A study in 2004 in Mexico, Kenya, Peru and the Philippines discovered higher exposure to antibiotics in urban areas of these countries, from both agricultural and medical fields (16). In addition, a study in China found that antibiotics were prescribed more often in urban settings than in rural settings. However, this study showed that more expensive and powerful antibiotics were being prescribed in rural areas (17). In addition, population density is a common way of measuring crowding or overcrowding of an area, and studies have shown that areas with greater densities harbor more antibiotic resistant organisms and have a higher overall consumption of antibiotics than less populated areas (18). Exposure to antibiotics can also vary geographically based on practices in the veterinary and agriculture industry. For example, areas devoted to farming have much higher exposures to antibiotics, as tetracyclines are given to animals in sub-therapeutic concentrations to aid in their growth. Additionally, fruit orchards are sprayed with tetracycline to prevent bacterial

blights. Persons in coastal areas have higher exposures to antibiotics such as tetracycline used to treat infections in lobster, catfish, and salmon (19;20). Environmental exposure to antibiotics for persons in these areas is often higher than what is usually encountered in soil and water.

In addition to the emergence of antibiotic resistant bacterial infections, antibiotic use has been suggested as a risk factor for human cancer. This hypothesis was first proposed by Setchell et al in 1981 (21) and has gained credibility through studies by Knekt et al in 2000 and Velicer et al in 2004 (22;23). Both studies found that antibiotic usage is a risk factor for breast cancer, yet it is also plausible that antibiotic use has a hand in causing cancers in other organs of the body. The liver and kidneys, being the major organs responsible for metabolizing and excreting antibiotics carry the greatest risk to be affected by antibiotic usage (24-30). Using aggregated data, this study aims to determine the risks of developing resistant bacterial infections, liver cancer, and kidney cancer by accounting for exposure to antibiotic prescriptions in populations in various census tract types in the state of South Carolina

Methods

Data Sources: This ecological study aimed to determine risk factors, specifically antibiotic usage and geographic and demographic factors, for outcomes of ARI, liver cancer, and kidney cancer at an aggregate level. It used existing demographic and pharmacy data and diagnostic codes from South Carolina Medicaid administrative claims merged with State Health Plan (SHP) claims data.

Specifically, pharmacy and drug files from Medicaid and SHP were used to ascertain antibiotic prescribing data. These appended datasets were integrated with liver and kidney cancer incidences from the South Carolina Central Cancer Registry (SCCCR), a division of the South Carolina Department of Health and Environmental Control (SCDHEC). The study period included dates from January 1, 2000 to December 31, 2009. Study participants represented both males and females aged 18 years or older. This study was approved by both the University of South Carolina and SCDHEC Institutional Review Boards.

Determination of Census Tract Types: United States Census Bureau data from 2000 and the Department of Natural Resources (DNR) GAP data and Landcover codes 54 were used to determine geographical aspects of the state of South Carolina, including racial composition of census tracts, agricultural or industrial, coastal or inland, and rural or urban census tract designations. Racial composition was determined by the percentage of black population within each tract, 0 to 33%, 34 to 66%, and 67 to 100%. Agricultural or industrial census tracts were determined by DNR Landcover Codes. Industrial areas were defined as those with Landcover codes 22 and 23 (Urban development and Urban residential). Census tracts which were more than 50% developed were classified as industrial. Census tracts with less than 50% development were classified as agricultural. Coastal census tracts were defined within counties in the coastal zone, or the counties of Beaufort, Berkeley, Charleston, Colleton, Dorchester, Horry, Jasper, and Georgetown. Census tracts in all other counties were defined as inland. Using census data, census tracts which had greater than or equal to

50% urban characteristics (urban areas with a population of 50,000 or more) were classified as urban, otherwise the tract was classified as rural.

Antibiotic Usage: Antibiotic usage was assessed by using Medicaid and SHP data. This study was limited to antimicrobial agents effective against bacterial infections and excluded antiviral, antifungal, and antiparasitic agents, and were chosen by using American Hospital Formulary System (AHFS) codes. Furthermore, only antibiotics prescribed for oral use were included. Topical and intravenous antibiotics were excluded. Each record was geocoded, allowing for placement into the appropriate census tract in the state. All antibiotic prescriptions during the study period were summed up by each census tract and the tract was later geographically and demographically defined by using the methods above.

Selection of Antibiotic Resistant Bacterial Infections: Incidences of antibiotic resistant infections (ARI) were determined using ICD-9 codes from Medicaid and SHP claims data. These ICD-9 codes included: V09.8 infection with microorganisms resistant to other specified drugs, 041.12 methicillin resistant *Staphylococcus aureus*, V09.0 infection with microorganisms resistant to penicillins, V09.1 infections with microorganisms resistant to cephalosporins and other β-lactam antibiotics, V09.2 infection with microorganisms resistant to macrolides, V09.3 infection with microorganisms to tetracyclines, V09.4 infection with microorganisms resistant to aminoglycosides, V09.5 infection with microorganisms resistant to quinolones and fluoroquinolones, V09.6 infection with microorganisms resistant to sulfonamides, 038.12 methicillin resistant

Staphylococcus aureus septicemia, 041.12 methicillin resistant *Staphylococcus aureus*, 482.42 methicillin resistant pneumonia due to *Staphylococcus aureus*. All geocoded cases of ARI during the study period were summed for each census tract, and the tract was then identified demographically and geographically using the criteria described above.

Selection of Liver and Kidney Cancers: Cases of kidney cancer were ascertained from SCCCR using North American Association of Central Cancer Registries (NAACCR) ICD-O-3 codes. Liver cancers included hepatic carcinomas and unspecified malignant hepatic tumors(ICD-O-3 codes C220 and C221), and kidney cancers included renal cell carcinomas and renal pelvis cancers (ICD-O-3 codes C649 and 659). Again, the total number of geocoded liver and kidney cancers was summed up by each census tract and the tract was later geographically and demographically defined by using the methods above.

Statistical Analyses: Two tailed Student's t-tests were used to compare the mean number of antibiotic prescriptions by census tract type. Multivariate Poisson regression using empirical errors was used to assess the incidence risk ratios (IRRs) of ARI, liver, and kidney cancer by census tract type. IRRs were used as estimators of relative risks ratios (RRs) in this study. An offset variable, the population of the census tract from the 2000 U.S. Census, was used in these analyses to adjust for the different population sizes in each census tract. A midpoint population was not able to be calculated and used as the census boundaries were redrawn for the 2010 U.S. Census, resulting in about 220 more census tracts in 2010 which had no equivalent in the 2000 Census. Confounding

was assessed at the aggregate level by census tracts. Only significant variables were included in each modeled outcome. Data retrieval, management, and analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC) and $P < 0.05$ was used to determine statistical significance.

Results

Tertiles of antibiotic exposure by total number of prescriptions per census tract are reflected in Table 1. Tertiles were used to give exposure level classifications of low, medium, and high. Low exposure includes 293 census tracts which received a total of 0 to 2994 antibiotic prescriptions during the study period. Medium exposure includes 290 tracts which received 2995 to 5297 total prescriptions, and 298 tracts received 5298 to 16,348 total prescriptions which were classified as having high exposure.

Table 2 shows the overall prescribing patterns for census tracts in South Carolina by tract type. The state is composed of almost 60% agricultural tracts and 40% industrial tracts and this is reflected in the total number of antibiotic prescriptions and the mean of prescriptions as well, as there are more antibiotic prescriptions in total and on average for agricultural than industrial tracts. Coastal tracts make up only about a quarter of tracts while inland tracts make up the other three quarters, this is also the trend with total antibiotic prescriptions per tract type and mean prescriptions. Fewer rural tracts are present in the state, and more than half are urban, with most total antibiotic prescriptions belonging to urban tracts. However, rural tracts have a higher mean number of prescriptions. Most tracts

have a black population of 0 to 33%, fewer of the tracts have a black population between 34 and 66%, and fewer tracts have a black population between 67 to 100% black. The total number of prescriptions for each census tract type descends according to percentage types, as does mean prescriptions for each tract. Student's t-tests show that the means between the tract types are significantly different from each other.

Relative risk ratios for antibiotic resistant infections by census tract type are displayed in Table 3. Multivariate analyses resulted in only 2 significant covariates, those being number of antibiotic prescriptions and racial composition of census tracts. Census tracts with 0 to 2994 prescriptions were the reference category, tracts with 2995 to 5297 prescriptions have an RR of 1.38 (1.10 – 1.73), and tract with 5298 to 16348 prescriptions have an RR of 1.38 (1.10 – 1.73). These RRs appear identical due to rounding, but without rounding are 1.3814 (1.1046 – 1.7275) and 1.3810 (1.1035 – 1.7283), respectively. These RRs are significant with a p-value of 0.0040 and show that increased amounts of antibiotic prescriptions in these census tracts is a risk factor for ARI outcomes. The racial composition of census tracts was also a significant variable in this association, with a p-value of 0.0269. Using census tracts with 0 to 33% black population as the referent level, tracts with 34 to 66% black population carried an RR of 1.09 (0.92 – 1.29), and tracts with 67 to 100% black population carried an RR of 1.49 (1.15 – 1.92). Although the middle level of black population is not significant as the RR crosses the null value, the high level is significant,

suggesting that, when adjusted for total number of prescriptions, census tracts with high black populations have increased risks for an ARI outcome.

Relative risk ratios for outcomes of liver cancer by census tract are shown in Table 4. In this Poisson model, only the racial compositions of the census tracts were associated with liver cancer outcomes. Increased black populations in census tracts carried increasing risks of liver cancer. Living in a tract composed of 34 to 66% black population gave a risk of 1.43 (1.13 – 1.81) and living in a tract with 67 to 100% black population carried a risk of 2.15 (1.64 – 2.80). All other covariates were not statistically significant in this model, therefore, antibiotic exposure cannot be associated with liver cancer outcomes at the aggregate level.

Modeling relative risk ratios for kidney cancer outcomes provided three significant covariates, number of antibiotic prescriptions per census tract, rural or urban designations of census tracts, and racial composition of the tract. Data for these analyses is shown in Table 5 and all RRs are adjusted for these variables. A medium level of exposure to antibiotics yielded an RR of 1.20 (0.98 – 1.47) and a high level showed an RR of 1.51 (1.25 – 1.82). Even though the middle level of exposure is insignificant, the high level of exposure carries an increased risk of kidney cancer outcomes. Urban tracts showed a protective effect with an RR of 0.86 (0.75 – 0.98). Census tracts with greater than 33% black population showed increased risks of kidney cancer outcomes, when adjusted for other variables in this model. Tracts composed of 34 to 66% black population gave a

risk of 1.51 (1.31 – 1.74) and living in a tract with 67 to 100% black population carried a risk of kidney cancer of 2.04 (1.68 – 2.48).

Discussion

Looking at relative risk ratios from our study, our findings indicate significant differences in outcomes of antibiotic resistant infections, liver cancers, and kidney cancers by antibiotic exposure and census tract types. Predictably, antibiotic exposure is associated with ARI outcomes. However, higher percentages of black populations are also associated with an increase in ARI outcomes. This effect is also seen when investigating liver cancer outcomes, but antibiotic exposure has no influence over liver cancer development. Kidney cancer is associated with higher levels of antibiotic exposure, residing in a rural census tract, as well as with higher percentages of black populations in the tract.

While it has been established that increased antibiotic use leads to the emergence of ARIs (12-15), this trend has not been investigated on a background of geographical and demographic differences. However, this only seems natural as differential prescribing patterns in various region types have been uncovered in previous studies (16;17). Our results show that the mean antibiotic prescriptions do vary based on geographical census tract type, as agricultural, inland, and rural areas receive more prescriptions, but this does not necessarily translate to increased risks for ARIs, or even liver or kidney cancers. From the RRs in this study, we have confirmed that antibiotic exposure is associated with ARI outcomes, but that geographical factors of the census tracts

do not influence this association. However, demographics do influence this relationship as higher black populations show increased risk of ARI when adjusted for total number of prescriptions.

Because of the hypotheses and studies by Setchell, Knekt, and Velicer, antibiotic usage in relationship to cancer is now also an avenue that requires examination. Metabolic pathways allow the liver and kidneys the most exposure to antibiotics in the body, and therefore make these organs more at risk for developing toxicities and cancers. This was seen in our analysis for kidney cancer, but not for liver cancer. It may be that the kidneys' continuous filtering and concentration of antibiotics in urine provides the kidney with more potent and constant exposure to antibiotic compounds in the body than the liver, leading to an increased risk in one organ but not the other. In addition to antibiotic exposure and racial composition, rural and urban designations of the census tract affect kidney cancer outcomes. Urban tracts carry a decreased risk for kidney cancer outcomes when adjusted for the other variables in the model. In this case, it is also important to remember antibiotic exposures from environmental sources, for example rural census tracts are exposed to antibiotics in runoff from farms in which animals are treated with antibiotics, as well as airborne and waterborne exposure from orchards in which fruiting trees are sprayed with antibiotics. This increased environmental exposure can also influence the relationship between kidney cancer and antibiotic usage.

