Pharmacoepidemiologic studies on antimicrobial safety

Nibell, Olof

2024

Document Version:
Förlagets slutgiltiga version

Pharmacoepidemiologic studies on antimicrobial safety

OLOF NIBELL
DEPARTMENT OF INFECTIOUS DISEASES | FACULTY OF MEDICINE | LUND UNIVERSITY

Pharmacoepidemiologic studies on antimicrobial safety

Pharmacoepidemiologic studies on antimicrobial safety

Olof Nibell M.D.

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on Friday, May 3, 2024 at 13.00 in Belfragesalen, BMC, Lund

Faculty opponent
Professor Reimar W. Thomsen, Aarhus University

Organization: LUND UNIVERSITY

Document name: Doctoral dissertation, 2024:55 **Date of issue** 2024-05-03

Author(s): Olof Nibell **Sponsoring organization:**

Title and subtitle: Pharmacoepidemiologic studies on antimicrobial safety

Abstract:

In the realm of drug development, the primary reason for post marketing drug withdrawal is adverse drug reactions (ADR). These are reactions that in most cases are so rare that the pre-clinical trials are unable to detect them. Antimicrobials represent the most common drug class associated with ADRs and hepatotoxicity and cardiotoxicity remain the most prevalent types of injury.

It is seldom financially nor logistically feasible to conduct large enough randomized controlled pre-clinical trials to detect rare ADRs, and carefully designed observational studies most likely represent the highest level of scientific evidence.

In **paper I**, based on national registry data from Denmark 1997 to 2011, we examined the risk of long-term cardiovascular death in a matched cohort of courses of penicillin V, clarithromycin, and roxithromycin. We found no increased delayed risk of cardiovascular death associated with clarithromycin or roxithromycin.

Paper II sought to estimate the risk of acute liver injury (ALI) associated with use of fluoroquinolones using a matched cohort of fluoroquinolone- and amoxicillin courses, sampled from all Swedish adults between 2006 to 2014. We found a twofold increased risk of ALI associated with fluoroquinolone treatment.

In **paper III** we examined the risk of heart valve regurgitation associated with use of fluoroquinolones in a matched cohort of courses of fluoroquinolones and penicillin V, collected from all Swedish adults between 2006 to 2018. We found that fluoroquinolone use was not associated with an increased risk of heart valve regurgitation.

In **paper IV** we investigated the magnitude of the association between exposure to flucloxacillin and the risk of ALI. Courses of flucloxacillin and clindamycin were collected from all Swedish adults in the 2006 to 2018 time period. We concluded that there was a seven-fold risk increase for ALI associated with flucloxacillin exposure.

This thesis aimed to quantify the risk of rare adverse events associated with use of antimicrobials by harnessing the national healthcare registers of Sweden and Denmark. Hopefully these reports can have a positive influence on antimicrobial prescription patterns by informing the clinicians on the potential risks (and risk factors) associated with the prescribed treatments.

Key words:

Classification system and/or index terms (if any) Supplementary bibliographical information

Language: English **ISSN and key title**: 1652-8220

ISBN: 978-91-8021-548-0

Recipient’s notes **Number of pages**: 86

Price Security classification

Signature Date 2024-03-20

Pharmacoepidemiologic studies on antimicrobial safety

Olof Nibell M.D.

LUND
UNIVERSITY

Coverphoto by Johan Sundberg

Faculty of Medicine
Department of Infectious Diseases

ISBN 978-91-8021-548-0 (Print)
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2024

For Sanne and for our children, Elsa and Dante

"At its best such a trial shows what can be accomplished with a medicine under careful observation and certain restricted conditions. The same results will not invariably or necessarily be observed when the medicine passes into general use"
– Sir Austin Bradford-Hill

Table of Contents

Abstract

In the realm of drug development, the primary reason for post marketing drug withdrawal is adverse drug reactions (ADR). These are reactions that in most cases are so rare that the pre-clinical trials are unable to detect them. Antimicrobials represent the most common drug class associated with ADRs and hepatotoxicity and cardiotoxicity remain the most prevalent types of injury.

It is seldom financially nor logistically feasible to conduct large enough randomized controlled pre-clinical trials to detect rare ADRs, and carefully designed observational studies most likely represent the highest level of scientific evidence.

In **paper I**, based on national registry data from Denmark 1997 to 2011, we examined the risk of long-term cardiovascular death in a matched cohort of courses of penicillin V, clarithromycin, and roxithromycin. We found no increased delayed risk of cardiovascular death associated with clarithromycin or roxithromycin.

Paper II sought to estimate the risk of acute liver injury (ALI) associated with use of fluoroquinolones using a matched cohort of fluoroquinolone- and amoxicillin courses, sampled from all Swedish adults between 2006 to 2014. We found a twofold increased risk of ALI associated with fluoroquinolone treatment.

In **paper III** we examined the risk of heart valve regurgitation associated with use of fluoroquinolones in a matched cohort of courses of fluoroquinolones and penicillin V, collected from all Swedish adults between 2006 to 2018. We found that fluoroquinolone use was not associated with an increased risk of heart valve regurgitation.

In **paper IV** we investigated the magnitude of the association between exposure to flucloxacillin and the risk of ALI. Courses of flucloxacillin and clindamycin were collected from all Swedish adults in the 2006 to 2018 time period. We concluded that there was a seven-fold risk increase for ALI associated with flucloxacillin exposure.

This thesis aimed to quantify the risk of rare adverse events associated with use of antimicrobials by harnessing the national healthcare registers of Sweden and Denmark. Hopefully these reports can have a positive influence on antimicrobial prescription patterns by informing the clinicians on the potential risks (and risk factors) associated with the prescribed treatments.

Populärvetenskaplig sammanfattning

Läkemedelsbiverkningar är den främsta orsaken till återkallning av ett lanserat läkemedel. De kliniska prövningar som ligger till grund för att ett läkemedel bedöms vara säkert att använda på människor är av finansiella och logistiska skäl sällan dimensionerade för att upptäcka sällsynta biverkningar. Ofta dröjer det till efter att läkemedlet lanserats, och en större mängd personer börjat använda medicinen, innan dessa ovanliga biverkningar påträffas. Detta är ett känt fenomen och ända sedan mitten på 1900-talet övervakar man därför biverkningar under en lång period efter att ett läkemedel lanserats på marknaden. Antibiotika är en av de grupper av läkemedel som ofta orsakar biverkningar. Exempel på biverkningar är leverpåverkan samt påverkan på hjärta och kärl.

De grupper av individer som läkemedelsföretag testar sina mediciner på är framtagna på ett sätt som innebär att individuella egenskaper (ålder, kön, bakomliggande sjukdomar, etc.) är slumpmässigt fördelade på den grupp som får det faktiska läkemedlet och den grupp som får ett effektlöst jämförelsepreparat (även kallat placebo). Den senare gruppen kallas även för kontrollgrupp och man kallar även detta slumpmässiga urval, eller lottning, benämns randomisering. Randomisering sker ofta med datorhjälp och ofta också helt utan studieansvarigas vetskap om vem som får vilket preparat. Om test- och kontrollgrupperna inom läkemedelsstudien är tillräckligt stora (ofta hundra- till tusentals individer) så kommer grupperna att vara helt jämförbara (ha samma genomsnittsålder, samma fördelning av män och kvinnor, osv.), vilket underlättar möjligheten att slutsatser om läkemedlets effekter och eventuella biverkningar. *Ovanliga* biverkningar däremot, som kanske drabbar en på 100 000, blir däremot i stort sett omöjliga att upptäcka i dessa läkemedelsstudier eftersom man då skulle behöva ha minst så många deltagare i studien. Detta är inte rimligt att genomföra av finansiella och logistiska skäl; det skulle helt enkelt kosta alldeles för mycket.

Först när ett godkänt läkemedel släpps på marknaden och kanske hundratusentals till miljontals patienter börjar använda det, kan man upptäcka dessa ovanliga biverkningar. Det är därför som det är viktigt att bevaka rapporter om biverkningar kopplade till olika läkemedel, framför allt de som nyligen lanserats. För att fånga upp dessa biverkningar kan man också ta hjälp av olika hälsoregister där det kan finnas detaljerad information om alla uthämtade läkemedelsrecept, alla kontakter inom sjukvården och alla dödsorsaker. I Sverige och Danmark finns nationella sådana register och tack vare att varje invånare i Sverige och Danmark har ett unikt personnummer går det att länka ihop alla dessa hälsoregister med varandra. Denna länkning möjliggör att man exempelvis kan få fram alla recept som en person hämtat ut på apotek, och samtidigt ta reda på om samma person behövt besöka akutmottagningen eller blivit inlagd på sjukhus i anslutning till detta.

Våra studier använder sig av dessa länkade hälsoregister för att se om vissa antibiotika går att koppla till olika typer av biverkningar. För att efterlikna läkemedelsföretagens randomisering, använde vi oss av avancerade statistiska metoder och gjorde studiegrupperna så lika som möjligt på de mätbara egenskaperna (ålder, kön, bakomliggande sjukdomar, osv).

I den första studien som är baserad på vuxna individer från danska hälsoregister från åren 1997 till 2006, undersökte vi om användandet av antibiotikan claritromycin och roxitromycin ökade risken för att död i hjärt- kärlsjukdom. Som kontrollgrupp använde vi oss av individer som behandlats med en annan antibiotika som heter penicillin V. Vi kunde inte se att claritromycin och roxitromycin ökade risken för hjärt- kärldöd. Vårt andra delarbete bygger på data från svenska hälsoregister (2006 till 2014) och syftade till att undersöka om det fanns en ökad risk för leverskador kopplat med användning av antibiotikan fluorokinoloner. I jämförelse med personer som använt sig av en annan antibiotika vid namn amoxicillin, kunde vi visa att de personer som fått fluorokinoloner hade dubbelt så stor risk att drabbas av leverskador. I det tredje arbetet jämförde vi risken för hjärtklaffskador mellan de vuxna individer i Sverige (åren 2006 till 2018) som fått fluorokinoloner med personer som fått penicillin V. Vi fann att risken för hjärtklaffskador var densamma i dessa två grupper och kunde därför dra slutsatsen att det inte fanns någon riskskillnad. Slutligen, i det fjärde arbetet, uppskattade vi risken för leverskador hos personer som fått förskrivet antibiotikan flukloxacillin jämfört med de som fått förskrivet en annan antibiotika, klindamycin. Även här använde vi svenska registerdata från åren 2006 till 2018 och kunde dra slutsatsen att de som fick flukloxacillin löpte sju gånger större risk att drabbas av leverskador.

Våra studier har med hjälp av skandinaviska hälsoregister samt moderna statistiska metoder kunnat uppskatta risken för olika typer av biverkningar associerade med ett antal antibiotika. Vi har inte bara kunnat bekräfta misstankar på biverkningar, så som leverskador vid fluorokinolon- och flukloxacillinanvändning, men även kunnat avfärda den misstänkta associationen mellan hjärt- kärlbiverkningar och användandet av antibiotika så som claritromycin och roxitromycin samt fluorokinoloner.

Förhoppningsvis kan resultaten från dessa studier hjälpa behandlande läkare och ansvariga myndigheter att fatta välgrundade beslut kring antibiotikaförskrivning och behandlingsrekommendationer.

List of Papers

Paper I

Inghammar M, **Nibell O**, Pasternak B, Melbye M, Svanström H, Hviid A. Long-Term Risk of Cardiovascular Death with Use of Clarithromycin and Roxithromycin: A Nationwide Cohort Study. Am J Epidemiol. 2018 Apr 1; 187(4): 777-785

Paper II

Nibell O, Svanström H, Inghammar M. Oral Fluoroquinolone Use and the Risk of Acute Liver Injury: A Nationwide Cohort Study. Clin Infect. Dis. 2022 Jul 6; 74(12): 2152-2158.

Paper III

Nibell O, Björk J, Inghammar M. Oral Fluoroquinolone Use and the Risk of Aortic- and Mitral Valve Regurgitation. *Manuscript undergoing peer-review in British Medical Journal (BMJ)*

Paper IV

Nibell O, Björk J, Nilsson A, Jacobsson G, Inghammar M. The Risk of Acute Liver Injury Associated with Flucloxacillin – A Nationwide, Entropy Balanced cohort Study. *Manuscript submitted to Clinical Microbiology and Infection.*

Papers I-II were reprinted with permission from the publishers.

Abbreviations

ADR	Adverse Drug Reaction
ALI	Acute Liver Injury
ATC	Anatomical Therapeutic Chemical
ATE	Average Treatment Effect
ATT	Average Treatment Effect in the treated
BMI	Body Mass Index
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
EB	Entropy Balancing
EMA	European Medicines Agency
DILI	Drug Induced Liver Injury
FDA	Food And Drug Administration
HERG	Human Ether-à-go-go-Related Gene
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
ICD	International Classification of Disease
IR	Incidence Rate
OR	Odds Ratio
PS	Propensity Score
PYRS	Person-years
RCT	Randomized Controlled Trial
RR	Rate Ratio
SD	Standard Deviation
SMD	Standardised Mean Differences
SSTI	Skin And Soft Tissue Infections
TDP	Torsade de pointes

Introduction

Adverse drug reaction (ADRs), defined by the WHO as a reaction that "is noxious, is unintended, and occurs in doses normally used in man", has been estimated to be a leading cause of death in a number of reports based on European and North American data (1-4). The European Commission reported in 2008 that an estimated 197,000 deaths were caused by ADRs, carrying an estimated cost of approximately 80 billion euros (4). Although the process of developing new drugs is painstaking and includes a rigorous, often years-long process before introducing it to the public, it often cannot account for very rare events that clinical trials simply are not designed to detect.

The US Food and Drug Administration (FDA) and European Medicines Agency (EMA) are examples of organizations that play an important role in monitoring post-marketing signals for drugs approved for human use. Reports on suspected drug reactions are communicated to these agencies via prescribers, patients, and drug companies (5). These signals are subsequently disseminated to the public and the scientific community, prompting vigilance and encouraging further research to elucidate potential correlations between the use of drugs and suspected ADRs.

Although randomized controlled trials (RCTs) upon which drug-development studies are based, remain the gold standard, they are not without limitations. For example. RCTs are not infrequently limited in size (difficult to detect rare ADRs), limited in temporal scope (reduced ability to capture long-term ADRs), and often do not include children or elderly, frail, or pregnant subjects (less generalizable) (6).

The Swedish and Scandinavian healthcare registers provide a unique opportunity to conduct observational studies, particularly those focusing on investigating drug safety issues. Large observational studies, based on the general population, play a significant role in providing high quality evidence for estimating treatment effects, with relatively less constraints on size, follow-up time, and cohort selection compared to RCTs.

The studies presented herein use Scandinavian healthcare registers to investigate the risk of ADRs related to use of antimicrobials.

Background

In the 1950s, thalidomide was introduced to the market as a sedative and antiemetic, primarily used to alleviate morning sickness during pregnancy. The drug was widely used but subsequently discovered to be teratogenic and responsible for causing phocomelia, a devastating birth defect characterized by severe limb deficiencies. In response to this tragedy, the WHO established the Programme for International Drug Monitoring (PIDM) in 1968, emphasizing the importance of drug safety on a global scale (1).

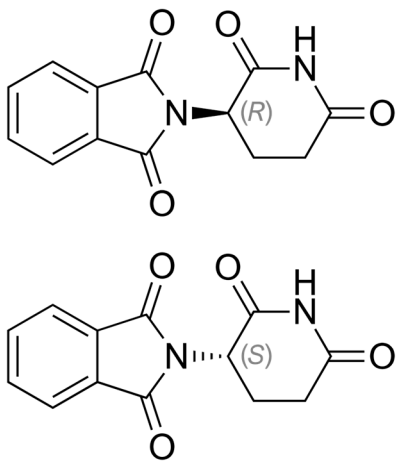

Figure 1: The two enantiomers of Thalidomide

It has been reported that ADRs account for almost 5% of hospital admissions throughout the European Union (7). The global incidence of non-fatal ADRs was estimated to be almost 35 million in the 2017 Global Burden and Diseases, Injuries, and Risk Factors study (8). A recent review of drug safety concluded that 133 drugs had been withdrawn from the market between 1990 and 2020 due to safety concerns, out of which 36 (27.1%) and 25 (18.8%) were due to hepatoxicity and cardiotoxicity respectively (9). The timing of drug withdrawals following reported incidents range from years to decades (10).

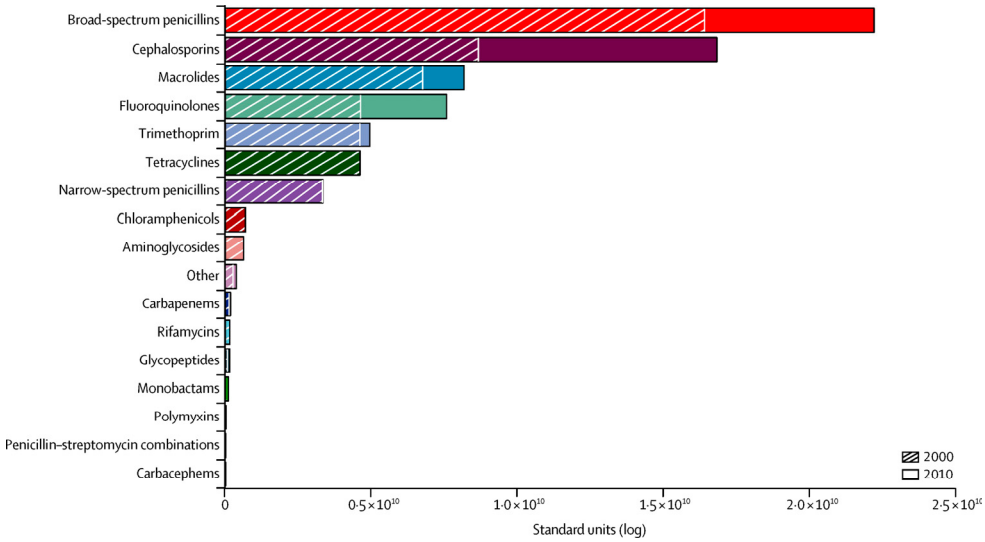

Figure 2: Global antibiotic use in 2000 and 2010. From Van Boeckel et al. (2014). Reproduced with permission. (11)

Antimicrobials are commonly associated with ADRs, although other drug classes such as non-steroidal anti-inflammatory drugs (NSAIDs), antidiabetics, antineoplastics, and anticoagulants have well-known potential to cause serious adverse effects (12).

Study drug overview

Fluoroquinolones are widely used broad spectrum antibiotics with excellent bioavailability and tissue penetration. They are used in a wide range of clinical situations but primarily used to treat infections caused by Gram negative bacteria such as members of the Enterobacterales family (*E.coli, Klebsiella pneumoniae,* etc.). Their antimicrobial property hinges on their ability to inhibit (causing bacterial cell death) two essential bacterial enzymes: Topoisomerase IV and DNA gyrase (13). There are several clinically relevant members of the fluoroquinolone class, and the prescription patterns differ between the Scandinavian countries. See table 1 for the use of fluoroquinolones in Denmark and Sweden. The pharmacologic effect of fluroquinolones is not exclusive to microbial cells, however. There is substantial interaction with host cells, explained in further detail below.

Table 1: Prescriptions / 1,000 individuals in 2023 (Source: Socialstyrelsen (Swe) / Sundhedsdatastyrelsen (Den). In Denmark, a total of 11.6 fluoroquinolone prescriptions / 1,000 individuals was recorded but only details on ciprofloxacin were available.

Drug	ATC	Denmark	Sweden
Ciprofloxacin	J01MA02	9.9	15.8
Norfloxacin	J01MA06	-	-
Moxifloxacin	J01MA14	-	0.4
Levofloxacin	J01MA12	-	0.3
Ofloxacin	J01MA01	-	-

Macrolides are a moderately broad spectrum group of antibiotics used to treat infections caused by both Gram negative and Gram positive bacteria (13). Common clinical scenarios include respiratory tract infections, genital- and urethral infections, etc. Macrolides bind to and inhibit the protein synthesis activity of the 50s ribosomal subunit, unique to prokaryotic cells, causing bacterial cell death (14). The most common side-effect of macrolide usage is gastrointestinal disturbance, although several serious side-effects have been described such as the prolongation of cardiac repolarization, described below (13).

Flucloxacillin is an oral beta-lactam antibiotic of the subgroup isoxazolylpenicillins used primarily for its anti-staphylococcal properties. *Staphylococcus aureus* is a Gram positive bacteria that is a major cause of skin- and soft tissue infections (SSTI), bone- and joint infections, and infections related to surgical procedures. Beta lactam antibiotics interfere with the bacterial cell wall, causing bacterial cell death. Adverse reactions related to beta lactam use are not uncommon and has been reported as occurring in up to 10% of users (15). Most adverse effects are hypersensitivity reactions such as rash and anaphylaxis. There is evidence of a propensity for flucloxacillin to cause severe liver injuries often with characteristics indicating an immunoallergic origin, explained in further detail below (13).

Cardiovascular events related to use of macrolides and fluoroquinolones

In recent years there have been several drug withdrawals due to reports on ADRs causing a multitude of severe cardiovascular events. Most (in-)famously, in the past decades, two of the most used COX-2-inhibitors on the market were withdrawn due to post-marketing analyses reporting an increased risk of myocardial infarction associated with their use, especially in patients with underlying cardiovascular disease (16, 17). Two members of the antimicrobial class of fluoroquinolones, temafloxacin and grepafloxacin, were recalled shortly after marketing due to reports of sudden cardiac death, presumably caused by the well-described property of QTc-prolongation associated with this drug class (18-20). Another class of antibiotics, the macrolides, is also described as a prolonger of the QTc-interval, and several

reports and warnings have been communicated regarding this issue (20-22). More recently, evidence of severe cardiovascular events such as aortic rupture, aortic aneurysm, aortic dissection, and heart valve regurgitation associated with fluoroquinolone use, have prompted both the EMA and the FDA to issue warnings, especially if used by patients with predisposing cardiovascular conditions (23-26).

