Non-Steroidal Anti-Inflammatory Drugs and the risk of
Clostridium Difficile-Associated Disease

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ABSTRACT

*Clostridium difficile* is a bacterium which causes gastro-intestinal infection. The association between non-steroidal anti-inflammatory drugs (NSAIDs) and *Clostridium difficile*-associated disease (CDAD) has not been studied. Several case reports link diclofenac, an NSAID, with CDAD.

In this thesis, we conducted a case-control study, using data from the United Kingdom’s General Practice Research Database (GPRD), to examine the risk of CDAD associated with NSAID use. We identified 1,360 cases and 13,072 matched controls from 1994 through 2005. Using conditional logistic regression, we found an increased risk of CDAD associated with diclofenac [adjusted rate ratio 1.35; 95% confidence interval: 1.10-1.67]. We did not observe an increased risk of CDAD with use of any other NSAID. In addition, no dose response for diclofenac was found.

In conclusion, diclofenac was associated with an increased risk of CDAD. Several NSAIDs could be prescribed in place of diclofenac, reducing the risk of CDAD without additional inconveniences.
RÉSUMÉ

Le Clostridium difficile est une bactérie qui cause des infections gastro-intestinales. L’association entre les anti-inflammatoires non-stéroïdiens (AINS) et le C. difficile n’a jamais été étudiée. Plusieurs rapports de cas lient le diclofenac (AINS) au C. difficile.

Dans cette étude cas-témoin, basée sur des données du United Kingdom’s General Practice Research Database, le risque de C. difficile suite à l’utilisation d’AINS a été évalué. 1360 cas et 13072 contrôles ont été identifiés entre 1994 et 2005. À l’aide de régression logistique, nous trouvons une augmentation du risque de C. difficile chez les utilisateurs de diclofenac [Risque relatif ajusté 1.35; intervalle de confiance 95%: 1.10-1.67]. Aucun autre AINS n’est associé à une augmentation d’infection par le C. difficile. De plus, le diclofenac n’a pas d’effet de dose.

En conclusion, le diclofenac est associé à un risque accru de C. difficile. Ce risque peut être éliminé en remplaçant le diclofenac par d’autres AINS.
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CHAPTER 1 - INTRODUCTION

*Clostridium difficile* (*C. difficile*) is a spore-forming, gram-positive anaerobic bacillus bacterium which was discovered in 1935.¹ Spore forming bacteria can remain dormant for years in their resistant spore-form until they are exposed to the optimal conditions at which point they begin to multiply. Anaerobic bacteria do not need oxygen to grow, making the human gastro-intestinal tract the perfect growth medium for infection by this type of bacteria.² Originally, *C. difficile* was isolated from healthy newborns' stool samples and was consequently believed to be non-pathogenic. It received its name from the difficulty scientists experienced when attempting to grow and isolate it.¹ *C. difficile* only resurfaced in the scientific literature over forty years later, when it was finally associated with pseudomembranous colitis, in the late 1970’s, several years after broad-spectrum antibiotics were introduced into the practice of medicine.³ We know today that *C. difficile* is known to produce a wide range of clinical manifestations from simple diarrhea to fulminant colitis with megacolon and perforation. CDAD is the acronym for the medical condition called *Clostridium difficile* Associated Disease, which refers to this broad range of gastro-intestinal illnesses.⁴

Over the past ten years, there has been a worldwide increase in the frequency and in the severity of CDAD.⁵ In the United States alone, there are over 3 million new cases of CDAD a year, leading to yearly health care costs of over $1.1 billion and a case-fatality rate as high as 2.5%.⁵,⁶ CDAD is the leading cause of hospital-acquired infectious diarrhea and the fourth most common hospital-acquired disease in developed countries.⁵
Broad-spectrum antibiotics have for several decades been recognized as a risk factor for CDAD. In a recent study of the association between proton pump inhibitor use and the risk of CDAD, an unexpected finding was made, implicating NSAID use as a risk factor for CDAD (adjusted rate ratio [RR], 1.3; 95% CI 1.2-1.5). In that study, however, NSAIDs were all grouped together. Furthermore, several case reports over the past 30 years have suggested an association between diclofenac use and CDAD. The question is therefore whether or not NSAIDs are in fact associated with the development of CDAD, and if so, is diclofenac any different from other NSAIDs with respect to this risk.
CHAPTER 2 - BACKGROUND AND LITERATURE REVIEW

2.1 Clostridium Difficile-Associated Disease (CDAD)

2.1.1 Etiology

CDAD is thought to be caused by the opportunistic proliferation of C. difficile following a disruption of the normal intestinal microflora. Antibiotics are an important factor in this disruption. In particular, classes of antibiotics that are used against gram-negative bacteria tend to have the most disruptive effect. Ampicillin, clindamycin, and cephalosporins are the most common antibiotics associated with C. difficile opportunistic proliferation and therefore with CDAD. When C. difficile proliferates and colonizes the intestinal tract, it produces and releases two protein exotoxins (toxins A and B). These toxins bind to intestinal epithelial cell receptors causing substantial secretion of fluid (diarrhea) and acute inflammatory infiltrate of the intestinal tract.

2.1.2 Epidemiology

C. difficile, as stated above, is the leading cause of hospital-acquired enteric infection in developed countries. Until recently, CDAD was believed to be important only as a nosocomial disease causing very few and mostly benign cases in the otherwise healthy community dwellers. However, recent studies have been implicating C. difficile as an ever more significant cause of diarrhea in the community. Indeed, there has been an exponential increase in the incidence of cases diagnosed in the community from less than 1 per 100,000 persons in 1994 to 22 per 100,000 in 2004, in the United Kingdom. A growing
number of these cases are emerging in the community from persons who are healthy, non-hospitalized and even unexposed to antibiotic treatment.\textsuperscript{4, 8} Furthermore, the cases arising from the community are reported to be increasingly more severe.\textsuperscript{10} The hypothesized reason for this change in CDAD disease patterns seems to point towards the newly discovered strains of \textit{C. difficile}, which are more virulent than those previously described in the literature.\textsuperscript{4}

\textbf{2.1.3 Risk factors for CDAD}

Antibiotic use is a major risk factor for CDAD.\textsuperscript{8, 17} Age and co-morbidity are the others.\textsuperscript{17} Typically, \textit{C. difficile} infects elderly, debilitated patients. The co-morbid diseases shown to be risk factors are: inflammatory bowel disease, diverticular disease, peptic ulcer disease, gastroesophageal reflux disease, \textit{Helicobacter pylori}–associated disease, pernicious anemia as a marker of gastric hypochlorhydria, renal failure, cancer, methicillin-resistant \textit{Staphylococcus aureus} positive, diabetes mellitus, chronic obstructive pulmonary disease, and cirrhosis.\textsuperscript{10} Additional risk factors for \textit{C. difficile} infection include gastro-intestinal surgery and extended exposure to a \textit{C. difficile} infected individual.\textsuperscript{17} A recent epidemiological study identified gastric acid-suppressive agents as being associated with CDAD.\textsuperscript{10} In this study, proton pump inhibitors (adjusted RR, 2.9; 95\% CI 2.4-3.4) and H2-receptor antagonists (adjusted RR, 2.0; 95\% CI 1.6-2.7) appeared to increase the risk of CDAD. This same study found an association between NSAID use and the risk of CDAD (adjusted RR, 1.3; 95\% CI 1.2-1.5).
2.1.4 Clinical manifestations

