Respiratory tract infections in primary care

- aspects of diagnosis and treatment

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Primum non nocere

First, do no harm.

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ABSTRACT

Background Primary health care is accountable for most of the antibiotic prescriptions in humans. In Sweden, most of these antibiotics are used for respiratory tract infections, and pharyngotonsillitis (acute sore throat) is the single respiratory tract infection that leads to most antibiotic prescriptions. The first line treatment for pharyngotonsillitis with presence of group A streptococci is penicillin V 1000 mg three times daily for 10 days. Though, effectiveness of shorter penicillin V treatment for pharyngotonsillitis is unknown. Antibiotic treatment can induce ecological changes in the intestinal microbiota, including emergence of antimicrobial resistance. Previous studies on side-effects from penicillin V therapy, including antimicrobial resistance in the intestinal microbiota, are lacking. To restrict the use of antibiotics, two point-of-care tests have become very popular in Swedish primary care: the C-reactive protein (CRP) and rapid antigen detection test for group A streptococci (GAS). They have capacity to restrict unnecessary use of antibiotics in primary care, when used according to the guidelines. However, concentration of CRP in primary care patients with influenza-like illness has not been investigated earlier, and the reliability of rapid tests for GAS after recent penicillin V treatment for pharyngotonsillitis has been questioned.

Aim The overall aim of the thesis was to contribute to a safe reduction in antibiotic use and to investigate the benefit of two popular near-patient tests in patients with common RTIs. We set out to: i) determine if a shorter but more intense penicillin V treatment could give a clinical cure rate of GAS pharyngotonsillitis comparable to the currently recommended treatment; ii) compare the presence of GAS in rapid antigen detection test (RADT) and throat culture among patients recently treated with penicillin V for pharyngotonsillitis; iii) evaluate penicillin V effects on the microbiota with a

focus on the emergence of β-lactam resistance; iv) examine if CRP can predict presence of influenza A in primary care patients presenting with influenza-like symptoms.

Methods The first paper was a randomised controlled non-inferiority trial, comparing penicillin V 800 mg x 4 for 5 days to 1000 mg x 3 for 10 days. The second paper was an observational study comparing the results from RADT and culture for GAS at a follow-up visit within 21 days from inclusion. In the third paper we explored some clinically relevant changes in cultures of faecal swab samples from patients, before and after pencillin V treatment for pharyngotonsillitis. The fourth paper was a cross-sectional study in patients with an influenza-like illness for less than 72 h. The main outcome measures were capillary blood CRP and PCR test for detection of influenza A or B in the upper respiratory tract.

Results and conclusions We found penicillin V for 5 days to be non-inferior to the 10-day treatment regarding clinical cure. Hence, it is possible to maintain the clinical effectiveness with a shorter penicillin V treatment for GAS pharyngotonsillitis. The most reported side-effects from treatment were diarrhoea and vulvovaginal symptoms. With the 10-day treatment 35% of patients reported gastrointestinal side-effects and 25% of female patients reported vulvovaginal symptoms. The incidence of these side-effects was lower with the 5-day treatment. We found penicillin V treatment to induce several alterations in the faecal microbiota that are generally considered signs of ecological disturbance, including a significant increase of resistance towards ampicillin and third generation cephalosporins. These findings emphasise the importance to restrict the use of penicillin V. Further, we found no significant difference between the results of RADT and throat culture for GAS after recent penicillin V treatment for pharyngotonsillitis. Thus, we conclude that RADT for GAS is reliable also after recent penicillin V treatment. Finally, we found that the CRP concentrations in patients with influenza-like illness of different confirmed aetiology had a great overlap. We found no association between CRP and confirmed influenza A. We conclude that the CRP concentration cannot predict influenza A in patients with influenza-like illness.

The results of this thesis is relevant in other primary care settings. It may influence the future treatment recommendation for pharyngotonsillitis and the clinical use of two popular rapid tests. The demonstrated ecological disturbances in the microbiota from penicillin V treatment ought to raise awareness of the risks from treatment and may guide the design of future studies in the field.

Keywords: group A streptococci, pencillin V, phenoxymethylpenicillin, rapid antigen detection test, antimicrobial resistance, faecal microbiota, C-reactive protein, influenza-like illness, influenza A, primary care.

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SAMMANFATTNING PÅ SVENSKA

Bakgrund Antimikrobiell resistens är ett växande hot mot människors hälsa. De senaste 80 årens vidlyftiga antibiotikaanvändning har drivit fram en ökande förekomst av resistens i de mikrobiella ekosystemen. Globalt sett används merparten av antibiotikan inom djurindustrin. Vad gäller antibiotikaförskrivning till människor så sker den till största del i primärvården, och i Sverige är det oftast till människor med luftvägsinfektioner. Halsfluss är den luftvägsinfektion som ger upphov till flest antibiotikarecept. Enligt aktuell behandlingsrekommendation är det vid halsfluss med uttalade besvär och påvisade grupp A streptokocker som behandling med pencillin V kan erbjudas. Den rekommenderade doseringen av penicillin V är 1000 mg tre gånger dagligen i 10 dagar och syftet med behandlingen är att lindra symtom. Effekten av kortare behandlingstider är okänd. Trots att pencillin V är vårt vanligast använda antibiotikum i Sverige så saknas tidigare studier av behandlingens effekter på den antimikrobiella resistensen hos patienternas tarmbakterier. Två patientnära snabbtest har rönt stor popularitet i svensk primärvård vid bedömning av luftvägsinfektioner: C-reaktivt protein (CRP, snabbsänka) och antigentest för grupp A streptokocker (RADT, strep A-test). Testen kan minska onödig antibiotikaförskrivning när de används enligt riktlinjerna. CRP hos primärvårdspatienter med influensalik sjukdom har inte undersökts tidigare, och tillförlitligheten hos strep A-test efter penicillinbehandling för halsfluss är ifrågasatt.

Syfte Det övergripande målet för avhandlingen var att bidra till en säker minskning av antibiotikaanvändning och att undersöka utfall hos de två vanligast använda snabbtesten i två olika kliniska situationer med luftvägsinfektioner. Syftet var att: I) undersöka om en 5-dagarsbehandling med penicillin V gav klinisk effekt likvärdig den rekommenderade 10-dagarskuren; II) jämföra utfall hos snabbtest och svalgodling för grupp A streptokocker hos patienter som nyligen behandlats med penicillin V för halsfluss; III) undersöka effekter av behandling med penicillin V på tarmens mikrobiota, med fokus på resistens mot betalaktam-antibiotika; IV) undersöka om CRP kan förutsäga förekomst av influensa A och B hos primärvårdspatienter med influensalik sjukdom.

Metod Det första delarbetet var en randomiserad kontrollerad prövning utan blindning. Vi testade om behandling av halsfluss med penicillin V 800 mg x 4 i 5 dagar var likvärdig 1000 mg x 3 i 10 dagar. Patienter som tillfrågades om att deltaga var $\geq$ 6 år, hade halsont med minst 3 Centorkriterier uppfyllda (feber, ömmande svullna lymfkörtlar i käkvinklarna, rodnade tonsiller med beläggningar och frånvaro av hosta) och dessutom positivt snabbtest för GAS.

Totalt inkluderades 433 patienter. Av dem hade 316 även positiv svalgodling för GAS vid studiens början, samt prov för både snabbtest och svalgodling tagna vid ett uppföljande besök inom 21 dagar från studiestart. I det andra delarbetet jämförde vi utfallet hos de båda testen vid uppföljning av dessa patienter. I det tredje delarbetet odlades avföringsprover från 31 patienter tagna före och efter pencillin V-behandling för halsfluss. I det fjärde delarbetet inkluderades 277 patienter ≥ 1 års ålder som haft symtom på influensalik sjukdom under högst 72 timmar. Utfallsmåtten var kapillärt prov för CRP och PCR-analys av prov från de övre luftvägarna för att fastställa förekomsten av influensa A eller B.

Resultat och slutsatser I första delarbetet fann vi att behandling med penicillin V i 5 dagar inte gav oacceptabelt sämre effekt än gängse 10 dagars behandling vad gäller klinisk utläkning av halsfluss. Det är således möjligt att bibehålla den kliniska effekten med en kortare behandlingstid. Bland patienterna som behandlades med penicillin V i 10 dagar rapporterade 35% biverkningar från magtarmkanalen, mestadels diarré och illamående. Av de kvinnliga patienterna med 10 dagars behandling rapporterade 25% att de fick underlivsbesvär vid behandling. Andelen patienter med dessa biverkningar var lägre i 5-dagarsgruppen. I andra delarbetet fann vi ingen signifikant skillnad mellan utfall av snabbtest och svalgodling för GAS hos patienter som nyligen behandlats med penicillin V för halsfluss. Vår slutsats var att snabbtest för GAS fungerar tillförlitligt även efter antibiotikabehandlad halsfluss. I tredje delarbetet visade odling av avföringsprover från patienter före och efter penicillinbehandling bland annat en signifikant ökning av resistens mot betalaktam-antibiotika. Fynden utmanar den gängse bilden av penicillin V som ett ekologiskt säkert preparat för den enskilda patienten och understryker vikten av återhållsam penicillinförskrivning. I fjärde delarbetet fann vi att CRP hos patienter med influensalik sjukdom överlappade stort för olika infektionsorsaker. Vi fann ingen association mellan CRP och bekräftad förekomst av influensa A-virus. Vår slutsats är att CRP inte kan identifiera patienter med hög sannolikhet för influensa A vid influensalik sjukdom.

Resultaten från avhandlingen kan komma till användning i primärvården: påverka behandlingsrekommendationen för halsfluss och användningen av två vanligaste snabbtester.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Skoog Ståhlgren G, Tyrstrup M, Edlund C, Giske CG, Mölstad S, Norman C, **Rystedt K**, Sundvall PD, Hedin K. Penicillin V four times daily for five days versus three times daily for 10 days in patients with pharyngotonsillitis caused by group A streptococci: randomised controlled, open label, non-inferiority study. *BMJ*. 2019 Oct 4;367: l5337.

II. **Rystedt K**, Hedin K, Tyrstrup M, Skoog-Ståhlgren G, Edlund C, Giske CG, Gunnarsson R, Sundvall PD. Agreement between rapid antigen detection test and culture for group A streptococcus in patients recently treated for pharyngotonsillitis - a prospective observational study in primary care. *Scandinavian Journal of Primary Health Care*. 2023: 41(1): 91-97.

III. **Rystedt K**, Edquist P, Giske CG, Hedin K, Tyrstrup M, Skoog Ståhlgren G, Sundvall PD, Edlund C. Effects of penicillin V on the faecal microbiota in patients with pharyngotonsillitis - an observational study. *JAC – Antimicrobial Resistance.* 2023;5(1).

IV. **Rystedt K**, Harbin NJ, Lindbaek M, Radzeviciene R, Gunnarsson R, Eggertsen R, C Butler C, van der Velden AW, J Verheij T, Sundvall PD. Is C-reactive protein associated with influenza A or B in primary care patients with influenza-like illness? A cross-sectional study. *Scandinavian Journal of Primary Health Care.* 2020 Dec;38(4):447-453.

CONTENT

ABBREVIATIONS

AMR	Antimicrobial Resistance
ARF	Acute Rheumatic Fever
CRP	C-Reactive Protein
ESBL	Extended Spectrum Beta-Lactamases
GABHS	Group A Beta-Haemolytic Streptococci
GAS	Group A Streptococci
HGT	Horizontal Gene Transfer
ILI	Influenza-Like Illness
MIC	Minimum Inhibitory Concentration
mITT	Modified Intention To Treat
PCR	Polymerase Chain Reaction
POCT	Point-of-Care Test
PP	Per Protocol
RADT	Rapid Antigen Detection Test
RCT	Randomized Controlled Trial
RTI	Respiratory Tract Infection

OVERVIEW OF THE THESIS

	TITLE	AIM
I	*Penicillin V four times daily for five days versus three times daily for 10 days in patients with pharyngotonsillitis caused by group A streptococci: randomised controlled, open label, non-inferiority study*	To compare treatment for GAS pharyngotonsillitis with pencillin V 800 mg x 4 for 5 days to 1 g x 3 for 10 days. The primary outcome was clinical cure at follow-up. Secondary outcomes were bacteriological eradication, relapses, complications, and adverse events.
II	*Agreement between rapid antigen detection test and culture for group A streptococcus in patients recently treated for pharyngotonsillitis - a prospective observational study in primary care*	To compare test results from rapid antigen detection test (RADT) and throat culture for group A streptococci (GAS) in patients recently treated with penicillin V for pharyngotonsillitis. To evaluate possible factors associated with agreement between RADT and throat culture for GAS.
III	*Effects of penicillin V on the faecal microbiota in patients with pharyngotonsillitis - an observational study*	To explore the effects of penicillin V on the faecal microbiota with focus on emergence of beta-lactam resistance. Ecological disturbances were also measured as increase of enterococci, shifts in species composition, and colonization with *C. difficile* and *Candida* spp.
IV	*Is C-reactive protein associated with influenza A or B in primary care patients with influenza-like illness? A cross-sectional study*	To examine if CRP can predict presence of influenza A or B in primary care patients with influenza-like illness (ILI). To examine the association between CRP and severity of influenza-like symptoms in patients with ILI.

	PATIENTS	STATISTICAL METHODS	RESULTS
I	433 sore throat patients, aged ≥6 years, with 3-4 Centor criteria**:** absence of cough, fever >38.5°C, tender anterior cervical lymph nodes, coatings of the tonsils (or inflamed tonsils if 6 years of age), and positive RADT for GAS were randomly allocated to 5 or 10 days pencillin V treatment.	Fisher's exact test and Mann-Whitney U-test. Kaplan-Meier survival analysis with log rank test.	The five-day treatment was non inferior to the 10-day regarding clinical cure: 89.6% (n=181/202) in the 5- day treatment arm and 93.3% (n=182/195) in the 10-day arm. The difference in clinical cure was −3.7 percentage points (95% CI −9.7 to 2.2)
II	316 patients from paper I, having both a positive RADT and a positive throat culture for GAS at inclusion, and also having conclusive RADT and throat culture for GAS taken at a follow-up within 21 days from inclusion.	Kaplan-Meier survival analysis with log rank test. Multivariable binary logistic regression analysis.	Log rank test did not reveal any significant difference of positive RADTs and positive throat cultures for GAS after penicillin V treatment for pharyngotonsillitis.
III	29 patients aged ≥10 years with pharyngotonsillitis contributed with 3 faecal swab samples each. The samples were collected before, at the last dose, and at follow-up 7-9 days after completed penicillin V treatment.	McNemar test and Wilcoxon signed rank test.	Enterobacterales with decreased susceptibility to ampicillin and third generation cephalosporins increased significantly after treatment. There was a significant increase in number of bacterial species resistant to ampicillin that was and remained during the study.
IV	277 patients aged ≥1 year, with ILI for less than 72 hours during seasonal influenza. ILI was defined as fever + at least one systemic symptom (headache, muscle ache, sweats or chills or tiredness) + respiratory symptom/symptoms (cough, sore throat or runny or congested nose).	Multivariable binary logistic regression analyses.	CRP was not associated with presence of influenza A. CRP concentrations showed a distinct overlap for different aetiological findings.