The types of cardiovascular ADRs associated with these drugs depends on, and varies with, the biological effect the drug has on the human cells. For instance, QTc-prolongation in the case of macrolides is related to the effect it exerts on potassium channels in cardiac cells, whereby the repolarization is delayed, potentially causing life threatening arrhythmias such as Torsade de Pointes (TdP) (18, 22). This potassium channel is encoded by the Human Ether-à-go-go-Related Gene (hERG). In addition to the effect on potassium channels, the macrolides are metabolized via the cytochrome P450 liver enzyme, in particular the subtype CYP3A4, and has the potential to cause interactions with drugs that are metabolized by the same enzyme (27-30). Consequently, co-administration of macrolides with drugs that also affect the hERG-associated potassium channel could potentially increase the risk of life-threatening arrhythmias.

Fluoroquinolones on the other hand, are known to have potential detrimental effects on connective tissues, classically described as Achilles tendon rupture and tendinopathy (31). The pathologic mechanism underlying this condition is believed to involve the remodelling of the extracellular matrix and the downregulation of type I collagen, which is abundant in tendons and other connective tissues (24, 31-33). The main structural components of the vascular tree (including the heart valves) include elastin and various types of collagens which provide tensile strength and elasticity (34). The destabilizing potential of fluoroquinolones on these components may thus predispose users to aortic aneurysm/dissection, and heart valve-regurgitation (35).

Hepatotoxicity related to use of fluoroquinolones and flucloxacillin

Numerous summary reports and reviews of ADRs have identified hepatotoxicity as the leading cause for the withdrawal of drugs from the market (36, 37). The rationale behind the hepatotoxic potential of different drugs is less difficult to comprehend than more intricate effects some medications have on biological processes, considering that a large proportion of drugs are metabolized by the liver (13).The process by which the drugs cause hepatic cellular damage, however, is more complicated.

Liver metabolization of drugs is mediated by cytochrome P450, a gene family comprised of hundreds of enzymes (38). As a result, there is significant enzyme polymorphism which further complicates the predictability of certain ADRs related to drug use. Some hepatotoxic reactions, however, are quite predictable such as the dose-dependent relationship between acetaminophen and hepatocellular damage

(39). A dose exceeding the capacity of enzymatic activity, leads to the formation of harmful compounds, with subsequent cell death (38, 39). Many cases of drug induced liver injury (DILI), however, are idiosyncratic, meaning they occur independent of dose and duration.

The injuries are often classified as hepatocellular, cholestatic, or mixed, depending on biochemical and biopsy characteristics (38). In addition to direct hepatotoxic effects, several compounds trigger allergic or immunological reactions that can lead to acute liver injuries of varying severity (40). This is often established by the presence of eosinophils and immunoglobulin complex deposits in biopsy material (40).

However, the diagnosis of DILI poses a significant challenge, primarily due to the absence of specific diagnostic tests. Establishing a causal link between the use of a drug and acute liver injury (ALI) is often a diagnosis of exclusion, although some tools have been developed to provide objective assessments in cases of suspected DILI. One such method of assessing causality is the Roussel-Ouclaf Causality Assessment Method (RUCAM) which relies on several datapoints, including liver enzyme tests (41-43). Unfortunately, this method has proven to be not only cumbersome even when used by experienced physicians, but also lacking in reproducibility, which limits its usability and leads to its infrequent application in clinical practice (44).

As a result, the recognition and diagnosis of DILI in a real-world clinical setting is at the discretion of the treating physician and their clinical acumen. The unpredictability, variability in severity, and diagnostic difficulty presents significant challenge in trying to establish true incidences for hepatotoxic ADRs.

Fluoroquinolones are primarily metabolized via the kidneys although an estimated 1/3 undergoes non-renal breakdown (13). The historical perspective on the hepatotoxic potential of fluoroquinolones have classified the reactions as exceedingly uncommon. Contemporary publications, however, have reported a significant potential for hepatotoxicity, prompting a re-evaluation of their safety profile (45-47). The mechanism of injury is not fully understood but histopathological analyses of suspected cases have displayed immunoallergic properties indicating a type of hypersensitivity reaction (45).

Flucloxacillin is a semi-synthetic compound derived from penicillin (13). It has a well-known potential for causing elevated liver enzymes as well as more severe hepatotoxic reactions such as cholestatic hepatitis (13, 48-50). Research indicates that the adaptive immune system plays a significant part in this process, with a potential link to the HLA-B-*57:01 allele (51). The presence of flucloxacillin-specific IgE antibodies activated CD4+ and CD8+ T lymphocytes have been observed in patients with flucloxacillin-related acute hepatic injury, which further suggests an immunologic process (52). One hypothesis is that flucloxacillin binds to, and modifies hepatocytes, leading to the formation of molecules that sets of an immunological response (53).

General drug safety aspects

Over the past several decades, the landscape of drug safety has been refined through the implementation of regulatory enhancements for both pre- and post-marketing of new compounds. This includes the implementation of mandatory randomized controlled trials aimed to create comparable and unbiased study groups. Another example is the concept of Good Clinical Practice (GCP), designed to help ensure the integrity and safety of drug trial participants (54).

Despite the progress made, there are inherent weaknesses in the current pharmacovigilance system, particularly in the early phases of drug development. The extrapolation of study group data to the general population is particularly challenging. For instance, the diversity of comorbidities and concomitant medications in the general population is rarely reflected in the study group selection, which often consists of generally healthy participants. This was particularly evident in the case of the withdrawal of several COX-2-inhibitors, in which myocardial infarction disproportionally affected users with underlying cardiovascular disease. Finally, the detection of very rare events also poses a significant challenge, primarily because the relative size of the study groups rarely provides sufficient statistical power.

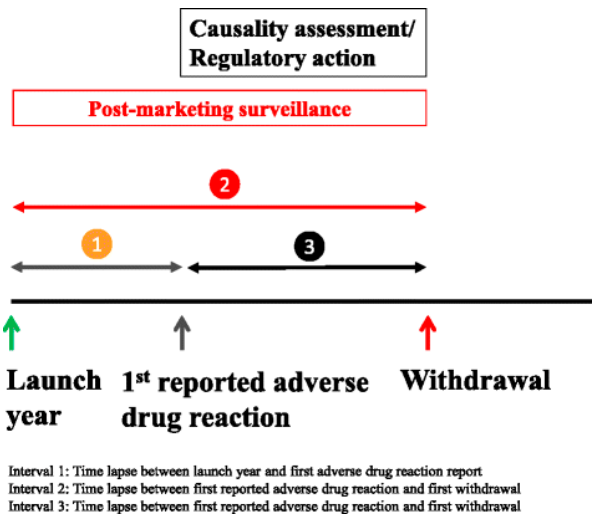

Figure 3: Example of post-marketing surveillance process (36). Creative Commons Licence: http://creativecommons.org/licenses/by/4.0/; no changes were made.

Considering the current state of drug development, there is a need for a robust system that can reliably detect and interpret the post-marketing signals associated with drugs released to the general public.

The role of observational, pharmacoepidemiologic studies

Pharmacoepidemiologic studies play an important role in interpreting the effects of drugs in a real-world setting. However, spurious results based on poorly designed studies in this field run a significant risk of incorrectly identifying or excluding causal relationships between drug exposure and outcomes.

Potential pitfalls

There are several ways that poor design can influence the results of an observational study. Some examples include:

- **Small study sample sizes** can lead to lack of power and a lessened ability to detect true associations.
- **Poor data quality**. Incorrect information or low-quality information can lead to misclassification of both exposure and outcome (see later chapters). An example of this is recall bias when conducting case-control studies of retrospective design, wherein an individual experiencing a negative outcome is more likely to report exposures or behaviours than those who did not experience the outcome.
- **Non-representative study cohort**. Conducting a study using a cohort that does not reflect the demographic and clinical characteristics of the general population utilizing the drug of interest may lead to several issues, including lack of generalization. Unbalanced study populations can lead to conclusions in either direction depending on the specific characteristics that are over- or underrepresented. For instance, disproportionally including healthy or frail individuals can skew the results and lead to incorrect conclusions.
- **Lack of confounding control**. Using inappropriate or simplistic statistical methods can mask a true effect or, conversely, create a false association. For instance, failing to use multivariate analysis to adjust for confounding factors.
- **Unbalanced study groups**. Lack of comparable (on baseline characteristics) case- and control groups may lead to invalid conclusions.

Therefore, signalled risks of adverse drug effects that arise from spontaneous reporting systems, case reports, or observational studies, must be approached with nuance and scrutiny trough well-designed pharmacoepidemiologic studies to distinguish true causal relationships from mere associations. Moreover, reproducing these studies across diverse populations increases the robustness of the results and ensures that reported associations can be either *established* or decisively *excluded*.

Scandinavian registers

The Scandinavian healthcare registers are comprised of data representative of the total population, providing an optimal environment for answering pharmacoepidemiologic questions such as relationships between a drug exposure and a certain outcome. The registers are linkable via a unique personal identifier which enables the collection of all filled prescriptions, hospital visits and healthcare contacts, causes of death, and demographic variables (age, sex, etc.). This data can subsequently be harnessed to define exposure, outcome, and help establish covariates that are then used to create comparable study groups.

The constraints of cost, follow up-time, participant numbers, and generalizability that limit clinical trials become less problematic when aggregating data from healthcare registers, which contain extensive personal data collected over long periods of time.

Navigating bias

The primary issue when conducting register-based studies concerns bias and confounding, which largely stems from the lack of randomization inherent in their design. A randomization process provides an unbiased allocation of individuals in the treatment and control groups, ensuring that baseline characteristics remain evenly dispersed between the groups. It is therefore imperative to implement methodological strategies to mitigate these issues effectively. These strategies often centre around applying data management and modern statistical methods designed to simulate the effects of randomization by controlling for confounders and minimizing different types of bias.

Risk signals associated with macrolides, fluoroquinolones, and flucloxacillin

Macrolide antibiotics are widely used in an array of clinical scenarios and have frequently been associated with acute cardiac events related to its effect on potassium channels and its metabolism via the cytochrome P450-system. In recent years, however, several publications have reported an increased risk of long-term cardiovascular effects supposedly related to short term macrolide use (55, 56). A randomized study in which participants with stable coronary heart disease were administered a two-week clarithromycin course reported an increased risk of cardiovascular mortality during a follow up of several years, compared to placebo (55). Likewise, a British study of patients with pneumonia and acute COPD exacerbations that received clarithromycin, reported an increased risk of cardiovascular events during a follow up of one year (56). Contrary to this, no such association was reported in a number of other publications (57-64).

Fluoroquinolones belong to one of the most prescribed classes of antibiotics worldwide (65). Established adverse effects of fluoroquinolone include tendinopathies, and arrhythmic potential related to its effect on the QTc-interval (18, 20, 32, 66). Less established adverse reactions related to its use are hepatotoxic reactions and effects on the vascular tree including the inner linings of the heart including the heart valves (24-26, 45-47, 67). As early as the 1990s and 2000s, two relatively new fluoroquinolones were taken off the market due to reports on severe hepatotoxic reactions (19, 68, 69). In addition, several publications based on north American cohorts have reported an increased risk of acute liver injury associated with commonly used fluoroquinolones such as levofloxacin, ciprofloxacin, and moxifloxacin (46, 47, 70).

More alarmingly, fluoroquinolone use has been associated with an increased risk of aortic dissection and aneurysm as well as heart valve regurgitation (25, 26). The risk of heart valve regurgitation was informed by EMA and FDA based on a publication from 2019 which found an increased risk for this outcome among fluoroquinolone users (26). Following this report, an observational study from Denmark reported no such association (71).

While the hepatotoxic potential of flucloxacillin is well established, the details on the magnitude, predisposing factors, and temporal patterns remain less clear. The incidence has been estimated in several studies including in the UK outpatient setting, although there remains a scarcity of population-based estimates (72).

Aims

The principal aim of this work centres around processing data from Scandinavian healthcare registers, applying modern statistical methods to reduce confounding and bias, to investigate cardiotoxic and hepatotoxic drug reactions related to use of antimicrobials. In detail, the questions we asked were:

- Is short-term use of the macrolides clarithromycin and roxithromycin associated with an increased risk of long-term cardiovascular death?
- Is fluroquinolone use associated with an increased risk of acute liver injury?
- Is fluroquinolone use associated with an increased risk of mitral- and aortic valve regurgitation?
- What is the magnitude of association between flucloxacillin exposure and the risk of acute liver injury?

Methods

The Scandinavian healthcare systems at a glance

The Scandinavian model of healthcare focuses on universal access, comprehensive coverage, with a significant emphasis on public health. It is publicly funded through taxes and provides high-quality care to all residents regardless of individual income or insurance status. The establishment of national healthcare registers, dating back several decades, reflects the early commitment of systematic data collection to improve public health.

In addition, all residents are assigned a unique personal identification number which unlocks the potential to cross-reference data between the registers.

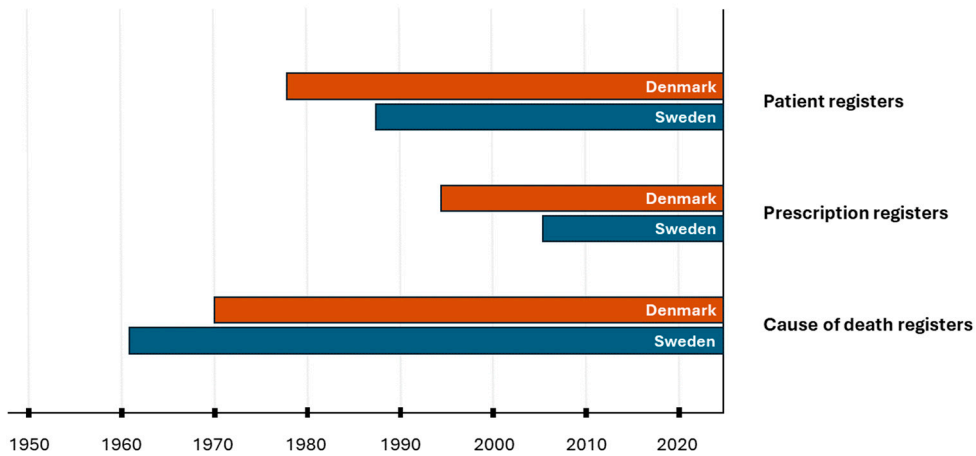

Figure 4: Timespan of data inclusion for Danish and Swedish healthcare registers.

The use of personal identifiers in Sweden and Denmark

In Sweden, the use of a personal identification number ("personnummer", PIN) was introduced in 1947. It contains the date of birth (YYMMDD) combined with a three digit "birth number", to which a fourth digit (control digit) was added in 1967. In general, an individual will keep the same PIN during their entire life and will not

change in case of moving in and out of the country. However, there are some rare exceptions. Until 2015 there have been about 83,000 instances where changes have been made to an assigned PIN. Likewise, until 2015, there have been approximately 23,000 cases of re-used PINs. The single most common reason for these exceptions is due to immigration (73). The estimated total number of PINs assigned between 1969 and 2007 is 13,500,000 (74).

The Danish Civil Registration System (CRS) applies a similar identification number (CPR) to all inhabitants, dating back to 1968 (1972 for inhabitants of Greenland) (75). The CPR is assigned for life and never changes except for in very rare instances (administrative errors, identity theft, change of sex, etc.). In case of CPR changes, the CRS maintains a link between the old and new CPR making it possible to keep tracking the individual. A CPR number is never reassigned to another individual (76). As of 2014 approximately 9,5 million unique CPR numbers have been assigned to residents of Denmark and Greenland (76).

Sources of data in Sweden and Denmark

In general, the Swedish and Danish register share more similarities than differences, but for the sake of clarity and examination, the registers are described separately in the following section.

Sources of data in Sweden

The Total Population Register (TPR)

The TPR (Swedish: Register över totalbefolkningen) is maintained by the government agency Statistics Sweden (SCB) and contains individual information on birth, death, name change, country of birth, marital status, movement within and out of the country, and family relationships (77).

The National Inpatient Register (IPR)

Also called the hospital discharge register, the IPR (Swedish: Patientregistret) contains discharge diagnoses (according to *The International Classification for Diseases*, ICD-7 to ICD-10) and related data for all patient visits since its inception in 1964 (including psychiatric diagnoses since 1973) (78). The data collection was initially limited in geographical scope, until 1987 when all counties were included (79). Since 1997 all surgical day care visits are also included. Private care visits (including private surgical day care) are characterized by a notable low degree of reporting and a consequence of this is a near 100% coverage of the inpatient register,

whereas the outpatient register coverage is estimated to be about 80% (80). In addition, since 2001, all emergency room- and hospital-based outpatient visits are also included in the register.

The IPR contains a large number of variables related to a patient visit including primary- and additional diagnoses (according to ICD), date of admission, date of discharge, etc. Diagnoses are registered by the discharging physician which is subsequently counter signed by a board-certified specialist.

The overall diagnostic accuracy in the IPR has been estimated to be in the range of 85-95%, with variation in positive predictive values (PPV) depending on diagnosis (78). In recent years, numerous validation studies of the IPR have been conducted, with encouraging results. For instance, one study reported a high accuracy of venous thromboembolism (VTE)-diagnoses with a reported PPV of 95% (81). Furthermore, the diagnoses of cirrhosis, oesophageal varices, and hepatocellular carcinoma (HCC) was reported as having PPVs in the range of 84-93% (82). Conversely, psychiatric diagnoses within the IPR have been observed to have overall low PPVs, assumingly due to diagnostic challenges (78).

The National Prescribed Drug Register (NPDR)

The NPDR (Swedish: Läkemedelsregistret) contains data for all filled prescriptions in Sweden from July 2005 and onward. The prescriptions recorded therein are classified in accordance with the Anatomical Therapeutic Classification (ATC) system. Additionally, a comprehensive list of individual variables is simultaneously recorded for each filled prescription which includes date of dispensing, dosage and amount, prescribing physician, and other pertinent details. Drugs that are used to treat hospital-admitted patients are not registered in the NPDR.

The National Cause of Death Register (NCDR)

The NCDR (Swedish: Dödsorsaksregistret) contains data on underlying and contributing causes of death according to ICD (version 6 to 10), since 1961 (with retrospectively compiled data from 1952-1960) (83). To facilitate international comparison of mortality statistics, the coding system of the NCDR adheres to WHO-standards which are closely aligned to the Swedish classification system on the three- (ICD-10) and four- (ICD-9) character level (83). The overall concordance between the physician registered cause of death and retrospectively reviewed medical records has been reported to be almost 90% (83, 84).

Sources of data in Denmark

The Danish Civil Registration System (CRS)

The CRS (Danish: Det centrale personregister) was established in 1968 and holds information on all people residing in Denmark. Individuals in the CRS are designated as either "active" (currently alive and residing in Denmark or Greenland) or "inactive" (dead, migrated, or disappeared). In addition, it holds information on demographic characteristics such as sex, date of birth, and birthplace (76).

The Danish National Patient Register (DNPR)

The DNPR (Danish: Landspatientregisteret) was established in 1977, with nationwide coverage since 1978, and holds personal and admission data on all hospital contacts. Primary and secondary diagnoses according to the ICD (version 8 to 10) are registered by the discharging physician. Furthermore, details on type of hospital contact (outpatient, emergency room, and inpatient), date of admission and discharge, treatments and examinations, are also included in the register (85). Outpatient- and emergency room contacts have been recorded in the register since 1995 (85).

The validity of the DNPR in general is high for most, but not all diagnoses (86). Similarly to the Swedish registers, several validation studies have explored the accuracy of several specific diagnoses within the DNPR. Diagnoses such as acute stroke (PPV 79%), atrial fibrillation / flutter (92%), acute myocardial infarction (100%), liver cirrhosis (85%), have been investigated with promising results (87-90).

The Danish National Prescription Register (NPR)

The NPR (Danish: Lægemiddelstatistikregisteret) contains detailed information on all filled prescriptions since 1995 (91). Available data include (not limited to) drug category according to ATC, dispensing date, dose and package size. As with its' Swedish counterpart, the Danish prescribed drug register does not hold information on drugs administered during hospital visits (91).

The Danish Register of Causes of Death (DRCD)

The DRCD (Danish: Dødsårsagsregisteret) holds electronic records for mortality data in Denmark has been available since 1970. As of late 1990 it is the physician verifying the death of a Danish resident, that also issues the death certificate in which immediate and contributing causes of death is registered (92). All entries are coded in accordance with the ICD-classification system. The coverage is near 100%

with only about 0.3-0.6% of deaths not fully reported (92). Overall validity of the diagnoses listed in the DRCD is not regularly reported, although validation of specific diagnoses report relatively high sensitivity (92, 93).

Methodological considerations

In an ideal scenario, random assignment of treatment ensures an equitable distribution of observed (and unobserved) characteristics, isolating treatment as the sole differing factor. This approach allows for an unbiased estimation of a treatments' causal effect on a specified outcome.

When conducting quantitative pharmacoepidemiologic studies with the goal to estimate causal effects of exposure on an outcome, however, we must find alternative methods to try to mimic the randomized process to address the issues with confounding and bias.

Traditional approaches such as stratification and multiple regression models serve as foundational techniques to control for confounding. Stratification involves dividing data into subgroups based on different confounders to allow for effect estimation within homogenous blocks, or strata. Multiple regression involves including confounding variables (covariates) in a statistical model to estimate the influence they have on the outcome.