Approximately 20% of all hospitalized patients, as well as 3% to 5% of the general population in North America, are asymptomatic carriers of \textit{C. difficile}.\textsuperscript{6} These carriers act as a reservoir for the spread and perpetuation of CDAD both in the hospital setting and in the community. As for the symptomatic cases, the invasion of the GI tract by \textit{C. difficile} can lead to gastro-intestinal manifestations of varying severity. Most often CDAD will manifest itself as mild to severe watery diarrhea. Less frequently but with more dire consequences, CDAD will evolve into pseudomembranous colitis.\textsuperscript{3, 9, 18} Pseudomembranous colitis is believed to result from an inflammatory reaction of the intestinal wall to \textit{C. difficile} toxins. These pseudomembranes are characterized by a mix of inflammatory cells, fibrin, and bacterial and cellular components, which exude from the intestinal mucosa.\textsuperscript{19} In 3% of cases of CDAD, fulminant colitis, the most severe form of pseudomembranous colitis, develops, and it has a case-fatality rate of up to 80% depending on the co-morbid characteristics of the patient.\textsuperscript{18}

2.1.5 Diagnosis

A history of antibiotic use is important in the diagnosis of CDAD.\textsuperscript{8, 17} Patients taking antibiotics (or recently having taken antibiotics) who develop abdominal pain, cramps and diarrhea are usually tested for \textit{C. difficile} infection. The gold standard for the laboratory diagnosis of \textit{C. difficile} is the cytotoxin stool assay.\textsuperscript{20} It consists of a culture of the organisms on selective medium, followed by testing for toxin production. It has a sensitivity of 94 to 100 percent and a
specificity of 99 percent. Unfortunately, this test is expensive and requires two to three days to complete. Newer, more rapid immunoassays are replacing the old cytotoxin assays. The new assays are enzyme linked immunosorbent assays (ELISAs), which detect both \textit{C. difficile} toxins (A and B) in under an hour, with a sensitivity of 70 to 90 percent and a specificity of 99 percent.\textsuperscript{20} When the diagnosis is in doubt or when it is needed immediately, colonoscopy can be used to provide confirmation of the presence of pseudomembranes, which are indicative of CDAD.\textsuperscript{21, 22}

2.1.6 Treatment

In general, if the symptoms are mild and the duration of the disease is short, CDAD can often go undiagnosed.\textsuperscript{7-9} If, on the contrary, the symptoms persist, then the first line of treatment is discontinuation of antibiotics (if the person is presently taking antibiotics). If the symptoms do not resolve, metronidazole is used as the first-line drug because vancomycin is expensive (the wholesale cost of a standard course of oral vancomycin is approximately $125, compared with $1 for metronidazole) and because widespread use of oral vancomycin could lead to the emergence of vancomycin-resistant bacteria.\textsuperscript{9, 23} Oral vancomycin therapy is therefore only recommended for patients who fail to respond to metronidazole, who do not tolerate metronidazole, or who have severe pseudomembranous colitis.\textsuperscript{23}
2.2 Anti-inflammatory drugs

2.2.1 Physiology of inflammation

Inflammation is an intricate biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a process by which the body attempts to remove the injurious stimuli as well as initiate the healing process of the affected tissue.

The inflammatory process begins with arachidonic acid, a dietary unsaturated fatty acid obtained from animal fats. This acid is converted by the enzyme cyclooxygenase to synthesize different prostaglandins. These prostaglandins regulate many bodily functions. Two types of cyclooxygenase enzymes exist: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Each of these produces different types of prostaglandins. COX-1 enzymes generate prostaglandins which are responsible for bodily functions such as production of a protective mucous lining by the stomach, diuresis, and platelet formation, to state a few. COX-2 enzymes are different in that they are induced, compared to COX-1 enzymes which are always present in the human body. The induction of COX-2 enzymes results in the release of specific prostaglandins which are responsible for the inflammatory response as we know it, causing the cardinals signs of inflammation: pain, redness, swelling, and heat.

These clinical signs are due to the infiltration of the tissues by plasma (the acellular part of blood) and leukocytes (white blood cells). This process continues until the injurious stimuli has been removed, broken down, or walled off by fibrosis (scarring). The process of acute inflammation is initiated by modification to the
blood vessels local to the injured tissue, which become permeable to plasma and leukocytes, promoting their extravasation into the surrounding tissue. This sudden increased flow of plasma fluid into the tissue causes the distinctive swelling associated with inflammation, and the increase in blood flow to the area causes the reddened color and increased heat. The extravasation of leukocytes toward the site of injury is essential because these are the cells that attempt to remove the injurious stimulus and repair the tissue.

The inflammatory response is controlled by several biochemical cascade systems, also known as inflammatory mediators:

- The complement system, removes pathogens by way of opsonisation and phagocytosis.
- The kinin system mainly causes vasodilation.
- The coagulation system stops the bleeding, if any, and forms a shield of protein mesh over sites of injury.
- The fibrinolysis system counterbalances the coagulation system to prevent inappropriate and excessive clotting.

Many inflammatory mediators have short half lives and are therefore rapidly degraded in the tissue. Consequently, removing the injurious stimuli hastily ends the inflammatory response.19

2.2.2 Steroidal anti-inflammatory drugs

Steroidal anti-inflammatory drugs, specifically glucocorticoids, reduce inflammation by binding to cortisol receptors. These drugs are often mistakenly
referred to as corticosteroids, which is a larger category that encompasses glucocorticoids. The most important human glucocorticoid, cortisol (or hydrocortisone), regulates an array of essential cardiovascular, metabolic, immunologic, and homeostatic functions. Indeed, cortisol receptors are found in the cells of almost all vertebrate tissues.

No matter what their cause, all inflammatory mechanisms are affected by cortisol, which induces the synthesis of lipocortin-1 (annexin-1), which in turn binds to cell membranes, preventing phospholipase A2 from coming into contact with arachidonic acid. As a result, eicosanoid production is reduced, and expression of cyclooxygenase (both COX-1 and COX-2) is suppressed. In other words, the action of cortisol inhibits the two major products in inflammation, prostaglandins and leukotrienes. In addition, cortisol stimulates lipocortin-1, which binds to the leukocyte membrane receptors in the extracellular space, and therefore inhibits various inflammatory events, such as: epithelial adhesion, chemotaxis, phagocytosis, and the release of various inflammatory mediators (lysosomal enzymes, cytokines, tissue plasminogen activator, chemokines etc.) from neutrophils, macrophages and mastocytes.

Because they act nonselectively, cortisol-like drugs may impair several healthy bodily processes over time. The known side effects of glucocorticoids are: Immunosuppression, hyperglycemia due to increased gluconeogenesis, insulin resistance and impaired glucose tolerance ("steroid diabetes"), increased skin fragility, easy bruising, reduced bone density (osteoporosis, higher fracture risk, slower fracture repair), weight gain due to increased visceral and truncal fat
deposition (central obesity) and appetite stimulation, adrenal insufficiency (if used for long time and stopped suddenly without a taper), muscle breakdown (proteolysis) with associated weakness, anovulation, irregularity of menstrual periods, growth failure, pubertal delay, increased plasma amino acids, increased urea formation, negative nitrogen balance, excitatory effect on central nervous system. The combination of clinical problems produced by prolonged, excess glucocorticoids, whether synthetic or endogenous, is termed Cushing's syndrome.24

2.2.3 Non-steroidal anti-inflammatory drugs

In 1829, salicin, the predecessor of acetylsalicylic acid, was isolated from the folk remedy willow bark. Non-steroidal anti-inflammatory drugs (NSAIDs) have since become a significant drug in the treatment of pain and inflammation (at low and high doses, respectively). NSAIDs have become very popular because, unlike opioids, they do not cause sedation or respiratory depression, and are, strictly speaking, not addictive.24

NSAIDs have analgesic, antipyretic and anti-inflammatory effects. In other words, they reduce pain, fever and inflammation.24 The term "non-steroidal" is used to distinguish these from steroidal anti-inflammatory drugs (known as glucocorticoids). NSAIDS are unusual in that they are non-narcotic drugs. NSAIDs are sometimes also referred to as non-steroidal anti-inflammatory agents/analgesics (NSAIAs). The most prominent members of this group of drugs are acetylsalicylic acid and ibuprofen. NSAIDs, such as acetylsalicylic acid and
ibuprofen, are deemed to be reasonably safe by the medical community when used as recommended, and are available over-the-counter in most countries.\textsuperscript{24} Acetaminophen (also know as paracetamol) has negligible anti-inflammatory activity, and is not considered as an NSAID.