1 INTRODUCTION

The smell is sweet, and present already in the waiting room. It is the smell of sickness and night sweats. The patient was overtaken by fever and a sore throat three days ago, and now the voice is thick, and it is so painful to swallow. The skin is hot and damp. It is the kind of feverish suffering that surrounds our existence – life in a vessel of meat.

Sore throat is a very common symptom. Generally, it remits spontaneously within one week, regardless of aetiology. As for other respiratory tract infections, viral origin of disease is common, and the signs and symptoms cannot distinguish between different aetiologies. However, the Swedish clinical guidelines for sore throat focus on finding patients with more pronounced symptoms and a higher likelihood of group A streptococcal (GAS) aetiology to the infection, as they may have benefits from antibiotics. The purpose of treatment is to speed up the resolution of symptoms. Penicillin V is the first line treatment for GAS pharyngotonsillitis, and the most prescribed antibiotic in Sweden. To target the use of antibiotics in patients with respiratory tract infections, two point-of-care tests have become popular in Swedish primary care: the rapid antigen detection test for GAS, and C-reactive protein.

Antibiotics save lives, and it is a challenge for primary health care to identify the potentially dangerous infections that occasionally show up. Nevertheless, most of the infections in primary care are uncomplicated and self-limiting conditions, where the makings of health care are not important to survival – people get well anyhow.

The widespread use of antimicrobials during the last 80 years has resulted in an emergence of antimicrobial resistance, and the presence of resistance genes is steadily increasing in the microbial ecosystems. An antibiotic treatment selects for resistant microbes in the individual patient's intestinal microbiota and enhances the spread of resistance genes between the bacterial species. With increasing resistance towards antimicrobial treatments, we are gradually losing the foundation for modern medicine. The main challenge for primary care in infections has become to limit the use of antibiotics.

This thesis comprises a randomized controlled trial comparing the currently recommended 10-day penicillin V treatment to a shorter but more intense treatment for GAS pharyngotonsillitis; an observational study comparing the results from rapid antigen detection test and throat culture for GAS after recent treatment for GAS pharyngotonsillitis; an explorative evaluation of ecological disturbances in the faecal microbiota from penicillin V treatment with focus on emergence of β-lactam resistance; and a cross-sectional study on C-reactive protein in patients with influenza-like illness. The overall aim of the thesis is to contribute to a safe reduction in the use of antibiotics.

1.1 THE MICROBES WERE HERE FIRST

Life on earth originated in the form of bacteria at least 3.4 billion years ago [1-3]. Most likely, the event unfolded in the deep ocean. Since the first trembling molecules of life were not well preserved, the exact passage of events remains unknown. However, the microbes were here first, and they have evolved for billions of years. Not only did they leave the primordial sea [4] and adapt to a multitude of environments. Also, their metabolic activity changed the chemical and physical conditions on the planet, creating and maintaining an atmospheric homeostasis suitable to life [5]. A perfect planet for the microbes.

1.2 BACTERIA GAIN NEW GENES THROUGH HORIZONTAL GENE TRANSFER

Reproduction through cell division is common to all procaryotes, including bacteria and archaea. Bacterial reproduction occurs by growth, chromosome replication and cell division, resulting in two daughter cells genetically identical to their single parent [6]. A species was originally defined as two individuals of different sexes or mating types able to produce a fertile offspring. Since this definition does not apply to organisms with asexual reproduction through cell division, other ways are needed to assort bacterial diversity [7]. In bacteria, 97% rDNA similarity is generally regarded as corresponding to a species variation [8], which gives a very large genetical variation within each bacterial species. For comparison, if 97% genetic similarity was applied to humans, we would be categorized in one species together with chimpanzees and a bunch of other primates [9]. The magnificent

genetical variation in bacteria is obtained through *horizontal gene transfer* - a capacity to gain and lose genes during life [10, 11]. Thus, bacteria can adapt to the environment continuously during life, in a rapid and radical manner. The horizontal gene transfer is not restricted within a bacterial species or genera; it enables a generous genetic exchange across the species definitions: an exchange also of genes coding for antimicrobial resistance [12].

1.3 HUMANS AND MICROBES DEVELOP IN SYMBIOSIS

The early animals on the planet were surrounded by microorganisms, and adapted to that [13]. When the first humans began to evolve on the African continent approximately 400 000 years ago [14] microbial symbiosis had long been a part of our development and physiology [15].

The term microbiota refers to all the colonising microorganisms that form complex ecosystems within our bodies: bacteria, fungi, archaea, viruses, and protozoans, [16]. To maintain a functional interaction, the human host should neither eliminate nor allow them to take over entire tissues [13]. Some of the symbiont microbes have co-evolved with us and influence our development and individual phenotype, through close extended interaction. Some of them are transient visitors with a limited impact on the host organism [17].

Both commensals - the friendly inhabitants - and some opportunistic pathogens can be symbionts to the human host. However, microbes that disappear from the body within a few days, and microbes that injure and kill the host, are generally not considered symbionts, but pathogens. The unit of host and microbial symbionts is referred to as a *holobiont*. As the holobiont functions as one entity in development and metabolism, it is considered a level of evolutionary selection [17, 18]. Recent research reveals the importance of microbes during human evolution [19]. In contrast, the evolution of the intestinal microbiota is largely unknown. Analyses of archaeological findings report major changes in the microbiota over the past 300 years, from industrialisation onwards. The trend is towards lower diversity in human microbiota [20, 21].

1.4 MICROBIOLOGICAL HABITATS OF THE HUMAN BODY

Human body habitats, such as skin, oral cavity, nose, airways, urogenital tract, and the gastrointestinal tract have largely different microbial compositions. However, the differences between microbiota compositions are even greater between individuals, than between different sampling habitats in one individual human [22]. There is a correlation of α-diversity levels across microbial communities in different body habitats [23], that means the degree of complexity in an individual's microbiota is rather constant in her different body sites. Although the species' composition and amount of microbes of the oral cavity and of the faecal microbiota are not the same they somehow predict each other's composition in the individual [24].

1.5 THE ORAL MICROBIOTA IS DOMINATED BY STREPTOCOCCI

Over 700 species of bacteria have been identified in the oral cavity [25]. As food and microbial organisms are frequently passing by, it is hard to differ transient visitors from endogenous species [26]. The variation among less dominant species is very high. Most of the microhabitats within the oral cavity are dominated by *Streptococcus spp,* within the phylum *Firmicutes*. The turbulent oral environment requires a talent for adhesion, and the streptococci use adhesin proteins to keep attachment to their host. After brushing the teeth the streptococci are the first organisms to attach to the clean tooth surfaces. [23, 25]. Streptococcal species have been successfully attached to the human mouth for a long time. Some streptococci have been identified in birch material chewed by a person 5700 years ago [27], and it is likely that the relationship has lasted much longer than that.

Gene sequencing has divided streptococcal species to six major groups. The α-haemolytic mitis group of streptococci are the most abundant in the human oral cavity [28]. This thesis focuses on *Streptococcus pyogenes*, within the pyrogenic group of streptococci. *S. pyogenes* is also known as group A streptococci (GAS), or group A β-haemolytic streptococci (GABHS). It is generally not regarded as a commensal member of the normal microbiota. However, GAS does not always cause symptoms, and in children transient colonisation is rather common [29]. Meta-analyses have reported 8-12% of

asymptomatic children having pharyngeal specimens positive for GAS [30, 31], though the carrier rate is lower for those under 5 years of age [30]. The carriage rate of GAS in adults is estimated to be around 2% [31]. However, the GAS carriage differs depending on method: the use of PCR analyses gives a higher prevalence of GAS in a healthy population than throat cultures [32]. Notably, there is no gold standard, or criterion standard for confirming presence of GAS in the throat [33, 34], and there is no reliable method to distinguish between the carrier state and the infection from GAS [29].

The α-haemolytic streptococci function as commensal inhabitants of the oral microbiota, and more seldom as opportunistic pathogens causing endocarditis and aspiration pneumonia in the elderly [28]. As commensals they can both enhance and protect against respiratory pathogens in humans [28, 35]. Some α-streptococci from the mitis group have been suggested to protect against GAS infection [36] and some to elevate the risk of influenza A infection [28]. A randomised controlled trial of patients with recurrent GAS pharyngotonsillitis has previously shown that using a spray containing four strains of α-streptococci after penicillin V treatment reduced the rate of relapses [37]. The pharyngeal microbiota of adults contains more bacteria with interfering and bactericidal effects on group A streptococci than the microbiota of children [38]. Penicillin V suppresses α-streptococci and other commensals within the oral microbiota [39], but the effects of penicillin V treatment on the susceptibility to new tonsillitis episodes is not well investigated.

1.6 THE GASTROINTESTINAL MICROBIOTA IS THE RICHEST AND MOST COMPLEX

The human gastrointestinal microbiota is associated with at least 40 000 bacterial species [8], the exact number depending on species classifications. However, an individual does not harbour all of them, but somewhere between hundreds or thousands different bacterial species.

The acidic milieu of the human stomach holds a rather scarce microbiome that is easily disturbed [40]. The small intestine collects a slightly greater diversity of microbes, although bacterial life in the small intestine is limited by low pH, short transit time, and in addition, secretion of antimicrobial peptides [41]. The terminal ileum harbours a large part of the human lymphoid tissue and it is

probably the most important habitat for the communication between the microbiota and the immune system [42]. Also, the terminal ileum is in close contact to the large intestine, where our richest microbiota is found [43]. The dense and diverse microbiota of the colon can be stratified into different microhabitats. The mucus layers of the colon more or less separate the microbiota from the epithelium and contain a gradient of species. The population density increases towards the lumen of the gut [41]. Most of the members of the colon microbiota are anaerobic [16], belonging mostly to one of two bacterial phyla: around 65% *Firmicutes* and 23% *Bacteroidetes*. The phyla *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia, Cyanobacteria* and *Spirochetes* are found in smaller proportions [8, 41, 44, 45]. High-risk pathogens are normally not found in the microbiota of healthy people [23]. However, small children can be colonised by *Clostridoides difficile* without symptoms, while this is not common in adults [46].

1.7 HUMAN MICROBIOTA IS ACQUIRED AND INDIVIDUAL

We acquire symbiotic microbes from our surroundings [15, 47]. Diet, age, geography, environment, body mass index, genetics and eventual antibiotic treatments influence the development of the microbiota [23, 48]. Consequently, there is no universal standard type of human microbiota, and each person collects their own unique set of symbionts [47]. In animals the diversity of faecal microbiota generally increases from carnivorous to omnivorous to herbivorous diets [49]. Even in humans, diet is a determining factor in the microbial composition of the gut [41], and the human microbiota often resembles the microbiota of other omnivorous primates [49]. People with a diet rich in animal protein, have a less diverse and more carnivorous-like microbiota [47]. In those under three years of age, the variation in faecal microbiota composition between samples is very high. Environmental changes, such as a new diet or an antibiotic treatment, can result in huge shifts in the infant gut microbiotas [47, 50]. In adults, the composition is rather stable over time, and the diversity continues to increase with age [47]. In elderly people changes and losses of microbiome diversity are associated with frailty and physiological ageing processes [51].

1.8 HUMAN MICROBIOTA HAS MULTIPLE FUNCTIONS

The human microbiota is important in both health and disease. The symbiotic microbes have profound effects on the normal physiology, development, maintaining of homeostasis and ageing of the human phenotype [17, 51-53]. The colonisation of mucosal surfaces with appropriate symbionts is needed for maturation of the innate and adaptive immune systems [54]. Also, the microbiota itself protects us against infectious diseases, as it constitutes a *colonisation resistance* that prevents invasion by pathological species [41]. The microbiota is involved in the development of different organs, including the brain and the nervous system [53, 55]. The gut microbiota may even influence human behaviour and cognitive functions [53]. The gut microbiota is important for the metabolism, including the breakdown of polysaccharides, and synthesis of amino acids, vitamins, and hormones [41, 56, 57]. Consequently, it plays a role in glycaemic control and the development of type 2 diabetes [57]. Alterations in the intestinal microbiota are associated with different intestinal diseases; irritable bowel syndrome (IBS) [58], ulcerative colitis, Crohn's disease and colorectal cancer [48], where alterations in the microbiota are likely a part of both the aetiology and pathophysiology. Also, cardiovascular disease [59], Parkinson's disease [60], bipolar disorder [61], autism spectrum disorder [62], childhood asthma [63, 64], and several other conditions are associated with changes in the intestinal microbiota. The multiple functions of the intestinal microbiota are subject to ecological disturbances from various antibiotic treatments with consequences for the health of the individual [65].

1.9 EMERGENCE OF ANTIMICROBIAL RESISTANCE

The substances that we call antimicrobials show different effects on bacterial cells, depending on the mode of action and concentration. Low subinhibitory concentrations may correspond to the natural occurrences of these substances [66]. In lower concentrations they can interact with different cellular processes without inhibiting cellular growth: they may mediate interactions between different bacterial species and modulate the structure of the microbial ecosystem, regulate genes for metabolism or stimulate horizontal gene transfer.

On the contrary, high concentrations can inhibit growth, or even kill other microbes, justifying the term "antimicrobials" [67]. So, antimicrobial resistance is a natural response from microbes, and just as antimicrobial substances are ancient, so are resistance mechanisms [68]. Many genes coding for antimicrobials and antimicrobial resistance have been reported, and beyond these reports is a reservoir of genes unknown to humans [69]. Human large-scale production of antimicrobials in the last 80 years has brought an enormous selective pressure upon microbes, leading to an increase of antimicrobial resistance genes within the natural microbial ecosystems [70, 71]. The consequences to these ecosystems due to the human production of antimicrobial substances are largely unknown [67, 72]. Still, the antibiotic consumption is increasing worldwide [73, 74].