Subsequent sections will build upon these concepts, beginning with methodological frameworks that can be used for causal inference. This will be followed by an exploration of different sources of bias and confounding that can distort the estimation of causal effects. Finally, we will go through modern statistical approaches used to minimize the influence of these biases.

The Bradford-Hill criteria

A conceptual tool for causality assessment, are the Bradford-Hill criteria (described below) (94). They are a set of nine principles set forth by Sir Austin Bradford Hill in 1965, which can be used as a framework to assess evidence and argue for causality in observational studies (94). Although not all criteria need to be fulfilled to suggest a causal relationship, they can be used to systematically evaluate the evidence.

The strength of association relates to the magnitude of risk or odds increase for the outcome in the exposed compared to the unexposed. The higher the risk, the higher the probability of a causal effect. The **consistency** refers to finding similar results across several studies, or populations which would support causality. **Specificity** refers to a one-to-one relationship between cause and effect and is

considered less useful due to the complexity in diseases. The criterion of **temporality** is non-negotiable and simply refers to the idea that the effect must follow the exposure; or as Sir Bradford-Hill states: "which is the cart and which is the horse?" (94). A dose-response curve, or **biological gradient**, would strengthen the argument for causality, likewise would **plausibility** and **coherence** as they refer to the biological plausibility of a suggested causal effect of the exposure. **Experiment** is seldom a realistic criterion to fulfil as it implies conducting human trials to assess causality. Finally, **analogy** refers to allowing for the extrapolation of causal inferences from similar associations found in other studies.

The counterfactual model

Also known as the potential outcome framework, seeks to provide a way to consider what would happen to the same individual in different scenarios, i.e. the outcome of a person that received treatment (factual scenario) if they did not experience the treatment (the unobserved counterfactual scenario). Under certain conditions and assumptions, the causal effect can be estimated by the difference between these scenarios (95).

Average Treatment Effect (ATE) and Average Treatment effect on the Treated (ATT)

Both the ATE and ATT rely on idea of potential outcomes – the factual outcomes we observe versus the counterfactual outcomes we do not. The ATE estimates the mean effect of treatment across the entire population, assessing the impact if everyone received treatment versus if no one did. In contrast, the ATT estimates the mean effect in those who actually received treatment, comparing their outcomes with a comparable group who did not receive the treatment (such as a matched or balanced group that received a comparator drug) (95). Propensity score-matching (PSM) and entropy balancing (EB) are examples of methods that can be used to estimate the ATT; explained in further detail later.

Internal and external validity

Internal validity refers to the degree to which the study allows for an accurate conclusion regarding a causal effect. In other words, it is directly related to the study design and the approaches made to: control for confounding variables, minimize systematic errors, and ensure reliable capture of exposure and outcome (96-98).

External validity answers the question of how representative the results are to the general population, or at least across diverse populations and circumstances (98).

High external validity allows for extrapolation of the study results beyond the confinements of the conducted study, ensuring its utility in real-world settings.

Sources of bias

Bias in an epidemiologic study refers to systematic errors that distort the true effect of an exposure on an outcome and can occur at any stage of the process (design, data collection, analysis) (95). Confounding occurs when the effect of the exposure on an outcome is mixed with the effect of another factor, which is correlated to both the exposure and the outcome but not on the causal pathway between them (95).

Confounding by indication

This occurs when allocation of treatment is related to the outcome of interest, complicating the ability to distinguish the effects of medication from the effects of the underlying condition (99). Confounding by indication is a common challenge in pharmacoepidemiologic studies especially since there rarely are any available data on the clinical reasoning behind treatment decisions (100). Consequently, it is difficult to discern if the observed outcome is solely due to the medication or, in fact, due to the underlying illness that led to the drug prescription in the first place. A closely related form of confounding by indication is confounding by severity in which the severity of the underlying disease determines the level of treatment. This type of confounding is especially insidious because it can affect several factors (and different levels) on the causal pathway (100).

An example of confounding by indication is the reported association between Acetaminophen use and exacerbations of asthma in asthmatics (101). Here, use of Acetaminophen is related to asthma because it is often the preferred analgetic and antipyretic instead of non-steroidal anti-inflammatory drugs (NSAIDs) since the latter is discouraged in asthmatics (102). This could lead to an incorrect conclusion that there is a causal association between Acetaminophen and asthma exacerbations.

Protopathic bias (reverse causation)

This type of bias has a temporal element because it occurs if study drug treatment is initiated based on a *symptom* of the studied outcome, before the actual outcome itself is diagnosed (103). It increases the risk of incorrectly inferring a causal relationship between the treatment and the outcome (104).

For instance, if a new drug is introduced and prescribed to patients experiencing pain – a symptom that may precede the onset of multiple sclerosis (MS) – and some of these patients are later diagnosed with MS, there is a potential for protopathic bias (105). If the researchers do not recognize the possibility of pain being a precursor for MS, they might incorrectly infer a causal relationship between the drug and MS.

Misclassification bias

When analysing observational data, one must consider the risk of them being incorrectly specified. Misclassification bias is a type of information bias which occurs when there are errors in the measurement or classification of exposure or outcome (95). In an exposure – outcome study this type of bias can be differential or non-differential depending on whether the misclassification is equally likely to occur in all study groups (cases/exposed and controls/unexposed) (106).

Following is an example of the effect of non-differential misclassification disease (95):

Table 2 (true distribution)

	Exposed	**Unexposed**	
Case	40	20	60
Control	60	180	240
	100	200	300

Table 2 reflects the "true" distribution of cases among the exposed and unexposed. The risk ratio is (40 / 100) / (20 / 200) = 0.4 / 0.1 = **4**, whereas the risk difference is (40 / 100) - (20 / 200) = 0.4 - 0.1 = **0.3**.

Now, consider instead a perfect disease detection with sensitivity 100%, but less than perfect specificity at 80%. Results shown in table 3.

Table 3 (100% sensitivity, 80% specificity)

	Exposed	**Unexposed**	
Case	52 *(40+((1-0.8)*(100-40))°*	56 *(20+((1-0.8)*(200-20))*	108
Control	48	144	292
	100	200	300

In this example the risk ratio is (52 / 100) / (56 / 200) = 0.52 / 0.28 = **1.9**, and the risk difference will be (52 / 100) - (56 / 200) = 0.52 - 0.28 = **0.24**.

*°80% specificity means that 20% of the non-diseased individuals (0.2 * 60 and 0.2 * 180 respectively) will be incorrectly classified as diseased, thereby subtracted from the row of controls and added to the row of cases.*

In conclusion the non-differential misclassification in exposure or outcome produces a bias toward (or, in extreme cases beyond) the null (95, 106, 107). Differential misclassification, however, can affect the bias in either direction, away from, or towards the null (106, 108). Misclassification may cause a variation in relative risk, which is a function of sensitivity and specificity as demonstrated in the example above (108).

Approaches to reduce bias and confounding

Rigorous efforts must be made to mitigate potential sources of bias stemming from the inherent complexities of register-based, observational data. The following section will explore the methodological approaches used in our work to temper the effect of potential biases.

Active Comparator, New User (ACNU)-design

In a randomized controlled trial, the indication for treatment is uniformly distributed between the study cohorts. In contrast, the subjects of the control arm in an observational study are considerably less likely to have a treatment indication. Unless observed and taken into consideration, this scenario introduces confounding by indication, especially if using a non-user cohort as the control group. A common approach to mitigate this risk is using the ACNU-design (109).

The active-comparator design can be used to compare the risk of an outcome in the drug of interest, with another drug used for the same or similar indications in clinical practice (110). This approach ensures a greater overlap of patient characteristics (including unmeasured characteristics such as frailty and lifestyle), and aligns the indications for treatment between the study cohorts (109, 110).

Selecting an appropriate comparator drug requires considerable deliberation and ideally, it should (main points) (100):

- Have the same or similar chance of being prescribed by a physician in a given clinical scenario
- Have the same or similar therapeutic effect on the disease it intends to treat or alleviate
- Not be known to have an increased propensity to cause the outcome in question
- Not differ in cost or availability

The concept of comparator drug design is particularly effective in reducing bias when combined with the new-user design, or incident-user design (111, 112). This approach hinges on identifying patients who have initiated drug treatment during a specified time-period (110). The start of treatment for each patient is referred to as the index date, which also serves as the start of follow-up. Patient baseline characteristics are ascertained in this moment which should also be preceded by a period of study drug non-use (washout period) (110, 111). A potential weakness in the new-user design is that only users within the specified time-period are included, limiting the study sample size (110). In the strictest form, only incident courses are included, and all other courses excluded (113).

Prevalent new user

An alternative to the new user design, which exclusively encompasses patients who have not previously received either the study drug or the comparator, is the prevalent new user design (114). The prevalent new user design includes all treatment initiations, regardless of previous use of either study drug or comparator drug, and therefore avoids severely restricting the sample size due to prevalent use. This method is particularly advantageous when investigating drugs that are frequently prescribed on multiple occasions, such as antibiotics. An extension of this method is the episode-based approach in which, for example, multiple filled prescriptions (or treatment courses) can be included given certain criteria that ensures no overlap of treatment courses (see figure 5 on next page for a typical description). Furthermore, in certain circumstances, it could be of interest to divide the cohort into users that are treatment naïve (true new users) and those who are prevalent users and perform separate analyses of these groups (113, 115).

Cohort restriction

When performing multivariate regression analysis or other methods such as propensity score-analyses and entropy balancing-methods, we are limited to using variables that are measurable and ascertainable from the observational data. In many scenarios there are unmeasured confounders, or at least variables that are not easily produced.

Age-restriction and frailty

Frailty refers to a condition associated with increased physical vulnerability and decreased ability to uphold homeostasis after experiencing physiological stress (116). It is a condition often observed in elderly and terminally ill patients, and that is not explicitly obtainable in observational data. When presented with illness in a frail patient, it is not uncommon to abstain from treatment or other therapeutic interventions, including primary and secondary prophylaxis (116, 117). Subsequently, including frail or severely ill patients can not only confound the initiation of treatment but also the risk of experiencing the outcome. One common approach to address this situation, is to restrict the inclusion of patients either by age or by some other proxy for frailty, such as diagnoses indicating severe comorbidities or end-stage illness (118, 119).

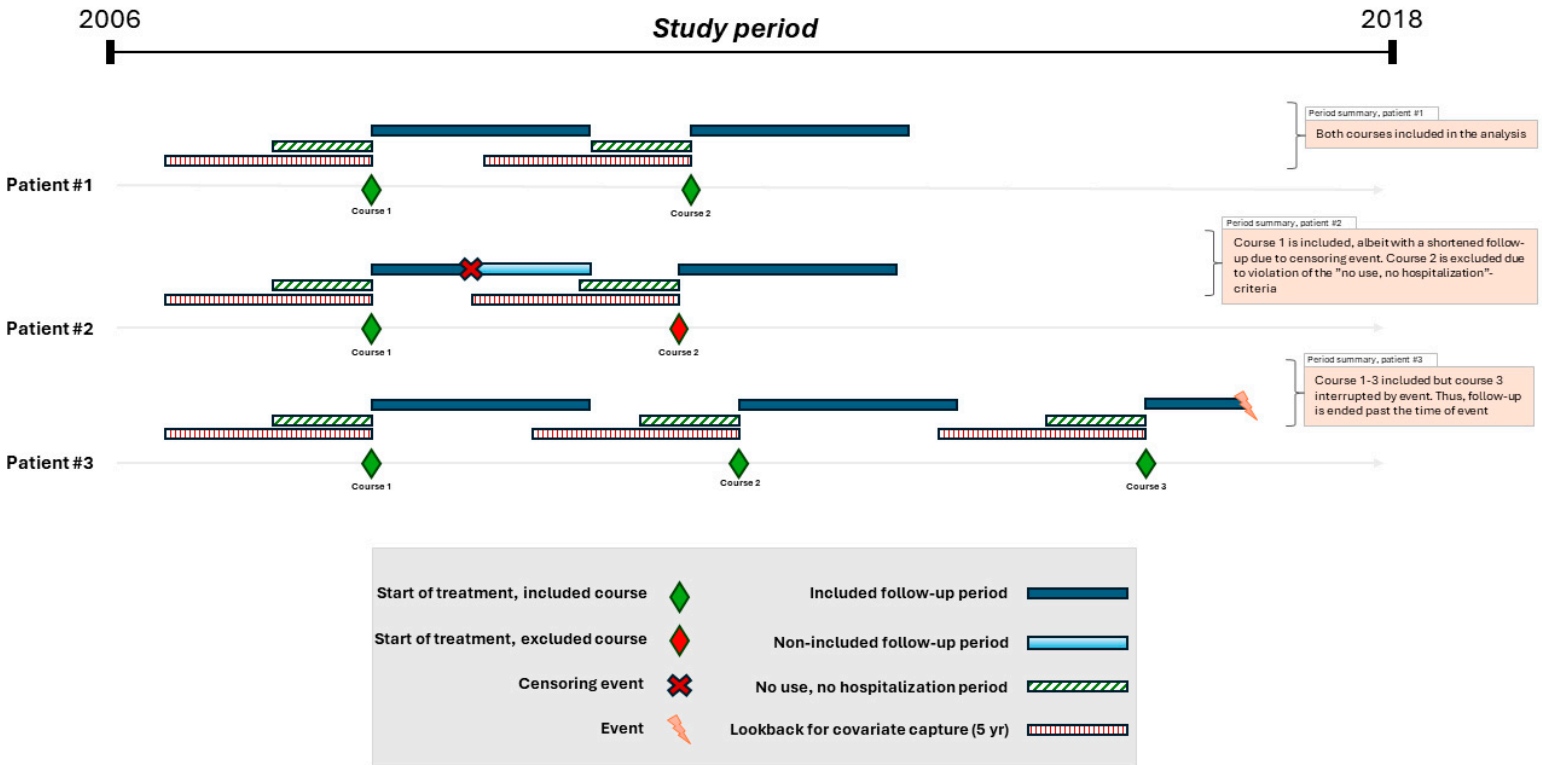

Figure 5: Typical prevalent new user-design (episode-based approach) used in papers I-IV (note: bars not scaled proportionally to actual durations)

High pre-treatment risk of the outcome

The practise of restricting study populations to mitigate the potential for specific or residual confounding extends beyond assessment of age and frailty. Another commonplace approach is to restrict the cohort on several other factors that can influence the risk of experiencing the outcome. Patients that are exposed to, or suffer from, risk factors that are known to be associated with the outcome are therefore often excluded. This restriction criteria includes patients that have previously experienced the outcome of interest. These techniques aim to minimize and/or homogenize the baseline risk between the study groups to enable a clearer interpretation of the relationship between treatment and outcome. Furthermore, the application of these criteria will likely improve the ascertainment of incident cases, as opposed to prevalent cases, improving the evaluation of new occurrences of the outcome.

Implications and trade-offs

However, the cost of attempting to increase the internal validity of the study by using restrictions, can be loss of external validity, or generalization, due to too severe restriction of study population (120). Various techniques can be used to address this tension between external and internal validity. A common technique is to test the robustness of the findings performing sensitivity analyses, including or excluding high risk individuals, etc.

Propensity scores (PS)

A hallmark of the RCT is the randomisation process which, in effect, provides an unbiased estimate of the effect of treatment on the study population (121). As discussed earlier, when designing an observational study, alternative techniques are needed, to mimic the RCT in attempting to estimate a causal effect. One popular method used to balance baseline covariates is the propensity score-method, introduced by Rosenbaum and Rubin in 1983 (122). They define the propensity score as the probability of treatment assignment given a set of variables (baseline covariates) (121, 122). The propensity score will thus be a summary score, or probability for treatment assignment, for a certain distribution of baseline characteristics that can be used to match exposed and unexposed individuals, thereby balancing on a large set of covariates simultaneously (121). By stratifying, adjusting, or matching on the propensity score it is therefore possible to estimate the treatment effect in an unbiased fashion.

One of the main advantages of propensity score-methods compared to traditional multivariate regression, is the methods' ability to handle dimensionality (123). Whereas multivariate regression struggles with reliable estimations in situations with a few outcomes and a large number of covariates, the propensity score method

reduces the dimensionality by collapsing the covariates into one value and enables the analysis to proceed even when outcomes are limited (123).

A common method to calculate the propensity score is to use logistic regression on treatment status (index drug) using ascertained baseline characteristics as covariates. Several other methods for estimating the propensity score have been described, such as recursive partitioning, random forest, and neural networks (124, 125). After estimating the propensity scores there are several ways to apply this to reduce the effects of confounding in the observational data. Examples include propensity score-matching, propensity score-adjusting, propensity score-stratification, and inverse probability weighting (IPW) (122, 126, 127). We will focus on exploring propensity score-matching since this is the primary method used in this thesis.

Matching on propensity score

Propensity score-matching (PSM) follows the estimation of the propensity score and is a method used to match cases and controls on their respective propensity score, creating a matched pair conditioned on their distribution of covariates (122). The most common form of matching is *one-to-one*, where each case is matched to one control based on the propensity score. Other approaches such as *many-to-one* or *one-to-many* can be considered, for instance if there is a need for expanding the study sample (128). We now explore the method for creating matched pairs.

Nearest-neighbour-matching (NNM) involves selecting a case and matching it with a control having the nearest propensity score. The selection process can be either greedy or optimal, with or without replacement, and with or without the use of calipers (121, 129, 130). With replacement means that after a match is found, the case or control is returned to the pool of available subjects to be matched again if possible. Greedy matching means that the selected case will be matched with the best possible control, without considering if there is a better match. Optimal matching does the opposite, that is, it matches with the intent to create matching pairs with as little total between-pair distance as possible. Another option to this matching is the use of calipers. If used, a caliper distance is set, representing the accepted difference threshold in propensity score between cases and controls (130). Within the caliper, the control that is nearest the case will be chosen and one picked at random if there are multiple matches with identical propensity scores (130). If no match can be made within the specified caliper distance, the "unmatchable" control will be excluded from the study sample (131). If, however, a caliper is *not* defined there is no restriction on the accepted difference in propensity score between cases and controls, which could lead to a poorly matched cohort (121).

Assessing balance

A common way to assess balance of the covariates in the matched cohort is the use of standardized mean difference (SMD). It represents the differences in means (or

proportions if binary) of a covariate between treatment groups, divided by the pooled standard deviation of the covariate across the groups (121). A SMD < 0.1 (10%) is often considered to indicate a negligible difference of the covariate between the groups (121, 132).

Covariate selection

Given that the propensity score reflects the likelihood of receiving treatment, it is implied that only those covariates (baseline characteristics) which influences treatment should be included in the model (121). This approach, however, has been demonstrated to produce larger standard errors and introduce bias, especially in small study samples (133-136). The optimal approach is, in short, not entirely conclusive.

A recent study performed Monte Carlo-simulations to estimate the effect of restricting covariate inclusion to: all covariates, only confounders (associated with both treatment and outcome), only variables predictive of outcome (137). Although there was covariate imbalance (due to exclusion of these covariates in the model) when restricting the included covariates to confounders only, this method resulted in a greater number of matched pairs and subsequently greater precision in estimating treatment effect (137). There was, however, no discernible difference in measurable bias between the different simulations (137). Another publication concluded (also based on simulations), as theorised by Rubin and Thomas, that only variables unrelated to exposure but related to outcome should be included in the model as this approach led to increased precision (130, 133, 138).

Including too many covariates can introduce problems with overfitting and issues with common support. The risk of overfitting increases in situations where the covariate to observation ratio is high, which reduces the ability for estimating the propensity for treatment due to noise in the statistical model (139). Lack of common support means that there is reduced overlap in propensity scores between cases and controls, leading to problems with matching and subsequently lack of generalizability (140). Balance checking methods such as SMD described above, complemented with visual representations of propensity score-distribution in among cases and controls, can assist in diagnosing imbalances in covariate characteristics. In addition it is of importance that the included variables are ascertained at baseline and not post-treatment (121).

In conclusion, there is no definitive consensus on the best approach. Still, the process of covariate selection is fundamentally anchored in *subject-matter expertise*, ensuring careful consideration of included variables.

The use (or not) of matching variables in Cox regression

The decision to include matching variables in the Cox regression model can be considered in situations where there is residual covariate imbalance between the

matched cohorts or if there are confounding variables unaccounted for in the model. Otherwise it has been shown that ignoring the matching variables in logistic models, has little to no effect on the estimates (on average), and yield the effect of the exposure on the exposed population (141).

Entropy balancing (EB)

A newer method of estimating causal inference in observational studies with binary treatment, is the entropy balancing method introduced by Jens Hainmueller in 2012 (142). Like propensity score-methods, entropy balancing seeks to balance the baseline characteristics of the case and control groups. It does this by executing a re-weighting scheme that minimizes the moments (mean, variance, kurtosis, etc.) of the distributions of selected covariates between the groups (142).

Traditional methods (including propensity score-estimation) start with calculating a probability of treatment given a set of covariates (i.e. propensity score) and subsequently checks the balance between cases and controls on the included covariates. The entropy balancing method turns this process around by setting the goal of balance as a first step. This is done by specifying which characteristics (covariates) should be balanced and what that balance should look like (moments). It then assigns weights for each observation based on these restrictions and moments to create a perfect balance between the cohorts, while maintaining efficiency (keeping the weights as close as possible). The weighted cohorts can subsequently be used in any statistical model to estimate treatment effects.

Entropy balancing has been shown to produce reduced model dependency in simulations with finite data, compared to a range of more traditional methods such as propensity scores-matching and weighting, difference in means, etc (142). Furthermore, entropy balancing possesses the property of double robustness, which ensures that an estimation of treatment effect is consistent if either the treatment model is correctly specified or if the outcome model is accurately adjusted, though not necessarily both (143).