Furthermore, our findings suggest higher risks of ARIs, liver, and kidney cancer outcomes in tracts with higher percentages of black population,

specifically 67 to 100% of the population. This may be due to socioeconomic statuses of these tracts, as areas such as these may be more rural and less likely to have routine access to health care, resulting in undesirable outcomes. In addition, less routine access to healthcare may actually increase antibiotic exposure, as doctors would be more willing to prescribe antibiotics for a patient who cannot easily come back for a follow up appointment. This difference may even indicate a biological or physiological phenomenon in which African Americans are unable to metabolize antibiotics as efficiently or completely as Caucasian populations, thus leading to these outcomes. This type of dissimilarity between ethnicities is not unheard of, as it has been found that Asian and Native American populations do not possess all of the functional and active enzymes to metabolize alcohol as efficiently as Caucasians, and that this may lead to the undesirable outcome of alcoholism (31;32).

Collectively, our results indicate that antibiotic usage is a risk factor for the development of antibiotic resistant bacterial infections and kidney cancers and that these risks can be magnified based on the types of census tracts in which people live. Various characteristics of these tracts may lead to these increased risks, including environmental exposures to antibiotics, regular access to health care, and genetic dispositions of the populations in these tracts. Our findings agree with the studies which show that antibiotics use is a major cause of resistance by bacteria and subsequent resistant infections (12-15), and in part, with studies by Knekt and Velicer, which show increased risks from antibiotics for breast cancer. Here, we found that kidney cancer is associated with antibiotic

usage, but liver cancer is not. Although the Knekt study briefly assessed these risks by urban, agricultural, and rural types, this study's region types were more varied and inclusive, relying on geocoded data and defined criteria for region types. The Velicer study did not address the effect of various region types. Also, both the Knekt and Velicer study focused on breast cancer in women, while our methods focused on both men and women at risk for liver and kidney cancers.

As an ecological study, there are limitations to these results. Temporality cannot be accurately determined with this study, as there was no time line between antibiotic exposure and the disease outcome, so a proper cause and effect situation cannot be outlined. As aggregate data and because our smallest unit of analysis is the census tract, risks at an individual level cannot be assessed. At an individual level, the risks uncovered in this study may exist on a different scale or may not exist at all. Additionally, confounding at an individual level cannot be assessed with this type of study and analysis.

Strengths of this study include the linkage between SHP and Medicaid claims data with SCCCR data to ascertain and confirm the diagnoses of liver and kidney cancers in the population. The claims data from SHP and Medicaid provided a relatively easy and inexpensive way to ascertain ARI diagnoses and the drug files allowed accurate antibiotic exposure data as well. This study was ideal for determining the rudimentary relationship between antibiotic usage and ARI, liver cancer, and kidney cancer outcomes in relation to census tract types, and offers hypotheses and conclusions for further exploration and investigation. From our study, it is important to now look at antibiotic usage not only as a whole

but against the background of varying geographical and demographic factors, and to realize that antibiotics do have a role in unwanted and potentially fatal outcomes. Further targeted studies using case-control or cohort methodology would be the next step in identifying and describing an accurate relationship between antibiotic usage, negative outcomes, and prescribing patterns and geographical areas in South Carolina.

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Table 4.1. Tertiles of Antibiotic Exposure by Total Number of Antibiotic Prescriptions per Census Tract from January 2000 to December 2009

Exposure Level Classification	Number of Total Antibiotic Prescriptions	# of Census Tracts (n = 881)
Low	0 – 2994	293 (33.3%)
Medium	2995 – 5297	290 (33.0 %)
High	5298 – 16348	298 (33.8%)

Table 4.2. Antibiotic Prescriptions by Census Tracts in South Carolina from January 2000 to December 2009*

Census Tract Characteristic	# of Census Tracts (n = 881)	# of Total Antibiotic Prescriptions (n = 3976569)	Mean Antibiotic Prescriptions Per Tract	t-test for Means
Agricultural/Industrial				
Agricultural	523 (59.8%)	2710736 (68.2%)	5202.9	<0.0001
Industrial	351 (40.2%)	1265833 (31.8%)	3606.4	
Coastal/Inland				
Coastal	213 (24.4%)	855269 (21.5%)	4015.3	0.0011
Inland	661 (75.6%)	3121300 (78.5%)	4736.4	
Rural/Urban				
Rural	322 (36.8%)	1634613 (41.1%)	5108.2	<0.0001
Urban	552 (63.2%)	2341956 (58.9%)	4242.7	
Racial Composition of Census Tracts				
0 to 33% Black Population	517 (59.2%)	2475274 (62.3%)	4787.8	<0.0001
34 to 66% Black Population	250 (28.6%)	1123890 (28.3%)	4495.6	
67 to 100% Black Population	107 (12.2%)	377405 (9.5%)	3594.3	

* Missing data for agricultural/industrial, coastal/inland, rural/urban and racial composition of census tracts include 7 total missing (0.008%) tracts. Variable stratum numbers may not equal total number of census tracts due to these missing data.

Table 4.3. Relative Risk Ratios for Outcomes of Antibiotic Resistant Infections by Census Tract Type

Census Tract Characteristic	Relative Risk Ratio (95% CI†)	P value for Trend
Number of Antibiotic Prescriptions		
0 – 2994	Reference	
2995 – 5297	1.38 (1.10 – 1.73)‡	0.0040
5298 – 16348	1.38 (1.10 – 1.73)‡	
Racial Composition of Census Tracts		
0 to 33% Black Population	Reference	0.0269
34 to 66% Black Population	1.09 (0.92 – 1.29)	
67 to 100% Black Population	1.49 (1.15 – 1.92)	

*RR estimates represent all significant variables in the multivariate model. †CI = Confidence Interval. ‡Due to rounding, these estimates seem identical, however the actual RR values for 2995 – 5297 and 5298 – 16348 prescriptions are 1.3814 (1.1046 – 1.7275) and 1.3810 (1.1035 – 1.7283), respectively.

Table 4.4. Relative Risk Ratios for Outcomes of Liver Cancer by Census Tract Type

Census Tract Characteristic	Relative Risk Ratio (95% CI†)	P value for Trend
Racial Compositions of Census Tracts		
0 to 33% Black Population	Reference	<0.0001
34 to 66% Black Population	1.43 (1.13 – 1.81)	
67 to 100% Black Population	2.15 (1.64 – 2.80)	

*RR estimates represent all significant variables in the multivariate model. †CI = Confidence Interval.

Table 4.5. Relative Risk Ratios for Outcomes of Kidney Cancer by Census Tract Type

Census Tract Characteristic	Relative Risk Ratio (95% CI†)	P value for Trend
Number of Antibiotic Prescriptions		
0 – 2994	Reference	
2995 – 5297	1.20 (0.98 – 1.47)	<0.0001
5298 – 16348	1.51 (1.25 – 1.82)	
Rural/Urban		
Rural	Reference	0.0244
Urban	0.86 (0.75 – 0.98)	
Racial Composition of Census Tracts		
0 to 33% Black Population	Reference	<0.0001
34 to 66% Black Population	1.51 (1.31 – 1.74)	
67 to 100% Black Population	2.04 (1.68 – 2.48)	

*RR estimates represent all significant variables in the multivariate model. †CI = Confidence Interval.

CHAPTER 5

Case-Control Study of Antibiotic Usage in Relation to Liver Cancer Outcomes in South Carolina[2]

Introduction

The overuse and misuse of antibiotics have caused various issues in today's clinical settings. Their unchecked use has led to the selection of drug resistant, in some cases, multiple drug resistant, bacterial strains and an increase in these types of infections. In these cases, conventional antibiotics become useless as a treatment option (1-5). In addition to causing an emergence of resistant bacterial infections, previous research has shown that the use of antibiotics can lead to the development of cancers. The hypothesis that the use of antibiotics may increase the risk of cancer in humans was first suggested in 1981 by Setchell et al (6). This study showed that antibiotics interfere with the ability of human intestinal microflora to metabolize plant estrogens into compounds which are protective against cancers. Since then, studies by Knekt et al and Velicer et al have uncovered associations between antibiotic usage and cancer (7;8). However, a lack of research currently exists examining the association between antibiotic use and cancer. Furthermore,

[2] Thathiah, P., S.A. Adams, A. Merchant, R. Moran, K. Bennett, R. S. Norman. 2014. To be submitted to the *Journal of the National Cancer Institute*.

while both the Knekt and Velicer studies demonstrated that antibiotics may indeed be a risk for breast cancer, no other cancer type has been studied in relation to antibiotics.

Physiologically, the liver is one of the body's organs that experience the most exposure to drugs in the bloodstream, and therefore would presumably be a site with a substantial amount of risk for antibiotic-induced cancers. The liver plays a vital role in the metabolism and processing of pharmaceuticals, including antibiotics, and is the most metabolically active tissue per unit of weight. The liver is also the largest organ in the body, is perfused by blood containing drugs from the digestive tract, and houses the majority of drug metabolizing enzymes found in organs of the body, further cementing its vast role in drug metabolism. The liver accomplishes two phases of drug metabolism, and depending on the drug, both of these phases will be completed in sequence, or only one of the two phases will be performed. Phase 1 reactions include oxidation, reduction, and hydrolysis of the drug which prepares it for the second phase to produce conjugation products. Phase 2 conjugation reactions, which include glucoronidation, sulphation, and acetylation, increase the water solubility of the drug permitting its excretion in bile or urine. Phase 2 reactions can also inactivate the drug or its active metabolites produced in phase 1 (4;9). As a major player in drug metabolism, the liver is also prone to hepatotoxicities as well as more serious drug induced liver injuries (DILI) (10), which may lead to more serious complications such as liver failure or the need for a liver transplant (11;12). It is reasonable to suggest that cancers can result in the liver due to its

continued processing and exposure to excessive or unneeded antibiotics. Velicer et al suggest that use of antibiotics can lead to cancer in a number of ways. Antibiotics affect the metabolism of the indigenous bacteria in the gastrointestinal tract, allowing for inefficient or incorrect processing of carcinogens, chemicals, and hormones. Antibiotics can also interfere with immune and inflammatory responses, permitting an increase in production of inflammatory cytokines, prostaglandins, and enzymes (6;8;13;14). These events can trigger somatic cells to proceed down a carcinogenic pathway and eventually produce fatal cancers.

Moreover, many studies have pointed to various prescribing patterns for antibiotics based on geographic locations, and this in turn could affect cancer development in these areas if antibiotics prove to be a risk factor for cancer. A global study found that urban areas, such as regions in Mexico and Kenya, have higher amounts of exposure to antibiotic when compared to more rural areas in these and other countries (15). This geographical trend was also uncovered in a study in China (16). Because of these disparities in prescribing patterns, it is feasible that cancer risk can vary in response to the levels of antibiotic exposure in these geographical regions.

The studies by Knekt and Velicer have shown associations between treatment of women with antibiotics and breast cancer, suggesting that these chemotherapeutics may have a much more serious role in disease development. This study primarily addresses the use of antibiotics and their relationship to liver cancer outcomes in both males and females in South Carolina. Additional

analyses including confounding and effect modification of demographic and geographic variables will be performed to describe this relationship more completely.

Methods

Data Sources: This matched case-control study used existing demographic and pharmacy data and diagnostic codes from South Carolina Medicaid administrative claims and State Health Plan (SHP) claims data. Specifically, pharmacy and drug files from Medicaid and SHP were used to ascertain antibiotic prescribing data. The pharmacy and drug files provided information for all individuals in this study for the duration of the study period which included dates from January 1, 2000 to December 31, 2009. They contained information such as days of therapy, drug strength, quantity provided, dispense date, National Drug Codes (NDC), and American Hospital Formulary System (AHFS) codes. These appended datasets were integrated with liver cancer incidences from the South Carolina Central Cancer Registry (SCCCR), a division of the South Carolina Department of Health and Environmental Control (SCDHEC). Study participants represented both males and females aged 18 years or older. All participants were selected from patients enrolled in Medicaid or SHP continuously for at least 1 year before the diagnosis of liver cancer. There were a total of 1620 participants in this study. This study was approved by both the University of South Carolina and SCDHEC Institutional Review Boards.

United States Census Bureau data from 2000 and the Department of Natural Resources(DNR) GAP data and Landcover codes 54 were used to determine geographical aspects of the state of South Carolina, including racial composition of census tracts, agricultural or industrial, coastal or inland, and rural or urban census tract designations. Racial composition was determined by the percentage of black population within each tract categorized by tertiles, 0 to 33%, 34 to 66%, and 67 to 100%. Agricultural or industrial census tracts were determined by DNR Landcover Codes. Industrial areas were defined as those with Landcover codes 22 and 23 (Urban development and Urban residential). Census tracts which were more than 50% developed were classified as industrial. Census tracts with less than 50% development were classified as agricultural. Coastal census tracts were defined within counties in the coastal zone, or the counties of Beaufort, Berkeley, Charleston, Colleton, Dorchester, Horry, Jasper, and Georgetown. Census tracts in all other counties were defined as inland. Using census data, census tracts which had greater than or equal to 50% urban characteristics (urban areas with a population of 50,000 or more) were classified as urban, otherwise the tract was classified as rural.