Globally, most antimicrobials are used in meat production. The proportion of antimicrobials used to promote growth in cattle, pigs, and poultry is estimated at 73% of the total world-wide consumption [75, 76]. The animals are fed with long term sub-therapeutic doses of antimicrobials to enhance weight gain by reducing the gut microbiota diversity, which at the same time promotes selection of antimicrobial resistance genes. Resistant microbes in animal droppings then spread to the environment and further to humans. The patterns of antimicrobial resistance (AMR) within a geographic area reflects the local use of antimicrobials in animal production, and in healthcare [77]. In Sweden, the use of antimicrobials to promote growth in animals ended in 1986 [78].

The unfortunate consequence of increasing AMR in the microbial communities is that antimicrobials used in human medicine become ineffective. Modern medicine, as we know it in high-income countries, with routine surgical treatments and chemotherapy for cancer, would not be possible without effective antimicrobial treatments [79, 80].

"Access to effective antimicrobial agents constitutes a prerequisite for most modern medicine", as it is stated in the World Health Organisation's Global action plan on antimicrobial resistance. Today, antimicrobial resistance is one of the leading causes of death in the world [81], and infections that were once treatable with several antimicrobial agents have now acquired resistance to most of the available drugs [80]. During 2019, 1.27 million deaths in humans were directly due to resistant infections. If all the mortality associated to AMR is included, the number of deaths for the same period was estimated to 4.95 million [81].

1.10 ANTIBIOTICS LESSEN DIVERSITY AND SELECT FOR ANTIMICROBIAL RESISTANCE

The effects of an antibiotic exerted on the microbiota depends on the type of antibiotic and its spectrum of activity [44, 82], dose and duration of treatment [83], and the resistance level in the microbial ecosystem [45, 64, 84]. Even a short antibiotic treatment can result in long-lasting changes [84-86]. While the composition of the microbiota can change drastically during antibiotic exposure, the microbiota functions can be partly maintained by the new composition of more resilient species. However, some level of functional diversity losses are common, particularly impairment of metabolic activity and reduction of colonisation resistance [45]. Changes in the microbiota associated with adverse effects to the host are termed *dysbiosis* [41].

Reduced diversity within the phyla *Firmicutes* and *Bacteroidetes*, and overgrowth of the *Proteobacteria* phylum *Enterobacterales* are common in post-antibiotic dysbiosis [44]. Weakening of colonisation resistance following antibiotic treatment may enable colonisation by *Salmonella* species [87] or *Clostridoides difficile.* In susceptible persons the release of *C. difficile* toxins can lead to diarrhoea and colitis. Symptoms of colitis do not develop in all colonised persons, especially not in infants. The symptoms of *C. difficile* disease can be mild and self-limiting, or severe and fatal [88].

Enterobacterales is a diverse order within the phylum *Proteobacteria*, including both commensal colonisers, and clinically relevant pathogens. *Escherichia coli* being the most famous and abundant member. Some of the potential pathogenic species within *Enterobacterales* can also function as commensals, given that the rest of the microbiota prevents overgrowth through colonisation resistance. Thus, in a healthy individual the *Enterobacterales* constitutes a minor component of the microbiota. An outburst of *Enterobacterales* and a shift to more pathogenic species within the order are reckoned as ecologically relevant disturbances reported after antibiotic treatment [89].

Also, species of the genus *Enterococcus*, within the phylum *Firmicutes*, can function as both commensals and opportunistic pathogens - causing infections when translocated from the gastrointestinal tract [90]. The enterococci are Gram-positive bacteria intrinsically insensitive to β-lactam antibiotics due to low affinity penicillin-binding proteins in the peptidoglycan layer. They often possess multiple acquired antimicrobial resistance mechanisms. Vancomycin-

resistant enterococci (VRE) has become an emerging health problem around the world, causing disease with limited treatment options [91].
The post-antibiotic dysbiosis promotes a flow of adaptive functions through horizontal gene transfer and a spreading of antimicrobial resistance within the microbiota [44]. Antibiotic treatment can also affect some viruses, mainly by interaction between viruses and bacterial species, which may further enhance horizontal gene transfer [45]. Repeated antibiotic treatments can lead to persistent changes in the microbiota composition, although these effects are unevenly distributed among patients; the microbiota resilience shows large variations between individuals [44, 48, 85]. The microbiota of infants is highly variable and individualised, as is the microbial response to antibiotics. The faecal microbiota of young children can show enormous changes due to a single or multiple antibiotic exposure. Notably, also very early in life, prior to any antibiotic treatment, antibiotic resistance genes are abundant in the microbiota [92].

1.11 PENICILLIN V – AN ORIGINAL NARROW SPECTRUM ANTIBIOTIC

"The colony appears as a white fluffy mass which rapidly increases in size and after a few days sporulates, the centre becoming dark green and later in old cultures darkens to almost black. In four or five days a bright yellow colour is produced which diffuses into the medium. In certain conditions a reddish colour can be observed in the growth."

This kaleidoscopic poetry describing a mould on an agar plate, was published by Alexander Fleming in 1929, along with the findings on the antibacterial action of *Penicillium notatum* [93]. However, the active substance was difficult to extract from the mould, and it took until the 1940s until penicillin was available for clinical use [94].

Penicillin V belongs to the beta-lactam antibiotics. The common denominator of this class of drugs is the beta-lactam ring, built from 3 carbon and 1 nitrogen atoms, which binds to enzymes involved in bacterial cell wall formation. The beta-lactam antibiotics inhibit the peptidoglycan synthesis and thereby hamper growth and induces lysis of the bacterial cells. Except for penicillins, the beta-lactams consist of cephalosporins, carbapenems, and monobactams.

Sometimes also the beta-lactamase inhibitors, such as clavulanic acid and tazobactam, are included in this class of drugs [95].

Gram-positive bacteria, with peptidoglycan on the surface, are generally susceptible to penicillins. Contrarily, Gram-negative bacteria having a double membrane envelope, with an outer lipid membrane covering the peptidoglycan, are generally considered less susceptible to penicillins [96].

The efficacy of penicillin V is dependent on time above minimum inhibitory concentration (MIC), and the drug has none or very limited persisting effects when the concentration goes below MIC [97, 98]. Penicillins have an effective tubular secretion in the kidneys and a short half-life in plasma. For patients with a normal kidney function the half-time is about one hour [99, 100]. Thus, a more frequent intake of adequate doses can give longer time above MIC, and be more efficient.

The expressions *broad- and narrow-spectrum antibiotics* were introduced in the mid-1950s when penicillins were compared to chloramphenicol and tetracyclines. The penicillins were considered narrow-spectrum in relation to these specific antibiotics, that were used to treat a broader spectrum of infectious diseases [101]. The terminology of narrow- and broad-spectrum antibiotics are still in use, but lacks an established definition [102]. Broad-spectrum antibiotics change the composition of the intestinal microbiota and increase antimicrobial resistance [82, 84, 103]. Narrow-spectrum antibiotics are thought to cause less ecological damage, since their action is restricted to certain bacterial genus or species [102]. However, investigations of the ecological effects of penicillin - the original narrow-spectrum agent - are sparse.

1.12 PENICILLIN V´S EFFECTS ON THE INTESTINAL MICROBIOTA

There are four earlier studies on the ecological effects of penicillin V on the faecal microbiota. Three studies used cultures and examined effects of lower daily dosages of penicillin V than are currently recommended. Two of them used 800 mg penicillin V twice a day for 7 days, in 10 and 6 adults, respectively [104, 105], and a third study used 1 g penicillin V twice a day for 10 days in

10 adults [39]. These studies found none or very small disturbances in cultures of stool samples, except from an increase in *Clostridoides* spp. A fourth, and more recent study, investigated oral and faecal samples in an infant, using metagenomic sequencing methods. The child received penicillin V for 5 days, but the dosage was not reported in the article [106]. The authors found disturbances in the microbiota, such as massive increase of *E. coli* and an emergence of antimicrobial resistance genes.

The recommended pencillin V dosages have changed in favour of a more frequent intake and higher daily doses. Thus, the modern pencillin V treatments give more time above MIC and improved clinical efficacy [107-109]. If the higher, modern penicillin V dosages are also enhancing the effects on the microbiota has not been investigated. The previous culture studies did not explore the emergence of antimicrobial resistance.

1.13 PENICILLIN V AND VULVOVAGINAL CANDIDIASIS

Yeast is found in a many natural habitats. The yeast species within the *Candida* genus are considered commensal members of the human microbiota, commonly colonising the surfaces of skin, nails, and the genital, urinary, respiratory, and gastrointestinal tracts. However, they are opportunistic. With certain changes in the host conditions, they can transform from harmless inhabitants to pathogenic troublemakers, or even invaders. A majority of women carry *Candida albicans* in their vagina at some time-point during life, with or without symptoms. If disease occurs, the most common symptoms are pruritus, vaginal soreness, and a sensation of burning irritation in the genital tract. Among the reported risk factors for developing vulvovaginal candidiasis are immunosuppression, high oestrogen levels/pregnancy, diabetes, and antibiotic treatment [110]. Antibiotics can lead to candidiasis by causing disturbances in the vaginal microbiota. Both antibiotic treatment and a longer duration of treatment is associated with vulvovaginal candida infection. No difference in risk for vulvovaginal candidiasis has been shown between penicillins and other antibiotics [111]. To our knowledge, the incidence of vulvovaginal candidiasis from penicillin V treatment for pharyngotonsillitis has not been previously reported.

1.14 THE HUMAN VIROME IS LARGELY UNIDENTIFIED

The origin of viruses is unclear. Because many viral proteins have no existing counterpart in modern cells, viruses are assumed to be ancient, evolving from primordial cells that no longer exist [112]. The viruses are parasitic to all processes associated with life; they need a host cell to perform any production of energy. When they are not infecting a living cell they consist of only genetic material in some sort of envelope or capsid, a *virion* [113].

The viral particles in the human body, *the virome*, have been approximated to be greater than the number of bacterial and human cells [114]. However, most viruses in the human body are bacteriophages, parasitic to the bacteria in our intestines. The interactions between the virome and the bacteria within the human body increase the complexity of the ecosystem. Some virome compositions are associated with human disease and others with health. Despite the metagenomic sequencing and other methods that have revealed new insights to the diversity of the human virome, the majority of the virome is not yet identified [115].

1.15 RESPIRATORY TRACT INFECTIONS ARE OFTEN OF VIRAL ORIGIN

The respiratory tract is the most common site for viruses infecting humans [116] and viral respiratory tract infections (RTIs) are probably more common than bacterial in all age groups [117, 118]. While some viral RTIs are severe and have fatal outcomes, most viral infections are mild, self-limiting [116], or even asymptomatic [119].

Upper respiratory tract infections steadily hold a top position among diseases in terms of global incidence, though the incidence is elusive since most do not attend health care when experiencing an upper respiratory tract infection. Respiratory tract infections of all types have a very high incidence in young children [120], and is the main course of morbidity and mortality in this age group [117].

The upper respiratory tract refers to the nose, mouth and pharynx, and the lower respiratory tract to the trachea, bronchi, and lungs. The division between upper and lower respiratory tract is situated around the level of the tonsils [116]. For many years the lungs were considered sterile locations. However,

metagenomics has revealed that microorganisms also colonise the lower airways, and that the microbiota derives mainly from the microbes present in the upper respiratory tract [121]. Accordingly, the composition of the nasopharyngeal microbiota in children are correlated to the risk of developing RTIs [122]. Furthermore, a viral RTI can affect the mucosal immunity in ways that facilitate bacterial adherence [116, 123] and promote bacterial invasion of the airways [123].

1.16 RESPIRATORY TRACT INFECTIONS IN SWEDISH PRIMARY CARE

In Swedish primary care, the consultation rate for infections has remained around 30 % for several years, and most frequent are the unspecific upper respiratory tract infections (RTIs), including the common cold. Pharyngotonsillitis has for many years been the second most common RTI diagnosis, followed by cough and bronchitis. Among the RTIs pharyngotonsillitis is the diagnosis with the highest number of antibiotic prescriptions in Sweden [124].

As an effect of the COVID-19 pandemic, there was a temporary decrease in all visits to primary care physicians in 2020 [125]. Due to social distancing, improved hand hygiene and several interventions, the common RTIs markedly decreased [126]. In addition, there was a change in health-seeking behaviour in the population [125]. The sale of antibiotics in Sweden initially decreased during the pandemic, but in 2021 both visits and sales in antibiotics for respiratory tract infections returned to normal [78].

Over the years, the clinical guidelines for infections have evolved towards more restrictive prescribing strategies, especially in the respiratory tract infections. If antibiotic treatment is in question, penicillin V is the first line treatment for most RTIs in Swedish primary care. Although the number of consultations has been fairly consistent over the years, the proportion of antibiotic prescriptions in Swedish primary care has continued to decrease since 1992 and penicillin V is consistently the most used antibiotic [124].

1.17 INFLUENZA A VIRUS

Typically, influenza A epidemics occur during the winter season and cause acute febrile illness with both systemic and respiratory symptoms [127]. Children, pregnant women, the elderly, and people with chronic medical conditions are at higher risk for severe illness [128, 129], and influenza A has a substantial mortality among persons aged 65 years and older. Globally, influenza deaths are estimated at 290 000- 646 000 annually [130].

The most effective method to prevent influenza A infections is vaccination. The challenge of vaccinating against influenza A infection is the rapid evolution of the virus [131]. Influenza A has two surface glycoproteins of importance to its virulent and changing nature: haemagglutinin (H) and neuraminidase (N). Haemagglutinin attaches to the host cell and initiates virus entry, neuraminidase is important in viral replication and further virion release [127]. Vaccines against influenza A targets the haemagglutinin.

The ongoing changes of influenza A virus is due to antigenic drift and shift. *Antigenic drift* stems from a high genetic instability that continuously gives minor changes in the viral RNA, where some of the changes occur in antigenic regions of the surface glycoproteins, haemagglutinin and neuraminidase. *Antigenic shift* stems from the segmented structure of the influenza A genome. When coinfection with different types of influenza A viruses occurs in one cell, there can be an exchange of genetic segments between viruses, rapidly giving rise to a new assortment of influenza A variants [131]. Influenza A are zoonotic pathogens and infect both humans and other mammals, mostly birds and sometimes swine [131]. Influenza coinfections and the antigen shift occur in these animal reservoirs.