Some limitations need to be taken into consideration, however. Weighting issues can occur if either the constraints are too extreme or in situations where the constraints are consistent but the underlying data are lacking in overlap (142). Finally, it is recommended to analyse the distribution of observational weights, as there could be extreme weights that may need to be pruned to avoid over-reliance of a few heavily weighted observations which in turn could unduly influence the estimated treatment effect (142, 144).

Disease risk score (DRS)

Another type of summary score is the disease risk score which can be used to divide the cohort into groups allowing for stratified or interaction analyses. The DRS is an aggregate of multiple risk factors into a single score that represents an estimation of the individual's risk of experiencing the outcome, independent of treatment assignment (145, 146). This independence makes the score particularly suitable in scenarios where there are multiple treatment options (146, 147).

Calculating the DRS involves modelling the probability of an outcome based on various predictors, removing the influence of treatment by setting treatment to zero (148, 149). The DRS is the total sum of the products of the coefficients and covariate values, which can be estimated in either all subjects or in the untreated subjects only (148). In certain cases, the "all subject"-approach has demonstrated superior performance (146).

Study design for papers I-IV

The following table contains an overview of the included papers:

Paper	Cohort setting	Exposure	Outcome	Method
I	All adults 40-74 years, Denmark 1997 to 2011	Penicillin V versus clarithromycin and roxithromycin	Cardiovascular death within 1 year	Prevalent new user*, PS-match 1:4
II	All adults 40-85 years, Sweden 2006 to 2014	Amoxicillin versus fluoroquinolones	Acute liver injury within 60 days	Prevalent new user*, PS-match 1:1
III	All adults 18-75 years, Sweden 2006 to 2018	Penicillin versus fluoroquinolones	Heart valve regurgitation within 30 days	Prevalent new user*, PS-match 1:1
IV	All adults 18-85 years, Sweden 2006 to 2018	Clindamycin versus fluclocaxillin	Acute liver injury within 45 days	Prevalent new user*, Entropy balance

* Episode-based approach (i.e. multiple courses can be included depending on certain criteria)

Paper I

The aim of this study was to investigate whether short-term use of roxithromycin and clarithromycin is associated with an increased risk of long-term cardiovascular death.

Study design

A historical cohort was created from the source population of all Danish adults aged 40-74 years in the time period 1997 to 2011. We used an Active Comparator Prevalent New User (ACPNU)-design, with penicillin V as the comparator drug.

All filled drug prescriptions for penicillin V, clarithromycin, and roxithromycin (ascertained from the Danish National Prescription Agency), by individuals included in the cohort were collected. Baseline characteristics, including medical history and drug use, for each course were collected from the Danish National Patient Register and Danish National Prescription Agency at the time of inclusion (baseline). Eligible courses after restrictions were subsequently matched on the propensity score. Two separate cohorts were created; one with penicillin V courses matched 1:4 with clarithromycin courses, and one with penicillin V courses matched 1:4 with roxithromycin courses.

Cohort restriction

Baseline restrictions were employed. Courses preceded by antibiotic use or hospitalization < 30 days were excluded (no available data on in-hospital drug use). As were courses filled by individuals suffering from serious disease (to reduce confounding due to high pre-treatment risk of the outcome) and by individuals who had no register activity < 2 years (to ensure valid ascertainment of baseline covariates). Filled prescriptions for multiple antibiotics (including study drugs) were excluded.

Courses were censored if the patient was hospitalized or filled an antibiotic prescription during follow-up.

Follow-up

Follow-up started on date of filled prescription and ended on the date of experiencing the outcome (death due to cardiovascular disease), reaching 75 years of age, loss to follow-up (emigration or disappearance), hospitalization, filling a prescription for any antibiotic, end of study (December 31, 2011), or 365 days after start of follow-up, whichever came first.

Outcome

The main outcome was death from cardiovascular disease within one year (from filled prescription), which in turn encompassed: ischemic heart disease, arrhythmic disorders, cardiac arrest or heart failure, cerebral infarction, and atherosclerosis.

Paper II

The aim of this study was to investigate if oral fluoroquinolone use is associated with an increased risk of acute liver injury.

Study design

We created a historical cohort consisting of all Swedish adults aged 40-85 years, from 2006 to 2014. An ACPNU-design was employed, using amoxicillin as the

comparator drug. All filled prescriptions for the study drugs (oral fluoroquinolones and amoxicillin) by individuals in the cohort were collected. At baseline (date of filled prescription), we collected baseline characteristics (drug use and medical history) from the National Drug Prescription register, and from the National Inpatient Register. Courses remaining after restrictions were matched 1:1 on propensity score, resulting in a matched cohort of courses of fluoroquinolones and amoxicillin.

Restrictions

At baseline, several restrictions were made. We excluded courses preceded by hospitalization or filling of any study drug past two months. If multiple antibiotics were filled on the index date, the course was excluded. Courses initiated by patients with a history of severe chronic disease or any of the outcome diagnoses were excluded. Likewise, courses preceded by diagnoses indicating acute hepatitis in the past two months were excluded (to avoid any lingering effects that could increase the risk of experiencing the outcome). To ensure that baseline characteristics were accurately ascertained, courses filled by individuals without register activity the past year were excluded.

Courses were censored if followed by hospitalization or switch to another antibiotic during follow-up.

Follow-up

Follow-up started on the date of filling a study drug prescription and ended if any of the following occurred: end of study (January 1, 2014), patient reaching 86 years of age, hospitalization or death due to any of the outcome diagnoses, or 60 days passed since start of follow-up (end of follow-up period), or emigration.

Outcome

The outcome was defined as any diagnosis indicating acute liver injury (toxic liver disease, toxic liver disease with hepatic necrosis, toxic liver disease with acute hepatitis, toxic liver disease with acute hepatitis not elsewhere specified, toxic liver disease unspecified, acute and subacute hepatic failure, hepatic failure unspecified) was registered in the Cause of Death- or Inpatient register.

Paper III

The aim of this study was to investigate if use of oral fluroquinolones is associated with an increased risk of aortic- or mitral valve regurgitation.

Study design

A historical cohort was established comprising all Swedish adult individuals aged 18-75 years, identified within the time period of 2006 to 2018. All filled prescriptions of oral fluoroquinolones and penicillin V made by the individuals of the cohort were collected from the National Drug Prescription Register. We employed an ACPNU-design with penicillin V as the comparator drug. Baseline characteristics were ascertained on the date of filled prescription by collecting individual data from the National Drug Prescription Register (drug use) and the National Inpatient Register (medical history). After restrictions, the eligible courses of penicillin V and oral fluoroquinolones were propensity score-matched 1:1.

Restrictions

At the time of filled prescription (baseline), several exclusions were made. We excluded courses that were preceded by hospitalization or study drug prescriptions the past 120 days. If a prescription was filled concurrently with other antibiotics, the course was excluded. Courses filled by individuals with a history of valve disease or suffering from severe chronic disease were also excluded. Finally, courses filled by individuals with no register activity in the past year were excluded.

Courses were censored if interrupted by hospitalization or switch to another antibiotic during follow-up.

Follow-up

The date of filled prescription for a study drug was designated the start of follow-up, or index date. Follow-up ended if either of the following occurred: end of study (December 31, 2018), participant reaching 76 years of age, hospitalization or death due to any of the primary outcome diagnoses, reaching the end of follow-up period (120 days after start of follow up), or emigration.

Outcome

The outcome of interest was heart valve regurgitation (mitral valve insufficiency, aortic valve insufficiency or rupture of chordae tendinae) within 120 days of filled prescription, recorded in either the Cause of Death Register or the National Inpatient Register.

Paper IV

The aim of this study was to estimate the magnitude of association between flucloxacillin use and the risk of acute liver injury.

Study design

We created a historical cohort of all Swedish adults aged 18-85 years in the 2006 to 2018 time period. All filled prescriptions of flucloxacillin and oral clindamycin by individuals in this cohort were collected from the National Prescribed Drug Register. We used the ACPNU-design using oral clindamycin as the comparator drug. Baseline characteristics were ascertained from the National Prescribed Drug Register (drug use history) and the National Inpatient Register (medical history) at the time of filled study drug prescription. After applying restrictions, the eligible courses of oral clindamycin and flucloxacillin were entropy balanced.

Restrictions

Restrictions were employed at the time of filled prescription (baseline). Courses that were preceded by hospitalization or filling of any study drug were excluded, as were courses filled by individuals with severe chronic disease or any history of liver disease. If multiple antibiotic prescriptions were filled on the index date, the course was not included.

Courses followed by hospitalization or switch to another antibiotic during follow-up, were censored.

Follow-up

Follow-up started on the date of filling a study drug prescription (index date) and ended if any of the following occurred: end of study (December 31, 2018), hospitalization or death due to any of the primary outcome diagnoses, participant reaching 86 years of age, end of follow up reached (180 days from start of follow-up), or emigration.

Outcome

Primary outcome of interest was defined as acute liver injury (toxic liver disease, toxic liver disease with hepatic necrosis, toxic liver disease with acute hepatitis, toxic liver disease with acute hepatitis not elsewhere specified, toxic liver disease unspecified, acute and subacute hepatic failure, hepatic failure unspecified) registered in either the National Inpatient Register or National Cause of Death Register.

Statistical Analyses

Statistical analyses for all papers were primarily performed by using SAS version 9.4 (SAS institute). For paper IV we used R-studio 2023.06.0 Build 421 with corresponding packages (ebal, cobolt, and GGplot2), to perform entropy balancing

and for creating plots and visualization. Some graphical components in papers I-IV were created using Microsoft PowerPoint.

All statistical tests were two-sided where a 95% confidence interval not overlapping 1.0, or equivalently having a p-value less than 0.05, was considered statistically significant. Likelihood ratio tests were performed to test for differences and interactions.

Poisson regression

Poisson regression is a fully parametric statistical method for modelling count data, particularly for examining the frequency of events within a specified time frame, often referred to as analysis of rates. It is adept at estimating the incidence rate or absolute risk of an event within a certain period. Being fully parametric implies that the data follow a specific distribution, namely the Poisson distribution (150). This distribution is determined by a fixed set of parameters which determines the probability of various counts (150).

The validity of Poisson regression is contingent on two key assumptions: the independence of events (each event occurs independently of others) and equidispersion (the event count's mean and variance are equal) (150). In cases of overdispersion, where variance exceeds the mean, modifications such as time-partitioned regression (piecewise Poisson regression) may be warranted (151).

In Poisson regression, time plays a critical role and is often treated as an exposure variable that influences the rate of events. This is handled by incorporating the *log(time at risk)* as an offset variable in the regression model, thus standardizing the event rate with respect to time at risk (150).

Similarly, when addressing censoring, provided it is non-informative, the *log(time to censoring*) can also be incorporated as an offset to account for the period individuals are at risk (150). This method ensures that the event rates are comparable, even when observation periods differ due to censoring.

Cox regression

Also known as proportional hazards regression is one of the most common models used for analyzing time-to-event (survival) data. The Cox model aims to understand how a set of covariates affects the instantaneous risk, or hazard, of a certain event at a certain point in time in the study period (95, 150). It does this while simultaneously considering that only a certain proportion of participants are at risk (the risk set) of experiencing the outcome at certain time points. In other words, the model accounts for participants that, for example, have experienced the outcome, withdrawn, been lost to follow-up, or been censored (95).

In contrast to the fully parametric Poisson regression, the Cox-model is semi-parametric, meaning it makes no assumption of the distribution of survival times

nor is it concerned with the absolute risk (95). Instead, it focuses on estimating the relative risk between individuals or groups, associated with a certain set of covariates (95). So, while Cox-regression informs us about the relative risk, for instance between exposed and unexposed, its semi-parametric nature does not directly provide an estimate of the baseline hazard function (95). Consequently, the model does not inherently give estimates of the *probability* of an event occurring at a certain time point (152, 153).

The likelihood function is used to estimate the covariate coefficients by comparing the instantaneous hazard for a participant experiencing the outcome (and its covariate set) with the cumulative hazard for all participants of the risk set at that specific moment in time (95).

The model works under the assumption that the effects of the covariates are multiplicative with respect to the hazard function and constant over time, also known as the proportional hazards assumption (95). This means that although the absolute risk, or hazard, can change over time, the relative risk does not.

This assumption can be tested using several methods. Graphical methods such as plotting the log(-log(survival)) against time for different strata can be used to check that the lines are parallel (95). The Schoenfeld residual (the difference between an observed covariate value and the expected covariate value) test, where the residuals are regressed on time to estimate whether there is a correlation or not, is another common way to assess proportionality (95). A Wald test can be used to evaluate if the effect of a covariate, or an interaction between covariate and time, differs significantly from zero (which should not be the case if the proportional hazards assumption is to hold true) (154).

Paper I

Poisson regression was used to estimate rate ratios for cardiovascular death in propensity score-matched (1:4) courses of clarithromycin and roxithromycin compared to penicillin V. The propensity score was estimated using logistic regression with covariates (ascertained at the time of filled prescription) such as medical history, drug use, demographics (age, sex, geographic location), and healthcare usage selected *a priori*. Courses in the two cohorts (clarithromycin vs penicillin V and roxithromycin vs penicillin V) were matched 1:4 using the greedy 5 to 1 digit algorithm. Covariate balance was assessed using standardized mean differences with values <0.1 indicating adequate balance.

A cardiovascular risk score (DRS) was calculated for each course by multiplying all coefficients and predictors while setting the treatment variable to zero. The courses were categorized according to distribution over deciles in three groups: low - medium - high (1-5, 6-8, 9-10). In addition to disease risk score, subgroup analyses included sex and age. Likelihood ratio test was performed to estimate homogeneity

across the subgroup strata. Main follow-up period was 365 days from filled prescription, with secondary analyses including the time periods 0-7, 8-89, and 90-365 days. Additional secondary analyses included analysis of rate ratios for other cardiovascular death and other non-cardiovascular death.

Paper II

Cox regression was used to estimate hazard ratios for acute liver injury in propensity score-matched (1:1) courses of oral fluoroquinolones and amoxicillin. Propensity score estimates were calculated using logistic regression with covariates (ascertained at the same time or just before a filled prescription) selected *a priori*, including medical history, drug use, sex, age, geographic location, and healthcare usage. Courses of oral fluoroquinolones and amoxicillin were matched on propensity score in a 1:1 fashion using the greedy 5 to 1 digit algorithm. Covariate balance was estimated using standardized mean differences where values <0.1 was considered adequate covariate balance.

Subgroup analyses included sex and age (40-64 and 65-85 years) where test of homogeneity across the strata was estimated using the likelihood ratio test. The main analysis period was 1-60 days. The absolute rate difference for the main period was estimated as [(hazard ratio – 1) x incidence in the amoxicillin group], presented as number of cases per 1 million treatment episode (155). Secondary analysis included hazard ratio for all-cause mortality in the two cohorts.

Paper III

Cox regression was used to estimate hazard ratio for the risk of heart valve regurgitation in propensity score-matched (1:1) courses of oral fluoroquinolones and penicillin V. Propensity scores were estimated using logistic regression with *a priori* selected covariates (medical history, drug use, sex, age, geographic location, healthcare usage) ascertained at the date of filled prescription. Courses were matched 1:1 using the greedy 5 to 1 digit algorithm and considered well balanced if the standardized mean difference for each covariate was estimated to be <0.1. Subgroup analyses included age (<65> years) and sex, where likelihood ratio tests were employed to test homogeneity across the strata. The main analysis period was 1-30 days including secondary analyses for periods 31-60 and 61-120 days.

Supplementary analyses included estimation of hazard ratios for:

- all-cause mortality
- oral fluoroquinolones and penicillin V and the risk of valve surgery or death

- risk of acute liver injury in oral fluoroquinolones and penicillin V using the first prescription only
- risk of acute liver injury in oral fluoroquinolones and amoxicillin
- risk of acute liver injury in oral fluoroquinolones and penicillin V in the 1 to 365-day time period

The absolute rate difference for the main period was estimated as [(hazard ratio – 1) x incidence in the penicillin V group], presented as number of cases per 1 million treatment episode (155).

Paper IV

Cox regression was used to estimate hazard ratios for the risk of acute liver injury in entropy balanced courses of flucloxacillin and oral clindamycin. *A priori*-selected covariates (medical history, drug history, geographic location, sex, age, healthcare usage) were assigned weights to achieve balance across the two cohorts. Inherently, the method achieves perfect balance although for completeness, balance was estimated using standardized mean differences.

Subgroup analyses included age (<75> years), sex, and number of exposures. Homogeneity across strata was estimated using interaction analysis. The main analysis period was 1-45 days with secondary analyses including 46-90, and 91-120 days.

Supplementary analyses included estimation of hazard ratios for:

- acute liver injury including only the first prescription
- acute liver injury excluding cases with the ICD-10 code for jaundice (R17) in the outcome
- all-cause mortality

The absolute rate difference for the main period was estimated as [(hazard ratio – 1) x incidence in the oral clindamycin group], presented as number of cases per 1 million treatment episode (155).

Results

Paper I

From the source population of 3,380,262 Danish adults aged 40-74 years, a total of 8,911,449 antibiotic courses were identified. There were 415,297 courses of clarithromycin, 1,150,387 courses of roxithromycin, and 7,345,765 courses of penicillin V. After exclusions there remained a total of 6,282,248 antibiotic courses (187,887 courses of clarithromycin, 698,899 courses of roxithromycin, and 5,395,462 courses of penicillin V).

Subsequently, 1:4 propensity score-matching was applied to create two separate study cohorts. One cohort with 187,887 courses of clarithromycin matched with 751,524 courses of penicillin V, and one with 698,899 courses of roxithromycin matched with 2,721,538 courses of penicillin V. Prior to propensity score-matching, users of clarithromycin and roxithromycin were more likely to be of female sex and having a history of respiratory disease. After matching, covariate balance was within acceptable limits (<0.1) according to estimation of standardized mean differences.

Key findings

We found no increase in the risk of cardiovascular death during a one year follow up period among courses of clarithromycin compared to penicillin V. There were 78 events (incidence rate [IR] 0.8 per 1,000 person-years [pyrs]) of cardiovascular death among clarithromycin courses compared to 259 events (IR 0.6 per 1,000 pyrs) among the penicillin V courses, resulting in a rate ratio (RR) of 1.26 (95% confidence interval [CI]: 0.96-1.59).

The risk was most pronounced in the 0 to 7-day "current use"-period, and attenuated in the subsequent time periods, 8 to 89-days and 90 to 365 days.

Similar results were found among courses of roxithromycin compared to penicillin V. We identified 211 events (IR 0.6 per 1,000 pyrs) of cardiovascular death compared to 858 events (IR 0.6 per 1,000 pyrs) among penicillin V courses. This yielded a RR of 0.99 (95% CI: 0.86-1.16).

We did not find support for an increased risk in the "current use" (0 to 7 days) period in the roxithromycin cohort.

Subgroup analyses

Use of clarithromycin among women was associated with an increased risk of cardiovascular death within one year (RR 1.69; 95% CI: 1.14-2.48) compared to men (RR 1.00; 95% CI: 0.71-1.40), with P=0.05 for the test of homogeneity. Test for homogeneity across strata for age <65> (P=0.82), and cardiac risk score (P=0.52) did not support any significant differences.

Among users of roxithromycin there were no significant differences in any of the subgroups: sex (P=0.84), age <65> (P=0.11), or disease risk score (P=0.90).

Supplementary analyses

In addition to the main outcome cardiovascular death within one year, we also evaluated the risk of other cardiovascular death (ICD-10 codes I10-I99 except those included in the main outcome definition) and non-cardiovascular death (all codes except I10-I99) within one year for both cohorts. We found no significant difference in the risk of other cardiovascular death when comparing clarithromycin to penicillin V (RR 0.86; 95% CI: 0.57-1.29) or when comparing roxithromycin to penicillin V (RR 0.97; 95% CI: 0.79-1.21). There was a slight difference in non-cardiovascular mortality when comparing clarithromycin with penicillin V (RR 1.10; 95% CI: 1.00-1.22) as well as when comparing roxithromycin with penicillin V (RR 1.20; 95% CI:1.14-1.27).

Finally, several sensitivity analyses were performed. Analysis only including the first course of antibiotic among courses of clarithromycin and penicillin V yielded a RR of 1.16 (95% CI: 0.75-1.79) and a RR of 0.95 (95% CI: 0.70-1.23) among courses of roxithromycin and penicillin V, in line with the main results. Likewise, the conclusion remained the same when removing the censoring criteria for hospitalization, RR 1.04 (95% CI: 0.87-1.26) and RR 1.0 (95% CI: 0.87-1.11) for respective courses of clarithromycin and roxithromycin compared to penicillin V.

There was a slight difference in all-cause mortality in users of roxithromycin compared to penicillin V (RR 1.12; 95% CI: 1.07-1.18) but not among users of clarithromycin compared to penicillin V (RR 1.06; 95% CI: 0.98-1.16).

Paper II

From the study population of all Swedish adults aged 40-85 years we identified 2,456,901 courses of oral fluoroquinolones (n=1,542,175) and amoxicillin (n=914,726). After applying exclusion criteria and 1:1 propensity score-matching the remaining cohort consisted of 419,930 courses of oral fluoroquinolones and amoxicillin respectively. The most common oral fluoroquinolone was ciprofloxacin (79.3%) followed by norfloxacin (17.4%), moxifloxacin (1.78%), levofloxacin (1.11%), and ofloxacin (0.47%). Covariate balance in the matched cohort was estimated, and within acceptable limits (<0.1), according to estimation of standardized mean differences.