\subsection*{2.2.3.1 Traditional NSAIDs: non-selective COX inhibitors}

The traditional NSAIDs (see appendix) are non selective COX inhibitors. They inhibit both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes to varying degrees. The traditional NSAIDs can generally be classified based on their chemical structure. NSAIDs within a class tend to have comparable characteristics and tolerability.\textsuperscript{24, 25}

NSAIDs are generally indicated for the treatment of medical conditions where pain and inflammation are present, such as: rheumatoid arthritis, osteoarthritis, inflammatory arthropathies (e.g. ankylosing spondylitis, psoriatic arthritis, and Reiter's syndrome), acute gout, dysmenorrhea, metastatic bone pain, headache and migraine, postoperative pain, mild-to-moderate pain due to inflammation and tissue injury, pyrexia, and renal colic.\textsuperscript{26} Research continues into their potential for prevention of diseases such as colorectal cancer.\textsuperscript{24, 25}

Acetylsalicylic acid, the only NSAID that irreversibly inhibits COX-1, is also used to inhibit platelet aggregation, therefore making it helpful in the management of arterial thrombosis and in the prevention of adverse cardiovascular events.\textsuperscript{24, 25} Since 2001, at least 70,000,000 prescriptions and 30 billion over-the-counter doses of NSAIDs have been sold annually in the United States.\textsuperscript{25} These numbers
can be expected to increase with the aging of the Baby Boomer generation and the associated rise in the incidence of conditions for which NSAIDs are indicated.\textsuperscript{25}

In addition, albeit them being relatively safe drugs, the ever-increasing use of NSAIDs has lead to a considerable increase in the prevalence of adverse effects. While NSAIDs have recently been associated with cardiovascular risks\textsuperscript{27}, their main adverse effects involve the gastrointestinal tract. Common gastrointestinal adverse effects include nausea, vomiting, dyspepsia, gastric ulceration/bleeding, and diarrhea.\textsuperscript{28} The risk of ulceration increases with duration of therapy and higher doses.\textsuperscript{26} An estimated 10-20\% of NSAID users experience dyspepsia. NSAID-associated gastrointestinal adverse events are estimated to result in 103,000 hospitalizations and 16,500 deaths per year in the United States, and represent 43\% of drug-related emergency visits.\textsuperscript{25} These adverse effects are secondary to NSAIDs’ dual insult on the gastrointestinal tract. First, the acidic molecules directly irritate the gastric mucosa. Second, inhibition of COX-1 reduces the levels of protective prostaglandins in the gastric mucosa.\textsuperscript{26, 29} However, in clinical practice, both enteric-coated NSAIDs and rectal formulations, have not been shown to cause less gastrointestinal adverse events.\textsuperscript{26} Furthermore, the risks seem to vary across different NSAIDs. The highest prevalence of gastric adverse events appear to be associated with use of indomethacin, ketoprofen and piroxicam, while ibuprofen and diclofenac appear to have lower rates.\textsuperscript{26}
2.2.3.2 Newer NSAIDs: selective COX-2 inhibitors

The discovery of the COX-2 enzyme in 1991 led to much research with a clear objective: finding an NSAID devoid of the gastric problems distinctive of these drugs. By selectively inhibiting COX-2, it would in theory be possible to obtain anti-inflammatory action without upsetting the balance of the gastro protective prostaglandins.

The relatively selective COX-2 inhibitors, oxicam and meloxicam, were the first attempt at manufacturing a true COX-2 selective inhibitor. The newest classes of NSAIDs, the coxibs, are highly selective COX-2 inhibitors, and include celecoxib, rofecoxib, valdecoxib, parecoxib and etoricoxib. Rofecoxib (brand name Vioxx) was shown to produce significantly fewer gastrointestinal adverse events compared to naproxen.\textsuperscript{30} This same study, the VIGOR trial, did not show any difference in the mortality rate from cardiovascular events and in the overall mortality rate. However, it did show that the incidence of myocardial infarction was higher in the rofecoxib (selective COX-2 inhibitor) group compared to the naproxen (traditional NSAID) group (0.4% vs. 0.1%). Further data, from the APPROVe trial, showed a two-fold increase in the risk of cardiovascular events compared to placebo, which led to the worldwide withdrawal of rofecoxib in October 2004.\textsuperscript{31}
2.3 NSAIDs and the risk of CDAD in the community

2.3.1 Biological plausibility

As documented above, NSAIDs affect the gastro-intestinal mucosa. They cause a dual insult on the gastro-intestinal tract: the acidic molecules directly irritate the gastric mucosa, and inhibition of COX-1 reduces the levels of protective prostaglandins. Consequently, the ensuing breach in the protective barrier, i.e. the gastro-intestinal lining, opens the door to opportunistic pathogens. A damaged protective barrier results in less protection against infection.32

In addition to the above well-studied effects of NSAIDs, another mechanism should be considered as biologically plausible in the risk of infection. The inflammatory response in the human body is in fact a non-specific immune response to an injury. This injury can be caused by either a trauma or by pathogenic invaders. The inflammatory response to invading bacteria in particular, involves the recruitment and subsequent activation of neutrophils to eliminate the invading microbes.33 Over the last two decades, several studies have repeatedly shown that NSAIDs decrease neutrophilic activation in the inflammatory response.34, 35 Consequently, this decreased neutrophilic activation should theoretically make it easier for pathogenic invaders to cause infection.35 These mechanisms are hypothetical and only offer a potential explanation with regards to the effects of NSAIDs on the digestive system.

Several case reports have suggested that diclofenac may be associated with the development of CDAD. In these case reports, patients received a
standard prescription of diclofenac (75-150mg per day), and had no recent history of antibiotic use.\textsuperscript{11-13}

\textbf{2.3.2 Summary of epidemiological studies to date}

A recent study observed an association between the use of NSAIDs and the occurrence of CDAD. Exposure to NSAIDs in the previous 90 days was associated with an increased rate of CDAD (RR, 1.3; 95\% CI, 1.2-1.5).\textsuperscript{10} This epidemiological study is the only one to date to report NSAIDs as a possible risk factor for CDAD. This study, in which NSAIDs were not the exposure of interest, used a case-control design to analyze data from the United Kingdom General Practice Research Database (GPRD). The controls were matched on physician practice, which dealt with a major potential source of selection bias. By matching on physician practice, the researchers insured that the controls were representative of the source population from which the cases occurred. Furthermore, confounding by indication is unlikely since prescribing an NSAID to a patient with gastro-intestinal symptoms is not protocolar. Additionally, the researchers controlled for all the appropriate potential confounders based on the literature on CDAD. On the other hand, misclassification may have occurred with regards to the exposure to NSAIDs. Since NSAIDs are an over-the-counter drug, it is probable that a certain proportion of the cases and controls may have self-administered NSAIDs. This likely non-differential misclassification would result in an under-estimation of the true effect. In addition, another bias would be the misclassification of the outcome. The authors of this study defined cases as
having either a clinical diagnosis of CDAD or a toxin positive assay. A clinical
diagnosis of CDAD may not be very specific, since many pathogens can cause
the same symptoms as CDAD. If this misclassification occurred, the researchers
would in fact be measuring the association between NSAIDs and any type of
infectious diarrhea. One third of the cases of CDAD in this study were by clinical
diagnosis alone. This point was however addressed by the authors, who analyzed
the toxin-positive cases and found the same medication risk factors.