Different subtypes of influenza A have caused several global pandemics: in 1918 (H1N1), 1957 (H2N2), and 1968 (H3N2). The 1918 H1N1 pandemic was a major killer, causing 40-50 million deaths worldwide. New variants of H1N1 also caused a pandemic in 1977, and a pandemic known as “swine flu” in 2009 [132, 133].

Many influenza A virions contain incomplete genomic material and are non-infectious on their own. When several incomplete virions enter the same cell they compensate each other and cause an infection [134]. Thus, the inoculum

size (the number of infectious virions) is important for transmission, and also correlates to the severity of infection. The incubation time is only 1-2 days [135].

Antiviral treatment may have effect on mortality in severely ill and hospitalised influenza A patients [136-138]. Elderly primary care patients with comorbidities may recover 2–3 days earlier from influenza A infection if treated with antivirals [129]. However, diagnosing influenza A infections on clinical grounds alone is difficult due to non-specific symptoms of confirmed influenza infections [139]. There is a multitude of viruses, including influenza A and B, that can cause a similar combination of symptoms [140]. During an influenza epidemic, the prevalence of influenza viruses in patients with influenza-like illness (defined as fever in combination with at least one respiratory and one systemic symptom) is estimated at 52% [129].

PCR testing for influenza viruses is not available as a rapid and near-patient test in Swedish primary care. Eventual samples for etiological confirmation must be sent to a laboratory for analysis, and the results are not available during the consultation. C-reactive protein (CRP) is a cheap, near-patient test where the result is at hand within minutes. CRP is readily available in Swedish primary care. It has been unclear whether CRP can facilitate the clinical assessment of ILI in outpatients.

1.18 USE OF C-REACTIVE PROTEIN IN PRIMARY CARE

C-reactive protein (CRP) was introduced as a point-of-care test in Scandinavian primary care during the 1990s. The point-of-care CRP test, taken as a few drops of capillary blood, was expected to help physicians distinguish between viral and bacterial infections during consultation [141]. Bacterial infections of the airways were assumed to induce higher CRP than the viral infections, and different cut-offs to differ bacterial and viral respiratory disease have been suggested, ranging from 10 mg/L to 100 mg/L. However, there is a great overlap of CRP concentrations for bacterial and viral RTIs [142]. Moderately increased concentration of CRP (10-60 mg/L) is a common finding in primary care patients with viral RTIs, mostly with a peak during day 2-4 of the illness [143, 144]. Even though community-acquired pneumonia of

bacterial origin is associated with high CRP concentrations, CRP alone cannot discriminate between pneumonia and other RTIs [145, 146]. The hope for CRP to differ viral and bacterial infections has not been fulfilled. The capacity of viral respiratory diseases to cause very high CRPs is known from studies in hospitalised patients. Among viruses reported to induce high CRP concentrations in hospital settings are COVID-19, adenovirus and influenza A [147, 148].

CRP is an acute-phase protein produced in the liver in response to cytokines from inflammation caused by infection, tissue injury or other inflammatory processes [149]. Its biological role is in the first line defence against pathogens, promoting different immunological defence mechanisms including the phagocytosis of bacteria [150]. CRP concentration changes rapidly in response to inflammation. The CRP concentration in plasma can increase a thousandfold within 24-48 hours [151], and decreases rapidly when the stimulus is reduced or removed. The CRP half-life in plasma is about 19 hours, and is not affected by disease or other circumstances [149]. Thus, the timing of testing during the course of disease is of great importance. A test is often used to trace the progress of infectious diseases in patients during hospitalisation. CRP is the most popular near-patient test in Swedish primary health care. An interview study from 2015 found it common for general practitioners to, despite lack of evidence, use CRP to distinguish between viral and bacterial infections. The threshold used for this was commonly 50 mg/L, with a range of 40 to 200 mg/L according to the GPs interviewed [152]. Besides the variation of CRP interpretation between physicians, there is also a striking variation between different health care centres regarding the use of CRP in patients with respiratory tract infection [153]. Currently, one fourth of patients with RTIs in Swedish primary care undergo CRP testing [124], which indicates a reduction in the use of CRP over the years [153]. CRP influences the GPs in their decision on antibiotic prescribing for RTIs [154, 155], and has probably a reducing effect on overall antibiotic prescriptions in primary care [156]. Nevertheless, this effect is dependent on the context. CRP testing does not help GPs who already have a significantly low prescription rate of antibiotics to lower it any further [155].

CRP has been associated with severity of disease and increased mortality in hospitalised influenza A patients [157-161]. The CRP response to influenza

infection has been suggested to be higher in elderly than in younger subjects [162], and in patients with induced experimental influenza A, the peak concentration of CRP has been reported to occur at day three [163]. However, CRP concentrations in primary care influenza patients are not well investigated. One previous study found that CRP >5 mg/L was strongly associated with influenza A or B infection, compared to other viruses in primary care patients [164]. However, they included 79 patients who were a mixture of influenza-like illness (ILI) and other milder acute upper RTIs such as the common cold. Thus, CRP in patients attending primary care with more severe symptoms of ILI has not been evaluated.

1.19 ACUTE SORE THROAT IS MOSTLY SELF-LIMITING

Sore throat is a very common symptom, with the highest incidence during winter season. Only a minority - around one in 10 persons - experiencing a sore throat attend health care [165, 166]. Mostly, people self-manage the condition. Women and young people are more likely to have sore throat symptoms [166]. Most pharyngotonsillitis (acute sore throat) resolve spontaneously within one week, regardless of aetiology. People who experience a fever during a sore throat episode are usually fever-free after three days, while symptoms of sore throat may persist longer [167]. The consultations for pharyngotonsillitis in Swedish primary care have been rather constant for many years and are estimated at 11-17% of all visits due to infection. The number of antibiotic prescriptions for pharyngotonsillitis has steadily decreased since the 1990s. Still, GAS pharyngotonsillitis is the respiratory tract infection with the highest number of antibiotic prescriptions in Swedish primary care [124, 168].

Viral findings are very common in throat swabs from people with febrile sore throat [169-171]. Among bacteria, group A streptococci is the most common finding, but the prevalence of GAS varies widely depending on the setting, both in symptomatic and asymptomatic persons. The highest prevalence of GAS is reported in children with sore throat. The proportion of GAS positive throat swabs in children presenting to health care for sore throat symptoms is estimated at 37%, while in asymptomatic children the GAS carriage rate is estimated at 8-12% [30]. Corresponding estimates for positive throat swabs in adults presenting with sore throat symptoms are 24.1%, and approximately 2%

in asymptomatic adults [31]. In addition, the non-infectious causes of sore throat are plentiful, but not well investigated or quantified. Smoking, snoring, and having sleep apnoea are some physico-chemical factors associated with sore throat episodes. Also, sore throat is common in teachers after straining their voices at work [172].

1.20 DIAGNOSING PHARYNGOTONSILLITIS WITH GROUP A STREPTOCOCCI

Signs and symptoms cannot distinguish between different bacterial or viral causes of sore throat [169, 173, 174]. Symptoms of cough and coryza are generally useful in ruling out group A streptococci [175], but among patients free of these symptoms, signs and symptoms still cannot differentiate between different aetiologies [167]. Guidelines for the management of sore throats vary between countries [176, 177]. The Swedish clinical guidelines for pharyngotonsillitis focuses on identifying patients with more severe symptoms and a confirmed presence of GAS in throat swabs, since these patients may have some relief of symptoms from treatment with penicillin V [178]. Until 2012, the guideline recommended a rapid test for GAS in patients with 2–4 Centor criteria. Subsequently, the recommendation was changed to patients with 3–4 Centor criteria. Patients with less severe symptoms have no effect of antibiotics, regardless of the cause of infection. Thus, in patients with Centor score 2 or less, testing and antibiotics are not recommended.

The Centor criteria is based on a logistic regression analysis that evaluated parameters for predicting a GAS positive throat culture in adults with sore throat. The model identified four variables of significance: fever, tender anterior cervical lymph nodes, coatings of the tonsils and lack of cough. Presence of tonsillar exudates (coating of the tonsils, pus) was the most important variable. According to the model, the probability of GAS in a throat culture when all four variables are present was 55.7%, and with three variables present the probability of a positive culture was 30.1-34.1% [173]. Patients with four Centor criteria report more severe throat pain than patients with three criteria [179]. The criteria are considered to select patients who have both a higher probability of GAS in throat swabs and who have more severe symptoms from pharyngotonsillitis.

According to the Swedish guidelines, it is recommended to confirm the presence of GAS in patients with 3-4 Centor criteria before treatment, and a negative test for GAS should be used as a stopping rule for antibiotics [180]. A rapid antigen detection test for GAS is the standard measure, and throat culture is restricted to patients with complicated disease. In spite of its simplicity, physicians' adherence to the guideline for pharyngotonsillitis is low [181], with large variations in adherence between different GPs [182]. Some physicians use RADT for GAS also in patients having 1-2 Centor criteria, and do not use a negative RADT for GAS as a stopping rule for antibiotics.

There are a few alternative scores for assessing sore throat patients, with roughly the similar type of criteria as the Centor score. The FeverPAIN score, employed in the UK, has a criteria also for rapid attendance (illness ≤3 days when attending health care) [109]. The McIsaac score, has included age as an additional criterion, thus giving patients between 3 and 14 years an additional point and those aged ≥45 years, one point off. However, a meta-analyses of Centor and MacIsaac scores have found them to be equally limited in performance to find GAS positive sore throat patients and concluded both scores need the augmentation of a rapid test for GAS in the diagnosis of GAS pharyngotonsillitis [183].

1.21 RAPID TESTS FOR GROUP A STREPTOCOCCI

For many years a throat culture on blood agar plate was the standard method for identification of GAS in patients with pharyngotonsillitis. The introduction of rapid antigen detection tests (RADTs) in the 1980s fundamentally changed the diagnostic procedure. While the cultures have a turnover time of 1-2 days in a laboratory, the RADTs for GAS are locally available giving results within minutes [184]. The first rapid tests for GAS used latex agglutination technique, which was later changed to different types of immuno-assay methods due to higher sensitivity. Since antibiotics are often prescribed while waiting for throat culture results, the employment of RADT for GAS reduces the use of antibiotics for pharyngotonsillitis [185-187].

When RADT for GAS was introduced the sensitivity and specificity for RADT were calculated using throat culture as a reference. However, it is unknown which of the tests is closest to the truth, and RADT may perform better than

routine throat culture for diagnosing GAS pharyngotonsillitis. The use of routine throat culture as a reference standard has been challenged by a Norwegian research group that used additional media for throat cultures and yielded an additional 15% GAS positive cultures [34]. Further, most of the positive RADTs with negative conventional throat culture results (76%) are associated with positive polymerase chain reaction (PCR) for GAS, suggesting a specificity for RADT close to 100% when compared to PCR [33]. Today, in some places, real-time PCR is considered a clinical laboratory routine for diagnosis of GAS infection [184]. Obviously, none of these tests can differ the asymptomatic carriage of GAS from disease.

In clinical practice, the reliability of RADT for GAS after recent antibiotic therapy for GAS pharyngotonsillitis has been questioned. The suspicion has been that antigens from dead bacteria remain a while and thus give false positive RADT for GAS [188, 189]. There are three earlier studies on RADT after antibiotic treatment for GAS pharyngotonsillitis. However, they used an older rapid test method (latex agglutination technique), low or unspecified treatment dosages, were conducted only in paediatric patients, had a very small sample size, did not specify the inclusion criteria, and/or reported inconclusive results [188-190]. Thus, previous studies have not determined if the modern immuno-assay RADT for GAS is reliable after recent penicillin V treatment for pharyngotonsillitis.

1.22 GROUP A STREPTOCOCCI

Streptococcus pyogenes, also known as group A streptococcus (GAS), is primarily adapted to the narrow ecological niche of human tissues [191]. It is a Gram-positive bacterium that causes a multitude of diseases: pharyngotonsillitis, scarlet fever, impetigo, erysipelas, and invasive infections including necrotising fasciitis and streptococcal toxic shock syndrome [192-194]. In addition, GAS pharyngotonsillitis may trigger immunologic disease, such as acute rheumatic fever and chronic rheumatic heart disease [192, 194]. The most common manifestation of GAS disease is pharyngotonsillitis [192]. Mortality from GAS disease is mainly due to the immunologic sequelae and the invasive diseases [195], together accounting for over 500 000 deaths each year and placing GAS among the top 10 infections causing human mortality [192].

The primary habitats of group A streptococci are the upper respiratory tract epithelium and the superficial layers of epidermis of humans. No environmental reservoir is known, and the transmission of GAS occurs person to person via droplets or via direct contact. Environmental risk factors for GAS disease are cold winter seasons for pharyngotonsillitis and tropical climate for impetigo [191].

The incidence of GAS infections is not fully known, though suspected to be several times higher in low-income than in high-income countries [192]. With improved living standards and available antibiotics the incidence of GAS diseases in high-income countries declined during the 20th century [193]. Being a plague of poverty and overcrowding, GAS diseases remain a significant health problem in many low-income countries, and in disadvantaged populations within high-income countries [192, 194]. At least 95% of the deaths from GAS infections are estimated to occur in the disadvantaged areas [192]. GAS pharyngotonsillitis affect people of all ages but is most common in school-aged children and adolescents. The youngest children may have profuse rhinitis when infected by GAS. Throat infections from GAS are the most infectious in the acute phase, and the incubation time is estimated at 2-5 days [196].

The cell surface antigens of GAS show high diversity due to a combination of horizontal gene transfer with genetic recombination and point mutations. There is also a strong selection pressure from the human immune system, and different GAS strains adapt to the microenvironments of either human throat or skin [191]. The classification of β-haemolytic streptococci is made from carbohydrate and protein surface antigens. Group A streptococci are recognised by the group A carbohydrate on the cell surface, as first described by Rebecca Lancefield in the late 1920s [197]. Further serological classification of GAS can be made from differences in the surface M protein [191]. The surface M protein is one of the factors important to the streptococcal virulence and encoded by the *emm* gene [194]. Over 220 different *emm* types are documented and known to have a varying distribution between different populations. The implication for the variation of *emm* types for GAS epidemiology in different parts of the world is not clear [193, 198, 199]. The human immune defence towards GAS infections is largely M type specific

[191]. The emergence of new strains of GAS, with new variants of surface virulence factors, can lead to increased rates of disease [193].