Antibiotic Usage: Antibiotic usage was assessed by using Medicaid and SHP data. This study was limited to antimicrobial agents effective against bacterial infections and excluded antiviral, antifungal, and antiparasitic agents, and were chosen by using American Hospital Formulary System (AHFS) codes. These included: 081200 antibiotics (systemic), 081202 aminoglycosides, 081206 cephalosporins, 081207 miscellaneous β-lactam antibiotics, 081208

chloramphenicol, 081212 macrolides, 081216 penicillins, 081218 quinolones, 081220 sulfonamides (systemic), 081224 tetracyclines, 081228 antibacterials (miscellaneous), 081600 antimycobacterials, 081604 antituberculosis agents, 081692 antimycobacterials (miscellaneous), 082200 quinolones, 082400 sulfonamides (systemic), 082600 sulfones, 361600 brucellosis, 362800 diphtheria, 367200 scarlet fever, and 368400 tuberculosis. Furthermore, only antibiotics prescribed for oral use were included. Topical and intravenous (IV) antibiotics were excluded. IV antibiotics are usually given on an inpatient basis in hospitals while all oral antibiotics included in this study were outpatient prescriptions. All prescriptions predated the diagnosis of liver cancer in the cases, which was used as the reference date. For the controls, prescriptions predated their reference date, which was the last date of service in the claims data.

Two measures of antibiotic usage were used, both having been adjusted (within the study design) for the time the participant was enrolled in Medicaid or SHP by matching on length of enrollment in the health plans. The first measure was the total number of antibiotic prescriptions per participant during the study period. Total numbers of prescriptions by AHFS categories mentioned above were summed up over the study period by each participant and divided into quartiles for exposure levels. The second predictor was the total days of antibiotic usage by each participant during the study period. These values were calculated by summing up days of therapy variable for each prescription of each participant and then were divided into quartiles as well. For days of use by antibiotic class,

the days of therapy variables for each prescription of each class was summed for each participant to generate the cumulative days of classes of antibiotic use. For analysis by antibiotic class, the 6 most prescribed antibiotics in the dataset were used, representing 99.5% of all prescriptions by participants. These were cephalosporins (31.9%), penicillins (22.4%), quinolones (19.8%), macrolides (13.0%), tetracyclines (6.6%), and miscellaneous antibacterials (5.9%).

Selection of Liver Cancer Cases and Controls: Cases of liver cancer were ascertained from SCCCR using North American Association of Central Cancer Registries (NAACCR) ICD-O-3 codes C220 and C221. Liver cancers included hepatic carcinomas and unspecified malignant hepatic tumors. Incidence density sampling was used as controls were randomly selected from Medicaid and SHP enrollees during the same years the cases were diagnosed. Controls were frequency matched to cases at a ratio of 3:1 on age and length of enrollment in Medicaid or SHP, and type of insurance program, either Medicaid or SHP. Frequency matching ensured that the characteristics of the population of controls were similar to the characteristics of the cases. Controls and cases with previous diagnoses of other cancers were restricted from the dataset, as were participants with multiple liver biopsies and diagnoses of liver cirrhosis, as these are usually associated with liver cancers. This was done by using Current Procedural Terminology (CPT) and International Classification of Disease 9th revision codes (ICD-9). CPT codes for biopsies included 4700, 47001, 47100, 37200, 36011, and 75970, ICD-9 codes for cirrhosis included 571.5, 571.2, and 571. Due to privacy concerns from Medicaid and SHP, these groups were pooled and could

not be analyzed separately. A total of 405 cases of liver cancer and 1215 controls were identified. All participants were given unique identification numbers to protect their anonymity.

Statistical Analyses: Conditional logistic regression was used to estimate the odds ratios of liver cancer associated with antibiotic use overall, by antibiotic class, sex, and population type. Calculating odds ratios by class of antibiotic allowed for ascertainment of additional cancer risk by type of antibiotic, as one class may be associated more strongly than another with a cancer outcome. Furthermore, odds ratios were calculated separately by sex and population to examine any gender or geographic disparities that may have existed between the association between antibiotic usage and cancer outcomes. The cancer outcome variable was categorical, either yes (1) or no (0), while the predictor variables of days of antibiotic use or number of prescriptions were divided into categories of exposure. Data retrieval, management, and analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC) and $P < 0.05$ was used to determine statistical significance. For effect modification, this was relaxed to $P < 0.10$ for interaction terms.

Results

As shown in Table 1, the exposure variables of total number of antibiotic prescriptions and total days of use were divided into quartiles. Quartiles were chosen to reflect clinical practices, as the referent level corresponds to common antibiotic exposure in most individuals. There were a total of 4374 antibiotic

prescriptions in the dataset and a total of 56245 days of use of antibiotics. The dataset contains slightly more females than males, and this is true for controls as well. However, there are more male cases of liver cancer than female cases. When looking at the racial composition of the census tracts in which the participants live, more than half of the controls and cases live in census tracts composed of 0 to 33 % African American population, with fewer living in a census tract composed of 34 to 66% black population, and even less living in a census tract with 67 to 100% black population. The controls and cases have a similar break down for all categories of racial makeup for census tracts. Most of the participants live in agricultural census tracts, inland census tracts, and urban census tracts. The controls and cases have a comparable break down for demographic categories.

The relationship between incident liver cancer and the total number of prescriptions, the first predictor variable, adjusted for sex, is displayed in Table 2. The reference level was between 0 to 4 prescriptions during the study period, and the results show odds ratios of 1.07 (0.77 – 1.49) for 5 to 63 total prescriptions, 1.03 (0.72 – 1.46) for 64 to 204 prescriptions, and 1.39 (0.98 – 1.98) for 205 to 4374 prescriptions. These ORs are not statistically significant as all 95% confidence intervals cross 1, suggesting that no risk exists between the use of antibiotics and liver cancer outcomes. No other variables, besides sex, were shown to be confounders between this relationship. This analysis was also performed with data divided into quintiles and showed similar results.

Effect modification by all demographic variables was investigated in these analyses. Coastal and inland census tracts were investigated as effect modifiers displayed in Table 3. While the p-value was 0.1020 for the interaction, none of the ORs were significant in that all of the 95% confidence intervals crossed 1. However, it is interesting to note the pattern of the ORs as the ORs for the lower and highest level of exposure for both coastal, 1.39 (0.40 0 4.78) and 2.35 (0.67 – 8.31), and inland census tracts, 1.02 (0.68 – 1.55) and 1.55 (0.99 – 2.44) show an increase in risk, but the middle levels of exposure, 0.59 (0.16 – 2.23) for coastal and 0.99 (0.63 – 1.55) for inland, show a protective effect. Sex, agricultural or industrial census tracts, and urban or rural census tracts were not shown to be effect modifiers between total number of antibiotic prescriptions and liver cancer development.

The relationship between the second predictor variable, days of use of antibiotics, and liver cancer is shown in Table 4. While sex was a confounder for the association between incident liver cancer and total number of antibiotic prescriptions, it was shown to be an effect modifier in the association between liver cancer and total days of antibiotic use. Table 4 shows a p-value of 0.0082 for sex as an effect modifier, and has the stratified ORs for this relationship. When comparing the ORs for each strata across sex, 27 to 448 days of use gives an OR of 1.34 (0.77 – 2.32) for females and 0.71 (0.41 – 1.21) for males, 449 to 1590 days gives an OR of 0.69 (0.37 – 1.28) for females and 1.36 (0.80 – 2.31) for males, and 1591 to 56246 days of use gives an OR of 1.75 (0.97 – 3.17) for females and 1.03 (0.60 – 1.767) for males. The ORs for each strata of female

and male are dissimilar from each other, but within each sex, they also cross the null value of 1. While none of these OR's are statistically significant, these patterns do show some effect modification by sex, resulting in differential risk estimates for each group. At the lower exposure level, women have an increased risk of cancer while men seem to be protected from cancer outcomes.

The middle level of exposure flips this trend, and men have an increased risk but women are protected. For the highest exposure level, both sexes are at risk, but females carry a higher risk than males. While the patterns of ORs are interesting, an association between antibiotic usage and liver cancer can not be confirmed. Yet, in spite of the lack of statistical significance for all stratified ORs, the significant p-value for the trend suggests that sex is an effect modifier between antibiotic usage by days and liver cancer outcomes.

All other demographic variables were investigated as potential effect modifiers for this relationship. Table 5 and 6 show the stratified results from these analyses. With a p-value of 0.0763, coastal or inland census tracts suggest effect modification between antibiotic usage by days and liver cancer, however none of the ORs are significant. For 27 to 448 days of antibiotic use, the ORs are 1.28 (0.41 – 4.01) for coastal and 1.01 (0.66 – 1.54) for inland, 1.20 (0.36 – 4.05) for coastal and 1.00 (0.64 – 1.53) for inland for 449 to 1590 days of use, and 1.63 (1.46 – 5.72) for coastal and 1.47 (0.95 – 2.27) for inland for 1591 to 56246 days of use. While these ORs are similar between the strata, indicating similar levels of risk, the p-value makes the observation that coastal or inland census tracts modify the association of days of usage and liver cancer plausible.

A strong effect modifier for this association may be the agricultural or industrial classification of the census tract, shown in Table 6. With a p-value of 0.0340 for the trend, it is statistically significant at less than 0.05. The ORs for 27 to 448 days of use are 1.50 (0.92 – 2.44) for agricultural and 0.65 (0.30 – 1.42) for industrial, for 449 to 1590 days of use 0.80 (0.48 – 1.34) for agricultural and 1.45 (0.68 – 3.12) for industrial, and for 1591 to 56246 days of use, the OR for agricultural is 1.60 (0.98 – 2.64) and 0.78 (0.34 – 1.76) for industrial. These ORs show differential risks of cancer for each stratum in agricultural and industrial census tracts, but none of these ORs are significant statistically. All other covariates were not found to be effect modifiers or confounders.

Analyses to discover the additional risk posed by specific classes of antibiotics were carried out as well. Table 7 illustrates the association between liver cancer outcomes and total number of prescriptions by antibiotic class. These antibiotics were the 6 most common classes prescribed in the dataset and composed 99.5% of all prescriptions. The reference levels were those participants who had 0 prescriptions of the antibiotic in question, but they could have had prescriptions of other antibiotics. This gives the additional risk of the antibiotic class and is in accordance with current prescribing practices, as patients are usually exposed to at least one antibiotic in any given year. Because of the small numbers of those who were exposed to these antibiotic classes, there is a dichotomous distribution among these exposure groups. ORs for use of cephalosporins, penicillins, quinolones, macrolides, and miscellaneous antibacterials were 1.02 (0.68 – 1.53), 1.03 (0.70 – 1.52), 1.27 (0.86 – 1.85), 1.01

(0.68 – 1.50), and 1.72 (0.88 – 3.34), respectively. The use of tetracyclines had an OR of 0.89 (0.52 – 1.54). All of the 95% confidence intervals for these findings cross 1, and therefore are not significant, suggesting that the use of individual classes of antibiotics have no effect on liver cancer outcomes.

Discussion

Our findings showed that antibiotic usage is not a risk factor for the development of liver cancer, as none of the ORs were statistically significant. The relationship between exposure and outcome, however, was confounded by sex when total number of prescriptions was used as the exposure variable or was effect modified when total days of use of antibiotics was used as the exposure variable during analysis. Specific classes of antibiotics were also investigated as potential cancer risks, both by total numbers of prescriptions and total days of use. Again, these results were not statistically significant and cannot be established as risks for liver cancer outcomes.

Several differences in the analyses were noted when using each predictor variable. When using total number of prescriptions as the exposure, sex was a positive confounder, resulting in a shift away from the null from the crude OR estimates. Sex itself is already a documented risk factor for liver cancer, as men are at least 2.4 times more likely than women to develop these cancers (17). However, when using days of antibiotic use, sex was an effect modifier, resulting in different risk estimates for males and females at each level of exposure. Even though sex was also a positive confounder in these analyses, the significance of

the interaction term points towards effect modification as a major player in this association. Effect modification by sex for cancer outcomes have been described previously in the context of alcohol consumption and renal cell carcinomas, resulting in lower risks of cancer with increased alcohol consumption in women, due in part to differences in alcohol and estrogen metabolism in women (23). In this study, our two predictor variables are highly correlated, but the days of use variable is more subjective. For example, men may follow the allotted course of antibiotics and thus receive more exposure than females who may cut the course of treatment short, or vice versa. This could result in the different risk estimates for each group as the exposure levels are changing. Total number of prescriptions does not rely on the behaviors of patients, but is reflected as a doctor prescribed constant variable.