2.3.3 Importance for study

NSAIDs are amongst the most commonly used medications in the world.\textsuperscript{36} They are used by up to 30% of the elderly population in developed countries.\textsuperscript{37} Also, since the frequency and severity of CDAD in the community has been
increasing exponentially, it is fair to say that CDAD has become an important
public health issue.\textsuperscript{4, 10} Clinical observations have identified diclofenac as a
potential culprit among the NSAIDs. It is thus timely to document in a proper
observational study the potential effect of NSAIDs and diclofenac in particular on
the risk of developing CDAD.
3.1 Objectives

Primary objective

Evaluate whether NSAIDs are associated with an increased risk of CDAD.

Secondary objectives

1. To quantify the association between the use of specific NSAIDs and the risk of CDAD.
2. To study the potential dose-response association between the number of prescriptions of specific NSAIDs and the risk of CDAD.

3.2 Study design

The United Kingdom General Practice Research Database (GPRD) was used to assemble the case-control series.

3.2.1 Data Source

The data for this study was obtained through online computer access of the United Kingdom General Practice Research Database (GPRD). The GPRD is the world's largest database of anonymized longitudinal medical records from primary care medicine. It is owned by the United Kingdom Department of Health and managed by the Office of National Statistics. It has more than 50 million patient years of high quality validated data from almost 11 million patient records, of
which 3 million are active, with more than 400 participating general practices. General practitioners have been trained to enter detailed medical information using a computer and special software. Information collected from participating general practitioners includes: demographics (including age and sex), medical symptoms, signs and diagnoses (including comments), therapy (all prescriptions of medicines, vaccines, devices), treatment outcomes, events leading to withdrawal of a drug or treatment, referrals to hospitals or specialists, laboratory tests, pathology results, lifestyle factors (height, weight, BMI, smoking and alcohol consumption), patient registration, practice and consultation details.

A modification of the Oxford Medical Information System classification (similar to the International Classification of Diseases, Eighth Revision) is used to standardize the medical diagnoses and a coded drug list based on the UK Prescription Pricing Authority Dictionary is used for the recording of prescriptions. All recorded data are sent anonymously to the Medicine Control Agency (MCA). Several studies have evaluated the recorded information on diagnoses and drug exposure, which have proven to be highly reliable and valid. Numerous epidemiological studies support its validity and data completeness.

3.2.2 Case definition

Using the GPRD, cases of CDAD were defined as having a first clinical diagnosis of CDAD, a first laboratory diagnosis of CDAD, or a first prescription of oral vancomycin (its only indication being CDAD) between January 1, 1994, and December 31, 2005. Only first events were included as cases to insure that the
patient was not being treated for a recurrence of CDAD.\textsuperscript{10,43} This “first time” case definition excludes the possibility that these patients were being treated for recurrences of CDAD. The index date for included cases was the date of their first CDAD event. Cases had to be aged 18 years or older and have at least 2 years of records in the GPRD prior to the index date to be entered into the study.

3.2.3 Controls

For each case, 10 controls aged 18 years and older were randomly selected from patients attending the same medical practice as the case, matched on age (±2 years), who had not received a prescription of oral vancomycin, were neither toxin positive nor had a clinical diagnosis of \textit{C. difficile} recorded by the time the case was diagnosed (index date). Cases and controls were matched on medical practice to control for possible physician-related and geographical variations in the exposure.

3.2.4 Exposure to NSAIDs

Patients were classified as exposed if they received a prescription for any NSAID in the 90-day period prior to the index date. Otherwise, they were considered unexposed.

NSAIDs are almost exclusively prescribed for inflammatory pain relief. They are usually prescribed for one month at a time for chronic pain or on an “as needed” basis for intermittent pain. 90 days is therefore a period of time which
seems reasonable to capture both of these types of uses (constant and intermittent).

3.2.5 Covariates

The following potential confounders were identified. Age and physician practice were already matching factors, to which sex was added. In addition, the presence of the following gastro-intestinal diseases in the 2 years prior to the index date was determined from diagnoses entered by the general practitioner: gastroesophageal reflux disease (acid reflux disease), inflammatory bowel disease, diverticular disease, gastro-intestinal bleed, and peptic ulcer disease. Other physician diagnoses for co-morbid conditions present at any time prior to the index date included: cancer, chronic obstructive pulmonary disease, congestive heart failure, dementia, diabetes mellitus, heavy alcohol consumption, liver failure, myocardial infarction, renal failure, and stroke.

Other covariates included medications associated with the risk of CDAD. These included all prescriptions for any antibiotic, H2 antagonist, proton-pump inhibitor, and oral corticosteroid, given in the 90 days prior to the index date. All of the above covariates are known risk factors for CDAD.10

3.2.6 Data analysis

This study examined the association between use NSAIDs, overall and individually, in the 90 days prior to the index date and the risk of acquiring CDAD. All analyses were performed using SAS statistical software, version 9.1.3.
All of the variables that were studied were dichotomous, except for age. The primary analysis was based on conditional logistic regression to obtain an odds ratio as an estimate of the rate ratio of CDAD with regards to NSAID use. The adjusted rate ratio of CDAD was estimated for current use of all NSAIDs after adjustment for sex, comorbidities, and prescription of antibiotics, H2 antagonists, proton pump inhibitors, and oral steroids.

In the secondary analysis, conditional logistic regression was again used to evaluate the association between current use of individual NSAIDs and development of CDAD. Furthermore, a dose-response analysis was performed, in order to assess whether heavy users of specific NSAIDs were more likely than light users to acquire CDAD. The numbers of prescriptions of each NSAID, in the 90 day period prior to the index date, were assessed with regards to the outcome. Since NSAID doses are fixed, we used number of prescriptions as a measure of the quantity of NSAIDs used. Only cases with at least one prescription of an NSAID in the 90 days prior to the index date were included in the dose-response analysis. Because of statistical power issues, we defined the exposure as either less than 5 or more than 5 prescriptions in the 180 days prior to the index date.

Finally, a sensitivity analysis was performed to verify whether hospitalization in the year prior to the index date might partially or fully explain the results. As previously discussed, CDAD is much more prevalent in hospitalized rather than non-hospitalized patients.
**Sample Size:**

Sample size was known before beginning the study. Therefore, these calculations are shown simply to illustrate the limits of this study.

M  = number of controls per case  
N  = number of cases  
Po  = probability of exposure to NSAIDs in controls  
PSI  = odds ratio of exposure to NSAIDs in cases relative to controls

<table>
<thead>
<tr>
<th>ALPHA</th>
<th>0.05</th>
<th>0.05</th>
<th>0.05</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>N</td>
<td>1,360</td>
<td>1,360</td>
<td>1,360</td>
<td>1,360</td>
</tr>
<tr>
<td>Po</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>PSI</td>
<td>1.9</td>
<td>1.7</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Power</strong></td>
<td><strong>79%</strong></td>
<td><strong>86%</strong></td>
<td><strong>79%</strong></td>
<td><strong>81%</strong></td>
</tr>
</tbody>
</table>

The numbers of cases and controls are 1,360 and 13,072, respectively.