Despite penicillin use of over 80 years, for a long time no resistance to penicillin V or other β-lactams was reported in group A streptococci [195, 200]. Contrarily, GAS with resistance to clindamycin, macrolides, tetracycline, and other antimicrobials has been well documented. The reason for this long-preserved GAS susceptibility to penicillin V is unclear, and so is its future [195]. There is a recent report of GAS isolates with elevated MICs for several β-lactams, with a documented mutation of the penicillin-binding proteins [201]. If GAS will follow the same path of mutations in penicillin-binding proteins as have *S. pneumoniae* the situation can escalate very quickly. There is as yet no vaccine for group A streptococci [195].

1.23 COMPLICATIONS FROM PHARYNGOTONSILLITIS WITH GROUP A STREPTOCOCCI

The immune complications from GAS pharyngotonsillitis, acute rheumatic fever (ARF) and its chronic sequelae rheumatic heart disease, accounts for the greatest burden of morbidity and mortality due to GAS [192]. Today, acute rheumatic fever is endemic mainly in low-income countries and in certain indigenous populations in high-income countries [192, 202, 203]. In Sweden ARF is extremely rare. The incidence was 6 patients per year during 2020 and 2021, in a total population of 10 million [204].

The risk for rheumatic fever is associated with socioeconomic deprivation, low birth weight, lack of medical care, poor sanitary conditions, and household crowding [205, 206] and the large-scale decrease of acute rheumatic fever seen in some countries is understood as a result of socioeconomic improvements in combination with preventive penicillin treatment for GAS pharyngotonsillitis [207]. Antibiotic treatment of GAS pharyngotonsillitis can prevent ARF [203], but possible only in the endemic areas.

The suppurative complications of sore throat - quinsy or peritonsillar abscess, otitis media, sinusitis, impetigo, and cellulitis - are uncommon in high-income countries. In fact, they are so rare that the possible effect of antibiotic treatment to prevent them is difficult to study [208, 209]. There is probably a preventive effect towards peritonsillitis and acute otitis media, but the numbers needed to

treat to prevent one complication is very high [167]. A prospective observational study in the UK found that the proportion of sore throat patients having a tonsillar abscess was 0.5% (30/5932) if receiving antibiotics and 0.2% (11/4974) if not receiving antibiotic treatment (not randomized). In this study, neither patient history nor findings was useful to predict the suppurative complications from a sore throat [208].
Scarlet fever, or scarlatina, affects mainly children and young people [210]. It often presents with high fever, sore throat, strawberry-red tongue, and a scarlet red rash that feels rough as sandpaper [211]. The higher incidence of scarlet fever in children may be due to a weaker acquired immunity to GAS [212]. For many years the incidence and severity of scarlet fever was declining. However, there has been an increasing number of cases in the last decade, with local outbreaks in different parts of the world [211]. In 2022, after the COVID-19 pandemic, Europe experienced some severe cases of scarlet fever and invasive GAS infections causing death in otherwise healthy children.

1.24 TREATMENTS FOR SORE THROAT

The main purpose for antibiotic treatment of sore throat in high-income countries is to reduce symptoms [107]. A Cochrane meta-analysis showed that benefit from antibiotics is seen only in sore throat patients with confirmed GAS aetiology and with more severe symptoms [202]. Ten days of penicillin V 1000 mg three times daily is the recommended treatment in several countries [107, 108, 213] including Sweden [180], but there are guideline variations between countries [109, 177, 214]. Some guidelines advise against antibiotics for pharyngotonsillitis, on the grounds that it is a self-limiting condition with very low risks of complications and severe disease [176]. In countries where rheumatic fever is prevalent antibiotic treatment for GAS pharyngotonsillitis is strongly recommended.

The first studies supporting a 10-day penicillin V treatment for pharyngotonsillitis was conducted when GAS infections had a different epidemiology, and the threat of acute rheumatic fever made bacteriological eradication important. The first studies used intramuscular depot injections of penicillin once daily [215-217], and the main outcome was bacteriological eradication of GAS in the throat. In some studies, patients were asymptomatic carriers of GAS. Later, the studies were made with tablets of low doses of

penicillin V once or twice daily (375 mg x 1, 750 mg x1, 250 mg x2, 800 mg x 2) [218-220].
Despite extensive research in treatments for pharyngotonsillitis the efficacy of short and long courses of penicillin V remains unclear. Meta-analyses have shown inconclusive results [167, 214, 221, 222]. The included RCTS are using different diagnostic criteria and lower dosages of penicillin V than the current recommendation. The trials are conducted in various patient populations: only children, adolescents or adults, and mixed age groups. Notably, some meta-analyses also included studies from the 1950s, so the included studies were conducted in different epidemiological situations regarding GAS disease.
A more recent study compared the effects of penicillin V with a daily dosage of 2000 mg (500 mg x 4, or 1000 mg x 2) for 5, 7 and 10 days and found no statistical differences in clinical outcome. However, this was a prospective observational study. Patients were not randomized, and the treatment groups differed in symptom severity, which means that the effects of the different durations of treatment are not comparable [223].

The number of young children included in these studies was small. Although the presence of GAS is higher in children with sore throat than in adults, the cause of the symptoms may be something else. A double blind and placebo controlled RCT found no effect from penicillin V treatment for pharyngotonsillitis in children. However, this study included patients with 2 or more Centor criteria and did not confirm the presence of GAS in the included patients [224]. The efficacy of modern dosage penicillin V for pharyngotonsillitis in children with a confirmed presence of GAS has not been evaluated.
Critical to the efficacy of penicillin V treatment is time above minimum inhibitory concentration (MIC). Due to rapid renal excretion the dose and frequency of penicillin V intake is decisive to achieve sufficient time over MIC [97-99]. The low dosages of penicillin V in the earlier studies gave none or a very short time over MIC which limited treatment efficacy. Theoretically a more frequent and adequate penicillin V dosage may be more effective, even when the duration of treatment is shorter.
There is no evidence that antibiotics prevent recurrent sore throat [225]. Regarding suppurative complications antibiotic treatment for pharyngotonsillitis may have some preventive effect to quinsy (peritonsillitis) and infections of the middle ear. However, the numbers needed to treat to

prevent one suppurative complication is high, with variation depending on context. Non-suppurative complication, glomerulonephritis, is so rare that a possible preventive effect from antibiotic treatment has not been determined [167].
Treatment with paracetamol and NSAID effectively reduce sore throat symptoms. The NSAID ibuprofen has turned out particularly effective due to rapid onset of the effects [226].

1.25 STRAMA

The Swedish strategic programme against antibiotic resistance (Strama) was founded in the 1990s. Today, Strama is as a union of organizations, including the Public Health Agency, Swedish medical product agency, National veterinary institute, and others. Stramas work has a One Health perspective and recognises that the connections between people, animals, plants, and the environments are important for the health of all beings. Among other things Strama has taken action to strengthen the rational use of antibiotics locally and to develop surveillance of antimicrobial resistance. The regional Strama organisations support the primary health care units with training and implementation of the clinical guidelines, and they offer feed-back to clinicians including local statistics on antibiotic prescribing [227].

Since 1992 the overall decrease in use of antimicrobials in Swedish patients is 43%, and the largest decrease is seen in Swedish children younger than 4 years where the reduction of antibiotic prescriptions has been 73% [227]. Compared to other European countries the prescription of antibiotics in Swedish primary care is relatively low [228], and subsequently the incidence of antimicrobial resistance in the bacteria is low compared to many other countries [78, 81]. Still, there is a wide variation in antibiotic prescription rates between different regions of the country [229] and between different prescribers [230] that has no equivalent variation in the prevalence of infections. Thus, there are opportunities for further improvements in the prescribing practice.

1.26 REMAINING DILEMMAS

Despite extensive previous research on treatments for GAS pharyngotonsillitis the effectiveness of shorter courses of penicillin V remains unknown. Neither

has the side-effects from long and short penicillin V therapy been thoroughly evaluated. Although penicillin V has been in clinical use for almost 80 years by now, the ecological effects on the intestinal microbiota from treatment remains largely unknown. Further, RADT for GAS is known to reduce prescription of antibiotics for pharyngotonsillitis in both children and adults, but the reliability of the test recently after penicillin V treatment for pharyngotonsillitis has remained unclear. C-reactive protein (CRP) is the most used point-of-care test in respiratory tract infections in Swedish primary care, and annual influenza A epidemics accounts for considerable morbidity and mortality, especially in the elderly. Nonetheless, CRP concentrations in primary care patients with confirmed influenza A and influenza-like illness is largely unknown.

2 AIM

The overall aim of the thesis was to contribute with new knowledge of respiratory tract infections and to a safe reduction of antibiotic use in primary health care.

2.1 SPECIFIC AIMS OF THE THESIS

PAPER I

- To determine if adequate clinical effectiveness was maintained with a reduced course of penicillin V for pharyngotonsillitis with GAS, 800 mg four times daily for five days, compared to the current recommended treatment of penicillin V 1000 mg three times daily for 10 days.
- To compare bacteriological eradication of GAS in the throat in patients after a 5- and 10-day penicillin V treatment.
- To compare the frequency of relapses within one month after GAS pharyngotonsillitis in patients with 5- and 10-day penicillin V treatment.
- To compare the frequency of complications and new pharyngotonsillitis in patients with 5- and 10-day penicillin V treatment for three months from the first episode of GAS pharyngotonsillitis.
- To compare patterns of adverse events in patients with 5- and 10-day penicillin V treatment for GAS pharyngotonsillitis.
- To compare time to relief of fever and throat symptoms in patients with 5- and 10-day penicillin V treatment for GAS pharyngotonsillitis.

PAPER II

- To compare the presence of GAS in RADT and throat culture among patients recently treated with penicillin V for GAS pharyngotonsillitis.
- To examine factors associated with agreement of test results of RADT and throat culture for GAS in patients recently treated with penicillin V for GAS pharyngotonsillitis.

PAPER III

- To evaluate penicillin V effects on the emergence of β-lactam resistance in the faecal microbiota directly after, and at follow-up 7-9 days after treatment.
- To evaluate ecological alterations in the faecal microbiota after penicillin V treatment measured as overgrowth and species shift within the phylum Enterobacterales, enterococci, and the presence of *Candida* spp., and *C. difficile*.

PAPER IV

- To examine if CRP can predict presence of influenza A or B in primary care patients presenting with influenza-like symptoms.
- To examine the association between CRP and influenza-like symptoms in patients with ILI.

3 PATIENTS AND METHODS

3.1 PATIENTS

In papers I-III patients were recruited in Swedish primary healthcare between September 2015 and February 2018. Patients with sore throat aged 6 years and over were consecutively assessed for inclusion at 17 healthcare centres. Patients were eligible if having three or four Centor criteria (fever ≥38.5°C; tender anterior cervical lymph nodes; coatings of the tonsils (for children ≤6 years inflamed tonsils); absence of cough); and a positive rapid antigen detection test for group A streptococcus.

Paper II had additional inclusion criteria: except from a positive RADT for GAS at the start, all patients had to have a positive throat culture for GAS at inclusion and also conclusive throat swabs for RADT and throat culture at follow-up within 21 days from randomisation. Since all patients in paper II had both tests positive at the beginning, we could compare the decline of positive tests at follow-up.

In paper III patients were recruited from six primary healthcare centres, a subset of the 17 centres from paper I. The study had two additional inclusion criteria: patients were eligible if aged 10 years and over, and if able to produce faecal samples regularly.

Exclusion criteria in paper I-III were: signs of serious illness or hypersensitivity to penicillins; immunomodulating treatment corresponding to at least 15 mg prednisolone; antibiotic treatment for pharyngotonsillitis in the past month (relapse); or any antibiotic treatment within 72 hours of inclusion. Paper III had an additional exclusion criterion: patients with antibiotic treatment during the previous month were excluded. The study population of paper III consisted of patients with both 5- and 10-day penicillin V treatment, thus the included patients had not the same duration at risk for selective pressure promoting emergence of resistance in the intestinal microbiota.

Paper IV was conducted in a subset of the patients participating in a randomised controlled trial (RCT) on the effectiveness of oseltamivir treatment of influenza-like illness [129]. The patients were recruited in 30 primary healthcare centres in Lithuania, Norway, and Sweden when the prevalence of influenza passed the threshold for a seasonal influenza epidemic in each nation during January 2016 and April 2018.

Patients were eligible for paper IV if aged 1 year or over and attending primary care with influenza-like illness within ≤72 hours of onset of symptoms. Influenza-like illness (ILI) was defined as self-reported fever, with at least one systemic symptom (headache, muscle ache, sweats or chills or tiredness) and one respiratory symptom (cough, sore throat or runny or congested nose). The main exclusion criterion in paper IV was need for urgent hospital admission. Additional exclusion criteria, mainly related to the clinical drug trial that paper IV was part of.

Table 1. Inclusion and exclusion criteria of paper I-IV.

	INCLUSION CRITERIA	**EXCLUSION CRITERIA**
I	• sore throat patients attending primary care • aged 6 years and over • 3-4 Centor criteria: fever ≥38.5°C, tender anterior cervical lymph nodes, coatings of the tonsils (in children 6 years inflamed tonsils), absence of cough) • positive rapid antigen detection test for group A streptococci	• serious illness • hypersensitivity to penicillins • immunomodulating treatment • antibiotic treatment for pharyngotonsillitis in the past month • any antibiotic treatment within 72 hours of inclusion.
II	Same inclusion criteria as paper I, and: • a positive RADT for GAS *and* a positive throat culture for GAS at inclusion • throat swabs for both RADT and throat culture at a follow-up visit within 21 days	Same exclusion criteria as paper I.
III	Same inclusion criteria as paper I, and: • aged 10 years and over • ability to produce timely faecal samples	Same exclusion criteria as paper I, and: • any antibiotic treatment during the previous month

	INCLUSION CRITERIA	EXCLUSION CRITERIA
IV	Inclusion took place when the prevalence of influenza passed the threshold for a seasonal influenza. • attending primary care for influenza-like illness (ILI) within ≤72 hours of onset of symptoms • aged 1 year or over	• need for urgent hospital admission • chronic renal failure • impaired immunity • need for immediate antiviral treatment • allergy to oseltamivir • planned general anaesthesia within 2 weeks • less than 6 months life expectancy • severe hepatic impairment • need for live viral vaccine within 7 days

3.2 ETHICAL CONSIDERATIONS

All participating patients and eventual guardians were informed of the studies, verbally and in writing, and provided written consent before participation. In the case of children under 15 years both the child and guardian/guardians gave consent before participation. All studies were conducted according to the ethical principles of the Declaration of Helsinki. Monitoring was performed according to Good Clinical Practice.