Another difference between the analyses using these two predictors is effect modification by demographic variables. For total prescriptions, only a coastal or inland census tract was a potential effect modifier, but for days of antibiotic use, in addition to sex, coastal or inland census tracts and agricultural or industrial census tracts were effect modifiers. Generally, living in a coastal and agricultural census tract provided greater risk. This could be due to the greater environmental exposure to antibiotics in these regions, for example from waste water runoff in coastal tracts and farming and veterinary use in agricultural tracts (18-22). Further research needs to be conducted to take into account this environmental exposure as a significant risk for the development of cancer outcomes.

Analyses by total prescriptions by classes of antibiotics did not result in increased risks of liver cancer from cephalosporins, penicillins, quinolones, macrolides, miscellaneous antibacterials, and tetracyclines. Further analysis using days of use was also carried out and led to identical results to those in Table 7. Due to the insufficient numbers in each level, we were unable to properly assess risks across varying exposure levels. In the future, with a larger dataset, more levels of exposure can be teased out, therefore leading to a better resolution for detecting these associations by each predictor.

Our findings agree with those from the Knekt et al study. While we detected increased risks from antibiotic usage for liver cancer, our findings were also not always statistically significant, as with the Knekt findings. However, the Knekt study was a cohort study which only investigated antibiotic usage in response to urinary tract infections as a risk for cancer, while our case-control study did not have the restriction of antibiotics prescribed for certain types of infections. By doing so, we studied antibiotic usage as the sole risk factor and not the original infections. While they did find increased risk associated with antibiotic usage and breast cancer, this was displayed in premenopausal women who received antibiotics for a urinary tract infection, our study indicates that this risk is present in men and women of various ages who were prescribed antibiotics for varying conditions. While they did uncover a weak protective association between agricultural and industrial areas compared to urban centers, this study found that coastal and agricultural regions bore greater risks for liver cancer with antibiotic use as an exposure.

However, the conclusions from our study did not agree with the results from the Velicer et al study. The Velicer study noted significant risks from antibiotic usage for liver cancer, but our finding were not significant. While the study by Velicer detected risks posed by all major antibiotics classes in their dataset for total prescriptions and days of use, we did not find these same risks for any of the most common antibiotics in our dataset. Our study differs from the Velicer study as well because their study included only women, as it was primarily a breast cancer study. Also, our study only included incident cases of liver cancer, we did not study fatal cases of liver cancer. We further investigated confounding and interaction by demographic and geographical variables, and uncovered these effects in our study.

The foremost limitation in this study is that even though antibiotics were prescribed to participants, there is no way of knowing if the prescriptions were actually filled or administered correctly, for example at the right times and for the full course of treatment. Inpatient use of antibiotics was not factored into this study. Furthermore, this analysis did not take into account the timing between multiple antibiotic courses. Additional studies need to be performed to examine if risk increases from shorter time intervals between antibiotic courses versus longer intervals. In addition, there was no data about other risk factors for liver cancer or hepatic carcinomas, such as genetic disposition or smoking and alcohol consumption. Residual confounding by socioeconomic status may also be influencing these results, but was not directly addressed in these analyses. Furthermore, due to the small sample size of this study, it was difficult to detect

an association between exposure and outcome. Repeated analyses with a larger sample size may yield more consistent findings with those of Velicer.

The strengths of this study include the unique linkage between Medicaid and SHP data administrative claims to data from SCCCR. This allowed for the accurate determination of pharmacy records for participants and the lack of recall bias as no interviews had to be performed to obtain exposure status. Data were complete for antibiotic prescriptions, including drug names, National Drug Codes, American Hospital Formulary System information, days supplied, and number of prescriptions for each class of antibiotic. Also, by linking to a disease registry, we had a defined cohort from which cases could be chosen, along with the demographic data and detailed cancer information which comes from SCCCR. We did not include individuals who switched from one health plan to another, as a participant had to be enrolled continuously in either plan to be eligible for this study. This avoided missing data from periods of time with no insurance coverage or before switching to a different plan. As a whole, this study addresses risks for liver cancer development in relation to antibiotic usage in a wider population, both men and women, in various regions in the state of South Carolina, allowing for greater generalizability of this study's findings. Drug metabolism pathways were also considered in this study, as the liver is the main organ in the body responsible for metabolizing, processing and breaking down of antibiotics. Therefore, this organ should be closely monitored and studied as a site for cancer development in light of use, overuse, and misuse of antibiotics. As exposure levels increase, this study aimed to divide the data into appropriate

sample sizes for each category, providing more powerful results. The fact that cumulative exposure results could be repeated by dividing the data into quintiles, as well as quartiles, lends credibility to these findings.

To conclude, we did not uncover evidence to suggest that the use of oral antibiotics is associated with the development of liver cancer. These results suggest that demographic and geographical variables may influence this relationship, but further analyses with a larger sample size are needed to accurately describe this association. In addition, specific classes of antibiotics were not shown to be more associated with cancer outcomes than others. Clinically, these findings are not pertinent. Yet in light of findings from previous studies and the toxic nature of antibiotics, safer prescribing of antibiotics as well as reducing the exposure to antibiotics in the generally healthy adult population seems prudent. While this family of drugs has proven its effectiveness and advantages over the past decades, recent medicine has been negatively affected by their use, including the emergence of resistant bacterial infections, and now as a potential risk factor for cancers.

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Table 5.1. Descriptive Characteristics of Controls and Liver Cancer Cases*

Characteristic	All Participants (n = 1620)	Controls (n = 1215)	Cases (n = 405)
Quartiles of Antibiotic Exposure by Total Number of Prescriptions			
0 – 4	398 (24.6%)	296 (24.4%)	102 (25.2%)
5 – 63	404 (24.9%)	306 (25.2%)	98 (24.2%)
64 – 204	415 (25.6%)	323 (26.6%)	92 (22.7%)
205 – 4374	403 (24.9%)	290 (23.9%)	113 (28.0%)
Quartiles of Antibiotic Exposure by Total Days of Use			
0 – 26	398 (24.6%)	295 (24.3%)	103 (25.4%)
27 – 448	411 (25.4%)	313 (25.8%)	98 (24.2%)
449 – 1590	407 (25.1%)	317 (26.1%)	90 (22.2%)
1591 - 56246	404 (24.9%)	290 (23.9%)	114 (28.2%)
Sex			
Female	945 (58.3%)	794 (65.4%)	151 (37.3%)
Male	675 (41.7%)	421 (34.6%)	254 (62.7%)
Racial Composition of Census Tract			
0 – 33% Black	880 (54.3%)	659 (54.2%)	221 (54.6%)
34 – 66% Black	496 (30.6%)	372 (30.6%)	124 (30.6%)
67 – 100% Black	244 (15.1%)	184 (15.1%)	60 (14.8%)
Agricultural/Industrial Census Tract			
Agricultural	1008 (65.2%)	757 (66.1%)	251 (62.7%)
Industrial	538 (34.8%)	389 (33.9%)	149 (37.3%)
Coastal/Inland Census Tract			
Coastal	361 (23.4%)	254 (22.2%)	107 (26.8%)
Inland	1185 (76.6%)	892 (77.8%)	293 (73.2%)
Urban/Rural Census Tract			
Rural	613 (39.6%)	466 (40.7%)	147 (36.7%)
Urban	933 (60.4%)	680 (59.3%)	253 (63.3%)

*Data were complete for exposures and sex and racial composition of census tracts. Missing data for all other variables include 74 (4.6%) total missing, 69

controls (5.7%) and 5 cases (1.2%). Variable stratum numbers may not equal total number of cases or controls due to these missing data.

Table 5.2. Relationship Between Incident Liver Cancer and Total Number of Prescriptions

Number of Prescriptions	Controls (n = 1215)	Cases (n = 405)	OR (95% CI)*	P value for Trend
0 – 4	296 (24.4%)	102 (25.2%)	Reference	
5 – 63	306 (25.2%)	98 (24.2%)	1.07 (0.77 – 1.49)	0.2280
64 – 204	323 (26.6%)	92 (22.7%)	1.03 (0.72 – 1.46)	
205 - 4374	290 (23.9%)	113 (27.9%)	1.39 (0.98 – 1.98)	

*Adjusted for sex. Abbreviations: OR = odds ratio; CI = confidence interval.

Table 5.3. Effect Modification of Coastal or Inland Census Tract on Incident Liver Cancer and Total Number of Prescriptions*

Number of Prescriptions	Controls (n = 1215)	Cases (n = 405)	OR (95% CI)	P value for Interaction
Coastal				
0 – 4	72 (6.3%)	27 (6.8%)	Reference	
5 – 63	57 (5.0%)	28 (7.0%)	1.39 (0.40 – 4.78)	
64 – 204	71 (6.2%)	26 (6.5%)	0.59 (0.16 – 2.23)	0.1020
205 - 4374	54 (4.7%)	26 (6.5%)	2.35 (0.67 – 8.31)	
Inland				
0 – 4	214 (18.7%)	74 (18.5%)	Reference	
5 – 63	231 (20.2%)	70 (17.5%)	1.02 (0.68 – 1.55)	
64 – 204	232 (20.2%)	64 (16.0%)	0.99 (0.63 – 1.55)	
205 - 4374	215 (18.8%)	85 (21.3%)	1.55 (0.99 – 2.44)	

*Missing data include 69 controls (5.7%) and 5 cases (1.2%). Variable stratum numbers may not equal total number of cases or controls due to these missing data. Abbreviations: OR = odds ratio; CI = confidence interval.

Table 5.4. Effect Modification of Sex on Incident Liver Cancer and Total Days of Antibiotic Use

Number of Days of Antibiotic Use	Controls (n = 1215)	Cases (n = 405)	OR (95% CI)	P value for Interaction
Female				
0 – 26	168 (13.8%)	30 (7.4%)	Reference	
27 – 448	211 (17.4%)	48 (11.9%)	1.34 (0.77 – 2.32)	
449 – 1590	229 (18.9%)	26 (6.4%)	0.69 (0.37 – 1.28)	0.0082
1591 - 56246	186 (15.3%)	47 (11.6%)	1.75 (0.97 – 3.17)	
Male				
0 – 26	127 (10.5%)	73 (18.0%)	Reference	
27 – 448	102 (8.4%)	50 (12.4%)	0.71 (0.41 – 1.21)	
449 – 1590	88 (7.2%)	64 (15.8%)	1.36 (0.80 – 2.31)	
1591 - 56246	104 (8.6%)	67 (16.5%)	1.03 (0.60 – 1.77)	

Abbreviations: OR = odds ratio; CI = confidence interval.

Table 5.5. Effect Modification of Coastal or Inland Census Tract on Incident Liver Cancer and Total Days of Antibiotic Use*

Number of Days of Antibiotic Use	Controls (n = 1215)	Cases (n = 405)	OR (95% CI)	P value for Interaction
Coastal				
0 – 26	70 (6.1%)	27 (6.8%)	Reference	
27 – 448	61 (5.3%)	30 (7.5%)	1.28 (0.41 – 4.01)	
449 – 1590	65 (5.7%)	26 (6.5%)	1.20 (0.36 – 4.05)	0.0763
1591 - 56246	58 (5.1%)	24 (6.0%)	1.63 (0.46 – 5.72)	
Inland				
0 – 26	215 (18.7%)	75 (18.8%)	Reference	
27 – 448	230 (20.1%)	67 (16.8%)	1.01 (0.66– 1.54)	
449 – 1590	234 (20.4%)	63 (15.8%)	1.00 (0.64 – 1.53)	
1591 - 56246	213 (18.6%)	88 (22.0%)	1.47 (0.95 – 2.27)	

*Missing data include 69 controls (5.7%) and 5 cases (1.2%). Variable stratum numbers may not equal total number of cases or controls due to these missing data. Abbreviations: OR = odds ratio; CI = confidence interval.

Table 5.6. Effect Modification of Agricultural or Industrial Census Tract on Incident Liver Cancer and Total Days of Antibiotic Use*

Number of Days of Antibiotic Use	Controls (n = 1215)	Cases (n = 405)	OR (95% CI)	P value for Interaction
Agricultural				
0 – 26	174 (15.2%)	60 (15.0%)	Reference	
27 – 448	194 (16.9%)	68 (17.0%)	1.50 (0.92 – 2.44)	
449 – 1590	211 (18.4%)	47 (11.8%)	0.80 (0.48 – 1.34)	0.0340
1591 - 56246	178 (15.5%)	76 (19.0%)	1.60 (0.98 – 2.64)	
Industrial				
0 – 26	111 (9.7%)	42 (10.5%)	Reference	
27 – 448	97 (8.5%)	29 (7.3%)	0.65 (0.30 – 1.42)	
449 – 1590	88 (7.7%)	42 (10.5%)	1.45 (0.68 – 3.11)	
1591 - 56246	93 (8.1%)	36 (9.0%)	0.78 (0.34 – 1.76)	

*Missing data include 69 controls (5.7%) and 5 cases (1.2%). Variable stratum numbers may not equal total number of cases or controls due to these missing data. Abbreviations: OR = odds ratio; CI = confidence interval.