Key findings

During the 60-day follow-up period, we found a doubled risk (HR 2.32; 95% CI: 1.01-5.35) of acute liver injury associated with oral fluoroquinolone treatment (18 events; IR 2.98 per 10,000 pyrs) compared to amoxicillin (8 events; IR 1.27 per 10,000 pyrs). The timing of events was analysed by breaking up the follow-up interval in 10-day periods, which revealed that most (67%) events occurred within the first 30 days following start of treatment (filled prescription).

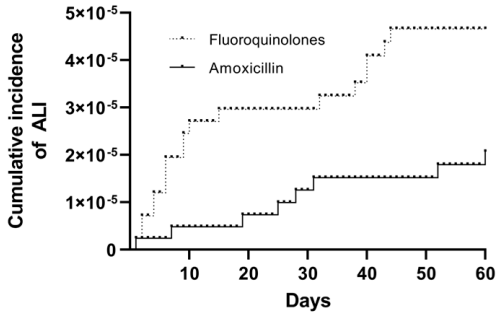

Figure 6: Cumulative incidence of acute liver injury, fluoroquinolones vs amoxicillin day 1-60.

Subgroup analyses

We observed a trend toward a higher risk of the outcome among older patients; however, the difference was not statistically significant (P=0.14 for the test of homogeneity across age groups <65>). No difference in risk was found in subgroup according to sex (P=0.42).

Supplementary analyses

We observed a difference in all-cause mortality between the two cohorts in the first 30 days (HR 0.70; 95% CI: 0.61-0.79) of follow-up, but not in the subsequent 30-day period (HR 1.02; 95% CI: 0.85-1.22).

Paper III

From the study population of all Swedish adults aged 18-75 years, a total of 9,830,008 courses of penicillin V (n=7,601,340) and oral fluoroquinolones (n=2,228,668) were identified. After exclusions and 1:1 propensity score-matching, there remained 794,588 courses of penicillin V and oral fluoroquinolones respectively. The main antibiotic in the oral fluoroquinolone cohort was ciprofloxacin (88%). We calculated standardized mean differences for the covariates to assess balance, which were all within acceptable range (<0.1). A visual estimation of covariate balance prior and post matching was included.

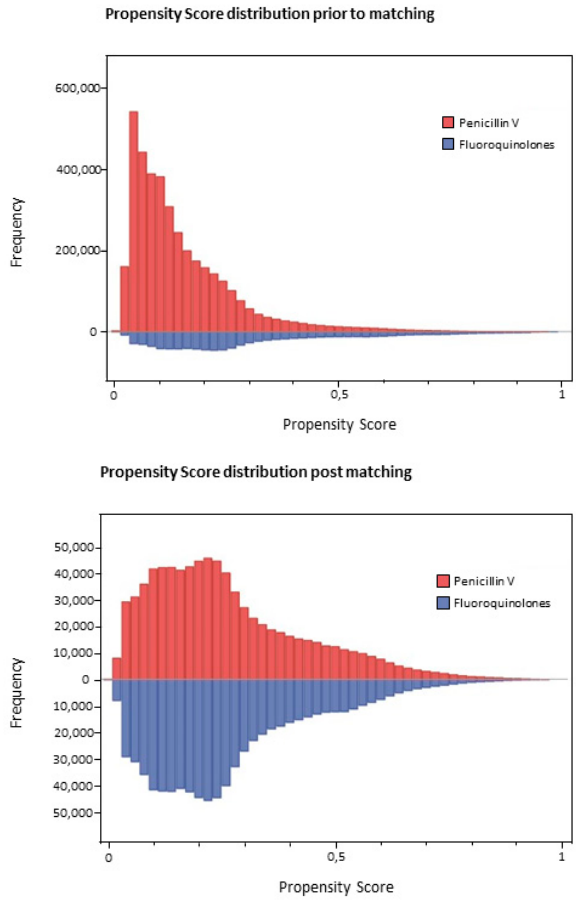

Figure 7: Distribution of propensity scores prior, and post matching

Key findings

We found no support for an increased risk of heart valve regurgitation associated with oral fluoroquinolone treatment compared to penicillin V (HR 0.70; 95% CI: 0.43-1.11) during a follow-up period of 30 days.

Subgroup analyses

No differences in sex or age group (<65>) were found; test of homogeneity P=0.83 and P=0.45 respectively.

Supplementary analyses

A small but statistically uncertain increased risk of all-cause mortality was detected among users of oral fluoroquinolones compared to penicillin V (HR 1.14; 95% CI: 0.94-1.40). None of the sensitivity analyses changed the main conclusion of no risk difference.

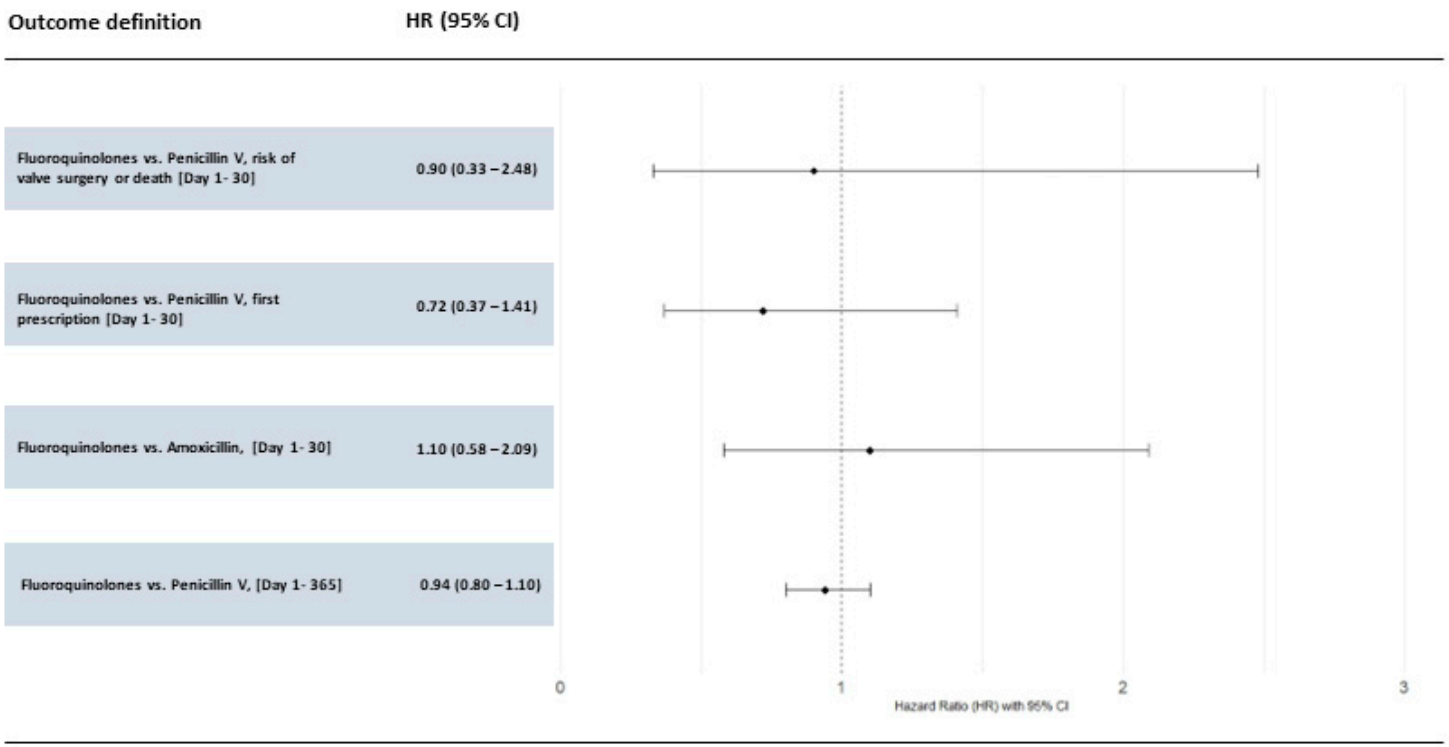

Figure 8: Forest plot of sensitivity analyses

Paper IV

From the study cohort of all Swedish adults aged 18-85 years during the 2006-2018 time period, a total of 4,298,093 courses of flucloxacillin (n=2,913,601) and oral clindamycin (n=1,354,492) were identified. After exclusions a total of 1,443,622 courses of flucloxacillin and 583,847 courses of oral clindamycin were available for entropy balancing. Although this method achieves perfects balance among the included covariates, we included a plot to visualise the balance before and after the method was applied. The range of weights for the two cohorts was visualised in a violin plot.

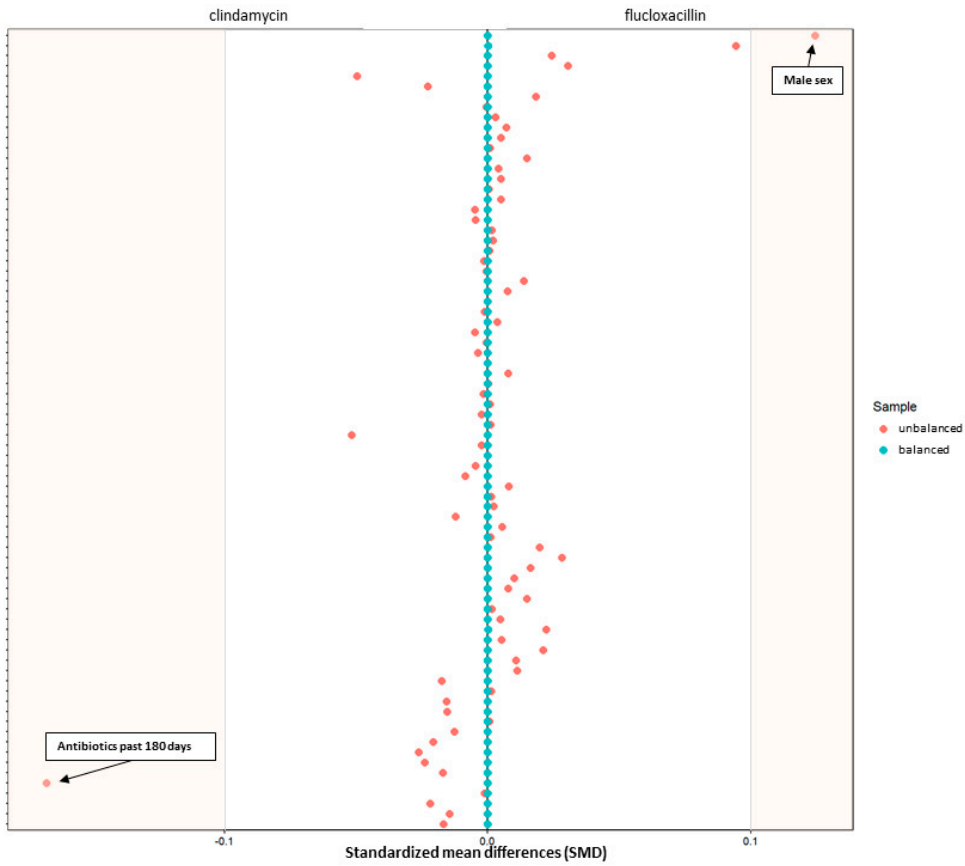

Figure 9: Covariate distribution in unadjusted and adjusted (unbalanced and balanced) cohorts

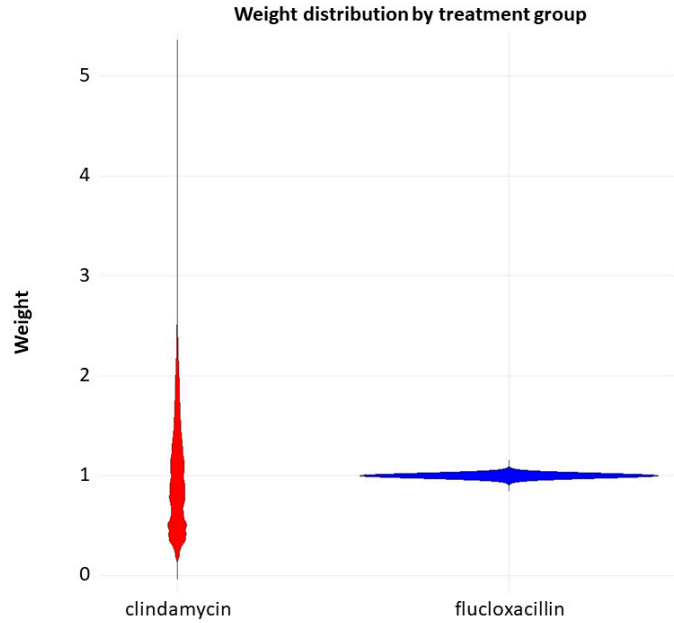

Figure 10: Violin plot visualising the weight distribution after entropy balancing. 2.5th percentile: 0.28 and 97.5th percentile: 2.1 (among courses of clindamycin). Courses of flucloxacillin all have the weight 1 (see section on Entropy balancing for explanation)

Key findings

A substantially increased risk of acute liver injury was identified among users of flucloxacillin compared to oral clindamycin, during a follow-up period of 45 days. There were 219 events among flucloxacillin users compared to 9 events among oral clindamycin users, corresponding to a HR of 7.32 (95% CI: 4.14-12.95).

Subgroup analyses

We found no statistical support for any differences across strata according to age (<70>), sex, or number of prescriptions (1, 2, 3+) according to test of interaction: P=0.72 and P=0.15 for age and sex respectively, and P=0.10 and P=0.36 for number of prescriptions (2, 3+).

Supplementary analyses

Supplementary analyses included: using only the first exposure, using a stricter outcome definition (i.e. excluding jaundice, ICD-10 code R17), and using a less restricted cohort (not excluding on "no-use"-courses). Neither of these sensitivity analyses changed the main conclusion. A small but statistically non-significant increased risk of all-cause mortality was noted in the flucloxacillin cohort (HR 1.07; 95% CI: 0.94-1.23).

Discussion

Adverse drug reactions (ADRs) not only pose a significant risk to patient health but also impart a considerable financial strain on healthcare systems. Several studies have estimated the prevalence of ADR-associated hospital admissions to be in the range of 2-12% and lead to estimated annual costs of hundreds of millions of euros (156-159).

Despite the advantage of randomization in clinical trials across the various phases of drug development, it is not uncommon for these trials to miss rare adverse drug reactions (ADRs) due to reasons described earlier in this text, such as scale, generalizability, and ability to follow patients over longer periods of time (160). To address these gaps, surveillance- and reporting systems have been established both internationally and on a national level to capture trends or signals for these rare ADRs. These systems enable the continuous monitoring of drugs as they enter the post-marketing phase and becomes available to the general public. In this context the field of pharmacoepidemiology plays a pivotal role and the application of pharmacoepidemiologic principles to analyse patterns, causes and effects within real-world settings contributes significantly to assessing drug-safety (161).

The following chapter will discuss the methodological strategies employed to minimize bias in the included papers and examine the principal limitations to each. We will revisit some concepts that were introduced earlier and elaborate on how they influenced the research design decisions in each respective papers.

Study design and methodological considerations

The use of active comparators

All papers (**I-IV**) made use of active comparators as part of the previously described ACPNU-design. The main concept is to use a comparator drug not known to affect the likelihood of the outcome of interest, while at the same time share treatment indications with the principal study drug. Employing this method aims to reduce not only unobserved confounders but also aims mitigate the risk of confounding by indication, in which the very reason for initiating drug treatment is associated with the outcome. This type of confounding introduces potential bias when comparing the risk of outcome among users of the drug of interest with instances of no-use,

where the baseline risk is not comparable due to the absence of infection. It is important to acknowledge that individuals with an infection can differ significantly from individuals who are uninfected. Infections stimulate the immune response, often leading to systemic inflammation and metabolic changes that can predispose an individual to a range of subsequent health issues.

Due to the absence of direct indication data in healthcare registers, we utilized national treatment recommendations to guide our selection of comparator drugs. We systematically analysed the all-cause mortality and especially the dispersion of ICD-codes that were registered as the primary cause of death, between the drug of interest and its comparator to make sure that there were no substantial differences which could indicate a significant difference in baseline risk between the cohorts.

In **paper I** we used penicillin V as comparator drug to estimate the risk of long-term cardiovascular death relative to macrolide use. Penicillin V has no known cardiotoxic effect and shares indications with macrolides in Denmark as both classes of drugs are used for respiratory tract infections.

Paper II, where the risk of acute liver injury related to fluroquinolone use was investigated, used amoxicillin as comparator drug. The drug has no known risk of acute liver injury and shares treatment indications with fluoroquinolones, albeit not perfectly aligned. Overlapping indications include respiratory- and urinary tract infections. An alternative comparator drug to amoxicillin was considered in trimethoprim-sulfamethoxazole (TMP-SMX). However, it was ultimately deemed unsuitable due to its relatively higher propensity for adverse drug reactions, which could confound the results. In addition, TMP-SMX was not prescribed in sufficient quantity to make for an adequately sized comparator cohort.

The comparator drug in **paper III**, where the risk of heart valve regurgitation associated with fluroquinolone use was investigated, was penicillin V. Penicillin V and fluroquinolones share indications mainly within respiratory tract-infections. However, several factors influenced the choice of penicillin V as the comparator drug: its non-association with the outcome of interest, the precedence of a Danish register-based study using the same comparator, and the general alignment of treatment guidelines between Denmark and Sweden. Confirmatory sensitivity analyses using amoxicillin as comparator upheld the primary conclusions, reinforcing the validity of our findings. Amoxicillin was considered as the primary comparator drug in this study but due to its use as endocarditis prophylaxis in dental work, and its relatively (to penicillin V) limited use, it was deemed as a less optimal candidate.

The choice of study drug in **paper IV** was more direct, comparing the risk of acute liver injury in users of flucloxacillin with oral clindamycin. These study drugs are in large completely interchangeable for most infections they are used to treat in the Swedish setting, namely skin- and soft tissue infections (SSTI), and prosthetic joint infections. Finally, clindamycin is not specifically linked to the risk of acute liver injury.

The prevalent new user design

A variation of the prevalent new user design (episode-based) was used for all **papers (I-IV).** This approach allowed for an individual to contribute with more than one episode of study drug use if the courses did not overlap, and the user did not experience the outcome. As a result, the study drug-free interval (or washout period depending on perspective) prior to filled prescription, varied from 30 days in **paper I** to 60 days in **paper II**, 120 days in **paper III**, and 180 days in **paper IV**.

In **paper I** we wanted to investigate whether a course of macrolide use (clarithromycin and roxithromycin) would increase the risk of long-term cardiovascular death. To isolate the influence of macrolides on this long-term outcome, we excluded courses that were preceded by any antibiotic use the past 30 days. This interval was chosen to mitigate the risk of confounding by severity, as multiple courses of antibiotics in a short period of time could indicate a more advanced and severe clinical course of infection which independently could influence the risk of long-term cardiovascular death. Multiple courses of antibiotics in the time period before start of follow up, would also make it difficult to elucidate which exposure was the potential cause of a subsequent outcome. Moreover, the literature suggests an acute and transient increased risk of cardiac toxicity, especially during the first week of treatment, subsiding to "baseline" thereafter (162). This supports the biological plausibility of an acute, pro-arrhythmic effect of macrolides described earlier. By implementing a 30-day antibiotic free lead-in period, we aimed to reduce the influence of such short-term effects, instead focusing on the long-term cardiovascular risk of macrolide use. In addition, courses were not allowed to overlap, to reduce the risk of misattributing the effect of prior treatment courses with the current one.

In **papers II-IV** we let the period of no-use mirror the follow-up period so that the courses naturally would not overlap. The period of no-use was restricted to use of study drugs instead of all antibiotics such as in **paper I**. This approach aimed to minimize confounding especially related to recent or repeated use of the drugs of interest, rather than confounding by antibiotic use in general, which may not be as relevant for acute outcomes as the long-term outcomes that were investigated in **paper I**. Furthermore, imposing too severe restrictions on the study cohort (i.e. all prior antibiotic use for extended durations) would often lead to a significant decrease in statistical power as well as generalizability of our findings.

The risk window

Risk window refers to the period of drug intake and the subsequent period of risk increase (163). The specification of the risk windows for **papers I-IV** were informed by several factors. In all papers the start drug intake was assumed to be anchored with date of filled prescription, also defined as the index date or start of

follow-up. The period of drug intake was established based on the typical duration of therapy as recommended in national guidelines, for the infections commonly treated with the study drugs.

A proposed risk trajectory is a period of high risk associated with the duration of drug administration ("current-use"-period), followed by a period of diminishing risk, and ending with a period of "return to baseline"-risk where the effect of the drug completely tapers off (164). The window must, of course, align with the research question at hand, the existing literature, as well as the pharmacokinetic and pharmacodynamic properties of the drug of interest.

In **paper I** we were less interested in the acute effects of macrolide use and instead focused on the long-term risk of cardiovascular death. The studies that prompted the investigation, reported an increased risk that extended months-to-years past a completed course of treatment. Therefore, a follow-up period of 365 days was deemed sufficient to capture late risk increased of the outcome. In complementary analyses, however, we estimated the rate ratio in the time periods 0-7 days, 8-89 days, and 90-365 days; periods that were chosen to mirror the risk trajectory described earlier.

Similar reasoning was used to define the risk windows in **paper II-IV**. **Paper II** explored the risk of acute liver injury associated with fluoroquinolone use, and a primary risk window of 60 days reflected the current body of evidence as well as the underlying mechanism of injury associated with its use. The timing of events was further granularized by including analyses of 10-day-intervals. **Paper III** defined the primary risk window to 30 days which also was supported by previous reports on the timing of association of fluroquinolone related connective tissue events (67). Two subsequent intervals of 31-60, and 61-120 days were included to capture potential late-presenting effects of fluoroquinolone exposure. In **study IV** we wanted to quantify the already known risk increase of flucloxacillin use on acute liver injury. The process is well described and entails an acute immunoallergic effect that takes place during or shortly after drug exposure. Therefore, the main risk window was set to 45 days.