Patient participation in these studies was voluntary. The patients were free to withdraw at any time. The data was coded and presented on a group level, and the results presented so that individual participants cannot be identified. The code keys were kept separate. All participating patients were included based on clinical indications and the negative side-effects of antibiotic treatments would have occurred regardless of study participation. The study on CRP and influenza was conducted as a sub study within a randomized controlled trial on the effectiveness of oseltamivir treatment for patients with ILI. Oseltamivir is a licensed medication and the study used only the standard dose of oseltamivir according to marketing authorisation. The extra samples and measures taken in these studies could have meant short discomfort for patients. Side effects and adverse events were reported according to Good Clinical Practice. All studies were initiated by researchers and clinicians. None of the studies have connections with any pharmaceutical company.

We published a study protocol [231] for paper I, and the study was registered in EudraCT 2015-001752-30; ClinicalTrials.gov NCT02712307 [231]. Definitions of outcome measures, correction of data and decisions on definitions and variables were made in advance. The randomised controlled trial was approved by the Swedish Medical Products Agency, MPA (Läkemedelsverket) 3 July 2015, reference number 5.1-2015-41783. The studies reported in paper I-III were approved by the Regional Ethical Review Board in Lund (reference number 2015/396) and monitored by Uppsala Clinical Research Centre and the Centre for Primary Health Care Research.
The cross-sectional study on CRP and influenza was approved by the Regional ethical review board in Gothenburg, Sweden (reference number 580-15), Regional committees for medical and health research ethics Southeast Norway (2015/932/REK sør-østA) and Lithuanian Bioethics Committee (2015-09-07 No P-15-73).

3.3 RANDOMISATION AND DATA COLLECTION

Paper I was a randomised controlled trial (RCT) on penicillin V treatment for GAS pharyngotonsillitis. The study had two parallel treatment arms: penicillin V 800 mg four times daily for five days and penicillin V 1000 mg three times daily for 10 days. All patients were examined by a physician at the inclusion visit. Computerised randomisation within fixed blocks was prepared in advance and distributed in opaque envelopes to be opened in consecutive order by the local investigators. The study was open: after randomisation both patients, physicians and research nurses knew which patients had 5- and 10-day treatments.
Both RADT for GAS and throat culture were taken at inclusion, and at the follow-up visit. In children, we used two swabs at one time rotated towards the tonsils and the pharynx to avoid the discomfort of two samplings. RADT for GAS was performed with the local available equipment. The available tests were either OSOM Strep A test manufactured by Sekisui diagnostics or QuickVue DipStick Strep A test manufactured by Quidel. Swabs for culture were sent to regional microbiological laboratories and incubated on blood agar plates in 35-37 ˚C overnight. Any growth of group A streptococci was regarded as a positive result.
In paper I-III physicians prescribed penicillin V according to randomisation, and the patients obtained the drugs from their local pharmacy. The penicillin

V treatment was given as oral tablets and the dosages for children were adjusted according to weight up to 40 kg: 10-20 kg 250 mg per dose, 20-40 kg 500 mg per dose. Patients or their guardians were asked to fill in a diary until the follow-up visit. The patients noted their symptoms graded in a Likert scale (no symptoms, mild, moderate, and severe symptoms) and each dose of penicillin V in the diary until the follow-up visit 5-7 days after the last dose. Follow-up took place at the same time after completed treatment in both randomisation arms with the intention of equal time without penicillin V protection at the *test-of-cure* in all patients. The primary outcome was if patients were clinically cured, defined as complete recovery without major residual symptoms or clinical findings of pharyngotonsillitis. Hence, the primary outcome was a combination of the patient's reporting and the physician's clinical examination. For each visit the physician recorded a clinical judgment of throat status in the case report. Eventual adverse events were recorded at the follow-up. Also, the patients (or their guardians) self-reported adverse events and side effects from treatment in the diary. Regional research nurses made telephone calls to patients or their guardians one month and three months after completion of penicillin V treatment. They asked for throat symptoms, relapses or new tonsillitis, complications, and adverse events. When patients had complications, the research nurses collected data from the medical records retrospectively.

In paper III we studied changes in the faecal microbiota in patients during penicillin V treatment for pharyngotonsillitis. The patients were asked to deliver three faecal swab samples: the first sample was collected before or within 18 hours of the first dose of penicillin V of penicillin V treatment, the second at the last dose, and the third and last sample at follow-up 7-9 days after completed treatment. If patients were unable to produce a faecal sample within the requested time frame (less than 18 hours from start of penicillin V treatment) the swab sample was instead taken rectally. The patients received a written and illustrated instruction on how to take a swab sample together with materials for faecal swab samples (1 ml Eswabs in a transport medium). All the faecal specimens were taken by the participating patients themselves and sent to the Public Health Agency of Sweden in prepaid envelopes. The faecal samples were stored at -70˚C pending analyses.

In paper IV we investigated CRP in patients with influenza-like illness within 72 hours from onset of symptoms. Information about temperature, pulse,

duration of symptoms, comorbidity and the severity of ILI-related symptoms was noted in a case report form during the inclusion visit. The main outcome measures were capillary blood CRP and presence of influenza A or B. For identification of the pathogens, we used an oropharyngeal and a nasal swab in patients under 16, and a nasopharyngeal swab for those 16 years and over. The swabs were sent to a central study lab for PCR analysis. The capillary blood CRP was analysed with the local available equipment at the primary health care centres. The 30 participating primary healthcare centres had devices of different brands for CRP testing.

3.4 SPECIFIC MICROBIOLOGICAL METHODS

In paper III the faecal samples were inoculated on selective agar media and incubated aerobic and anaerobic according to table 2. Samples from the first five patients were inoculated on a duplicate set of agar media to ascertain that the analyses were reproducible. Ecological changes were measured as:

- reduced susceptibility to beta-lactams (ampicillin and third generation cephalosporins)
- species shift among Enterobacterales (from *E. coli* to non-*E. coli* Enterobacterales)
- overgrowth of and species shift among enterococci (*Enterococcus faecalis* towards non-*E. faecalis)*
- new-colonization with *Candida* spp.
- new-colonization with *C. difficile*

The different colony types were counted and semi-quantitatively assessed in relation to growth on a non-selective control plate. Semi-quantitative assessment was made according to a 0-3 score: 0 corresponded to no growth, 1 to mild growth, 2 to moderate growth and 3 to rich growth. To analyse species shifts we approximated the fraction of the total colony counts, and a 0-3 score corresponded to a fraction of 0%, 25%, 50% and >75% of the total counts. Representative colony types of the bacteria were isolated from the selective agar media, isolated in a pure culture, and then identified by MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight).

Table 2. Selective agar media and incubation methods used in paper III.

SELECTIVE AGAR MEDIA	**TARGETED ORGANISMS**	**INCUBATION**
CHROMagar MH Orientation	Enterobacterales	Aerobic 35±1ºC for 18-24 hours
CHROMagar MH Orientation + 2 ampicillin discs (10µg) + 1 linezolid disc (30µg)	Enterobacterales with resistance to ampicillin (linezolid was used to supress Gram-positive bacteria)	Aerobic 35±1ºC for 18-24 hours
CHROMagar C3GR	Enterobacterales with reduced susceptibility to third generation cephalosporins	Aerobic 35±1ºC for 18-24 hours
CHROMagar C. difficile	*Clostridioides difficile*	Anaerobic 35±1ºC for 48 hours
CHROMagar Candida	*Candida spp.*	Aerobic 35±1ºC for 48 hours
Enterococcus faecium ChromoSelect Agar Base	*Enterococcus spp.*	Aerobic 35±1ºC for 18-24 hours
BKVagar	*Bacteroides spp.*	Anaerobic 35±1ºC for 48 hours

3.5 STATISTICAL METHODS

PAPER I

The primary outcome of paper I was clinical cure five to seven days after completed penicillin V, comparing 5- and 10-day treatment regimens. The inferiority margin was set to 10%. Thus, the *null hypothesis* was that the 5-day treatment with penicillin V would have at least 10% less clinical efficacy than the 10-day treatment. A rejection of the null hypothesis would imply a conclusion of non-inferiority.

The secondary outcomes in paper I were bacteriological eradication; frequency of relapses within one month after diagnosis; frequency of complications and new pharyngotonsillitis during the three-month study period; and patterns of adverse events. We also assessed the time to relief of fever and throat symptoms graded on a Likert scale (no symptoms, mild, moderate and severe) from the patients' diaries. Adherence to treatment was evaluated as the number of doses recorded in the diaries. We made a sensitivity analysis to evaluate the primary outcome. Analysis was made at five, seven and nine days after randomisation. The sensitivity analysis was based on a "yes" to a question in the diaries: "Do you consider yourself or your child cured from the current infection?".

In paper I the populations in the analysis were:

- all randomized patients
- the modified intention to treat population (patients who received at least one dose of penicillin V)
- the per protocol population (patients who met the inclusion criteria and did not fulfil the exclusion criteria; had no major deviations from the study protocol; took at least 80% of the study drug doses; had a test of cure evaluation eg assessment by a physician at follow-up).

The categorical variables were analysed with Fischer´s exact test and the continuous variables with the Mann-Whitney U test. The difference in primary outcome between the two treatment arms was presented as numbers and percentages and with a two-sided 95% confidence interval. We performed analyses for the primary outcome on the per protocol population, on the modified intention to treat population and on all randomized patients. We made supplementary analyses on the modified intention to treat population, both with the missing values imputed, and without imputation. Subgroup analyses were

made for the primary outcome regarding gender, age (under or over 18 years), and Centor score 3 and 4. Time to relief of symptoms was visualised in a Kaplan-Meier curve and the difference between the treatment arms was analysed with a log rank test.

PAPER II

In paper II we presented the results of RADT and culture for GAS taken at follow-up in a cross-tabulation. The decline in positive tests for GAS was presented in a Kaplan-Meier curve and differences between the distribution of positive RADT and positive culture for GAS was analysed with a log rank test. The *null hypothesis* was that there was no difference between positive RADT and a positive culture at any point during follow-up. We made a multivariable binary logistic regression analysis to evaluate certain characteristics for association with agreement between RADT and throat culture for GAS at the follow-up visits. The evaluated variables were treatment regimen (5 or 10 days), number of days from inclusion, clinical cure at follow-up (yes/no), gender and age (children 6-15 years versus 16 years and older).

PAPER III

In paper III we used entirely non-parametric tests. The *null hypothesis* was that there would be no difference in the distribution of resistance in Enterobacterales before and after penicillin V treatment, regardless of treatment duration. The analysis was made separately for Enterobacterales with ampicillin resistance respective decreased susceptibility to third generation cephalosporins. We also analysed the number of ampicillin-resistant Enterobacterales and non-Enterobacterales Gram-negative species in the whole study population before and after penicillin V treatment. Our focus was possible changes and new colonisations; thus species present in all three samples were not included in the analysis.

Since there is vast variation between individual microbiota all the faecal samples from the same individual were analysed as paired data using the McNemar test. The semi-quantitative assessment was not used in the statistical testing, we instead used dichotomised culture results: whether colonised or not. Number of resistant Enterobacterales and non-Enterobacterales Gram-negative species, between samples 1 and 2, and between samples 1 and 3, were analysed with paired Wilcoxon signed rank test.

PAPER IV

In paper IV we made multivariable binary logistic regression models. In the first model we tested patient variables for association with CRP ≥30 mg/L, which was used as the dependent variable in the model. The *null hypothesis* was that there was no association between variables. We chose 30 mg/L as a compromise cut-off, since previous studies on CRP have used cut-offs ranging between 5-50 mg/L. The variables significantly associated with a CRP ≥30 mg/L underwent a second multivariable binary logistic regression.

We made multivariable binary logistic regression models also to analyse factors associated with either the presence of influenza A or the presence of influenza B. In these analyses CRP was used as a continuous independent variable and was divided by 10 to provide the odds ratio for CRP concentration in incremental steps of 10.

4 RESULTS

4.1 STUDY POPULATIONS

PAPER I

In paper I we included and randomised 433 patients to either 5 or 10 days of penicillin V. However, 7 randomised patients were not meeting the inclusion criteria and 4 declined to participate after randomisation. Hence, 422 patients who received at least one dose of the allocated penicillin V treatment and constituted the *modified intention to treat* (mITT) population. We found no significant differences in the demographic data between the two treatments groups. The median age in the 5-day arm was 30.0 years (IQR 17-38) and in the 10-day arm 31.0 years (IQR 18-38). Patients <18 years constituted 25.9% (55/212) of the 5-day arm and 23.8% (50/210) of the 10-day arm. There was a predominance of female patients in both treatment arms, with 65.1% (138/212) women among the patients receiving 5-day and 62.9% (132/210) receiving 10-day penicillin V treatment. The median days of sore throat was 3.0 in both arms, and both treatment groups had similar numbers of patients presenting with 3 and 4 Centor criteria. The number of Centor criteria present in the mITT population is presented in table 3.

Table 3. The number of Centor criteria present in the mITT population.

CENTOR CRITERIA AT INCLUSION (422 patients)		
Centor criteria:	1-2	2
	3	207
	4	213
Fever (≥38.5°C)		318 (75.4%)
Tender anterior cervical lymph nodes		389 (92.2%)
Coatings of the tonsils		367 (87.0%)
Absence of cough		401 (95.0%)

Of the 422 patients in the mITT population, we excluded 25 more patients from the *per protocol* (PP) population for the following reasons: 6 patients were not meeting the inclusion criteria, 4 took less than 80% of the study drug, 7 declined follow-up contact, 6 chose to cancel participation, and 2 were excluded due to adverse events. Hence, 397 patients represented the PP population.