Table 5.7. Relationship between Incident Liver Cancer and Total Number of Prescriptions by Antibiotic Class*

Number of Prescriptions‡	Controls (n = 3705)	Cases (n = 1235)	OR (95% CI)	P value for Trend
Cephalosporins				
0	1074 (88.4%)	357 (88.2%)	Reference	0.9097
1 – 480	141 (11.6%)	48 (11.8%)	1.02 (0.68 – 1.53)	
Penicillins				
0	1031 (84.9%)	343 (84.7%)	Reference	0.8664
1 – 1001	184 (15.1%)	62 (15.3%)	1.03 (0.70 – 1.52)	
Quinolones				
0	1036 (85.3%)	336 (83.0%)	Reference	0.2077
1 – 837	179 (14.7%)	69 (17.0%)	1.27 (0.86 – 1.85)	
Macrolides				
0	1057 (87.0%)	352 (86.9%)	Reference	0.9439
1 – 650	158 (13.0%)	53 (13.1%)	1.01 (0.68 – 1.50)	
Tetracyclines				
0	1148 (94.5%)	385 (95.1%)	Reference	0.6819
1 – 799	67 (5.5%)	20 (4.9%)	0.89 (0.52 – 1.54)	
Miscellaneous Antibacterials				
0	1188 (97.8%)	390 (96.3%)	Reference	
1 – 850	27 (2.2%)	15 (3.7%)	1.72 (0.88 – 3.34)	0.1113

*The reference group for these analyses includes cases and controls with zero prescriptions of the specific antibiotic class in question during the regression. However, these participants may have had prescriptions for one of the other antibiotic classes in these analyses or an antibiotic which was not represented above. ‡Representing the 6 most prescribed antibiotic classes in the dataset (99.5% of all antibiotic prescriptions). Abbreviations: OR = odds ratio; CI = confidence interval.

CHAPTER 6

Case-Control Study of Antibiotic Usage in Relation to Kidney Cancer Outcomes in South Carolina[3]

Introduction

Antibiotics have been hailed as one of the most beneficial discoveries in medicine and public health, however the cytotoxic effects of these drugs may play a role in cancer development. A paucity of literature currently exists examining the association between antibiotic usage and cancer. The hypothesis that the use of antibiotics may increase the risk of cancer in humans was first suggested in 1981 by Setchell et al (1). This study determined antibiotics have a direct negative impact on human intestinal flora when metabolizing plant estrogens into compounds which are protective against cancer. To date, only two studies have been published investigating this link, and both studies focused on antibiotic utilization and breast cancer (2; 3). While these studies by Knekt et al and Velicer et al demonstrated that antibiotics may indeed be a risk for breast cancer, no other cancer type has been evaluated in relation to antibiotic usage. Furthermore, these studies focused mainly on women and only the Knekt study investigated basic geographic regions as a covariate.

[3] Thathiah, P., S.A. Adams, A. Merchant, R. Moran, K. Bennett, R. S. Norman. 2014. To be submitted to *Cancer Epidemiology,Biomarkers & Prevention*.

Antibiotics are very powerful drugs and have many known adverse side effects. These range from relatively mild, even asymptomatic, reactions to serious and life threatening conditions. Some of the most serious include toxicities of the body's organs or organ systems resulting in use of antibiotics (9;10). Among the organs within the body, the kidneys play a leading role in metabolizing and excreting chemotherapeutics, and are therefore prone to developing toxicities (7;11-14). Although toxicities can be induced by antibiotics, it is feasible that even more serious complications, such as cancers, can arise. Velicer et al suggest that antibiotics increase the risk of cancer in a number of ways. First, antibiotics affect the metabolism of the microflora in the gastrointestinal tract, allowing for inefficient or incorrect processing of carcinogens, chemicals, and hormones. Antibiotics can also interfere with immune and inflammatory responses, permitting an increase in production of inflammatory cytokines, prostaglandins, and enzymes (1;3;15;16). These events can trigger somatic cells to proceed down a carcinogenic pathway and eventually produce fatal cancers.

Physiologically, the kidneys experience the largest amount of exposure, except for the liver, to drugs in the bloodstream, and therefore would presumably carry a substantial amount of risk for antibiotic induced cancers. The kidneys not only mediate drug excretion but also play a role in the metabolism of drugs. Studies have shown that the kidneys carry out certain metabolic functions at a faster rate than the liver (4-6), and its metabolic repertoire has thus expanded in the past few decades. The kidneys receive a large amount of blood during

circulation, about 25% of resting cardiac output (7) and are therefore routinely and directly exposed to drugs and compounds in the bloodstream (6). Biochemical reactions in the kidneys, which include the same types of phase 1 and phase 2 reactions that occur in the liver, are capable of activating or inactivating drug compounds and may produce metabolic byproducts which are toxic to the organ. Blood flow through the kidneys, the pH of urine, and urine filtration pathways provide increased concentrations of drugs in various sections of the kidney, which may subsequently lead to damage of the organ (8).

Moreover, many studies have pointed to various prescribing patterns for antibiotics based on geographic locations, and this in turn could affect cancer development in these areas if antibiotics prove to a risk factor for cancer. A global study found that urban areas, such as regions in Mexico and Kenya, have higher amounts of exposure to antibiotic when compared to more rural areas in these and other countries (17). This geographical trend was also uncovered in a study in China (18). Because of these disparities in prescribing patterns, it is feasible that cancer risk can vary in response to the levels of antibiotic exposure in these geographical regions.

The studies by Knekt and Velicer have shown associations between treatment of women with antibiotics and breast cancer, suggesting that these chemotherapeutics may have a much more serious role in disease development. This study aims to fill the gaps in the research regarding antibiotic usage and its relationship to kidney cancer outcomes in both males and females. Confounding and effect modification by demographic and geographic variables will be further

investigated to describe the relationship between development of kidney cancer and antibiotic usage.

Methods

Data Sources: This matched case-control study used existing demographic and pharmacy data and diagnostic codes from South Carolina Medicaid administrative claims and State Health Plan (SHP) claims data. SHP is a comprehensive health plan offered to all government employees in the state of South Carolina. Specifically, pharmacy and drug files from Medicaid and SHP were used to ascertain antibiotic prescribing data. The pharmacy and drug files provided information for all individuals in this study for the duration of the study period which included dates from January 1, 2000 to December 31, 2009. They contained information such as days of therapy, drug strength, quantity provided, dispense date, National Drug Codes (NDC), and American Hospital Formulary System (AHFS) codes. These appended datasets were integrated with kidney cancer incidences from the South Carolina Central Cancer Registry (SCCCR), a division of the South Carolina Department of Health and Environmental Control (SCDHEC). Study participants represented both males and females aged 18 years or older. All participants were selected from patients enrolled in Medicaid or SHP continuously for at least 1 year before the diagnosis of kidney cancer. There were a total of 4940 participants in this study. This study was approved by both the University of South Carolina and SCDHEC Institutional Review Boards.

United States Census Bureau data from 2000 and the Department of Natural Resources(DNR) GAP data, including corresponding 54 class land cover codes, were used to determine geographical aspects of the state of South Carolina. These included racial composition of census tracts, agricultural or industrial, coastal or inland, and rural or urban census tract designations. Racial composition was determined by the percentage of black population within each tract categorized by tertiles, 0 to 33%, 34 to 66%, and 67 to 100%. Agricultural or industrial census tracts were determined by DNR Landcover Codes. Industrial areas were defined as those with Landcover codes 22 and 23 (Urban development and Urban residential). Census tracts which were more than 50% developed were classified as industrial. Census tracts with less than 50% development were classified as agricultural. Coastal census tracts were defined within counties in the coastal zone, or the counties of Beaufort, Berkeley, Charleston, Colleton, Dorchester, Horry, Jasper, and Georgetown. Census tracts in all other counties were defined as inland. Using census data, census tracts which had greater than or equal to 50% urban characteristics (urban areas with a population of 50,000 or more) were classified as urban, otherwise the tract was classified as rural.

Antibiotic Usage: Antibiotic usage was assessed by using Medicaid and SHP data. This study was limited to antimicrobial agents effective against bacterial infections and excluded antiviral, antifungal, and antiparasitic agents, and were chosen by using AHFS codes. Furthermore, only antibiotics prescribed for oral use were included. Topical and intravenous (IV) antibiotics were excluded. IV

antibiotics are usually given on an inpatient basis in hospitals while all oral antibiotics included in this study were outpatient prescriptions. All prescriptions predated the diagnosis of kidney cancer in the cases, which was used as the reference date. For the controls, prescriptions predated their reference date, which was the last date of service in the claims data.

Two measures of antibiotic usage were used, both having been adjusted for the time the participant was enrolled in Medicaid or SHP by matching on length of enrollment in the health plans. The first measure was the total number of antibiotic prescriptions per participant during the study period. Total number of prescription categories were summed up over the study period by each participant and divided into tertiles for exposure levels. The second predictor was the total days of antibiotic usage by each participant during the study period. These values were calculated by summing up days of therapy variable for each participant for each prescription to generate the cumulative days of antibiotic use, and were also divided into tertiles. For analysis by antibiotic class, the 6 most prescribed antibiotics in the dataset were used, representing 81.3% of all prescriptions by participants. These were aminoglycosides (20.7%), cephalosporins (17.0%), penicillins (14.4%), quinolones (19.1%) tetracyclines (5.4%), and sulfonamides (4.9%). These were divided into levels of exposure by natural breaks in the data, but also allowing for an appropriate sample size in each level.

Selection of Kidney Cancer Cases and Controls: Cases of kidney cancer were ascertained from SCCCR using North American Association of Central Cancer

Registries (NAACCR) ICD-O-3 codes. Kidney cancers included renal cell carcinomas and renal pelvic cancers. Incidence density sampling was used as controls were randomly selected from Medicaid and SHP enrollees during the same years the cases were diagnosed. Controls were frequency matched to cases at a ratio of 3:1 on age, length of enrollment in Medicaid or SHP, and type of insurance program, as the controls either used Medicaid or SHP, but not both. Frequency matching ensured that the characteristics of the population of controls are similar to the characteristics of the cases. Controls and cases with previous diagnoses of other cancers were restricted from the dataset. Due to privacy concerns from Medicaid and SHP, these groups were pooled and could not be analyzed separately. A total of 1235 cases of kidney cancer and 3705 controls were identified. All participants were given unique identification numbers to protect their anonymity.

Statistical Analyses: Conditional logistic regression was used to estimate the odds ratios of kidney cancer associated with antibiotic use overall, by antibiotic class, sex, and population type. Calculating odds ratios by class of antibiotic allowed for ascertainment of additional cancer risk by type of antibiotic, as one class may be associated more strongly than another with a cancer outcome. Confounding and effect modification were evaluated for all geographical and demographic variables. Furthermore, odds ratios were calculated separately by sex and population to examine any gender or geographic disparities that may have existed between the association between antibiotic usage and cancer outcomes. The cancer outcome variable was categorical, either yes (1) or no (0),

while the predictor variables of days of antibiotic use or number of prescriptions were divided into categories of exposure. Other covariates in the models included racial composition of census tract, and if the census tract was rural or urban, coastal or inland, and agricultural or industrial. These were used as categorical variables in the multivariate analyses and were defined using the data sources and methods above. Data retrieval, management, and analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC) and $P < 0.05$ was used to determine statistical significance.

Results

The total dataset included 4940 participants, comprised of 3705 controls and 1235 cases of kidney cancer. The primary focus of these analyses was to assess the relationship between antibiotic usage and kidney cancer outcomes. Additional analyses including confounding and effect modification of demographic and geographic variables were performed to describe this relationship more completely. The study had slightly more females than males but the composition was similar in respect to cases and controls. More participants resided in census tracts composed of 0 to 33% black population, and again the break down was similar in regard to cases and controls. In addition, most participants lived in agricultural versus industrial, inland versus coastal, and urban versus rural census tract types. These percentages were mirrored in the composition of cases and controls (Table 1).

Increased risks between kidney cancer development and two different predictor variables, total number of prescriptions and total days of use of antibiotics, were utilized in this study. These predictors were highly correlated, bearing a Pearson correlation coefficient of 0.83. The relationship between the total number of antibiotic prescriptions and incident kidney cancer is displayed in Table 2. A total of 12362 antibiotic prescriptions were dispensed to this study group and for the purpose of analysis were divided into tertiles; a 0 to 17 prescriptions referent group, 18 to 131 prescriptions, and 132 and above. An increase in cumulative number of prescriptions by participants showed statistically significant increased risks to the development of kidney cancer, after adjusting for age and length of enrollment in Medicaid or SHP. Having been prescribed 18 to 131 prescriptions yielded an odds ratio of 1.50 (1.27 – 1.78) and having 132 and above carried an odds ratio of 1.43 (1.20 – 1.70). Results were similar when examining the effect of the total number of days of antibiotic use in relation to kidney cancer (Table 3). A total of 123588 days of use of antibiotics were reported in this dataset, and was also divided into tertiles for analysis. An increase in risk for kidney cancer was found with increased levels of cumulative days of antibiotic usage. The reference group used 0 to 115 total days of use, while the category of 116 to 950 days of use produced an odds ratio of 1.41 (1.20 – 1.67), and the highest level of 951 and above days of antibiotic use yielded an OR of 1.46 (1.23 – 1.73). In addition, categories for both total prescriptions and days of antibiotic use were analyzed by dividing the data into quartiles. These analyses showed similar increased risks with higher levels of exposure as seen

with the tertile analysis described above. Geographical and demographic variables from Table 1 were investigated for confounding and effect modification during these analyses, but had no effect on the association between antibiotic usage and kidney cancer.