The Type 1 error probability (alpha) is 5%, to obtain a standard 95% confidence interval. With these set parameters, we will have 80% power of detection for a 40% increase in the risk.
CHAPTER 4 - RESULTS

4.1 Descriptive analysis comparing cases and controls

In all, 1,360 cases were obtained from the GPRD using the three case definitions previously described. The distribution of cases by definition can be seen in Table 1, in which the majority of the cases (63.5%) were identified through clinical diagnosis. Each case was matched to a maximum of 10 controls, for a total of 13,072 controls. Table 2 shows that there was no significant difference ($P = 0.35$) in sex between cases (48%) and controls (49%). The age distribution and distribution of index dates were identical in the cases and controls, since the controls were matched for these factors.

Table 1: Proportion of diagnosis by type in the General Practice Research database for CDAD; 1992 to 2005

<table>
<thead>
<tr>
<th>Oral Vancomycin</th>
<th>Clinical Diagnosis</th>
<th>CDAD positive test</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td>x</td>
<td>x</td>
<td>25.6</td>
</tr>
<tr>
<td>x</td>
<td></td>
<td>x</td>
<td>63.5</td>
</tr>
<tr>
<td>x</td>
<td></td>
<td>x</td>
<td>3.5</td>
</tr>
<tr>
<td>x</td>
<td>x</td>
<td></td>
<td>5.7</td>
</tr>
<tr>
<td>x</td>
<td>x</td>
<td>x</td>
<td>0.8</td>
</tr>
<tr>
<td>x</td>
<td></td>
<td>x</td>
<td>0.5</td>
</tr>
<tr>
<td>x</td>
<td>x</td>
<td>x</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Table 2: Demographic characteristics of cases and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>1,360</td>
<td>13,072</td>
<td></td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>648 (47.7%)</td>
<td>6,403 (49.0%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Age mean (SD)</td>
<td>71.2 (16.5)</td>
<td>71.1 (16.4)</td>
<td>*</td>
</tr>
<tr>
<td>18-37</td>
<td>5.9 %</td>
<td>5.8 %</td>
<td>*</td>
</tr>
<tr>
<td>38-57</td>
<td>13.0 %</td>
<td>12.9 %</td>
<td>*</td>
</tr>
<tr>
<td>58-77</td>
<td>37.1 %</td>
<td>38.5 %</td>
<td>*</td>
</tr>
<tr>
<td>78+</td>
<td>44.0 %</td>
<td>42.8 %</td>
<td>*</td>
</tr>
<tr>
<td>Index year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1994-1996</td>
<td>5.8 %</td>
<td>5.9 %</td>
<td>*</td>
</tr>
<tr>
<td>1997-1999</td>
<td>13.8 %</td>
<td>13.8 %</td>
<td>*</td>
</tr>
<tr>
<td>2000-2002</td>
<td>27.7 %</td>
<td>28.0 %</td>
<td>*</td>
</tr>
<tr>
<td>2003-2005</td>
<td>52.8 %</td>
<td>52.3 %</td>
<td>*</td>
</tr>
</tbody>
</table>

* None were significant at the p=.05 level

Table 3 compares the covariate distributions in the cases and controls. Overall, cases had a higher prevalence of gastrointestinal diseases and conditions when compared to controls. In particular, cases were significantly and much more likely to have inflammatory bowel disease and gastrointestinal bleeding than controls.

Cases were also more prone to other diseases (Table 3). The most notable differences were seen for cancer, chronic obstructive pulmonary disease (COPD), congestive heart failure, myocardial infarction, and renal failure. Diabetes was also significantly more common in cases.
Finally, cases had a much higher exposure to antibiotics, H2 blockers, and proton-pump inhibitors, in the 90 days preceding the index date. Oral corticosteroids, even though rarely prescribed in this study population, were also used significantly more by cases (Table 3).

**Table 3: Clinical characteristics of cases and controls**

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of subjects</strong></td>
<td>1,360</td>
<td>13,072</td>
<td></td>
</tr>
<tr>
<td><strong>Gastrointestinal diseases in the two years prior to the index date</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid Reflux</td>
<td>78 (5.7%)</td>
<td>475 (3.6%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Inflammatory Bowel Disease</td>
<td>67 (4.9%)</td>
<td>73 (0.56%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diverticular Disease</td>
<td>53 (3.9%)</td>
<td>345 (2.6%)</td>
<td>0.0070</td>
</tr>
<tr>
<td>Gastrointestinal Bleeding</td>
<td>44 (3.2%)</td>
<td>128 (0.98%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peptic Ulcer</td>
<td>3 (0.22%)</td>
<td>18 (0.14%)</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Other diseases any time prior to the index date</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>60 (4.4%)</td>
<td>234 (1.8%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>COPD</td>
<td>133 (9.8%)</td>
<td>542 (4.2%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>158 (11.6%)</td>
<td>596 (4.6%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dementia</td>
<td>43 (3.2%)</td>
<td>244 (1.9%)</td>
<td>0.0011</td>
</tr>
<tr>
<td>Diabetes</td>
<td>150 (11.0%)</td>
<td>1,055 (8.1%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Heavy Alcohol Consumption</td>
<td>22 (1.6%)</td>
<td>94 (0.72%)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Liver Failure</td>
<td>5 (0.37%)</td>
<td>15 (0.11%)</td>
<td>0.017</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>49 (3.6%)</td>
<td>221 (1.7%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Renal Failure</td>
<td>71 (5.2%)</td>
<td>145 (1.1%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stroke</td>
<td>59 (4.3%)</td>
<td>267 (2.0%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Medications in the 90 days prior to the index date</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Antibiotic</td>
<td>681 (50.1%)</td>
<td>2,218 (17.0%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Any H2 Blocker</td>
<td>86 (6.3%)</td>
<td>515 (3.9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Any Proton-Pump Inhibitors</td>
<td>356 (26.2%)</td>
<td>1,463 (11.2%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Oral Corticosteroids</td>
<td>3 (0.22%)</td>
<td>5 (0.04%)</td>
<td>0.0065</td>
</tr>
</tbody>
</table>
To assess further the potential confounding effects of the covariates, Table 4 describes these risk factors according to exposure. These comparisons were made in the controls that are representative of the study population. Among the 13,072 controls, 9,363 were not prescribed an NSAID in the 90 days prior to the index date, while 3,709 were. Of all the NSAIDs being studied, acetylsalicylic acid was the by far the most frequent drug exposure with 2,656 controls having received at least one prescription during the 90 day time window, followed by diclofenac with 547 exposed controls.

NSAID users were significantly more likely than non-users to have acid reflux, diverticular disease, cancer, chronic obstructive pulmonary disease (COPD), congestive heart failure, dementia, diabetes, myocardial infarction, and stroke. NSAID users were also more likely than non-users to have been prescribed an antibiotic, an H2-blocker, or a proton-pump inhibitor in the 90 days prior to the index date.

Diclofenac users were much more likely than unexposed controls to present with cancer, congestive heart failure, diabetes, myocardial infarction, renal failure and stroke; and were also more likely to have been prescribed an antibiotic, an H2-blocker or a proton-pump inhibitor in the 90 days prior to the index date.

Acid reflux was found to be more prevalent in ibuprofen and naproxen users. Cancer, chronic obstructive pulmonary disease, congestive heart failure, dementia, diabetes, myocardial infarction, and stroke, were highly prevalent among acetylsalicylic acid users. Acetylsalicylic acid is commonly prescribed to patients with cardiovascular disease and risk of stroke. Smoking and diabetes are
known risk factors for cardiovascular disease and stroke, which could explain the increased frequencies enumerated above.