Identifying an elevated risk associated with the period of active drug exposure that diminishes after discontinuation, supports a causal effect of the drug of interest on the outcome. However, a persistent long-term difference in risk, might reflect not only a causal effect but also pre-existing differences in baseline risk between the compared cohorts. This observation underscores the importance of the steps that need to be taken to ensure comparable cohorts with respect to baseline covariates.

The survival (time-to-event) methods

In **paper II-IV** Cox proportional hazards was used to estimate the hazard ratios for the outcomes of interest. This method was preferred due to its ability to handle

censoring effectively and allowing for hazard ratio estimation without the need to specify the underlying risk over time (baseline hazard). If the proportional hazards-assumption is not violated, the method is particularly suited for survival analysis, where the interest lies in assessing the effect of the covariates on the hazard over time. Moreover, the model is suitable in scenarios where the baseline hazard is assumed to vary over time.

In **paper II-IV** we anchored time scale to the date of entry, defined as date of filled prescription, to estimate hazard ratios. This approach ensured that the timing of drug exposure and subsequent risk of the outcomes could be accurately captured.

In **paper I**, however, Poisson regression was used to calculate rate ratios for the risk (incidence) of long-term cardiovascular death in the two cohorts. The primary reason for the use of Poisson regression in **paper I** was due to technical constraints at the time (2018), where attempting to perform Cox regression was met with computational issues due to the sheer number of observations in the cohorts.

Although the increase in events during the first 7 days of treatment was notable, the overall rate and the within-period (0-7, 8-89, 90-365 days) rates remained within the boundaries the Poisson distribution and did not violate the mean-variance equivalence.

The two methods can in many situations be used interchangeably given that the assumptions for each hold true, but the interpretation of the results differs. The hazard (ratio) derived from Cox regression refers to the instantaneous risk of an event occurring at a certain moment in time, given that the individual has survived up to that point (95, 150). This is inherently time relative as it reflects the change in risk over the duration of the time period.

In contrast, the rate ratio from Poisson regression is an estimation of the overall risk or incidence rate, reflecting an aggregated measure over time without capturing time-varying risks (assuming Poisson distribution) (95, 150).

In short (and simplified): hazard ratios are valuable for understanding the timing of the risk and rate ratio for assessing the overall risk burden.

The use of restrictions

Restricting the cohort by defining precise inclusion- and exclusion criteria is an effective method to reduce possible confounding factors that influence the internal validity of the study results. However, too much restriction has the potential to significantly hamper the external validity, or generalizability, of the results. Consequently, the inclusion- and exclusion criteria requires careful consideration and choices must be informed by the study objectives, expected disease epidemiology in different age intervals, drug profiles, etc.

In all papers, we used an age-based inclusion criteria, with the specific intervals varying to suit the research question addressed in each paper. In **paper IV**, a broad age range of 18-85 years was chosen. We used this interval to ensure a generalizable and heterogenous cohort to capture the incident cases of acute liver injury across the full spectrum of adult individuals to be able to estimate the overall magnitude of association between flucloxacillin and the outcome. In addition, flucloxacillin and oral clindamycin are used in a wide range of infections that affect young and old people alike and their usage is not typically restricted by age. We also wanted to estimate if advanced age was a modifying factor, as was implied in previous studies. The study cohort in **study III** was also based on a relatively wide age interval of 18-75 years. The main reason for using this interval was that we wanted to be able to add valid and generalizable data to the body of evidence regarding the proposed association between fluoroquinolone use and the risk of the potentially life-threatening event of heart valve regurgitation. Prior studies in similar settings had studied the risk in an even wider age range (up to 100 years of age) but we determined that adopting a similar interval would risk introducing confounding factors related to frailty and advanced age. Moreover, our data demonstrated that incident cases occurred predominately within the older population segment.

In **paper I** and **II** we included adults within a slightly narrower age span, 40-to-74 and 40-85 years respectively. In **paper I** our objective was to assess the long-term risk of cardiovascular death within a generalizable population, having a low baseline risk for this outcome. We noted that some existing studies suggesting a link between fluoroquinolone use and increased long-time risk of cardiovascular death were conducted among populations with a relatively high baseline risk. Consequently, we hypothesized that this risk increase could be confounded by elevated baseline risk characteristics in the study samples and used a slightly narrower interval.

The age-interval in **paper II** followed a similar reasoning as in paper I and the underlying data was determined to allow for inclusion of slightly older patients. The expansion allowed for an increase in statistical power and ensured the accrual of a sufficient number of outcome events, enhancing the ability to detect potential associations between fluoroquinolone use and the risk of acute liver injury. Furthermore, we determined that the prescription patterns for fluoroquinolones and amoxicillin would exhibit a less age-related bias compared to the patterns between macrolides and penicillin V. In particular with respect to respiratory infections in patients with COPD, where age may confound the choice of treatment due to increasing prevalence in older individuals.

In **papers I-IV** we also excluded on medical history. A five year-lookback was used for *a priori*-ascertainment of drug use- and medical history at the time of filled prescription. To ensure a representative and accurate covariate ascertainment, we excluded patients with no register activity the past year (two years in **paper I**).

In general, the exclusions on drug use and medical history aim to reduce the risk of confounding by including patients with a relatively higher risk of the outcome at baseline. Therefore, patients with covariates indicative of serious disease such as end-stage illnesses (end-stage renal disease, cancer, severe dementia, serious respiratory- or neurologic disease, etc.), substance abuse, HIV/AIDS, congenital anomalies, organ transplant, etc., were excluded. Any history (∞ lookback) of the outcome diagnoses was used as exclusion criteria in **papers II-IV**. These restrictions were implemented to exclude patients with pre-existing conditions related to the outcome of interest, such as patients with a history of heart valve regurgitation (**paper III**) or those with a history of liver disease (**paper II and IV**), and therefore isolate the incident cases.

As we had no information on in-hospital drug use, we excluded patients that had been hospitalized for any reason prior to filling a study drug prescription. The "hospitalization-free" interval varied from 30 days in **paper I**, to 60-, 365-, and 180-day intervals for **papers II-IV**. The variation was mirrored by the different follow-up periods in the papers.

Likewise, we excluded courses where multiple antibiotic prescriptions were filled simultaneously, as it would be impossible to discern the independent causal effect attributable to a specific agent.

The use of propensity score matching and entropy balancing

All papers used some form of summary score. **Paper I-III** made use of propensity score-matching and in **paper IV** entropy balancing methods were employed.

Covariate selection was based on an *a priori* selection of variables with the potential to influence the outcome, collected from the different healthcare registers in Denmark (**paper I**) and Sweden (**paper II-IV**) (*see section on covariate selection for the reasoning behind this approach*) (133). Although the absolute number of covariates used in the summary scores varied for each study, the categories remained consistent. The categories included demographic characteristics (age, sex, geographic- and temporal specifics), medical history, drug use, and healthcare usage. The selection of covariates was aimed to capture a wide range of baseline characteristics that would reflect an individual's overall health status, including frailty aspects, patterns of drug utilization, and healthcare usage. This approach significantly reduced the risk of confounding by differences in baseline health status between cases and controls.

For **paper III and IV** we expanded the number of covariates ascertained for the medical history-category, based on a recently published reclassification of infectious disease-related ICD-10-codes (165).

Paper I included subgroup analyses based on a third type of summary score, the disease risk score (DRS), explained in detail in previous sections. This score was not included as a predictor in the propensity score-estimation, but instead used to stratify the cohorts into low – moderate – high-risk individuals.

The external validity

Given the completeness of both Danish and Swedish register data, coupled with the nationwide coverage and indiscriminate access that is the hallmark of Scandinavian healthcare, these results are considered representative of the overall population. As discussed earlier, however, there are always some compromises between external and internal validity that need to be considered.

As previously discussed, focusing too much on internal validity and elucidating a causal inference by, for example, applying "aggressive" restrictions can lead to an obvious loss of generalizability by leaving a study cohort that is highly selective and not representative of the broader population.

The purpose of restrictions and matching procedures in **papers I-III** was aimed at creating study cohorts with as similar background characteristics as possible, enabling the isolation of a causal effect of the treatment on the outcome. Given the population-based selection of the source population for **papers I-III** we believe that we could afford a significant focus on the internal validity without compromising too much on the external validity of the results. **Paper IV** had a somewhat different focus which lay on estimating the magnitude of risk, or incidence, in the general population. Thus, a less restrictive age-interval was chosen, and the sensitivity analyses included a model with less strict exclusion criteria.

The misclassification of exposure and outcome

The concept of non-differential and differential misclassification has been discussed earlier. In summary, a non-differential misclassification in exposure (or outcome) produces a bias toward (or, in extreme cases beyond) the null (95, 106, 107). Differential misclassification, however, can affect the bias in either direction, away from, or towards the null (106, 108).

Exposure

In **papers I-IV,** exposure was defined as a filled prescription by any of the study drugs. Courses were treated according to the *per-protocol*, or "*as-treated*"-principle where we censored courses that were interrupted, during follow-up, by the filling of a new antibiotic or by being hospitalised. Otherwise, the patient was presumed to adhere to the initiated regimen. Non-adherence during follow-up due to differences in baseline characteristics has the potential to introduce bias, but considering the

efforts made in our studies to minimize these differences, any exposure misclassification would be non-differential and as such occur similarly across the treatment cohorts. A significant difference in rates of antibiotic switch and hospitalization between cohorts could be an indicator and source of differential misclassification, although none of the studies displayed such patterns.

Outcome

In **papers I-IV** we used Danish and Swedish inpatient-, and cause of death registers to capture outcome diagnoses. None of the ICD-codes used for outcome capture have been formally validated. This will be discussed in more detail in the section on study limitations. Several studies on the validity of diagnoses registered in the Danish and Swedish registers have been published (78, 83, 85, 92, 93, 166). In particular, the overall validity (PPV) of the Swedish National Inpatient Register has been estimated to 85-95% (78). The overall validity of causes of death in both Sweden and Denmark has not been formally investigated but has historically been regarded as high, yet the recent and notable decrease in autopsy rates in both countries could compromise the reliability of this data (83, 92).

In **paper II and IV** in which we found an increased risk of the outcome in users of the study drug (oral fluoroquinolones and flucloxacillin respectively), we reasoned that any misclassification would be non-differential and as such bias the estimate towards the null. In contrast, the results of **paper I and III** indicated no certain difference in risk of the outcome between the compared treatments, and using the same reasoning could influence the interpretation of these null results. However, misclassification to a degree that would completely reduce any risk differences seems unlikely as this would require an unreasonably low diagnostic performance of the diagnostic tests involved.

Study limitations

The validity of outcome diagnoses

A key limitation of all included **papers (I-IV)** is the lack of validation of the outcome diagnoses. **Paper I** estimated the risk for the outcome of cardiovascular death, and although the diagnoses had not been formally validated, a study examining the validity of the diagnosis of definite and possible myocardial infarction as a cause of death in the Danish Registry of Causes of Death estimated the PPV to be 86% (93). Likewise, in **paper III** we estimated the risk of heart valve regurgitation based on diagnoses captured in the Swedish Inpatient Register and Causes of Death register. Echocardiography, the gold standard for diagnosing valve-related disease, is widely accessible in the Swedish healthcare system. A 2018 study on cardiomyopathy in Gothenburg, Sweden, reported that echocardiography was

performed in 95% of the cases (as of 2009) and it can be reasonably presumed that this percentage has been maintained or even increased to date (167). Furthermore, Doppler ultrasound which is the primary method to assessing valve insufficiency, has demonstrated a sensitivity of 94% for detecting both aortic- and mitral valve regurgitations (168). Considering this, it can be assumed that most heart valve regurgitation diagnoses registered in healthcare databases are based on highly sensitive methods, implying high PPVs.

The difficulties in diagnosing drug-induced liver injuries have been discussed earlier. In **papers II and IV** we used a combination of ICD-codes to capture suspected cases of acute liver injury, that have been estimated to have a PPV of 74% (95% CI: 60%-85%) in a Danish setting (169). In **paper II** we sought to estimate a causal effect of oral fluoroquinolones on the risk of acute liver injury, whereas in **paper IV** the causal effect of flucloxacillin on the risk of acute liver injury was not in question. In **paper II**, we therefore purposefully left out the ICD-code for jaundice (R17). Considering the relatively high PPV for the selected outcome diagnoses used in **paper II**, we would avoid capturing diagnoses specifically related to non-toxic hepatobiliary conditions.

In summary, the absence of validated diagnoses for acute liver injury in a Swedish setting is one of the major limitations of **paper II and IV**, and a target for future research endeavours.

Choice of comparator drug and the risk of confounding by indication/severity

In **paper II and III** we used amoxicillin and penicillin V as comparator drugs to oral fluoroquinolones to minimise the risk of confounding by indication, reasoning that there was sufficient indication overlap to justify this choice. Although there are overlapping indications such as respiratory- and urinary tract infections, the drugs are, inarguably, not completely interchangeable (170). Finding a suitable comparator drug is a multifaceted process that involves several considerations, not only overlapping indications. Some potential comparators, despite being clinically relevant, were prescribed too infrequently and did generate large enough data for robust analysis. In addition, some candidates proved much more difficult to match with the study drug than expected, for reasons that seemed to stem from a combination of age and frailty bias that was difficult to ameliorate by restrictions alone. Also, in some situations the comparator candidate (or the study drug) was on the same treatment path, i.e. used if the other drug proved ineffective, opening for potential biases.

However, several measures were taken to elucidate if the underlying infection for which the drug was prescribed, systematically influenced the outcome. In both **paper II and III** we noted similar mortality patterns, as indicated by ICD-codes, which served as an indirect indicator that our efforts to homogenize the baseline characteristics of the treatment and control courses were successful, and that the

underlying infection for which the drug was prescribed was equally severe. Especially in **paper III** we were concerned about lacking overlap of treatment indications between oral fluoroquinolones and penicillin V. We estimated hazard ratios for the outcome of heart valve regurgitation using amoxicillin instead of penicillin V as the comparator, which did not change the main conclusion. Similarly, analyses using outcomes of unquestionable severity such as heart valve surgery, did not alter the main conclusion.

In all **papers** (**I-IV**), exclusions on recent use of study drugs and hospitalization should reduce the risk of including courses that were prescribed due to lack of effect from the alternate study drug. We also excluded instances where multiple antibiotic prescriptions were filled on the same day. This approach ensured that our studies did not inadvertently capture treatments with combination therapy such as oral fluroquinolone or amoxicillin, combined with oral metronidazole. Such combinations could, especially in an outpatient setting, indicate a strong suspicion of gastrointestinal or biliary infection, which in turn could independently increase the risk of acute liver injury (especially relevant in **paper II**). Additionally, patients with any prior hospital contact indicating liver injury were excluded which would reduce the risk of including patients with a propensity for liver related diagnoses.

In summary, selecting a comparator drug presented significant challenges, and a *perfect* match in any of the **papers I-IV** was not possible due to reasons previously described. Consequently, the conclusions drawn from our results should be interpreted as components of a broader investigative framework and as such, contribute to a cumulative understanding rather than definitive on their own.

Recently, there have been developments surrounding the active comparator-approach, which could open for alternative methods in situations where finding an appropriate comparator drug proves difficult (113).

Unmeasured confounders

In all included **papers (I-IV),** we lacked data on critical lifestyle factors such as smoking, alcohol- and drug consumption, body mass index (BMI), and exercise patterns. Given that these factors are known to potentially influence the outcomes in epidemiological research, their omission likely introduces some degree of unmeasured confounding in each study, potentially affecting the validity of the results.

More specifically, smoking has a known detrimental effect on the cardiovascular system due to oxidative stress and systemic inflammation (171). Likewise, alcohol- and drug consumption can confound the outcomes due to their toxic effect on both the liver and the cardiovascular system. BMI and obesity, as well as exercise patterns, are significant confounders that are independently associated with metabolic disturbances, which increases the risk of hepatic and cardiovascular conditions, such as hepatic steatosis and atherosclerosis.

Comparison with other studies

The findings in **paper I** present a contrast to the outcomes of two publications which reported an increase in long term risk of cardiovascular events (including mortality) (55, 56). The first, a randomized study comparing the risk of cardiovascular outcomes in patients with stable coronary heart disease that received a short-term course of clarithromycin or placebo, reported an increased risk of cardiovascular mortality (HR 1.45, 95% CI: 1.09 to 1.92) in clarithromycin users that seemed to persist several years after drug exposure (55, 172, 173). However, only 32% of eligible patients were included and despite randomization, an imbalance in smoking status between the two groups was noted. This could imply that there were confounding factors related to differences in baseline health, especially with respect to pre-existing cardiac disease.

The second study reported an increased 1-year risk of cardiovascular events in users of clarithromycin compared to users of two other antibiotics, in two cohorts of patients presenting with acute exacerbations of COPD and community-acquired pneumonia; HR 1.50 (95% CI: 1.13 to 1.97) and HR 1.67 (95% CI: 1.04 to 2.68) (56). However, the study population was presumed to have a high baseline risk of cardiovascular risk, which could have biased the estimates.

Several other publications examining the risk of cardiovascular events among users of both clarithromycin and roxithromycin, reported no association (57-64).

More recently, a nationwide cohort study among COPD patients treated with macrolides, found no increased risk of cardiovascular events during a three year follow up period (174).

Based on the large sample size of our study, and given the upper limits of the confidence intervals, we could rule out an increased relative risk of cardiovascular death of 60% for clarithromycin and 16% for roxithromycin.

The results in **paper II** align with at least three other publications that have investigated the risk of acute liver injury in users of oral fluoroquinolones (46, 47, 70). The first study reported an OR of 1.9 for acute liver injury among elderly users of levofloxacin compared to clarithromycin (47). There was, however, limited information on concurrent drug use and causes of death in the cohorts, why confounding factors affecting the baseline risk could not be excluded.

The second study reported a RR for acute liver injury of 3.2 and 2.3 respectively for use of levofloxacin and moxifloxacin; however, no active comparator was used, which increases the risk of confounding by indication (70).

The third study used a case-control design and reported an OR of 1.3 for acute liver injury among users of ciprofloxacin (46). They reported no such difference among users of levofloxacin or moxifloxacin. There was a significant difference in baseline

characteristics between cases and controls in this study, which could have biased the results.

Paper III was initiated due to reports on the association between heart valve regurgitation and oral fluoroquinolone use (26, 175). The study, based on data from north American insurance claims databases, reported an increased risk of heart valve regurgitation between users of oral fluoroquinolones compared to users of amoxicillin and azithromycin, corresponding to a RR of 2.40 and 1.75 respectively (26). Although attempts were made to control for differences in baseline characteristics between cases and controls, there was notable imbalance. In addition, populations ascertained from insurance claims databases may be subject of a certain selection bias.

A Danish study compared the risk of heart valve regurgitation among users of oral fluoroquinolones with penicillin V and, like our study, found no significant association (HR 1.0, 95% CI: 0.95 to 1.23) (71).

Paper IV explored the magnitude of risk for acute liver injury associated with use of flucloxacillin in a Scandinavian setting. We compared our results to a UK-based study which reported a risk of 8.5 / 100,000 individuals when comparing use of flucloxacillin with oxytetracycline (72). The study is based on data from the UK Clinical Practice Datalink (CPRD), which encompasses the majority of primary care visits in the UK (176). Consequently, an underestimation of the risk is plausible due to the indirect exclusion of cases of greater severity, which would likely seek specialist or hospital-based care instead. In addition, the use of oxytetracycline as the comparator may also influence the results, given that it has fewer indications in common with clindamycin, the antibiotic utilized as comparator in our study.

Conclusions

Paper I

In this nationwide cohort study of Danish adults from 1997 to 2011, we found no increased risk of long-term cardiovascular death among users of clarithromycin or roxithromycin compared to penicillin V. A transient risk increase was observed among clarithromycin users during the first week of treatment, which attenuated in subsequent time periods.

Paper II

In this nationwide cohort study of Swedish adults from 2006 to 2014, we found an increased risk of acute liver injury among users of oral fluoroquinolones compared to amoxicillin. The absolute risk difference was estimated to be 5 events per 1 million courses.

Paper III

In this nationwide cohort study of Swedish adults from 2006 to 2018, we found no increased risk of heart valve regurgitation among users of oral fluoroquinolones compared to penicillin V. The absolute risk difference was estimated to be -13 events per 1 million courses.

Paper IV

In this nationwide cohort study of Swedish adults from 2006 to 2018, we estimated the risk of liver injury associated with flucloxacillin use to be seven times greater compared to clindamycin, corresponding to an absolute risk difference of 11 cases per 100,000 treatment courses.

Further implications

The global consumption of antibiotics has increased dramatically between the years 2000 and 2015 and without immediate policy intervention, it is projected to rise by as much as 200% by 2030 (65). This surge in antibiotic use is a major contributing factor to the increase in antibiotic resistance, which is considered one of the top threats to global health according to the WHO (177). Drug-resistant pathogens are likely to increase dramatically, severely limiting the treatment options. Previously manageable infections could become more deadly and medical procedures that depend on prophylactic treatment with antibiotics, such as surgery or chemotherapy, may face increased risks.