Further analyses were performed to investigate the additional risk provided by various classes of the most prescribed antibiotics in the dataset during the study period, both by total number of prescriptions and days of use. Six classes composed 81.3% of prescriptions in the dataset, 20.7% aminoglycosides, 19.1% quinolones, 16.9% cephalosporins, 14.4% penicillins, 5.4% tetracyclines, and 4.9% sulfonamides. Pearson correlation coefficients for each predictor variable for each class were high, 0.78 for prescriptions of aminoglycosides and days use of aminoglycosides, 0.94 for prescriptions and days of use of cephalosporins, 0.97 for penicillin predictors, 0.90 for quinolones, 0.70 for tetracyclines, and 0.85 for sulfonamides. Table 4 shows the association of kidney cancer development with the total number of prescriptions by antibiotic class. Each class of antibiotic was divided into categories which reflected intrinsic breaks in the data, with 0 prescriptions for each class serving as the reference group. However, participants with 0 prescriptions of a specific antibiotic class may still have prescriptions for one of the other 5 classes or a different antibiotic not included in these analyses. This allowed for the determination of additional risk from these specific classes and did not restrict the dataset to those with 0 prescriptions of antibiotics for the entire study period, as this does not reflect current clinical prescribing patterns. For aminoglycosides,

an increase in risk was seen for each level of exposure, 1.21 (1.01 – 1.45) for 1 to 3 total prescriptions of aminoglycosides, 1.16 (0.93 – 1.43) for 4 to 85 prescriptions, and 1.39 (1.12 – 1.73) for 86 or more prescriptions of aminoglycosides. Only the lowest and highest levels of exposure were significant for this antibiotic class, the middle level of exposure was not significant. Cephalosporins showed significant risk for 1 to 3 prescriptions with an OR of 1.26 (1.06 – 1.50) and 4 to 100 total prescriptions with an OR of 1.37 (1.20 – 1.72). The highest level of exposure for cephalosporins with greater than 100 prescriptions was not significant at 1.12 (0.90 – 1.41). Penicillins showed a significant increased risk of cancer for only the middle level of exposure, 3 to 30 total penicillin prescriptions, with an OR of 1.24 (1.00 – 1.54). The lowest level of 1 to 2 prescriptions and highest level of greater than 30 prescriptions showed insignificant risk with ORs of 1.17 (0.98 – 1.39) and 1.03 (0.28 – 1.27), respectively. The quinolones exhibited significant risk of kidney cancer for all levels of exposure, with an OR of 1.44 (1.02 – 1.71) for 1 to 2 total prescriptions, 1.96 (1.62 – 2.37) for 3 to 30 prescriptions, and 1.61 (1.32 – 1.95) for greater than 30 total prescriptions. No significance was found for increased additional risk from tetracyclines or sulfonamides. Both classes of these antibiotics comprised the smallest percentages of the dataset, and therefore had only one level of exposure above the reference group. The corresponding ORs for these classes were 1.19 (0.99 – 1.43) for 1 to 4738 total prescriptions of tetracyclines and 0.85 (0.64 – 1.13) for 1 to 3818 total sulfonamide prescriptions.

Exposure classified by days of use by antibiotic class and its association with kidney cancer risk is shown in Table 5. Once again, levels of exposure were determined by natural breaks in the data, as well as clinical prescribing guidelines. Zero days of use of the specific antibiotic in each analysis was used as reference, even though participants could have days of use of another antibiotic. Aminoglycosides showed an elevated risk for the higher levels of exposure, 15 to 30 days of use and greater than 300 days of use with ORs of 1.25 (1.02 – 1.53) and 1.33 (1.09 – 1.3), respectively. The lowest level of exposure of 1 to 14 days of use was insignificant with an OR of 1.16 (0.94 – 1.41). The opposite trend was seen with cephalosporins, as the two lower categories of exposure showed significant risks with ORs of 1.28 (1.05 – 1.56) for 1 to 14 days of use and 1.35 (1.10 – 1.67) for 15 to 400 days of use. The highest level of exposure, greater than 400 days had an OR of 1.14 (0.93 – 1.40) and was not statistically significant. The only risk provided by the use of penicillins was between 1 to 14 days with an OR of 1.03 – 1.53). Using penicillins between 15 and 150 days and more than 150 days did not show significant risks with ORs of 1.16 (0.95 – 1.41) and 1.02 (0.83 – 1.26). The quinolones, as shown above with cumulative number of prescriptions, exhibited significant risks for each level of exposure. The OR for use between 1 to 14 days was 1.38 (1.14 – 1.68), for 15 to 35 days was 1.86 (1.50 – 2.31), for 36 to 400 days was 1.99 (1.61 – 2.46), and for greater than 400 days was 1.48 (1.20 – 1.83). All of these risks were statistically significant. Due to the smaller percentages of tetracyclines and sulfonamides in the dataset, these classes only had one level of exposure.

Neither of these antibiotic classes had a significant risk on the development of kidney cancer, as using tetracyclines for 1 to 71970 days provided an OR of 1.20 (0.99 – 1.43) and using sulfonamides for 1 to 79891 days produced an OR of 0.85 (0.64 – 1.13). We further investigated confounding and interaction by geographical variables, but did not uncover any effects from these variables.

Discussion

Antibiotic usage was positively associated with kidney cancer, in terms of both the total number of antibiotic prescriptions given to a patient and the total number of days of use of antibiotics. Using case-control methodology, we found that kidney cancer cases had higher exposures to both measures of antibiotic use than controls after multivariate adjustment, although not necessarily in a dose response manner. These findings were robust for both tertile and quartile exposure analysis. Furthermore, our findings suggested that specific classes of antibiotics at varying levels of exposure can pose as a risk factor for kidney cancer outcomes, but once again, not necessarily in a dose response manner. For aminoglycosides, cephalosporins, and penicillins, it is evident that only some levels of exposure led to increased risks, this may result from cellular mechanisms of drug absorption or processing that is only present at certain concentrations of drugs, or maybe even the sequential timing between the prescribing of these antibiotics. Varying modes of actions of these classes may also contribute to the differential risk they provide based on how they affect host immune and inflammatory responses and metabolism and byproducts of gastrointestinal bacteria (2;3). It is important to note that the quinolones showed

increased risks for all categories of exposure with both total prescriptions and days of use, and these can be described as the class of antibiotics which carry the most risk for kidney cancers in this study. Tetracyclines and sulfonamides were not prescribed in high amounts in this dataset, and separate analysis focusing on these two classes may be needed to tease out their real association with cancer development.

Results from our study are in accordance with studies by Knekt et al and Velicer et al which examined the risk of antibiotic usage in relation to breast cancer. However, the Knekt study was a cohort study and only investigated antibiotic usage in response to urinary tract infections as a risk for cancer, while our case-control study did not have the restriction of antibiotics prescribed for certain types of infections. In this way, we studied antibiotic usage as the sole risk factor and not the original infections. While they did find increased risk associated with antibiotic usage and breast cancer, this study was limited to premenopausal women who received antibiotics for a urinary tract infection, while our study finds risk in men and women of various ages who were prescribed antibiotics for varying conditions. While they did uncover a weak association between the types of region in which these women resided, urban, agricultural, or industrial areas, this study could not confirm those findings. Nor could our study detect an association between antibiotic usage and cancer from living in coastal or inland areas.

The conclusions from our study also agree with the results from the Velicer et al study. Both studies found that antibiotic usage is a significant risk

factor for cancer development. While the study by Velicer detected risks posed by all major antibiotics classes in their dataset for total prescriptions and days of use, we did not find these same risks for all of the most common antibiotics in our dataset. It is important to note that our dataset represented different antibiotics than those in the Velicer study. For example, the quinolones were not commonly dispensed in the Velicer dataset and so were not included in their analyses. Velicer et al also noticed this risk for fatal breast cancers as well as incident breast cancers. Our study differs from the Velicer study because their study included only women, as it was primarily a breast cancer study. Also, our study only included incident cases of kidney cancer, we did not study fatal cases of kidney cancer.

The foremost limitation in this study is that even though antibiotic were prescribed to participants, there is no way of knowing if the prescriptions were actually filled or administered correctly, for example at the right times and for the full course of treatment. Inpatient use of antibiotics was not factored into this study because we did not want the primary reason for hospitalization to be considered a risk factor for cancer development. Furthermore, this analysis did not take into account the timing between multiple antibiotic courses. Additional studies need to be performed to examine if risk increases from shorter time intervals between antibiotic courses versus longer intervals. Another limitation is that, even though both SHP and Medicaid enrollees were included in this study, we could not determine risks for individuals in each health plan separately. In addition, there was no data about other risk factors for kidney cancer or renal

pelvic cancers, such as genetic disposition or smoking and alcohol consumption. Residual confounding by socioeconomic status may also be influencing these results, but was not directly addressed in these analyses.

The strengths of this study include the unique linkage between Medicaid and SHP data administrative claims to data from SCCCR. This allowed for the accurate determination of pharmacy records for participants and the lack of recall bias as no interviews had to be performed to obtain exposure status. Data were complete for antibiotic prescriptions, including drug names, National Drug Codes, American Hospital Formulary System information, days supplied, and number of prescriptions for each class of antibiotic. Also, by linking to a disease registry, we had a defined cohort from which cases could be chosen, along with the demographic data and detailed cancer information which comes from SCCCR. We did not include individuals who switched from one health plan to another, as a participant had to be enrolled continuously in either plan to be eligible for this study. This avoided missing data from periods of time with no insurance coverage or before switching to a different plan. As a whole, this study addresses risk for kidney cancer development in relation to antibiotic usage in a wider population, both men and women, in various regions in the state of South Carolina, allowing for greater external validity of our findings. Drug metabolism pathways were also considered in this study, as the kidneys are vital in the processing, break down, and excretion of antibiotics. Therefore, these organs should be closely monitored and studied as a site for cancer development in light of use, overuse, and misuse of antibiotics. As exposure levels increase, this

study aimed to divide the data into appropriate sample sizes for each category, providing more powerful results. The fact that cumulative exposure results could be repeated by dividing the data into quartiles, as well as tertiles, lends credence to these findings. Lastly, our findings were confirmed with an adequate sample size for this case-control study, with 3705 controls and 1235 cases.

In conclusion, we uncovered evidence which suggests that the use of antibiotics is linked to the development of kidney and renal pelvic cancers, however, further epidemiological and biological studies must be conducted to verify these effects. These results were consistent for all levels of exposure for total number of prescriptions and days of use during this study period, which provided strong associations. While the days of use covariate provided increasing risk for increasing use, total prescriptions did not, indicating that days of antibiotic exposure is more strongly related to cancer outcomes. Conversely, higher doses of antibiotics may not increase risk, but overall use and exposure may be enough to lead to cancer. Moreover, certain classes of antibiotics at varying levels of exposure can also contribute to cancer development. There was also consistency in our findings due to their agreement with other studies performed in various parts of the world with varying populations. Clinically, these findings caution the prescribing of unneeded antibiotics as well as reducing the exposure to antibiotics in generally healthy adult patients. While this class of drugs has proven its effectiveness and advantages over the past decades, a detrimental side to them may exist as well, and in the mean time, they should be administered only with great carefulness and prudence.