An important fraction of Cox-2 inhibitor users were prescribed a proton-pump inhibitor. This could be explained by the tendency to prescribe gastric acid suppressants and Cox-2 inhibitors to patients who need anti-inflammatory medications, but are at risk of gastro-intestinal bleed.
Table 4: Clinical characteristics of controls according to NSAID exposure in the 90 days prior to the 
index date

<table>
<thead>
<tr>
<th></th>
<th>Unexposed</th>
<th>Exposed</th>
<th>Any traditional NSAID</th>
<th>Traditional NSAIDs</th>
<th>Other traditional NSAIDs</th>
<th>Cox-2 Inhibitors</th>
<th>acetylsalicylic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of controls (13,072)</strong></td>
<td>9,363</td>
<td>3,709</td>
<td>547</td>
<td>446</td>
<td>108</td>
<td>262</td>
<td>116</td>
</tr>
<tr>
<td><strong>Gastrointestinal diseases in the two years prior to the index date</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid Reflux</td>
<td>309 (3.3%)</td>
<td>166 (4.5%)</td>
<td>19 (3.5%)</td>
<td>25 (5.6%)</td>
<td>6 (5.6%)</td>
<td>17 (6.5%)</td>
<td>4 (3.5%)</td>
</tr>
<tr>
<td>Inflammatory Bowel Disease</td>
<td>52 (0.56%)</td>
<td>21 (0.57%)</td>
<td>3 (0.55%)</td>
<td>2 (0.45%)</td>
<td>0 (0.0%)</td>
<td>3 (1.2%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Diverticular Disease</td>
<td>220 (2.4%)</td>
<td>125 (3.4%)</td>
<td>18 (3.3%)</td>
<td>11 (2.5%)</td>
<td>2 (1.85%)</td>
<td>14 (5.3%)</td>
<td>5 (4.3%)</td>
</tr>
<tr>
<td>Gastrointestinal Bleeding</td>
<td>90 (0.96%)</td>
<td>38 (1.0%)</td>
<td>2 (0.37%)</td>
<td>4 (0.90%)</td>
<td>0 (0.0%)</td>
<td>3 (1.2%)</td>
<td>1 (0.86%)</td>
</tr>
<tr>
<td>Peptic Ulcer</td>
<td>12 (0.13%)</td>
<td>6 (0.16%)</td>
<td>0 (0.0%)</td>
<td>2 (0.45%)</td>
<td>0 (0.0%)</td>
<td>1 (0.38%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td><strong>Other diseases any time prior to the index date</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>149 (1.6%)</td>
<td>85 (2.3%)</td>
<td>15 (2.7%)</td>
<td>14 (3.1%)</td>
<td>0 (0.0%)</td>
<td>4 (1.5%)</td>
<td>1 (0.86%)</td>
</tr>
<tr>
<td>COPD</td>
<td>342 (3.7%)</td>
<td>200 (5.4%)</td>
<td>20 (3.7%)</td>
<td>13 (2.9%)</td>
<td>8 (7.4%)</td>
<td>5 (1.9%)</td>
<td>6 (5.2%)</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>296 (3.2%)</td>
<td>300 (8.1%)</td>
<td>24 (4.4%)</td>
<td>19 (4.3%)</td>
<td>5 (4.6%)</td>
<td>14 (5.3%)</td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Dementia</td>
<td>153 (1.6%)</td>
<td>91 (2.5%)</td>
<td>5 (0.91%)</td>
<td>7 (1.6%)</td>
<td>1 (0.93%)</td>
<td>5 (1.9%)</td>
<td>1 (0.86%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>556 (5.9%)</td>
<td>499 (13.5%)</td>
<td>58 (10.6%)</td>
<td>44 (9.9%)</td>
<td>10 (9.3%)</td>
<td>24 (9.2%)</td>
<td>10 (8.6%)</td>
</tr>
<tr>
<td>Heavy Alcohol Consumption</td>
<td>69 (0.74%)</td>
<td>25 (0.67%)</td>
<td>3 (0.55%)</td>
<td>3 (0.67%)</td>
<td>4 (3.7%)</td>
<td>0 (0.0%)</td>
<td>1 (0.86%)</td>
</tr>
<tr>
<td>Liver Failure</td>
<td>14 (0.15%)</td>
<td>1 (0.03%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>51 (0.54%)</td>
<td>170 (4.6%)</td>
<td>10 (1.8%)</td>
<td>9 (2.0%)</td>
<td>1 (0.93%)</td>
<td>8 (3.1%)</td>
<td>5 (4.3%)</td>
</tr>
<tr>
<td>Renal Failure</td>
<td>67 (0.72%)</td>
<td>78 (2.1%)</td>
<td>9 (1.7%)</td>
<td>7 (1.6%)</td>
<td>1 (0.93%)</td>
<td>5 (1.9%)</td>
<td>4 (3.5%)</td>
</tr>
<tr>
<td>Stroke</td>
<td>95 (1.0%)</td>
<td>172 (4.6%)</td>
<td>13 (2.4%)</td>
<td>4 (0.90%)</td>
<td>2 (1.9%)</td>
<td>6 (2.3%)</td>
<td>1 (0.86%)</td>
</tr>
<tr>
<td><strong>Medications in the 90 days prior to the index date</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Antibiotic</td>
<td>1,391 (14.9%)</td>
<td>827 (22.3%)</td>
<td>123 (22.5%)</td>
<td>94 (21.1%)</td>
<td>26 (24.1%)</td>
<td>72 (27.5%)</td>
<td>29 (25.0%)</td>
</tr>
<tr>
<td>Any H2 Blocker</td>
<td>296 (3.2%)</td>
<td>219 (5.9%)</td>
<td>36 (6.6%)</td>
<td>18 (4.0%)</td>
<td>12 (11.1%)</td>
<td>34 (13.0%)</td>
<td>8 (6.9%)</td>
</tr>
<tr>
<td>Any Proton-Pump Inhibitors</td>
<td>831 (8.9%)</td>
<td>632 (17.0%)</td>
<td>99 (18.1%)</td>
<td>72 (16.1%)</td>
<td>8 (7.4%)</td>
<td>44 (16.8%)</td>
<td>28 (24.1%)</td>
</tr>
<tr>
<td>Oral Corticosteroids</td>
<td>4 (0.04%)</td>
<td>1 (0.03%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>
Table 5 presents the results of the analysis that estimates the odds ratios for the different NSAIDs. The crude rate ratio (RR) of CDAD associated with any NSAID exposure during the 90 days prior to the index date was 1.27, while after adjustment for the confounders became 0.97 (95% CI: 0.86-1.10). Of all NSAIDS, only diclofenac was found to be associated with an increased risk of CDAD (adjusted RR 1.35; 95% CI 1.10-1.67).

### Table 5: Crude and adjusted rate ratios of CDAD associated with NSAID exposure in the 90 days prior to the index date

<table>
<thead>
<tr>
<th>NSAID exposure in the 90 days prior to the index date</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude RR</th>
<th>Adjusted RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any traditional NSAID</td>
<td>462 (34.0)</td>
<td>3,709 (28.4)</td>
<td>1.27</td>
<td>0.97</td>
<td>0.86-1.10</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>96 (7.1)</td>
<td>547 (4.2)</td>
<td>1.63</td>
<td>1.35</td>
<td>1.10-1.67</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>42 (3.1)</td>
<td>446 (3.4)</td>
<td>0.91</td>
<td>0.85</td>
<td>0.62-1.15</td>
</tr>
<tr>
<td>Naproxen</td>
<td>12 (0.88)</td>
<td>108 (0.83)</td>
<td>1.06</td>
<td>0.99</td>
<td>0.56-1.75</td>
</tr>
<tr>
<td>Other traditional NSAIDs</td>
<td>38 (2.8)</td>
<td>262 (2.0)</td>
<td>1.36</td>
<td>1.10</td>
<td>0.79-1.51</td>
</tr>
<tr>
<td>Cox-2 Inhibitors</td>
<td>11 (0.81)</td>
<td>116 (0.89)</td>
<td>0.92</td>
<td>0.77</td>
<td>0.42-1.39</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>319 (23.5)</td>
<td>2,656 (20.3)</td>
<td>1.18</td>
<td>0.88</td>
<td>0.77-1.01</td>
</tr>
</tbody>
</table>

Adjusted for age and physician practice by matching, and for sex, gastroesophageal reflux disease (acid reflux disease), inflammatory bowel disease, diverticular disease, gastro-intestinal bleed, peptic ulcer disease, cancer, chronic obstructive pulmonary disease, congestive heart failure, dementia, diabetes mellitus, heavy alcohol consumption, liver failure, myocardial infarction, renal failure, stroke, antibiotics, H2 antagonists, proton-pump inhibitors, and oral corticosteroids.