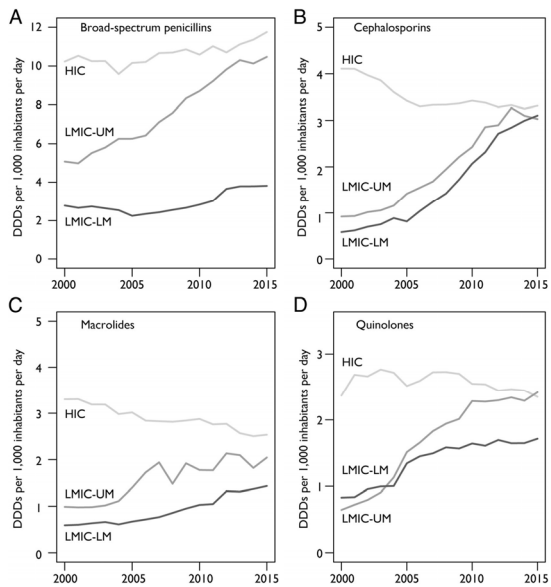

Figure 9: Consumption trends for the four most used antibiotics globally 2000 to 2015 in DDDs per 1,000 inhabitants per day. HIC = high income countries; LMIC = low- and middle income countries; UM = upper middle; LM = lower middle (65). Creative Commons Licence: http://creativecommons.org/licenses/by/4.0/; no changes were made.

Treating infections caused by multi-resistant bacteria often requires the use of antibiotic combination therapy, in which drugs are selected for their synergistic effects to enhance the probability of bacterial eradication. The antibiotics studied in this thesis – fluroquinolones, macrolides, and beta lactams such as flucloxacillin – are commonly included in these regimens.

While it is of importance to monitor antibiotic consumption to limit the development of resistance, it is of equal importance not to unduly restrict effective antibiotics due to reports on adverse reactions.

Paper I explored whether the use of the macrolides clarithromycin and roxithromycin increased the risk of long-term cardiovascular death, and found no such association. Most prior reports that had reported an association between these drugs and cardiovascular death were conducted in populations with a presumed high baseline risk of the outcome (55, 56, 172, 173). We therefore concluded that our results should be considered a stepping stone to repeated studies in the matter, preferably in more select populations such as in those with a high baseline risk of cardiovascular disease.

In **paper II** we found an increased risk of acute liver injury associated with oral fluoroquinolone use. We estimated the absolute risk to be 5 additional cases per one million episodes. Our results align with several other studies (46, 47, 70). Considering the small absolute increase in risk, these findings should be integrated into a nuanced clinical decision-making process, instead of resulting in broader changes in prescription patterns or public policies regarding its use. Nonetheless, in specific clinical scenarios – such as in patients with pre-existing liver disease, or those concurrently using drugs with hepatotoxic potential – these findings may warrant heightened awareness.

Paper III was conducted in response to communications from both the FDA and the EMA regarding potential risk of cardiovascular (blood vessel and heart valve) events related to use of fluroquinolones (175, 178, 179). Following a study published in 2019, in which the authors reported an increased risk of aortic- and mitral valve regurgitation among users of fluoroquinolones, a Danish study investigating the issue found no such association (26, 71). In line with the Danish study, our results could not confirm an association between heart valve regurgitation and the use of fluoroquinolones. Based on the large study sample of our study and the upper limit of the confidence interval, we estimated that we could rule out an increased relative risk of 11% of heart valve regurgitation.

Although our results provide a measure of reassurance to healthcare providers regarding the cardiovascular safety of fluoroquinolones, these results should be viewed as part of the body of evidence that informs a more comprehensive understanding of fluoroquinolone safety. The absence of an association with heart valve regurgitation found in our study supports the continued use of fluroquinolones when clinically appropriate, but also underscores the need for continued pharmacovigilance.

The results from **paper IV** indicated that there was a markedly increased risk of acute liver injury associated with flucloxacillin use. We estimated the incidence of acute liver injury to be 15 per 100,000 treatment courses. In Sweden, approximately 350,000 prescriptions of flucloxacillin were filled in 2023, indicating that roughly 53 cases of acute liver injury could be attributable to this antibiotic therapy annually, underlining the necessity for continued vigilance among clinicians (180).

Acknowledgements

Min huvudhandledare **Malin Inghammar**. Jag tror att jag egentligen skulle behövt skriva en hel bok till för att uttrycka min tacksamhet på ett rättvist sätt. Du är en inspiration och en förebild på flera plan och det är en ynnest att få ha lärt känna dig under alla dessa år. Ditt enastående skarpsinne kan bara överträffas av den omtänksamhet och värme som du alltid utstrålar. Du hittar lösningar i stället för att se problem och lyfter alltid upp när man känner sig som mest nere och bara ser det där oöverstigliga berget framför sig. Så, tack Malin, för att du ledsagat mig genom detta maraton. Jag kan bara hoppas att en smula av ditt stjärndamm har landat på mig.

Min bihandledare **Bengt** (inledningsvis även min handledare). Du har varit med från början av denna resa och skulle nu kunna säga som Elrond i Sagan om Ringen: "I was there Gandalf, I was there three thousand years ago"... Det har varit en trygghet att ha dig i bakgrunden och jag är ytterst tacksam för detta. Tack också för att du lät mig komma på jobbintervju på infektionskliniken den där hösten 2010!

Min bihandledare **Jonas**. Jag är så tacksam för att du tackade ja till bihandledarskapet och att jag har fått ta hjälp av din enorma expertis inom området.

Tack till **Henrik** och **Björn**. Även om allt inte gick som planerat så är jag väldigt glad över att få varit i Er omloppsbana under något år under början av denna maratonresa. Ett särskilt stort tack till dig **Henrik**, för att du alltid varit så hjälpsam och ställt upp när jag, trots idoga försök, inte fått rätt på saker och ting. Du har ett imponerande lugn och vansinnigt bra koll på statistiken. Förhoppningsvis kan vi snart se MFF på stadion tillsammans igen; it's on me.

Tack till dig **Anton**, för att du svarat på tekniska frågor om statistiska kluriheter, och ställt upp med kort varsel när tiden varit knapp.

Tack **Axel**, för att du har hjälpt mig att styra upp de registerdatafiler som alltid har olika format trots att databeställningarna ser exakt likadana ut…

Till **ALLA kollegor** på infektionskliniken i Lund. Sekreterare, sjuksköterskor och undersköterskor, läkare, kökspersonal, städare, fysioterapeuter, mfl. Jag längtar tillbaka till kliniken och till den underbara gemenskapen vi har där! Jag vill också tacka "kliniken" och schemaläggare **Fredrik** för att Ni har gett mig all forskningstid jag behövt.

Tack till min bästa vän **Johan** för det snygga omslaget, och för att du låtit mig sitta på ditt kontor och skriva boken. Det har varit en inspirerande miljö att arbeta i och inte minst, “damn good coffee”. Nu ser jag fram emot fortsatt umgänge och livsnjuteri tillsammans. Stort tack också till din kollega **Miruna** som också jobbat hårt med omslaget!

Tack till **Alex** och resten av det härliga gänget på **Reko Sushi**, som försett mig med tonvis av sushi som jag använt som högoktanigt bränsle under tiden jag skrivit min bok.

Tack till mamma **Anita** och pappa **Ulf**. Tack för att ni stöttat mig när jag helt plötsligt, efter flera år utomlands och nyss hemkommen med en universitetsexamen, fick för mig att läsa medicin. OK kanske inte omedelbart populärt, men när ni insåg att jag menade allvar så har ni aldrig varit annat än stöttande. Tack pappa, för att du (på gott och ont) lärt mig att aldrig ge upp. Tack mamma, för att du alltid betonat vikten av att vara en ödmjuk människa. Tack till min syster **Anna** som alltid varit en inspiration och förebild för mig. Jag vet att du kommer att skratta till jag säger så, men jag menar varje ord. Du är smart, snygg, och har hjärtat på rätt ställe. Inte helt lätt att vara din lillebror alltid om man säger så…

Slutligen, tack till min fru **Sanne** och till våra barn **Elsa** och **Dante**. Ni utgör en gränslös källa av glädje och energi för mig, och något mer värdefullt kan jag inte tänka mig. Jag älskar er!

References

1. Organization WH. International drug monitoring: the role of national centres, report of a WHO meeting [held in Geneva from 20 to 25 September 1971]: World Health Organization; 1972.
2. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. JAMA. 1998;279(15):1200-5.
3. Montane E, Castells X. Epidemiology of drug-related deaths in European hospitals: A systematic review and meta-analysis of observational studies. Br J Clin Pharmacol. 2021;87(10):3659-71.
4. Commission E. Strengthening pharmacovigilance to reduce adverse effects of medicines. European Commission Brussels; 2008.
5. Moore N, Bégaud B. Improving pharmacovigilance in Europe. British Medical Journal Publishing Group; 2010.
6. Avorn J. The promise of pharmacoepidemiology in helping clinicians assess drug risk. Circulation. 2013;128(7):745-8.
7. Bouvy JC, De Bruin ML, Koopmanschap MA. Epidemiology of adverse drug reactions in Europe: a review of recent observational studies. Drug safety. 2015;38:437-53.
8. Collaborators G. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. 2018.
9. Craveiro NS, Lopes BS, Tomás L, Almeida SF. Drug withdrawal due to safety: a review of the data supporting withdrawal decision. Current drug safety. 2020;15(1):4-12.
10. Onakpoya IJ, Heneghan CJ, Aronson JK. Delays in the post-marketing withdrawal of drugs to which deaths have been attributed: a systematic investigation and analysis. BMC medicine. 2015;13:1-11.
11. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, et al. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. The Lancet infectious diseases. 2014;14(8):742-50.
12. Leape LL, Brennan TA, Laird N, Lawthers AG, Localio AR, Barnes BA, et al. The nature of adverse events in hospitalized patients: results of the Harvard Medical Practice Study II. New England journal of medicine. 1991;324(6):377-84.

13. Grayson ML, Cosgrove SE, Crowe S, Hope W, McCarthy JS, Mills J, et al. Kucers' The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic, and Antiviral Drugs, -Three Volume Set: CRC Press; 2017.
14. Poehlsgaard J, Douthwaite S. The bacterial ribosome as a target for antibiotics. Nature Reviews Microbiology. 2005;3(11):870-81.
15. Macy E. Penicillin and beta-lactam allergy: epidemiology and diagnosis. Current allergy and asthma reports. 2014;14:1-7.
16. Jüni P, Nartey L, Reichenbach S, Sterchi R, Dieppe PA, Egger M. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. The lancet. 2004;364(9450):2021-9.
17. Ray WA, Griffin MR, Stein CM. Cardiovascular toxicity of valdecoxib. New England Journal of Medicine. 2004;351(26):2767-.
18. Owens RC, Jr., Nolin TD. Antimicrobial-associated QT interval prolongation: pointes of interest. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2006;43(12):1603-11.
19. Mandell L, Tillotson G. Safety of fluoroquinolones: An update. Can J Infect Dis. 2002;13(1):54-61.
20. Cornett E, Novitch MB, Kaye AD, Pann CA, Bangalore HS, Allred G, et al. Macrolide and fluoroquinolone mediated cardiac arrhythmias: clinical considerations and comprehensive review. Postgraduate medicine. 2017;129(7):715-24.
21. Ray WA, Murray KT, Meredith S, Narasimhulu SS, Hall K, Stein CM. Oral erythromycin and the risk of sudden death from cardiac causes. The New England journal of medicine. 2004;351(11):1089-96.
22. Albert RK, Schuller JL, Network CCR. Macrolide antibiotics and the risk of cardiac arrhythmias. American journal of respiratory and critical care medicine. 2014;189(10):1173-80.
23. Daneman N, Lu H, Redelmeier DA. Fluoroquinolones and collagen associated severe adverse events: a longitudinal cohort study. BMJ open. 2015;5(11).
24. Lee C-C, Lee M-tG, Chen Y-S, Lee S-H, Chen Y-S, Chen S-C, et al. Risk of aortic dissection and aortic aneurysm in patients taking oral fluoroquinolone. JAMA internal medicine. 2015;175(11):1839-47.
25. Pasternak B, Inghammar M, Svanström H. Fluoroquinolone use and risk of aortic aneurysm and dissection: nationwide cohort study. BMJ (Clinical research ed). 2018;360.
26. Etminan M, Sodhi M, Ganjizadeh-Zavareh S, Carleton B, Kezouh A, Brophy JM. Oral Fluoroquinolones and Risk of Mitral and Aortic Regurgitation. J Am Coll Cardiol. 2019;74(11):1444-50.
27. Pai MP, Graci DM, Amsden GW. Macrolide drug interactions: an update. Annals of Pharmacotherapy. 2000;34(4):495-513.
28. Westphal JF. Macrolide–induced clinically relevant drug interactions with cytochrome P-450A (CYP) 3A4: an update focused on clarithromycin, azithromycin and dirithromycin. British journal of clinical pharmacology. 2000;50(4):285.

29. Periti P, Mazzei T, Mini E, Novelli A. Pharmacokinetic drug interactions of macrolides. Clinical pharmacokinetics. 1992;23:106-31.
30. Volberg WA, Koci BJ, Su W, Lin J, Zhou J. Blockade of human cardiac potassium channel human ether-a-go-go-related gene (HERG) by macrolide antibiotics. Journal of Pharmacology and Experimental Therapeutics. 2002;302(1):320-7.
31. Wise BL, Peloquin C, Choi H, Lane NE, Zhang Y. Impact of age, sex, obesity, and steroid use on quinolone-associated tendon disorders. The American journal of medicine. 2012;125(12):1228. e23-. e28.
32. Zabraniecki L, Negrier I, Vergne P, Arnaud M, Bonnet C, Bertin P, et al. Fluoroquinolone induced tendinopathy: report of 6 cases. Journal of rheumatology. 1996;23(3):516-20.
33. Guzzardi DG, Teng G, Kang S, Geeraert PJ, Pattar SS, Svystonyuk DA, et al. Induction of human aortic myofibroblast-mediated extracellular matrix dysregulation: A potential mechanism of fluoroquinolone-associated aortopathy. J Thorac Cardiovasc Surg. 2019;157(1):109-19 e2.
34. Tsamis A, Krawiec JT, Vorp DA. Elastin and collagen fibre microstructure of the human aorta in ageing and disease: a review. Journal of the royal society interface. 2013;10(83):20121004.
35. Tamargo J, Agewall S. Fluoroquinolone use and valvular heart disease: is the jury still out? Eur Heart J. 2021;42(30):2909-11.
36. Onakpoya IJ, Heneghan CJ, Aronson JK. Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: a systematic review of the world literature. BMC medicine. 2016;14(1):1-11.
37. Ballet F. Hepatotoxicity in drug development: detection, significance and solutions. Journal of hepatology. 1997;26:26-36.
38. Lee WM. Drug-induced hepatotoxicity. New England Journal of Medicine. 1995;333(17):1118-27.
39. Larson AM. Acetaminophen hepatotoxicity. Clinics in liver disease. 2007;11(3):525-48.
40. Lee WM. Drug-induced hepatotoxicity. The New England journal of medicine. 2003;349(5):474-85.
41. Lucena MI, Garcia-Cortes M, Cueto R, Lopez-Duran J, Andrade RJ. Assessment of drug-induced liver injury in clinical practice. Fundamental & clinical pharmacology. 2008;22(2):141-58.
42. Benichou C, Danan G, Flahault A. Causality assessment of adverse reactions to drugs--II. An original model for validation of drug causality assessment methods: case reports with positive rechallenge. Journal of clinical epidemiology. 1993;46(11):1331-6.
43. Rochon J, Protiva P, Seeff LB, Fontana RJ, Liangpunsakul S, Watkins PB, et al. Reliability of the Roussel Uclaf Causality Assessment Method for assessing causality in drug-induced liver injury. Hepatology. 2008;48(4):1175-83.

44. Rockey DC, Seeff LB, Rochon J, Freston J, Chalasani N, Bonacini M, et al. Causality assessment in drug-induced liver injury using a structured expert opinion process: comparison to the Roussel-Uclaf causality assessment method. Hepatology. 2010;51(6):2117-26.
45. Orman ES, Conjeevaram HS, Vuppalanchi R, Freston JW, Rochon J, Kleiner DE, et al. Clinical and histopathologic features of fluoroquinolone-induced liver injury. Clin Gastroenterol Hepatol. 2011;9(6):517-23 e3.
46. Alshammari TM, Larrat EP, Morrill HJ, Caffrey AR, Quilliam BJ, LaPlante KL. Risk of hepatotoxicity associated with fluoroquinolones: a national case-control safety study. Am J Health Syst Pharm. 2014;71(1):37-43.
47. Paterson JM, Mamdani MM, Manno M, Juurlink DN, Canadian Drug S, Effectiveness Research N. Fluoroquinolone therapy and idiosyncratic acute liver injury: a population-based study. CMAJ. 2012;184(14):1565-70.
48. Crawford D, Roeser H, Devereaux B, Powell L, Purcell P. Flucloxacillin associated cholestatic hepatitis: an Australian and Swedish epidemic? European journal of clinical pharmacology. 1995;49:81-5.
49. Andrews E, Daly AK. Flucloxacillin-induced liver injury. Toxicology. 2008;254(3):158-63.
50. Derby LE, Jick H, Henry DA, Dean AD. Cholestatic hepatitis associated with flucloxacillin. Med J Aust. 1993;158(9):596-600.
51. Monshi MM, Faulkner L, Gibson A, Jenkins RE, Farrell J, Earnshaw CJ, et al. Human leukocyte antigen (HLA)-B*57:01-restricted activation of drug-specific T cells provides the immunological basis for flucloxacillin-induced liver injury. Hepatology. 2013;57(2):727-39.
52. Baldo BA, Pham NH, Weiner J. Detection and side-chain specificity of IgE antibodies to flucloxicillin in allergic subjects. Journal of Molecular Recognition. 1995;8(3):171-7.
53. Ali SE, Waddington JC, Lister A, Sison-Young R, Jones RP, Rehman AH, et al. Identification of flucloxacillin-modified hepatocellular proteins: implications in flucloxacillin-induced liver injury. Toxicol Sci. 2023;192(1):106-16.
54. Dixon JR. The international conference on harmonization good clinical practice guideline. Quality Assurance. 1999;6(2):65-74.
55. Jespersen CM, Als-Nielsen B, Damgaard M, Hansen JF, Hansen S, Helo OH, et al. Randomised placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. BMJ (Clinical research ed). 2006;332(7532):22-7.
56. Schembri S, Williamson PA, Short PM, Singanayagam A, Akram A, Taylor J, et al. Cardiovascular events after clarithromycin use in lower respiratory tract infections: analysis of two prospective cohort studies. BMJ (Clinical research ed). 2013;346:f1235.
57. Leowattana W, Mahanonda N, Bhuripanyo K, Samranthin M, Singhaviranon L, Pokum S, et al. Roxithromycin in prevention of acute coronary syndrome associated with Chlamydia pneumoniae infection: a randomized placebo controlled trial. JOURNAL-MEDICAL ASSOCIATION OF THAILAND. 2001;84:S669-S75.

58. Gurfinkel E, Bozovich G, Beck E, Testa E, Livellara B, Mautner B, et al. Treatment with the antibiotic roxithromycin in patients with acute non-Q-wave coronary syndromes. The final report of the ROXIS Study. European Heart Journal. 1999;20(2):121-7.
59. Zahn R, Schneider S, Frilling B, Seidl K, Tebbe U, Weber M, et al. Antibiotic therapy after acute myocardial infarction: a prospective randomized study. Circulation. 2003;107(9):1253-9.
60. Neumann F-J, Kastrati A, Miethke T, Pogatsa-Murray G, Mehilli J, Valina C, et al. Treatment of Chlamydia pneumoniae infection with roxithromycin and effect on neointima proliferation after coronary stent placement (ISAR-3): a randomised, double-blind, placebo-controlled trial. The Lancet. 2001;357(9274):2085-9.
61. Sinisalo J, Mattila K, Valtonen V, Anttonen O, Juvonen J, Melin J, et al. Effect of 3 months of antimicrobial treatment with clarithromycin in acute non–Q-wave coronary syndrome. Circulation. 2002;105(13):1555-60.
62. Andersen SS, Hansen ML, Norgaard ML, Folke F, Fosbøl EL, Abildstrøm SZ, et al. Clarithromycin use and risk of death in patients with ischemic heart disease. Cardiology. 2010;116(2):89-97.
63. Root AA, Wong AY, Ghebremichael-Weldeselassie Y, Smeeth L, Bhaskaran K, Evans SJ, et al. Evaluation of the risk of cardiovascular events with clarithromycin using both propensity score and self-controlled study designs. British journal of clinical pharmacology. 2016;82(2):512-21.
64. Wong AY, Root A, Douglas IJ, Chui CS, Chan EW, Ghebremichael-Weldeselassie Y, et al. Cardiovascular outcomes associated with use of clarithromycin: population based study. BMJ (Clinical research ed). 2016;352.
65. Klein EY, Van Boeckel TP, Martinez EM, Pant S, Gandra S, Levin SA, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proceedings of the National Academy of Sciences. 2018;115(15):E3463-E70.
66. Fernandez-Cuadros ME, Casique-Bocanegra LO, Albaladejo-Florin MJ, Gomez-Duenas S, Ramos-Gonzalez C, Perez-Moro OS. Bilateral Levofloxacin-Induced Achilles Tendon Rupture: An Uncommon Case Report and Review of the Literature. Clin Med Insights Arthritis Musculoskelet Disord. 2019;12:1179544119835222.
67. Schjott J, Messner T. Ciprofloxacin and acute aortic valve damage. Med Hypotheses. 2018;121:35.
68. Andrade RJ, Tulkens PM. Hepatic safety of antibiotics used in primary care. The Journal of antimicrobial chemotherapy. 2011;66(7):1431-46.
69. Lucena MI, Andrade RJ, Rodrigo L, Salmeron J, Alvarez A, Lopez-Garrido MJ, et al. Trovafloxacin-induced acute hepatitis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2000;30(2):400-1.
70. Kaye JA, Castellsague J, Bui CL, Calingaert B, McQuay LJ, Riera-Guardia N, et al. Risk of acute liver injury associated with the use of moxifloxacin and other oral antimicrobials: a retrospective, population-based cohort study. Pharmacotherapy. 2014;34(4):336-49.