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Table 6.1. Descriptive Characteristics of Controls and Kidney Cancer Cases*

Characteristic	All Participants (n = 4940)	Controls (n = 3705)	Cases (n = 1235)
Tertiles of Antibiotic Exposure by Total Number of Prescriptions			
0 – 17	1645 (33.3%)	1302 (35.1%)	343 (27.8%)
18 – 131	1618 (32.8%)	1170 (31.6%)	448 (36.3%)
132 – 12362	1677 (34.0%)	1233 (33.3%)	444 (35.9%)
Tertiles of Antibiotic Exposure by Total Days of Use			
0 – 115	1632 (33.0%)	1289 (34.8%)	343 (27.8%)
116 – 950	1629 (33.0%)	1192 (32.2%)	437 (35.4%)
951 – 123588	1679 (34.0%)	1224 (33.0%)	455 (36.8%)
Sex			
Female	2824 (57.2%)	2173 (58.7%)	651 (52.7%)
Male	2116 (42.8%)	1532 (41.3%)	584 (47.3%)
Racial Composition of Census Tract			
0 – 33% Black	3006 (60.9%)	2340 (63.2%)	666 (53.9%)
34 – 66% Black	1366 (27.7%)	960 (25.9%)	406 (32.9%)
67 – 100% Black	568 (11.4%)	405 (10.9%)	163 (13.2%)
Agricultural/Industrial Census Tract			
Agricultural	3163 (66.4%)	2337 (66.0%)	826 (67.4%)
Industrial	1604 (33.6%)	1204 (34.0%)	400 (32.6%)
Coastal/Inland Census Tract			
Coastal	1079 (22.6%)	787 (22.2%)	292 (23.8%)
Inland	3688 (77.4%)	2754 (77.8%)	934 (76.2%)
Urban/Rural Census Tract			
Rural	1909 (40.1%)	1391 (39.3%)	518 (42.3%)
Urban	2858 (59.9%)	2150 (60.7%)	708 (57.7%)

*Data were complete for exposures and sex and racial composition of census tracts. Missing data for all other variables include 173 (3.5%) total missing, 164 controls (4.4%) and 9 cases (0.7%). Variable stratum numbers may not equal total number of cases or controls due to these missing data.

Table 6.2. Relationship Between Incident Kidney Cancer and Total Number of Prescriptions

Number of Prescriptions	Controls (n = 3705)	Cases (n = 1235)	OR (95% CI)	P value for Trend
0 – 17	1302 (35.1%)	343 (27.8%)	Reference	
18 – 131	1170 (31.6%)	448 (36.3%)	1.50 (1.27 – 1.78)	<.0001
132 - 12362	1233 (33.3%)	444 (36.0%)	1.43 (1.20 – 1.69)	

Abbreviations: OR = odds ratio; CI = confidence interval.

Table 6.3. Relationship Between Incident Kidney Cancer and Total Days of Antibiotic Use

Number of Days of Antibiotic Use	Controls (n = 3705)	Cases (n = 1235)	OR (95% CI)	P value for Trend
0 – 115	1289 (34.8%)	343 (27.8%)	Reference	
116 – 950	1192 (32.2%)	437 (35.4%)	1.41 (1.20 – 1.67)	<.0001
951 – 123588	1224 (33.0%)	455 (36.8%)	1.46 (1.23 – 1.73)	

Abbreviations: OR = odds ratio; CI = confidence interval.

Table 6.4. Relationship between Incident Kidney Cancer and Total Number of Prescriptions by Antibiotic Class*

Number of Prescriptions‡	Controls (n = 3705)	Cases (n = 1235)	OR (95% CI)	P value for Trend
Aminoglycosides				
0	2412 (65.1%)	744 (60.2%)	Reference	
1 – 3	566 (15.3%)	209 (16.9%)	1.21 (1.01 – 1.45)	0.0084
4 – 85	375 (10.1%)	133 (10.8%)	1.16 (0.93 – 1.43)	
≥86	352 (9.5%)	149 (12.1%)	1.39 (1.12 – 1.73)	
Cephalosporins				
0	2517 (67.9%)	777 (62.9%)	Reference	
1 – 3	577 (15.6%)	224 (18.1%)	1.26 (1.06 – 1.50)	0.0064
4 – 100	285 (7.7%)	121 (9.8%)	1.37 (1.20 – 1.72)	
≥101	326 (8.8%)	113 (9.2%)	1.12 (0.89 – 1.41)	
Penicillins				
0	2326 (62.8%)	737 (59.7%)	Reference	
1 – 2	594 (16.0%)	220 (17.8%)	1.17 (0.98 – 1.39)	0.1163
3 – 30	366 (9.9%)	143 (11.6%)	1.24 (1.00 – 1.54)	
≥31	419 (11.3%)	135 (10.9%)	1.03 (0.83 – 1.27)	
Quinolones				
0	2197 (59.3%)	589 (47.7%)	Reference	
1 – 2	634 (17.1%)	239 (19.4%)	1.44 (1.02 – 1.71)	<.0001
3 – 30	430 (11.6%)	220 (17.8%)	1.96 (1.62 – 2.37)	
≥31	444 (12.0%)	187 (15.1%)	1.61 (1.32 – 1.95)	
Tetracyclines				
0	3212 (86.7%)	1045 (84.6%)	Reference	0.0664
1 – 4738	493 (13.3%)	190 (15.4%)	1.19 (0.99 – 1.43)	
Sulfonamides				
0	3469 (93.6%)	1167 (94.5%)	Reference	0.2604
1 – 3818	236 (6.4%)	68 (5.5%)	0.85 (0.64 – 1.13)	

Abbreviations: OR = odds ratio; CI = confidence interval. *The reference group for these analyses includes cases and controls with zero prescriptions of the specific antibiotic class in question during the regression. However, these participants may have had prescriptions for one of the other antibiotic classes in these analyses or an antibiotic which was not represented above. ‡Representing the 6 most prescribed antibiotic classes in the dataset (81.3% of all antibiotic prescriptions).

Table 6.5. Relationship between Incident Kidney Cancer and Total Days of Use by Antibiotic Class*

Number of Days of Antibiotic Use‡	Controls (n = 3705)	Cases (n = 1235)	OR (95% CI)	P value for Trend
Aminoglycosides				
0	2413 (65.1%)	744 (60.2%)	Reference	
1 – 14	455 (12.3%)	160 (13.0%)	1.16 (0.94 -1.41)	0.0113
15 – 300	418 (11.3%)	161 (13.0%)	1.25 (1.02 – 1.53)	
≥301	419 (11.3%)	170 (13.8%)	1.33 (1.09 – 1.63)	
Cephalosporins				
0	2518 (68.0%)	777 (62.9%)	Reference	
1 – 14	420 (11.3%)	165 (13.4%)	1.28 (1.05 -1.56)	0.0064
15 – 400	349 (9.4%)	146 (11.8%)	1.35 (1.10 – 1.67)	
≥401	418 (11.3%)	147 (11.9%)	1.14 (0.93 – 1.40)	
Penicillins				
0	2328 (62.8%)	738 (59.8%)	Reference	
1 – 14	431 (11.6%)	172 (13.9%)	1.26 (1.03 – 1.53)	0.0885
15 – 150	487 (13.1%)	178 (14.4%)	1.16 (0.95 – 1.41)	
≥151	459 (12.4%)	147 (11.9%)	1.02 (0.83 – 1.26)	
Quinolones				
0	2198 (59.3%)	589 (47.7%)	Reference	
1 – 14	483 (13.0%)	176 (14.3%)	1.38 (1.14 – 1.68)	<.0001
15 – 35	323 (8.7%)	156 (12.6%)	1.86 (1.50 – 2.31)	
36 – 400	328 (8.9%)	169 (13.7%)	1.99 (1.61 – 2.46)	
≥401	373 (10.1%)	145 (11.7%)	1.48 (1.20 – 1.83)	
Tetracyclines				
0	3212 (86.7%)	1045 (84.6%)	Reference	0.0664
1 – 71970	493 (13.3%)	190 (15.4%)	1.19 (0.99 – 1.43)	
Sulfonamides				
0	3469 (93.6%)	1167 (94.5%)	Reference	0.2604
1 – 79891	236 (6.4%)	68 (5.5%)	0.85 (0.64 – 1.13)	

Abbreviations: OR = odds ratio; CI = confidence interval. *The reference group for these analyses includes cases and controls with zero prescriptions of the specific antibiotic class in question during the regression. However, these participants may have had prescriptions for one of the other antibiotic classes in these analyses or an antibiotic which was not represented above. ‡Representing the 6 most prescribed antibiotic classes in the dataset (81.3% of all antibiotic prescriptions).

CHAPTER 7

Conclusion

Collectively, the findings from this study show that antibiotic usage is related to ARI and kidney cancer outcomes in South Carolina. Looking at relative risk ratios from our study, our findings indicate significant differences in outcomes of antibiotic resistant infections, liver cancers, and kidney cancers by antibiotic exposure and census tract types. Predictably, antibiotic exposure is associated with ARI outcomes. However, higher percentages of black populations are also associated with an increase in ARI outcomes. This effect is also seen when investigating liver cancer outcomes, but antibiotic exposure has no influence over liver cancer development. Kidney cancer is associated with higher levels of antibiotic exposure, residing in a rural census tract, as well as with higher percentages of black populations in the tract.

While it has been established that increased antibiotic use leads to the emergence of ARIs (12-15), this trend has not been investigated on a background of geographical and demographic differences. However, this only seems natural as differential prescribing patterns in various region types have been uncovered in previous studies (16;17). Our results show that the mean antibiotic prescriptions do vary based on geographical census tract type, as agricultural, inland, and rural areas receive more prescriptions, but this does not

necessarily translate to increased risks for ARIs, or even liver or kidney cancers. From the RRs in this study, we have confirmed that antibiotic exposure is associated with ARI outcomes, but that geographical factors of the census tracts do not influence this association. However, demographics do influence this relationship as higher black populations show increased risk of ARI when adjusted for total number of prescriptions.

Because of the hypotheses and studies by Setchell, Knekt, and Velicer, antibiotic usage in relationship to cancer is now also an avenue that requires examination. Metabolic pathways allow the liver and kidneys the most exposure to antibiotics in the body, and therefore make these organs more at risk for developing toxicities and cancers. This was seen in our analysis for kidney cancer, but not for liver cancer. It may be that the kidneys' continuous filtering and concentration of antibiotics in urine provides the kidney with more potent and constant exposure to antibiotic compounds in the body than the liver, leading to an increased risk in one organ but not the other. In addition to antibiotic exposure and racial composition, rural and urban designations of the census tract affect kidney cancer outcomes. Urban tracts carry a decreased risk for kidney cancer outcomes when adjusted for the other variables in the model. In this case, it is also important to remember antibiotic exposures from environmental sources, for example rural census tracts are exposed to antibiotics in runoff from farms in which animals are treated with antibiotics, as well as airborne and waterborne exposure from orchards in which fruiting trees are

sprayed with antibiotics. This increased environmental exposure can also influence the relationship between kidney cancer and antibiotic usage.

Furthermore, our findings suggest higher risks of ARIs, liver, and kidney cancer outcomes in tracts with higher percentages of black population, specifically 67 to 100% of the population. This may be due to socioeconomic statuses of these tracts, as areas such as these may be more rural and less likely to have routine access to health care, resulting in undesirable outcomes. In addition, less routine access to healthcare may actually increase antibiotic exposure, as doctors would be more willing to prescribe antibiotics for a patient who cannot easily come back for a follow up appointment. This difference may even indicate a biological or physiological phenomenon in which African Americans are unable to metabolize antibiotics as efficiently or completely as Caucasian populations, thus leading to these outcomes. This type of dissimilarity between ethnicities is not unheard of, as it has been found that Asian and Native American populations do not possess all of the functional and active enzymes to metabolize alcohol as efficiently as Caucasians, and that this may lead to the undesirable outcome of alcoholism (31;32).

These results indicate that antibiotic usage is a risk factor for the development of antibiotic resistant bacterial infections and kidney cancers and that these risks can be magnified based on the types of census tracts in which people live. Various characteristics of these tracts may lead to these increased risks, including environmental exposures to antibiotics, regular access to health care, and genetic dispositions of the populations in these tracts. Our findings

agree with the studies which show that antibiotics use is a major cause of resistance by bacteria and subsequent resistant infections (12-15), and in part, with studies by Knekt and Velicer, which show increased risks from antibiotics for breast cancer. Here, we found that kidney cancer is associated with antibiotic usage, but liver cancer is not. Although the Knekt study briefly assessed these risks by urban, agricultural, and rural types, this study's region types were more varied and inclusive, relying on geocoded data and defined criteria for region types. The Velicer study did not address the effect of various region types. Also, both the Knekt and Velicer study focused on breast cancer in women, while our methods focused on both men and women at risk for liver and kidney cancers.

As an ecological study, there are limitations to these results. Temporality cannot be accurately determined with this study, as there was no time line between antibiotic exposure and the disease outcome, so a proper cause and effect situation cannot be outlined. As aggregate data and because our smallest unit of analysis is the census tract, risks at an individual level cannot be assessed. At an individual level, the risks uncovered in this study may exist on a different scale or may not exist at all. Additionally, confounding at an individual level cannot be assessed with this type of study and analysis.

Strengths of this study include the linkage between SHP and Medicaid claims data with SCCCR data to ascertain and confirm the diagnoses of liver and kidney cancers in the population. The claims data from SHP and Medicaid provided a relatively easy and inexpensive way to ascertain ARI diagnoses and the drug files allowed accurate antibiotic exposure data as well. This study was

ideal for determining the rudimentary relationship between antibiotic usage and ARI, liver cancer, and kidney cancer outcomes in relation to census tract types, and offers hypotheses and conclusions for further exploration and investigation. From our study, it is important to now look at antibiotic usage not only as a whole but against the background of varying geographical and demographic factors, and to realize that antibiotics do have a role in unwanted and potentially fatal outcomes. Further targeted studies using case-control or cohort methodology would be the next step in identifying and describing an accurate relationship between antibiotic usage, negative outcomes, and prescribing patterns and geographical areas in South Carolina.