PAPER II

In paper II we analysed a subset of the 422 patients in the mITT population of paper I. To compare the results from RADT and throat culture for GAS at follow-up after treatment we included the patients who had both tests positive from the start. Therefore, 66 patients having a negative throat culture at the inclusion in paper I were excluded in this study. We also excluded 33 patients who did not have a follow-up visit within 21 days from inclusion, and a further seven patients were excluded for having no RADT taken at follow-up. Hence, 316 patients were included in the analyses of paper II. The median time to follow-up visit was 13 days from inclusion. Characteristics of the patient population in paper II are presented in table 4.

Table 4. Characteristics of 316 patients in paper II.

Women	66% (210/316)
Median age (years)	31 (IQR 17-38)
Children 6-15 years	24% (77/316)
5-day treatment	56% (176/316)
10-day treatment	44% (140/316)
Some level of remaining symptoms at follow-up	9.5% (30/316)

PAPER III

In paper III we included 31 patients also participating in the RCT of paper I. Of these, 29 patients delivered three faecal samples each. Two patients lacked the second sample. One patient had received an antibiotic treatment within 3 months before participation in the study. However, the first sample from this patient gathered no ampicillin resistant organisms. Eighteen patients were randomized to the 5-day penicillin V treatment and 13 to the 10-day treatment. All patients were part of the PP population of paper I and reported 100% adherence to penicillin V treatment. There was a female predominance of participants also in paper III: 79% (23/29) were women and 21% (6/29) men. The median age was 38 years (IQR 25-45).

PAPER IV

In paper IV we included 281 primary care patients with influenza-like illness during the seasonal influenza epidemic. In 277 of the patients the results from both PCR and CRP were available. Hence, we included 277 patients in the analyses. Characteristics of the patients in paper IV are presented in table 5.

Table 5. Characteristics of 277 patients in paper IV.

Women		57% (159/277)
Median age (years)		30 (IQR 18-46)
Children <12 years		18% (49/277)
12-65 years		78% (215/277)
>65 years		4.7% (13/277)
Country:	Lithuania	56% (154/277)
	Sweden	25% (69/277)
	Norway	19% (54/277)

4.2 FINDINGS

PAPER I

Primary endpoint: clinical cure

The primary endpoint of paper I was clinical cure at test-of-cure 5-7 days after completed treatment in patients with GAS pharyngotonsillitis. We showed that penicillin V 800 mg x 4 for 5 days was non-inferior to 1000 mg x 3 for 10 days to obtain clinical cure. The difference in clinical cure for the PP population was -3.7% in favour of the 10-day treatment. The 95% confidence interval was -9.7 to 2.2 percentage points, which was just within the 10% inferiority margin. Non-inferiority was also showed in supplementary analyses of the modified intention to treat population.

We made a sensitivity analysis of the primary endpoint, using the patients´ reports in the diaries at five, seven, and nine days after randomisation. The sensitivity analysis showed a faster resolution of symptoms in patients taking the 5-day treatment arm having four penicillin V doses a day, compared to patients taking the 10-day treatment arm having three doses a day.
A subgroup analysis of the clinical cure in patients aged <18 years showed a difference of -5.3% in favour of the 10-day treatment, with a 95% confidence interval of −16.9 to 6.4 percentage points. Thus, the confidence interval was outside of the 10% inferiority margin and the 5-day treatment was inferior in this analysis of the younger patients. However, paper I was not dimensioned for subgroup analyses. Sample size considerations and a discussion on type II error are further addressed in the statistical discussion section.

Secondary endpoints

A secondary endpoint of paper I was bacteriological eradication of group A streptococci in the throat 5-7 days after completed penicillin V treatment. We found that the 5-day treatment was inferior to the 10-day treatment regarding bacteriological eradication of GAS. The difference in the PP population was −10.2% with a 95% confidence interval of −17.8 to −2.7, which was outside of the 10% inferiority margin.

The most frequently reported adverse event was diarrhoea, followed by nausea, and vaginal discharge or itching. There were 16% respective 21% of the patients having diarrhoea in the 5- and 10-day treatment groups, according to physicians' reports. However, the side-effects were more frequent when reported in the diaries by the patients themselves. Also, the events had longer duration when reported by patients. The incidence of self-reported diarrhoea was 25.6% and 34.7% in the 5- respective 10-day treatment arms. When reported by the patients in the diaries, 10.1% in the 5-day and 16.3% of patients in the 10-day treatment arm had symptoms of vaginal discharge or itching from the penicillin V treatment. However, this percentages of vaginal symptoms reported in the paper was calculated with both female and male patients in the denominator. Among the female patients the proportions of vulvovaginal symptoms were 15.4% (20/130) and 25% (31/125) in the 5- respective 10-day treatment arms. The incidences of side-effects were higher, and the duration longer, in the 10-day treatment arm, both when assessed by the physicians and when reported by the patients in the diaries.

Within one month from treatment start there were eight and seven cases of relapses within the 5- respective 10- day treatment groups. Among these fifteen patients with relapsing symptoms within one month, twelve had negative tests for GAS at the follow-up visit. There were four patients having complications, and three of them experienced peritonsillitis. All four patients with complications had received a 10-day course of penicillin V. At the telephone follow-up 43 patients in each treatment arm were asked which, if any, of the treatments they would have preferred. In this subset of patients 63% stated they would have preferred the five-day treatment, 22% the 10-day and 15% of the patients had no preference. The median adherence to treatment was 100% in both randomisation arms. Statistical analysis with Mann-Whitney U test showed a significantly better adherence in the 5-day group. Differences for some of the secondary endpoints are presented in table 6.

Table 6. Some secondary endpoints of paper I.

	5-day penicillin V	**10-day penicillin V**	**Difference (95% CI)**
Bacteriological eradication	156/194 (80.4%)	165/182 (90.7%)	-10.2 (-17.8 to -2.7)
Relapse within one month	8/179 (4.5%)	7/180 (3.9%)	0.6 (-4.1 to 5.3)
Complications within 3 months	0/198 (0%)	4/189 (2.1%)	-2.1 (-4.7 to 0.5)
New tonsillitis within 3 months	6/197 (3.0%)	13/189 (6.9%)	-3.8 (-8.7 to 1.0)
Self-reported diarrhoea	51/199 (25.6%)	66/190 (34.7%)	
Self-reported nausea or vomiting	51/199 (25.6%)	60/190 (31.6%)	
Self-reported vaginal itching or discharge	20/199 (10.1%)	31/190 (16.3%)	

PAPER II

In paper II we compared the results from a rapid antigen detection test and throat culture for GAS after penicillin V treatment. We found the tests to be in agreement in 91% (286/316) of the study participants. There were more positive RADTs than positive cultures: 21% (66/316) of the RADTs, and 13% (42/316) of the cultures were positive for GAS at the follow-up after treatment. The result from RADT and throat culture for GAS are presented in table 7.

Table 7. Results from rapid antigen detection tests and throat cultures at follow-up within 21 days from start of penicillin V treatment for pharyngotonsillitis.

	Positive culture *n*	**Negative culture** *n*	**Total** *n*
Positive RADT	39	27	66
Negative RADT	3	247	250
Total	42	274	**316**

The decline of positive test results for RADT and throat culture showed no significant difference with log rank test (p = 0.24). In the multivariable logistic regression model there was no correlation between agreement of RADT and throat culture for GAS at the follow-up visit, nor any of the independent variables: five days´ treatment OR 0.90 (0.34-2.4; p=0.83), number of days from inclusion until follow-up OR 1.0 (0.89-1.2; p=0.64), throat symptoms at follow-up OR 1.1 (0.30-3.8; p=0.92), male gender OR 0.81 (0.36-1.94; p=0.62) and age ≥ 15 years OR 0.53 (0.24-1.2; p=0.12).

PAPER III

In paper III we found a significant increase in the proportion of *Enterobacterales* resistant to ampicillin between the first and second faecal sample, taken directly after the last dose of penicillin V (p=0.007). In the third sample, taken 7-9 days after completed treatment there was no significant difference regarding resistance to ampicillin within the order *Enterobacterales*. We also found a significant increase in the proportion of *Enterobacterales* with decreased susceptibility to third generation cephalosporins between the first and second faecal sample (p=0.034). In the third faecal sample the increase was no longer significant. Further, we found a new colonisation of ampicillin resistant bacteria after penicillin V treatment. The number of unique ampicillin resistant species within each patient (including both *Enterobacterales* and *non-Enterobacterales* Gram-negative species) significantly increased from first to second faecal sample (p=0.003) and remained significant in the third and last

sample (p=0.008). These bacteria belonged to species that are generally considered to have pathogenic potential: *Klebsiella* spp, *Citrobacter* spp., *Enterobacter* spp., *Hafnia* spp., *Raoultella* spp., *Pantoea* spp., *Kluyvera* spp. *Acinetobacter* spp., *Pseudomonas* spp., *Stenotrophomonas* spp., and *Aeromonas* spp. All the available faecal samples from the patients were included in the analyses.

We found no significant differences between the 5- and 10-day treatment groups regarding *Enterobacterales* with ampicillin resistance or reduced susceptibility to third generation cephalosporins. The growth of enterococci increased in 13 of 29 patients in the second sample, and in 11 of those the increase remained in the last sample. In three patients the growth of enterococci was reduced in sample two. No shift among different enterococcal species was detected. We found that three patients were colonised with *C. albicans* in the second sample. One patient had growth of toxin producing *C. difficile* in sample 3 and had symptoms of diarrhoea and fatigue as well at the follow-up visit. The patient recovered spontaneously within a few weeks.

PAPER IV

In paper IV we assessed CRP and influenza viruses in 277 primary care patients with influenza-like illness during seasonal influenza epidemic. The prevalence of influenza A was 44% (121/277 patients) and of influenza B 21% (58/277 patients), 8.7% (24/277) of the patients had the H1N1 type of influenza A. Median CRP for all included ILI patients was 10 mg/L, median CRP for influenza A positive patients was 13 mg/L and for influenza B positive patients 5.0 mg/L. The CRP concentration in patients with influenza-like illness due to different etiologic agents had a considerable overlap.

Fever, feeling generally unwell, and having low energy/being tired were the most common symptoms reported as moderate or major severe. We found that the 20% of patients (55/277) having a CRP ≥30 mg/L were more likely to report following symptoms as moderate or major severe: shortness of breath adjusted OR 3.1 (1.4-6.7; p = 0.0037), sweats or chills adjusted OR 4.2 (1.6-11; p = 0.0036) and dizziness adjusted OR 3.0 (1.5-6.3; p = 0.0029). Increased CRP concentrations were also associated with illness duration 48–72 h.

We found no association between CRP and confirmed influenza A, adjusted OR 1.0 (0.91–1.2; p=0.61). However, increasing CRP concentration in steps of 10 mg/L was associated with a lower risk for influenza B, adjusted OR 0.42 (0.25–0.70; p<.001). There was no correlation between the independent variables in the multivariable regression model when tested with Spearman's rank correlation.

5 DISCUSSION

We found that penicillin V 800 mg four times daily for 5 days was non-inferior compared to 1000 mg three times daily for 10 days, regarding clinical cure for pharyngotonsillitis with group A streptococci. Contrarily, the 5-day treatment seemed to be inferior regarding bacteriological eradication. There were fewer complications, fewer adverse events, and shorter durations of adverse events with the 5-day treatment. There was a small number of relapses within one month, with no difference between treatment groups.

We found no significant difference in the results of rapid tests and cultures for GAS taken at follow-up after treatment, within 21 days from start of treatment. There were no associations between agreement of results from RADT and throat culture for GAS, with treatment duration, number of days from inclusion, throat symptoms at follow-up, gender, or age.

Directly after the penicillin V treatment we found a significant increase in ampicillin-resistant Gram-negative bacteria in the patients' faecal samples. A further increase in ampicillin-resistant species was found at a third sample taken 7-9 days after completed treatment. We also found a significant increase in the proportion of Enterobacterales species resistant to ampicillin and the third generation cephalosporins, an increased growth of enterococci, and new-colonisation with *C. albicans* in the second sample. One in 29 patients had a *C. difficile* infection at follow up after penicillin V treatment.

In patients with influenza-like illness we found no association between concentrations of CRP and confirmed influenza A. The CRP concentrations in ILI patients with different aetiology had a significant overlap.

5.1 METHODOLOGICAL DISCUSSION

The thesis encompasses different quantitative study designs: one randomised controlled trial, two prospective observational studies and one cross-sectional study. All studies have a pragmatic approach: addressing research questions arise from clinical work, recruiting real world patients, and employing inclusion criteria accordant to current clinical guidelines. The outcome measures are clinically relevant, such as clinical cure from treatment of

pharyngotonsillitis, emergence of resistance within the gut microbiota and other side-effects from penicillin V treatment, and some aspects of the commonly used point-of-care tests CRP and RADT for GAS. The pragmatic approach of the work meant we aimed at testing the hypotheses in the full range of real-world patients, with all existing diversity and difficulties, to strengthen applicability. Thus, the results can be useful in clinical practice within primary health care. The included papers are researcher-initiated academic studies with no connection to the pharmaceutical industry.

RISKS OF BIAS

Bias refers to a systematic error that can affect observations and conclusions of a study. In this thesis one risk for bias was a possible systematic difference between study participants and patients fulfilling inclusion criteria but declining participation. There is a risk for patients with more severe symptoms from infectious disease, or earlier experience of side-effects from the treatment, to decline participation. In the case of pharyngotonsillitis, patients with more severe symptoms are known to have greater benefit from antibiotic treatment. However, due to randomisation this possible selection bias was equally distributed between the two treatment arms. Also, in the fourth paper on CRP concentrations in patients with influenza-like illness was a risk for more severely ill patients to decline participation.

The randomisation procedure of the first paper aimed to reduce the risk of different systematic errors. The patients were allocated by chance to respective treatment arms to avoid selection bias. However, there are additional risks of bias in randomized trials, such as drop-outs and missing data. There is also a risk for study participation to enhance adherence to treatment, and studies may overestimate the effectivity compared to what is achievable in routine practice. The first paper was an open label trial; thus, the patients and physicians were aware of the allocated treatment after randomisation. The knowledge of penicillin V dosage may have influenced the perception of treatment effects and the reported outcome. Among the study participants reporting that they would prefer one of the allocated treatments, most of them preferred the shorter five-day course. However, it is likely that the patients´ experiences of the treatment as such, will affect the outcome also in the real world. An open label trial, with clinical cure as the primary outcome, includes that treatment effect

in the analysis. Concerning the endpoints of papers II and III results from throat swabs and faecal samples were more difficult to influence for both patients and investigators. The laboratory personnel conducting the microbiological analyses were blinded to the patients' randomized allocation.