71. Strange JE, Holt A, Blanche P, Gislason G, Torp-Pedersen C, Christensen DM, et al. Oral fluoroquinolones and risk of aortic or mitral regurgitation: a nationwide nested case-control study. Eur Heart J. 2021;42(30):2899-908.
72. Wing K, Bhaskaran K, Pealing L, Root A, Smeeth L, van Staa TP, et al. Quantification of the risk of liver injury associated with flucloxacillin: a UK population-based cohort study. The Journal of antimicrobial chemotherapy. 2017;72(9):2636-46.
73. Personnummer2016 240217 [cited 2024 240217].
74. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. European journal of epidemiology. 2009;24:659-67.
75. Pedersen CB. The Danish civil registration system. Scandinavian journal of public health. 2011;39(7_suppl):22-5.
76. Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. European journal of epidemiology. 2014;29:541-9.
77. Ludvigsson JF, Almqvist C, Bonamy A-KE, Ljung R, Michaëlsson K, Neovius M, et al. Registers of the Swedish total population and their use in medical research. European journal of epidemiology. 2016;31:125-36.
78. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC Public Health. 2011;11:450.
79. Socialstyrelsen. the National Patient Register [Available from: https://www.socialstyrelsen.se/en/statistics-and-data/registers/national-patient-register/.
80. Forsberg L, Rydh H, Jacobsson A, Nyqvist K, Heurgren M. Kvalitet och innehåll i patientregistret. Utskrivningar från slutenvården 1964-2007 och besök i specialiserad öppenvård (exklusive primärvårdsbesök) 1997-2007.(Quality and content of the Patient Register)(2009-125-15). Book Kvalitet och innehåll i patientregistret Utskrivningar från slutenvården 1964-2007 och besök i specialiserad öppenvård (exklusive primärvårdsbesök) 1997-2007(Quality and content of the Patient Register)(2009-125-15)(Editor ed^ eds) City. 2009.
81. Molander V, Bower H, Askling J. Validation and characterization of venous thromboembolism diagnoses in the Swedish National Patient Register among patients with rheumatoid arthritis. Scandinavian Journal of Rheumatology. 2023;52(2):111-7.
82. Bengtsson B, Askling J, Ludvigsson JF, Hagström H. Validity of administrative codes associated with cirrhosis in Sweden. Scandinavian journal of gastroenterology. 2020;55(10):1205-10.
83. Brooke HL, Talbäck M, Hörnblad J, Johansson LA, Ludvigsson JF, Druid H, et al. The Swedish cause of death register. European journal of epidemiology. 2017;32:765-73.
84. Fall K, Strömberg F, Rosell J, Andrèn O, Varenhorst E, Group S-ERPC. Reliability of death certificates in prostate cancer patients. Scandinavian journal of urology and nephrology. 2008;42(4):352-7.

85. Schmidt M, Schmidt SAJ, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clinical epidemiology. 2015:449-90.
86. Laugesen K, Ludvigsson JF, Schmidt M, Gissler M, Valdimarsdottir UA, Lunde A, et al. Nordic health registry-based research: a review of health care systems and key registries. Clinical epidemiology. 2021:533-54.
87. Wildenschild C, Mehnert F, Thomsen RW, Iversen HK, Vestergaard K, Ingeman A, et al. Registration of acute stroke: validity in the Danish Stroke Registry and the Danish National Registry of Patients. Clinical epidemiology. 2013:27-36.
88. Rix TA, Riahi S, Overvad K, Lundbye-Christensen S, Schmidt EB, Joensen AM. Validity of the diagnoses atrial fibrillation and atrial flutter in a Danish patient registry. Scandinavian Cardiovascular Journal. 2012;46(3):149-53.
89. Vestberg K, Thulstrup AM, Sørensen HT, Ottesen P, Sabroe S, Vilstrup H. Data quality of administratively collected hospital discharge data for liver cirrhosis epidemiology. Journal of medical systems. 1997;21:11-20.
90. Coloma PM, Valkhoff VE, Mazzaglia G, Nielsson MS, Pedersen L, Molokhia M, et al. Identification of acute myocardial infarction from electronic healthcare records using different disease coding systems: a validation study in three European countries. BMJ open. 2013;3(6):e002862.
91. Pottegård A, Schmidt SAJ, Wallach-Kildemoes H, Sørensen HT, Hallas J, Schmidt M. Data resource profile: the Danish national prescription registry. International journal of epidemiology. 2017;46(3):798-f.
92. Helweg-Larsen K. The Danish register of causes of death. Scandinavian journal of public health. 2011;39(7_suppl):26-9.
93. Madsen M, Davidsen M, Rasmussen S, Abildstrom SZ, Osler M. The validity of the diagnosis of acute myocardial infarction in routine statistics: a comparison of mortality and hospital discharge data with the Danish MONICA registry. Journal of clinical epidemiology. 2003;56(2):124-30.
94. Hill AB. The environment and disease: association or causation? : Sage Publications; 1965.
95. Rothman KJ, Greenland S, Lash TL. Modern epidemiology: Wolters Kluwer Health/Lippincott Williams & Wilkins Philadelphia; 2008.
96. Pocock SJ. Clinical trials: a practical approach: John Wiley & Sons; 2013.
97. Friedman LM, Furberg CD, DeMets DL, Reboussin DM, Granger CB. Fundamentals of clinical trials: Springer; 2015.
98. Rothwell PM. External validity of randomised controlled trials:"to whom do the results of this trial apply?". The Lancet. 2005;365(9453):82-93.
99. Freemantle N, Marston L, Walters K, Wood J, Reynolds MR, Petersen I. Making inferences on treatment effects from real world data: propensity scores, confounding by indication, and other perils for the unwary in observational research. BMJ (Clinical research ed). 2013;347.

100. Sendor R, Stürmer T. Core concepts in pharmacoepidemiology: confounding by indication and the role of active comparators. Pharmacoepidemiology and drug safety. 2022;31(3):261-9.
101. Shaheen S, Sterne J, Songhurst C, Burney P. Frequent paracetamol use and asthma in adults. Thorax. 2000;55(4):266.
102. Signorello LB, McLaughlin JK, Lipworth L, Friis S, Sørensen HT, Blot WJ. Confounding by indication in epidemiologic studies of commonly used analgesics. American journal of therapeutics. 2002;9(3):199-205.
103. Gerhard T. Bias: considerations for research practice. American Journal of Health-System Pharmacy. 2008;65(22):2159-68.
104. Horwitz RI, Feinstein AR. The problem of "protopathic bias" in case-control studies. The American journal of medicine. 1980;68(2):255-8.
105. Disanto G, Zecca C, MacLachlan S, Sacco R, Handunnetthi L, Meier UC, et al. Prodromal symptoms of multiple sclerosis in primary care. Annals of neurology. 2018;83(6):1162-73.
106. Chen Q, Galfalvy H, Duan N. Effects of disease misclassification on exposure–disease association. American journal of public health. 2013;103(5):e67-e73.
107. Wacholder S, Hartge P, Lubin JH, Dosemeci M. Non-differential misclassification and bias towards the null: a clarification. Occupational and environmental medicine. 1995;52(8):557.
108. Flegal KM, BROWNIE C, HAAS J. The effects of exposure misclassification on estimates of relatwe risk. American journal of epidemiology. 1986;123(4):736-51.
109. Lund JL, Richardson DB, Stürmer T. The active comparator, new user study design in pharmacoepidemiology: historical foundations and contemporary application. Current epidemiology reports. 2015;2:221-8.
110. Yoshida K, Solomon DH, Kim SC. Active-comparator design and new-user design in observational studies. Nature Reviews Rheumatology. 2015;11(7):437-41.
111. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. American journal of epidemiology. 2003;158(9):915-20.
112. Johnson ES, Bartman BA, Briesacher BA, Fleming NS, Gerhard T, Kornegay CJ, et al. The incident user design in comparative effectiveness research. Pharmacoepidemiology and drug safety. 2013;22(1):1-6.
113. Wintzell V, Svanström H, Pasternak B. Selection of comparator group in observational drug safety studies: alternatives to the active comparator new user design. Epidemiology. 2022;33(5):707-14.
114. Suissa S, Moodie EE, Dell'Aniello S. Prevalent new-user cohort designs for comparative drug effect studies by time-conditional propensity scores. Pharmacoepidemiology and drug safety. 2017;26(4):459-68.
115. Webster-Clark M, Ross RK, Lund JL. Initiator types and the causal question of the prevalent new-user design: a simulation study. American journal of epidemiology. 2021;190(7):1341-8.

116. Kim DH, Schneeweiss S. Measuring frailty using claims data for pharmacoepidemiologic studies of mortality in older adults: evidence and recommendations. Pharmacoepidemiology and drug safety. 2014;23(9):891-901.
117. Glynn RJ, Knight EL, Levin R, Avorn J. Paradoxical relations of drug treatment with mortality in older persons. Epidemiology. 2001:682-9.
118. Schneeweiss S, Patrick AR, Stürmer T, Brookhart MA, Avorn J, Maclure M, et al. Increasing levels of restriction in pharmacoepidemiologic database studies of elderly and comparison with randomized trial results. Medical care. 2007;45(10 SUPL):S131.
119. Browner WS, Newman TB, Cummings SR, Grady DG. Designing clinical research: Lippincott Williams & Wilkins; 2022.
120. Grimes DA, Schulz KF. Bias and causal associations in observational research. The lancet. 2002;359(9302):248-52.
121. Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. Multivariate behavioral research. 2011;46(3):399-424.
122. Rosenbaum PR, Rubin DB. The central role of the propensity score in observational studies for causal effects. Biometrika. 1983;70(1):41-55.
123. Glynn RJ, Schneeweiss S, Sturmer T. Indications for propensity scores and review of their use in pharmacoepidemiology. Basic & clinical pharmacology & toxicology. 2006;98(3):253-9.
124. Lee BK, Lessler J, Stuart EA. Improving propensity score weighting using machine learning. Statistics in medicine. 2010;29(3):337-46.
125. Setoguchi S, Schneeweiss S, Brookhart MA, Glynn RJ, Cook EF. Evaluating uses of data mining techniques in propensity score estimation: a simulation study. Pharmacoepidemiology and drug safety. 2008;17(6):546-55.
126. Austin PC, Mamdani MM. A comparison of propensity score methods: a case-study estimating the effectiveness of post-AMI statin use. Statistics in medicine. 2006;25(12):2084-106.
127. Rosenbaum PR. Model-based direct adjustment. Journal of the American statistical Association. 1987;82(398):387-94.
128. Ming K, Rosenbaum PR. Substantial gains in bias reduction from matching with a variable number of controls. Biometrics. 2000;56(1):118-24.
129. Rosenbaum PR, Rosenbaum PB, Briskman. Design of observational studies: Springer; 2010.
130. Rosenbaum PR, Rubin DB. Constructing a control group using multivariate matched sampling methods that incorporate the propensity score. The American Statistician. 1985;39(1):33-8.
131. Austin PC. Propensity-score matching in the cardiovascular surgery literature from 2004 to 2006: a systematic review and suggestions for improvement. The Journal of thoracic and cardiovascular surgery. 2007;134(5):1128-35. e3.

132. Normand S-LT, Landrum MB, Guadagnoli E, Ayanian JZ, Ryan TJ, Cleary PD, et al. Validating recommendations for coronary angiography following acute myocardial infarction in the elderly: a matched analysis using propensity scores. Journal of clinical epidemiology. 2001;54(4):387-98.
133. Brookhart MA, Schneeweiss S, Rothman KJ, Glynn RJ, Avorn J, Stürmer T. Variable selection for propensity score models. American journal of epidemiology. 2006;163(12):1149-56.
134. Lefebvre G, Delaney JA, Platt RW. Impact of mis-specification of the treatment model on estimates from a marginal structural model. Statistics in medicine. 2008;27(18):3629-42.
135. Fu AZ, Li L. Thinking of having a higher predictive power for your first-stage model in propensity score analysis? Think again. Health Services and Outcomes Research Methodology. 2008;8:115-7.
136. Schneeweiss S, Rassen JA, Glynn RJ, Avorn J, Mogun H, Brookhart MA. High-dimensional propensity score adjustment in studies of treatment effects using health care claims data. Epidemiology (Cambridge, Mass). 2009;20(4):512.
137. Austin PC, Grootendorst P, Anderson GM. A comparison of the ability of different propensity score models to balance measured variables between treated and untreated subjects: a Monte Carlo study. Statistics in medicine. 2007;26(4):734-53.
138. Brookhart MA, Stürmer T, Glynn RJ, Rassen J, Schneeweiss S. Confounding control in healthcare database research: challenges and potential approaches. Medical care. 2010;48(6 0):S114.
139. Hill J, Weiss C, Zhai F. Challenges with propensity score strategies in a high-dimensional setting and a potential alternative. Multivariate Behavioral Research. 2011;46(3):477-513.
140. Garrido MM, Kelley AS, Paris J, Roza K, Meier DE, Morrison RS, et al. Methods for constructing and assessing propensity scores. Health services research. 2014;49(5):1701-20.
141. Sjölander A, Greenland S. Ignoring the matching variables in cohort studies–when is it valid and why? Statistics in medicine. 2013;32(27):4696-708.
142. Hainmueller J. Entropy balancing for causal effects: A multivariate reweighting method to produce balanced samples in observational studies2012. 25-46 p.
143. Zhao Q, Percival D. Entropy balancing is doubly robust. De Gruyter; 2016. p. 20160010.
144. McMullin J, Schonberger B. When good balance goes bad: A discussion of common pitfalls when using entropy balancing. Journal of Financial Reporting. 2022;7(1):167-96.
145. Glynn RJ, Gagne JJ, Schneeweiss S. Role of disease risk scores in comparative effectiveness research with emerging therapies. Pharmacoepidemiology and drug safety. 2012;21:138-47.
146. Arbogast PG, Kaltenbach L, Ding H, Ray WA. Adjustment for multiple cardiovascular risk factors using a summary risk score. Epidemiology. 2008:30-7.

147. Cadarette SM, Gagne JJ, Solomon DH, Katz JN, Stürmer T. Confounder summary scores when comparing the effects of multiple drug exposures. Pharmacoepidemiology and drug safety. 2010;19(1):2-9.
148. Stürmer T, Schneeweiss S, Brookhart MA, Rothman KJ, Avorn J, Glynn RJ. Analytic strategies to adjust confounding using exposure propensity scores and disease risk scores: nonsteroidal antiinflammatory drugs and short-term mortality in the elderly. American journal of epidemiology. 2005;161(9):891-8.
149. Arbogast PG, Ray WA. Performance of disease risk scores, propensity scores, and traditional multivariable outcome regression in the presence of multiple confounders. American journal of epidemiology. 2011;174(5):613-20.
150. Kirkwood BR, Sterne JA. Essential medical statistics: John wiley & sons; 2010.
151. Luque-Fernandez MA, Belot A, Quaresma M, Maringe C, Coleman MP, Rachet B. Adjusting for overdispersion in piecewise exponential regression models to estimate excess mortality rate in population-based research. BMC medical research methodology. 2016;16:1-8.
152. Nardi A, Schemper M. Comparing Cox and parametric models in clinical studies. Statistics in medicine. 2003;22(23):3597-610.
153. Pourhoseingholi MA, Hajizadeh E, Moghimi Dehkordi B, Safaee A, Abadi A, Zali MR. Comparing Cox regression and parametric models for survival of patients with gastric carcinoma. Asian Pacific Journal of Cancer Prevention. 2007;8(3):412.
154. Collett D. Modelling survival data in medical research. Third ed: CRC press; 2013.
155. Svanstrom H, Pasternak B, Hviid A. Use of azithromycin and death from cardiovascular causes. The New England journal of medicine. 2013;368(18):1704-12.
156. Pirmohamed M, James S, Meakin S, Green C, Scott AK, Walley TJ, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. BMJ (Clinical research ed). 2004;329(7456):15-9.
157. Schneeweiss S, Hasford J, Göttler M, Hoffmann A, Riethling A-K, Avorn J. Admissions caused by adverse drug events to internal medicine and emergency departments in hospitals: a longitudinal population-based study. European journal of clinical pharmacology. 2002;58:285-91.
158. Mjörndal T, Boman MD, Hägg S, Bäckström M, Wiholm BE, Wahlin A, et al. Adverse drug reactions as a cause for admissions to a department of internal medicine. Pharmacoepidemiology and drug safety. 2002;11(1):65-72.
159. Wester K, Jönsson AK, Spigset O, Druid H, Hägg S. Incidence of fatal adverse drug reactions: a population based study. British journal of clinical pharmacology. 2008;65(4):573-9.
160. Rossi AC, Knapp DE, Anello C, O'Neill RT, Graham CF, Mendelis PS, et al. Discovery of adverse drug reactions: a comparison of selected phase IV studies with spontaneous reporting methods. Jama. 1983;249(16):2226-8.
161. Brewer T, Colditz GA. Postmarketing surveillance and adverse drug reactions: current perspectives and future needs. Jama. 1999;281(9):824-9.

162. Svanstrom H, Pasternak B, Hviid A. Use of clarithromycin and roxithromycin and risk of cardiac death: cohort study. BMJ (Clinical research ed). 2014;349:g4930.
163. McMahon AD, Evans JM, McGilchrist MM, McDevitt DG, Macdonald TM. Drug exposure risk windows and unexposed comparator groups for cohort studies in pharmacoepidemiology. Pharmacoepidemiology and drug safety. 1998;7(4):275-80.
164. Maclure M. The case-crossover design: a method for studying transient effects on the risk of acute events. American journal of epidemiology. 1991;133(2):144-53.
165. Torisson G, Rosenqvist M, Melander O, Resman F. Hospitalisations with infectious disease diagnoses in somatic healthcare between 1998 and 2019: A nationwide, register-based study in Swedish adults. The Lancet Regional Health–Europe. 2022;16.
166. Thygesen SK, Christiansen CF, Christensen S, Lash TL, Sørensen HT. The predictive value of ICD-10 diagnostic coding used to assess Charlson comorbidity index conditions in the population-based Danish National Registry of Patients. BMC medical research methodology. 2011;11(1):1-6.
167. Basic C, Rosengren A, Lindström S, Schaufelberger M. High validity of cardiomyopathy diagnoses in western Sweden (1989–2009). ESC heart failure. 2018;5(2):233-40.
168. Quiñones MA, Young JB, Waggoner A, Ostojic M, Ribeiro L, Miller R. Assessment of pulsed Doppler echocardiography in detection and quantification of aortic and mitral regurgitation. Heart. 1980;44(6):612-20.
169. Forns J, Cainzos-Achirica M, Hellfritzsch M, Morros R, Poblador-Plou B, Hallas J, et al. Validity of ICD-9 and ICD-10 codes used to identify acute liver injury: A study in three European data sources. Pharmacoepidemiol Drug Saf. 2019;28(7):965-75.
170. Folkhälsomyndigheten. Behandlingsrekommendationer för vanliga infektioner i öppenvård: Folkhälsomyndigheten; 2024 [Available from: https://www.folkhalsomyndigheten.se/publikationer-och-material/publikationsarkiv/b/behandlingsrekommendationer-for-vanliga-infektioner-i-oppenvard/.
171. Burke A, FitzGerald GA. Oxidative stress and smoking-induced vascular injury. Progress in cardiovascular diseases. 2003;46(1):79-90.
172. Winkel P, Hilden J, Fischer Hansen J, Hildebrandt P, Kastrup J, Kolmos HJ, et al. Excess sudden cardiac deaths after short-term clarithromycin administration in the CLARICOR trial: why is this so, and why are statins protective? Cardiology. 2011;118(1):63-7.
173. Gluud C, Als-Nielsen B, Damgaard M, Fischer Hansen J, Hansen S, Helo OH, et al. Clarithromycin for 2 weeks for stable coronary heart disease: 6-year follow-up of the CLARICOR randomized trial and updated meta-analysis of antibiotics for coronary heart disease. Cardiology. 2008;111(4):280-7.
174. Alispahic I, Eklof J, Jordan AS, Sivapalan P, Harboe ZB, Jensen J-US. Risk of mortality and cardiovascular events in patients with chronic obstructive pulmonary disease treated with azithromycin, roxithromycin, clarithromycin and amoxicillin. Eur Respiratory Soc; 2023.

175. Agency EM. Systemic and inhaled fluoroquinolones: risk of heart valve regurgitation/incompetence: EMA; 2020 [updated 2020-10-29. Available from: https://www.ema.europa.eu/en/medicines/dhpc/systemic-inhaled-fluoroquinolones-risk-heart-valve-regurgitationincompetence.
176. Herrett E, Gallagher AM, Bhaskaran K, Forbes H, Mathur R, Van Staa T, et al. Data resource profile: clinical practice research datalink (CPRD). International journal of epidemiology. 2015;44(3):827-36.
177. Friedrich M. WHO's top health threats for 2019. Jama. 2019;321(11):1041-.
178. Meeting highlights from the Pharmacovigilance Risk Assessment Committee (PRAC) 10-13 May 2016 [press release]. Eurpean Medicines Agency, 160513 2016.
179. FDA warns about increased risk of ruptures or tears in the aorta blood vessel with fluoroquinolone antibiotics in certain patients [press release]. Food and Drug Administration, 181220 2018.
180. Folkhälsomyndigheten. Försäljning i öppenvård 2024 [Available from: http://fohm-app.folkhalsomyndigheten.se/Folkhalsodata/pxweb/sv/A_Folkhalsodata/A_Folkhalsodata__D_Antibiotika__Forsaljning/.

Printed by Media-Tryck, Lund 2024 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Infectious Diseases

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2024:55
ISBN 978-91-8021-548-0
ISSN 1652-8220