Findings from this aim showed that antibiotic usage is not a risk factor for the development of liver cancer, as none of the ORs were statistically significant. The relationship between exposure and outcome, however, was confounded by sex when total number of prescriptions was used as the exposure variable or was effect modified when total days of use of antibiotics was used as the exposure variable during analysis. Specific classes of antibiotics were also investigated as potential cancer risks, both by total numbers of prescriptions and total days of use. Again, these results were not statistically significant and cannot be established as risks for liver cancer outcomes.

Several differences in the analyses were noted when using each predictor variable. When using total number of prescriptions as the exposure, sex was a positive confounder, resulting in a shift away from the null from the crude OR estimates. Sex itself is already a documented risk factor for liver cancer, as men

are at least 2.4 times more likely than women to develop these cancers (17). However, when using days of antibiotic use, sex was an effect modifier, resulting in different risk estimates for males and females at each level of exposure. Even though sex was also a positive confounder in these analyses, the significance of the interaction term points towards effect modification as a major player in this association. Effect modification by sex for cancer outcomes have been described previously in the context of alcohol consumption and renal cell carcinomas, resulting in lower risks of cancer with increased alcohol consumption in women, due in part to differences in alcohol and estrogen metabolism in women (23). In this study, our two predictor variables are highly correlated, but the days of use variable is more subjective. For example, men may follow the allotted course of antibiotics and thus receive more exposure than females who may cut the course of treatment short, or vice versa. This could result in the different risk estimates for each group as the exposure levels are changing. Total number of prescriptions does not rely on the behaviors of patients, but is reflected as a doctor prescribed constant variable.

Another difference between the analyses using these two predictors is effect modification by demographic variables. For total prescriptions, only a coastal or inland census tract was a potential effect modifier, but for days of antibiotic use, in addition to sex, coastal or inland census tracts and agricultural or industrial census tracts were effect modifiers. Generally, living in a coastal and agricultural census tract provided greater risk. This could be due to the greater environmental exposure to antibiotics in these regions, for example from

waste water runoff in coastal tracts and farming and veterinary use in agricultural tracts (18-22). Further research needs to be conducted to take into account this environmental exposure as a significant risk for the development of cancer outcomes.

Analyses by total prescriptions by classes of antibiotics did not result in increased risks of liver cancer from cephalosporins, penicillins, quinolones, macrolides, miscellaneous antibacterials, and tetracyclines. Further analysis using days of use was also carried out and led to identical results to those in Table 7. Due to the insufficient numbers in each level, we were unable to properly assess risks across varying exposure levels. In the future, with a larger dataset, more levels of exposure can be teased out, therefore leading to a better resolution for detecting these associations by each predictor.

Findings from this aim agree with those from the Knekt et al study. While we detected increased risks from antibiotic usage for liver cancer, our findings were also not always statistically significant, as with the Knekt findings. However, the Knekt study was a cohort study which only investigated antibiotic usage in response to urinary tract infections as a risk for cancer, while our case-control study did not have the restriction of antibiotics prescribed for certain types of infections. By doing so, we studied antibiotic usage as the sole risk factor and not the original infections. While they did find increased risk associated with antibiotic usage and breast cancer, this was displayed in premenopausal women who received antibiotics for a urinary tract infection, our study indicates that this risk is present in men and women of various ages who were prescribed

antibiotics for varying conditions. While they did uncover a weak protective association between agricultural and industrial areas compared to urban centers, this study found that coastal and agricultural regions bore greater risks for liver cancer with antibiotic use as an exposure.

However, the conclusions from this aim did not agree with the results from the Velicer et al study. The Velicer study noted significant risks from antibiotic usage for liver cancer, but our finding were not significant. While the study by Velicer detected risks posed by all major antibiotics classes in their dataset for total prescriptions and days of use, we did not find these same risks for any of the most common antibiotics in our dataset. Our study differs from the Velicer study as well because their study included only women, as it was primarily a breast cancer study. Also, our study only included incident cases of liver cancer, we did not study fatal cases of liver cancer. We further investigated confounding and interaction by demographic and geographical variables, and uncovered these effects in our study.

The foremost limitation in this study is that even though antibiotics were prescribed to participants, there is no way of knowing if the prescriptions were actually filled or administered correctly, for example at the right times and for the full course of treatment. Inpatient use of antibiotics was not factored into this study. Furthermore, this analysis did not take into account the timing between multiple antibiotic courses. Additional studies need to be performed to examine if risk increases from shorter time intervals between antibiotic courses versus longer intervals. In addition, there was no data about other risk factors for liver

cancer or hepatic carcinomas, such as genetic disposition or smoking and alcohol consumption. Residual confounding by socioeconomic status may also be influencing these results, but was not directly addressed in these analyses. Furthermore, due to the small sample size of this study, it was difficult to detect an association between exposure and outcome. Repeated analyses with a larger sample size may yield more consistent findings with those of Velicer.

The strengths of this study include the unique linkage between Medicaid and SHP data administrative claims to data from SCCCR. This allowed for the accurate determination of pharmacy records for participants and the lack of recall bias as no interviews had to be performed to obtain exposure status. Data were complete for antibiotic prescriptions, including drug names, National Drug Codes, American Hospital Formulary System information, days supplied, and number of prescriptions for each class of antibiotic. Also, by linking to a disease registry, we had a defined cohort from which cases could be chosen, along with the demographic data and detailed cancer information which comes from SCCCR. We did not include individuals who switched from one health plan to another, as a participant had to be enrolled continuously in either plan to be eligible for this study. This avoided missing data from periods of time with no insurance coverage or before switching to a different plan. As a whole, this study addresses risks for liver cancer development in relation to antibiotic usage in a wider population, both men and women, in various regions in the state of South Carolina, allowing for greater generalizability of this study's findings. Drug metabolism pathways were also considered in this study, as the liver is the main

organ in the body responsible for metabolizing, processing and breaking down of antibiotics. Therefore, this organ should be closely monitored and studied as a site for cancer development in light of use, overuse, and misuse of antibiotics. As exposure levels increase, this study aimed to divide the data into appropriate sample sizes for each category, providing more powerful results. The fact that cumulative exposure results could be repeated by dividing the data into quintiles, as well as quartiles, lends credibility to these findings.

We did not uncover evidence to suggest that the use of oral antibiotics is associated with the development of liver cancer. These results suggest that demographic and geographical variables may influence this relationship, but further analyses with a larger sample size are needed to accurately describe this association. In addition, specific classes of antibiotics were not shown to be more associated with cancer outcomes than others. Clinically, these findings are not pertinent. Yet in light of findings from previous studies and the toxic nature of antibiotics, safer prescribing of antibiotics as well as reducing the exposure to antibiotics in the generally healthy adult population seems prudent. While this family of drugs has proven its effectiveness and advantages over the past decades, recent medicine has been negatively affected by their use, including the emergence of resistant bacterial infections, and now as a potential risk factor for cancers.

Antibiotic usage was positively associated with kidney cancer, in terms of both the total number of antibiotic prescriptions given to a patient and the total number of days of use of antibiotics. Using case-control methodology, we found

that kidney cancer cases had higher exposures to both measures of antibiotic use than controls after multivariate adjustment, although not necessarily in a dose response manner. These findings were robust for both tertile and quartile exposure analysis. Furthermore, our findings suggested that specific classes of antibiotics at varying levels of exposure can pose as a risk factor for kidney cancer outcomes, but once again, not necessarily in a dose response manner. For aminoglycosides, cephalosporins, and penicillins, it is evident that only some levels of exposure led to increased risks, this may result from cellular mechanisms of drug absorption or processing that is only present at certain concentrations of drugs, or maybe even the sequential timing between the prescribing of these antibiotics. Varying modes of actions of these classes may also contribute to the differential risk they provide based on how they affect host immune and inflammatory responses and metabolism and byproducts of gastrointestinal bacteria (2;3). It is important to note that the quinolones showed increased risks for all categories of exposure with both total prescriptions and days of use, and these can be described as the class of antibiotics which carry the most risk for kidney cancers in this study. Tetracyclines and sulfonamides were not prescribed in high amounts in this dataset, and separate analysis focusing on these two classes may be needed to tease out their real association with cancer development.

Results from our study are in accordance with studies by Knekt et al and Velicer et al which examined the risk of antibiotic usage in relation to breast cancer. However, the Knekt study was a cohort study and only investigated

antibiotic usage in response to urinary tract infections as a risk for cancer, while our case-control study did not have the restriction of antibiotics prescribed for certain types of infections. In this way, we studied antibiotic usage as the sole risk factor and not the original infections. While they did find increased risk associated with antibiotic usage and breast cancer, this study was limited to premenopausal women who received antibiotics for a urinary tract infection, while our study finds risk in men and women of various ages who were prescribed antibiotics for varying conditions. While they did uncover a weak association between the types of region in which these women resided, urban, agricultural, or industrial areas, this study could not confirm those findings. Nor could our study detect an association between antibiotic usage and cancer from living in coastal or inland areas.

The conclusions from our study also agree with the results from the Velicer et al study. Both studies found that antibiotic usage is a significant risk factor for cancer development. While the study by Velicer detected risks posed by all major antibiotics classes in their dataset for total prescriptions and days of use, we did not find these same risks for all of the most common antibiotics in our dataset. It is important to note that our dataset represented different antibiotics than those in the Velicer study. For example, the quinolones were not commonly dispensed in the Velicer dataset and so were not included in their analyses. Velicer et al also noticed this risk for fatal breast cancers as well as incident breast cancers. Our study differs from the Velicer study because their study included only women, as it was primarily a breast cancer study. Also, our study

only included incident cases of kidney cancer, we did not study fatal cases of kidney cancer.

The foremost limitation in this study is that even though antibiotic were prescribed to participants, there is no way of knowing if the prescriptions were actually filled or administered correctly, for example at the right times and for the full course of treatment. Inpatient use of antibiotics was not factored into this study because we did not want the primary reason for hospitalization to be considered a risk factor for cancer development. Furthermore, this analysis did not take into account the timing between multiple antibiotic courses. Additional studies need to be performed to examine if risk increases from shorter time intervals between antibiotic courses versus longer intervals. Another limitation is that, even though both SHP and Medicaid enrollees were included in this study, we could not determine risks for individuals in each health plan separately. In addition, there was no data about other risk factors for kidney cancer or renal pelvic cancers, such as genetic disposition or smoking and alcohol consumption. Residual confounding by socioeconomic status may also be influencing these results, but was not directly addressed in these analyses.

The strengths of this study include the unique linkage between Medicaid and SHP data administrative claims to data from SCCCR. This allowed for the accurate determination of pharmacy records for participants and the lack of recall bias as no interviews had to be performed to obtain exposure status. Data were complete for antibiotic prescriptions, including drug names, National Drug Codes, American Hospital Formulary System information, days supplied, and number of

prescriptions for each class of antibiotic. Also, by linking to a disease registry, we had a defined cohort from which cases could be chosen, along with the demographic data and detailed cancer information which comes from SCCCR. We did not include individuals who switched from one health plan to another, as a participant had to be enrolled continuously in either plan to be eligible for this study. This avoided missing data from periods of time with no insurance coverage or before switching to a different plan. As a whole, this study addresses risk for kidney cancer development in relation to antibiotic usage in a wider population, both men and women, in various regions in the state of South Carolina, allowing for greater external validity of our findings. Drug metabolism pathways were also considered in this study, as the kidneys are vital in the processing, break down, and excretion of antibiotics. Therefore, these organs should be closely monitored and studied as a site for cancer development in light of use, overuse, and misuse of antibiotics. As exposure levels increase, this study aimed to divide the data into appropriate sample sizes for each category, providing more powerful results. The fact that cumulative exposure results could be repeated by dividing the data into quartiles, as well as tertiles, lends credence to these findings. Lastly, our findings were confirmed with an adequate sample size for this case-control study, with 3705 controls and 1235 cases.

In conclusion, we uncovered evidence which suggests that the use of antibiotics is linked to the development of kidney and renal pelvic cancers, however, further epidemiological and biological studies must be conducted to verify these effects. These results were consistent for all levels of exposure for

total number of prescriptions and days of use during this study period, which provided strong associations. While the days of use covariate provided increasing risk for increasing use, total prescriptions did not, indicating that days of antibiotic exposure is more strongly related to cancer outcomes. Conversely, higher doses of antibiotics may not increase risk, but overall use and exposure may be enough to lead to cancer. Moreover, certain classes of antibiotics at varying levels of exposure can also contribute to cancer development. There was also consistency in our findings due to their agreement with other studies performed in various parts of the world with varying populations. Clinically, these findings caution the prescribing of unneeded antibiotics as well as reducing the exposure to antibiotics in generally healthy adult patients. While this class of drugs has proven its effectiveness and advantages over the past decades, a detrimental side to them may exist as well, and in the mean time, they should be administered only with great carefulness and prudence.

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