In Table 6, diclofenac, the only NSAID found to be significantly associated with CDAD, was further examined for a potential dose-
response association. Only users of diclofenac in the 90 days prior to the index date were included. For these users, the number of prescriptions of diclofenac in the 180 days prior to the index date was divided into two categories based on their distribution, namely less than 5 and 5 or more prescriptions. Both categories of diclofenac users were significantly associated with CDAD. Less than 5 prescriptions (adjusted RR 1.35; 95% CI: 1.02-1.79) as well as those with five or more prescriptions (adjusted RR 1.35; 95% CI: 1.00-1.82) had an increased risk of CDAD.

Table 6: Crude and adjusted rate ratios of CDAD associated with the number of prescriptions of diclofenac in the 180 days prior to the index date among the users of diclofenac in the 90 days prior to the index date

<table>
<thead>
<tr>
<th>Prescriptions in the past 180 days</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude RR</th>
<th>Adjusted RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>51</td>
<td>291</td>
<td>1.63</td>
<td>1.35</td>
<td>1.02-1.79</td>
</tr>
<tr>
<td>≥5</td>
<td>45</td>
<td>256</td>
<td>1.63</td>
<td>1.35</td>
<td>1.00-1.82</td>
</tr>
</tbody>
</table>

Adjusted for age and physician practice by matching, and for sex, gastroesophageal reflux disease (acid reflux disease), inflammatory bowel disease, diverticular disease, gastro-intestinal bleed, peptic ulcer disease, cancer, chronic obstructive pulmonary disease, congestive heart failure, dementia, diabetes mellitus, heavy alcohol consumption, liver failure, myocardial infarction, renal failure, stroke, antibiotics, H2 antagonists, proton-pump inhibitors, and oral corticosteroids.

In Table 7, the analysis estimating the odds ratios of CDAD associated with NSAIDs in non-hospitalized patients is presented. The
crude rate ratio of CDAD associated with any NSAID exposure in the 90 days prior to the index date in non-hospitalized patients during the year prior to index date was 1.43, while after adjustment for the confounders became 1.04 (95% CI: 0.90-1.20). In non-hospitalized patients, as was the case in all patients irrespective of hospitalization status, only diclofenac was found to be associated with an increased risk of CDAD (adjusted RR 1.43; 95% CI 1.11-1.84).

Table 7: Crude and adjusted rate ratios of CDAD associated with NSAID exposure in the 90 days prior to the index date in non-hospitalized patients

<table>
<thead>
<tr>
<th>NSAID exposure in the 90 days prior to the index date</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude RR</th>
<th>Adjusted RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any traditional NSAID</td>
<td>303 (32.6)</td>
<td>2,284 (22.3)</td>
<td>1.43</td>
<td>1.04</td>
<td>0.90-1.20</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>66 (7.1)</td>
<td>351 (3.8)</td>
<td>1.80</td>
<td>1.43</td>
<td>1.11-1.84</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>26 (2.8)</td>
<td>302 (3.3)</td>
<td>0.87</td>
<td>0.80</td>
<td>0.54-1.18</td>
</tr>
<tr>
<td>Naproxen</td>
<td>10 (1.1)</td>
<td>69 (0.7)</td>
<td>1.40</td>
<td>1.12</td>
<td>0.60-2.10</td>
</tr>
<tr>
<td>Other traditional NSAIDs</td>
<td>29 (3.1)</td>
<td>180 (1.9)</td>
<td>1.54</td>
<td>1.13</td>
<td>0.78-1.64</td>
</tr>
<tr>
<td>Cox-2 Inhibitors</td>
<td>10 (1.1)</td>
<td>73 (0.8)</td>
<td>1.33</td>
<td>1.03</td>
<td>0.55-1.92</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>196 (21.1)</td>
<td>1,569 (16.9)</td>
<td>1.28</td>
<td>0.91</td>
<td>0.77-1.08</td>
</tr>
</tbody>
</table>

Adjusted for age and physician practice by matching, and for sex, gastroesophageal reflux disease (acid reflux disease), inflammatory bowel disease, diverticular disease, gastro-intestinal bleed, peptic ulcer disease, cancer, chronic obstructive pulmonary disease, congestive heart failure, dementia, diabetes mellitus, heavy alcohol consumption, liver failure, myocardial infarction, renal failure, stroke, antibiotics, H2 antagonists, proton-pump inhibitors, and oral corticosteroids.
In Table 8, diclofenac, the only NSAID found to be significantly associated with CDAD in non-hospitalized patients, was analyzed for a potential dose-response association. Only non-hospitalized users of diclofenac in the 90 days prior to the index date were included. For these users, the number of prescriptions of diclofenac in the 180 days prior to the index date was divided into two categories based on their distribution, namely less than 5 and 5 or more prescriptions. Diclofenac users with less than 5 prescriptions (adjusted RR 1.39; 95% CI: 1.00-1.95) as well as those with five or more prescriptions (adjusted RR 1.47; 95% CI: 1.01-2.12) had an increased risk of CDAD.

Table 8: Crude and adjusted rate ratios of CDAD associated with the number of prescriptions of diclofenac in the 180 days prior to the index date among the users of diclofenac in the 90 days prior to the index date in non-hospitalized patients

<table>
<thead>
<tr>
<th>Prescriptions in the past 180 days</th>
<th>Crude RR</th>
<th>Adjusted RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>36</td>
<td>196</td>
<td>1.76</td>
</tr>
<tr>
<td>≥5</td>
<td>30</td>
<td>155</td>
<td>1.84</td>
</tr>
</tbody>
</table>

Adjusted for age and physician practice by matching, and for sex, gastroesophageal reflux disease (acid reflux disease), inflammatory bowel disease, diverticular disease, gastro-intestinal bleed, peptic ulcer disease, cancer, chronic obstructive pulmonary disease, congestive heart failure, dementia, diabetes mellitus, heavy alcohol consumption, liver failure, myocardial infarction, renal failure, stroke, antibiotics, H2 antagonists, proton-pump inhibitors, and oral corticosteroids.
CHAPTER 5 – DISCUSSION

In this population based study, use of diclofenac was associated with a 35% increase in the risk of developing CDAD. This same magnitude of association was found when limiting the analysis to non-hospitalized patients. No association was found between the use of all or any other NSAIDs and the risk of CDAD.

The results of this study differ from those of Dial et al., which found all NSAIDs combined to be associated with CDAD (adjusted RR, 1.3; 95% CI 1.2-1.5). This could be explained by the different case definition, which for Dial et al. only included CDAD clinical diagnoses and toxin-positive assays. It is possible that the inclusion of additional cases who received a prescription of vancomycin as in our study may have altered the results. One potential explanation is that those who were prescribed vancomycin were less likely to receive a prescription for diclofenac. If this were indeed the case, the positive association in the previous study for all NSAIDs combined would be diluted and diclofenac could then remain the only significant risk factor for CDAD. It is also possible that those with more severe disease, requiring a prescription of vancomycin which is the second line of treatment, have a predisposition to gastro-intestinal disease, preventing them from taking more potent NSAIDs like diclofenac. Our findings are consistent with previous case reports that link diclofenac to the development of CDAD. No other case reports or studies linking
other NSAIDs than Diclofenac to CDAD was found in the literature. It therefore seems very unlikely that this is only a chance finding.

Three possible types of biases must be discussed in this study: selection bias, confounding, and information bias. Selection bias is not an issue in this case because the database used comprises the entire population.

Confounding could have occurred for several reasons. Firstly, even though a broad list of covariates were controlled for, it is possible that a few were overlooked or simply were not available in the database. Some of these, such as smoking status and obesity, are not available accurately in the GPRD and could be accounting for part of the variation observed in the effect of diclofenac on the risk of CDAD. Information concerning chemotherapy also is not available in the GPRD but diagnoses of various cancers and tumors are and so were controlled for in the analysis. In addition, other unknown risk factors for CDAD might account for some for the residual confounding in this study. Secondly, if the indications for diclofenac are different than for the rest of the NSAIDs, there is a possibility of confounding by indication. No distinct indications for diclofenac are established in the literature. Thirdly, confounding was at first unclear with regards to gastro-intestinal bleeding, which behaves more like a variable in the causal pathway between NSAIDs and CDAD, rather than as a confounder. NSAIDs have both a direct effect on CDAD and an indirect effect through gastro-intestinal bleeding. The indirect
effect could in fact be through the intermediate effect of proton-pump inhibitors (PPIs), which are used to treat gastro-intestinal bleeding and which are also associated with a three-fold increase in risk of developing CDAD.\textsuperscript{10}

Indirect pathway:

\textbf{NSAIDs} $\rightarrow$ \textit{gastro-intestinal bleeding} $\rightarrow$ PPIs $\rightarrow$ CDAD

Indeed, gastro-intestinal bleeding seems to be an important risk factor to control for when studying CDAD (adjusted RR 1.58; 95\% CI 1.16-2.15). However, when gastro-intestinal bleeding is removed from the logistic regression model, the association between diclofenac and CDAD is not significantly altered (1.35$\rightarrow$1.34).

Information bias in this study is related to both the case definitions of CDAD and the exposure to NSAIDs. It must be noted that in 2002-2003, CDAD case reporting became compulsory in the United Kingdom. It is therefore probable that the data capture has improved from that point in time. To eliminate this expected temporal effect bias in this study, calendar time matching is used. To further decrease the possibility of misclassification, three case definitions were used: physician diagnosis, toxin positive assay, or prescription of vancomycin (for which the only indication is CDAD).\textsuperscript{42} Due to lack of power (see Table 1 for distribution by case definition), arising primarily from the low frequency of diclofenac
use, a sensitivity analysis comparing these case definitions was not feasible. A sensitivity analysis comparing physician diagnoses of CDAD to toxin positive assays was performed by Dial and showed no difference between the two case definitions with regards to risk of CDAD in proton pump inhibitor users.\textsuperscript{10} In another study by Dial which evaluated the same risk but in a cohort of vancomycin users only, found a very similar result.\textsuperscript{43} This leads us to believe that the three case definitions capture comparable cases.

Additionally, if a patient was prescribed oral vancomycin, we know that this patient had symptoms that led to a clinical diagnosis of CDAD and/or was confirmed by a \textit{C. difficile} toxin stool test. Additionally, if oral vancomycin was prescribed, it is very likely that metronidazole was tried and probably didn’t work or was not tolerated by the patient. The contraindications for metronidazole are rare and so it is almost always the first line of defense against CDAD.\textsuperscript{42} This case definition will likely reduce diagnostic misclassification since oral vancomycin, being an expensive drug, is only given when CDAD is the confirmed diagnosis and alternative treatments have failed or are contraindicated.\textsuperscript{9,23} Oral vancomycin may therefore be a more valid and reliable measure of CDAD, including only clinically significant cases.

One noteworthy limitation of the GPRD database is its incomplete case ascertainment for hospitalizations. We therefore might be missing important information about patients hospitalized for CDAD. This issue
was addressed by examining non-hospitalized patients in a secondary analysis in which diclofenac remained the only NSAID significantly associated with the risk of CDAD.

With regards to the exposure to NSAIDs, one potential source of misclassification is the non-filling of prescriptions as well as prescriptions filled but not used. Additionally, over-the-counter NSAIDs cannot be adjusted for, and therefore some misclassification is inevitable. However, this misclassification should be non-differential with respect to the CDAD outcome and thus result in an underestimation of the actual effect.\textsuperscript{44} Finally, there is no scientific basis to believe that patients at risk for CDAD are more or less likely than the general population to self-administer over-the-counter NSAIDs.

International generalizability is difficult since the GPRD is a United Kingdom database, which is not necessarily representative of other populations of the world. Generalizability of these results is made even more difficult by the composite end-point of this study. Firstly, it is not clear whether all three capture the same disease severity. Secondly, since oral vancomycin is prescribed as a second line of treatment, we may be capturing more severe cases of CDAD and/or more resistant strains of \textit{C. difficile}. In addition, the chance and type of misclassification might be different for each of the three case definitions.
CHAPTER 6 – CONCLUSION

In this study no association was found between the use of any NSAID and the risk of developing CDAD. However, in our subanalyses, a clear association exists between diclofenac use and CDAD, regardless of previous hospitalization status. This finding is consistent with previous case reports which all point towards diclofenac as being the NSAID linked to CDAD.

No dose response was observed for diclofenac and CDAD. This might be due to methodological limitations. Using prescriptions rather than recording actual usage of medications could lead to this type of counterintuitive finding.

This study is the first to assess the risk of CDAD with regards to NSAID use using a population based approach. To fully evaluate the extent of this finding, this question should be studied in other settings, such as in hospitals. In addition, with a larger study, a sensitivity analysis using various case definitions would be possible and this potential bias could be better elucidated.
Appendix I: Ethics Approval from the United Kingdom SEAG

Patients in the GPRD have an anonymous identification number attributed to them. Even so, ethics approval from both the McGill University Institutional Review Board and the United Kingdom SEAG were obtained.
Appendix II: List of Non-Steroidal Anti-Inflammatory Drugs by Class

**Salicylates**
- Aspirin
- Amoxiprin
- Benorilate
- Choline magnesium salicylate
- Diflunisal
- Faislamine
- Methyl salicylate
- Magnesium Salicylate
- Salicyl salicylate (salsalate)

**Arylalkanoic acids**
- Diclofenac
- Aceclofenac
- Acemetacin
- Bromfenac
- Etodolac
- Indometacin
- Ketorolac
- Nabumetone
- Sulindac
- Tolmetin

**2-Arylpropionic acids (profens)**
- Ibuprofen
- Carprofen
- Fenbufen
- Fenoprofen
- Flurbiprofen
• Ketoprofen
• Loxoprofen
• Naproxen
• Tiaprofenic acid
• Suprofen

Oxicams
• Piroxicam
• Lornoxicam
• Meloxicam
• Tenoxicam

COX-2 Inhibitors
• Celecoxib (FDA alert in March 2005)
• Etoricoxib
• Lumiracoxib
• Parecoxib
• Rofecoxib (withdrawn from the market in 2004)
• Valdecoxib (withdrawn from the market in 2005)
REFERENCES


37. Barat I, Andreasen F, Damsgaard EM. The consumption of drugs by 75-year-old individuals living in their own homes.[see comment]. Eur J Clin Pharmacol 2000; 56(6-7):501-509.