The risk of bias due to possible systematically missing data, for example patient drop-outs, patients not receiving the study drug or not reporting certain outcomes, was handled by comparing separate analyses of a modified intention to treat population and of a per protocol population. The modified intention to treat (mITT) population was defined as all included patients who received at least one dose of penicillin V within the study. The per protocol (PP) population were patients having no major deviations from the study protocol, took at least 80% of the penicillin doses, came back for a follow-up visit, and had no other antibiotics than penicillin V before the follow-up evaluation. The mITT- and the PP- analyses were in agreement, which strengthen the validity of the first paper. However, when the analysis of the mITT-population was conducted without imputation of missing data it gave a confidence interval of −10.04 to 1.9 passing the non-inferiority margin with 0.04.

Notably, in all studies of the thesis was a predominance of female participants. Although women are generally more prone to have sore throat episodes than are men there is no data supporting gender differences regarding GAS pharyngotonsillitis [166, 232]. The bulk of study patients being women is a selection bias with unknown cause. However, we used gender as an independent variable in the logistic regression models and found no correlation between gender and outcomes.

MONITORING

The data collection of papers I-III in this thesis were monitored in accordance with Good Clinical Practice. The fourth paper was conducted within a randomised controlled trial of oseltamivir treatment for patients with influenza-like illness. The main study on oseltamivir treatment was monitored according to Good Clinical Practice, but there was no specific monitoring of the collection of the CRP data. The purpose of monitoring was to keep participating patients safe and respected, get good quality data and improve the ongoing conduct of the trials. The monitoring procedures included specifically

trained persons regularly visiting the study sites, reading case report forms (CRF) and checking that the reported data was corresponding to the patient record at the health care unit. The persons responsible for monitoring also discussed relevant questions related to Good Clinical Practice with the local investigators, such as the practical procedure of inclusion and informed consent.

SAMPLE SIZE

In statistical testing, not rejecting a null hypothesis that is actually false is known as a *type II error*. In this thesis, the third paper is at risk for a type II error due to the small sample size. We aimed at recruiting at least 50 patients but ended up with 31. This illustrates one of the challenges conducting clinical research: recruiting patients takes great time and effort. Despite the small sample size, the third paper showed some statistically significant results. If there had been more participants, we cannot exclude the possibility that there may have been additional statistically significant differences.

In the first paper, the initial power calculation was based on the primary outcome. The study was not dimensioned to demonstrate statistical significances for the secondary variables or to analyse the primary outcome in patient subgroups. Children are known to differ from adults in respect to asymptomatic carriage of GAS, and signs and symptoms of GAS pharyngotonsillitis. Given this context it was a limitation to the study not being powered for a subgroup analysis in children. However, the effectiveness of the established 10-day penicillin V regimen in children has not been previously identified, and a study defining the effectivity of penicillin V in younger patients experiencing a GAS pharyngotonsillitis could advantageously be placebo-controlled. Also, the study was not dimensioned for the comparison of possible statistically significant differences in treatment effectivity in patients with 3, respective 4, Centor criteria.

On the other hand, an overly large investigation is not necessarily the best. It is unethical to include more patients than necessary, and in a very large study population, there may be statistical significance also for very small and unimportant differences. For example, a very large sample size in the second paper could have provided a statistically significant, but possibly clinically

insignificant difference between RADT and throat culture for group A streptococci. We estimated 316 patients to be a large enough sample to unveil a clinically relevant difference between the tests. Notable, the absence of a significant difference between RADT and throat culture does not prove similarity between the tests.

NON-INFERIORITY

A non-inferiority trial means in practice that a new experimental treatment is not compared to a placebo, but to an already established treatment. In the first paper we compared a shorter but hypothetically more efficient 5-day course of penicillin V, to the longer and currently recommended 10-day treatment. The non-inferiority margin of the first paper was set to minus 10% in advance, which meant that the efficacy from the 5-day treatment would be non-inferior if its confidence interval was within 10% less than the clinical efficacy of the 10-day treatment. The inferiority margin was chosen in advanced and published in a study protocol paper [231]. One risk with non-inferiority trial design is setting an inferiority margin that is too wide related to the effect of the reference treatment. The risk is to prove a new and truly ineffective treatment to be non-inferior to the established treatment [233]. Two arguments for a placebo-controlled superiority trial of pharyngotonsillitis therapy is i) the limited benefit from 10-day penicillin V and ii) that the epidemiology of GAS pharyngotonsillitis has changed since earlier placebo-controlled trials supporting a 10-day treatment [167]. Another aspect of the non-inferiority design is the binary hypothesis - the most efficient penicillin V treatment for GAS pharyngotonsillitis may be neither 5 nor 10 days. There are new innovative trial designs to identify an optimal duration of treatment: randomising patients to several treatment options (which may include placebo) and estimating a duration-response curve [234], or a two-step process involving the ranking of patients both from clinical outcomes and from duration of the antimicrobial treatments [235]. However, these trial designs may require substantially larger study populations. The need for a smaller sample size is considered an advantage of the non-inferiority trial design. However, with a very narrow confidence interval of the non-inferiority margin, this may not be the case. Notably, a placebo arm in a trial of antimicrobial treatments is very expensive. Being affordable and doable is also a strength of a trial.

STATISTICAL SIGNIFICANCE AND MULTIPLE TESTING

In statistical testing, rejecting a null hypothesis that is actually true is known as a *type I error*. The setting of a statistical significance level indicates the accepted risk of a type I error: the risk of a false positive finding. We set the significance level to 0.05 in advance, which is also the commonly used threshold for statistical significance.

Making multiple statistical tests in a data set increases the risk of making a type I error. Logistic regression models tend to generate a lot of statistical significance testing. However, we had few significant findings in these models in Paper II and IV and made no adjustments or corrections due to multiple testing.

THE POWER OF EXPLANATION

The power of explanation equals the proportion of the total variation of the dependent variable that is explained by the independent variables put into a specific statistical model. In the fourth paper we used the Nagelkerke R^2 test to measure the power of the explanation in the regression models. Nagelkerke R^2 is specified between 0 and 1, and the higher the score, the higher the power of explanation. For the regression model of factors associated with CRP >30 mg/L in patients with influenza-like illness the Nagelkerke R^2 was over 0.8. The regression models analysing factors associated with influenza A or B gave a low index score. The independent variables used in those models did not well predict the presence of influenza A or B.

LABORATORY METHODS

In the first, second, and fourth papers the tests investigated presence of specific microbes, group A streptococci and influenza A and B. However, there is no evidence for causality between the presence of these microbiological findings and the symptoms. We cannot rule out that some included patients may have presented with symptoms caused by alternative agents.

A causal relationship between the presence of microbes and current symptoms is not easily demonstrated. The Henle-Koch postulates from the 1880s suggested that a microbe, after being isolated and grown in pure culture, should be able to reinduce the same disease [236, 237]. This strategy for identifying a

causal relationship between an infectious agent and disease is unsuitable for both ethical and practical reasons.

The dilemma regarding causality is highly relevant in research on pharyngotonsillitis with presence of group A streptococci in the throat. Viral pharyngotonsillitis is common [169-171] and so is asymptomatic carriage of GAS in the throat, especially in children [30, 31]. A positive test for GAS in a symptomatic person does not necessarily predict that the current symptoms are due to a GAS infection. However, this reflects the clinical situation: during the few minutes of consultation in primary care there is often a need for a pragmatic interpretation of causality.

The situation is further complicated by the lack of criterion standard - or gold standard - method for confirming the presence of GAS in the throat [238]. In the second paper we evaluated RADT and conventional throat culture for GAS after penicillin V treatment. Since we do not know which test is closest to the ground truth for identification of GAS in the throat, we avoided calculation of sensitivity and specificity with throat culture as a reference. Instead, we used the log rank test for the statistical analysis. It would have been a strength to have PCR tests, for GAS and other microbes, in the comparison. Also examining the patterns of cytokine production could possibly have revealed some insights into causality.

The third paper was an explorative study, and to our knowledge the first to focus on emergence of β-lactam resistance in the faecal microbiota of penicillin V treated patients. The study modelled microbiological methods which may be a foundation for further studies in the field. However, the explorative nature of this study gave a high level of uncertainty in the selection of culture methods. It was a weakness of the study to not use metagenomic methods. Metagenomic analyses are suitable for microorganisms that are difficult to cultivate and can offer a broader apprehension of changes within the microbiota. Nevertheless, culture methods have the strength to detect emergence of resistance. The semi-quantification culture is able to approximate changes of abundance that may not be detected by metagenomics. A combination of culture and metagenomic methods will probably be rewarding for future research in this area. Findings in metagenomic analysis can guide the choice of culture methods.

The use of faecal samples as a proxy for the gastrointestinal microbiota has been debated [22]. The objection is that faecal sampling neither captures the different microbial compositions along the gastrointestinal passage, nor the microhabitats of the intestinal lumen, mucus layers and colon crypts [41]. In any case, the different microhabitats of the intestines are poorly accessible for sampling. Stool samples, on the other hand, are very easy and comfortable to collect [42] and still provide clinically relevant information about changes in the gut microbiota.

Earlier studies on CRP and influenza viruses have mainly recruited hospitalised patients retrospectively due to aetiological laboratory findings. A strength of the fourth paper was recruiting patients prospectively because of symptoms and not retrospectively because of laboratory findings of influenza A or B. This makes the findings of the paper more relevant for clinical work in primary care.

PAIRED SAMPLES

In the third paper each individual had her own unique microbiota composition prior treatment, and thus was impacted differently by the exposure to penicillin V. This high subject-to-subject variability means that grouping the data may mask changes and result in loss of statistical significance. Therefore, we used a statistical test for clusters of paired data, McNemar test, to measure divergences from baselines after treatment. In this way, each patient became her own control. The semi-quantitative assessment of the cultures was not used in the statistical testing. Instead, we used dichotomised culture results - presence of resistance or no presence of resistance - when analysing the antimicrobial resistance data. We found this approach more relevant from a clinical point of view.

FOLLOW-UP

A weakness of the second paper was having only one follow-up visit for RADT and throat culture for GAS. Repeated testing for a longer period would have clarified the duration of GAS positivity and given more information on the decline of GAS in RADT and throat cultures over time. In the third paper the short follow-up period limited the exploration of ecological effects in the microbiota from penicillin V treatment. At the third and last faecal sample

there was still an ongoing increase in ampicillin-resistant species. Additional faecal samples taken for a longer period would have provided more information on the duration of the observed disturbances after penicillin V treatment. The fourth paper was a cross-sectional investigation with one single CRP taken per patient within the first 72 hours of influenza-like illness. CRP concentration changes rapidly and having repeated CRP measures could have provided interesting information of the course of CRP over time in patients with ILI.

5.2 ETHICAL DISCUSSION

Patient participation in all studies of this thesis was voluntary. The patients were free to decline and cancel their participation at any time. Aside from this, it is still ethically complicated to ask a person seeking health care for an acute medical condition to participate in a scientific study. Despite a clear and stated intention for patient participation to be voluntary there were psychological aspects that may have confounded the patients´ decisions. The patients were in a dependent relationship with the caregiver, and most had a fever at the time of the decision. The perceived need to receive care may have influenced the patients 'perception of voluntariness. Although the extra samples taken were quick, and physically not or only moderately distressing to the patients, study participation may have raised concerns and caused anxiety.

On the other hand, it would be deeply unethical never to conduct clinical trials in acutely ill patients, or to neglect improvement of antibiotic stewardship in primary care. It is unethical to neglect the possible hazardous side effects that patients may suffer from a treatment. For a therapy aimed at limiting the duration of symptoms in a self-limiting disease possible harmful side effects from treatment should be carefully considered from an ethical point of view. The expression *primum non nocere* is used in ethical discussions within medical and healthcare education. The meaning is not to harm patients, to realise when ethically best in some cases may be not to act, or not to treat. Considering the limited benefit of certain antibiotic treatments in primary care the potentially harmful effects are indeed of importance from an ethical standpoint.

Sometimes clinical questions need to be answered with research involving real-world primary care patients. Questions about respiratory tract infections in primary care can seldom be answered by studies conducted in the highly

selected respiratory tract infection patients in hospitals. From an ethical point of view, it is remarkable that most patient visits in Sweden take place in primary care, and yet relatively little medical research is conducted in the primary care setting. Pragmatic studies in primary care, for example pharmacological phase IV studies such as the first paper of this thesis, or other types of clinical and pragmatic studies improving patient care, may have a large impact for health over time. Even minor adjustments in the primary care practice may be of importance, as they affect so many people.
A further ethical reflection is that equality data is conspicuous by its absence in Swedish healthcare. To identify any discrimination in quality of care and outcomes due to racism, homophobia, and other circumstances, such parameters must be measured. Equal care for all is an ethical issue that needs to be considered in future research.

5.3 FINDINGS IN RELATION TO LITERATURE

TREATMENT FOR PHARYNGOTONSILLITIS

Penicillin V has a short half-life in plasma and its antimicrobial effect is dependent on time above MIC [98]. Thus, a higher and more frequent dosing regimen can be more efficient [100]. This thesis studied efficacy of a shorter but more intense penicillin V treatment compared with the currently recommended treatment. We found the intense five-day treatment (800 mg penicillin V four times daily) to be non-inferior to the currently recommended ten-day treatment (1000 mg penicillin V three times daily) for clinical cure from GAS pharyngotonsillitis.

Contrarily to our findings, a meta-analysis by Holm et al found penicillin V therapy for ≤5 days to be less efficient than longer penicillin V therapies regarding clinical cure [214]. They included the first paper of this thesis in their analysis, together with three other RCTs comparing different durations of penicillin V treatments. The other RCTs used lower and less frequent dosages of penicillin V: 250 mg three times daily [219], 800 mg twice daily [218], and 250 mg three times daily [239]. An earlier meta-analysis by Falagas et al found different types of shorter antibiotic treatment for GAS pharyngotonsillitis to be inferior compared to longer treatments, regarding bacteriological eradication [221]. They made a subset analysis in penicillin V treated patients with the

same result. Just like the meta-analysis by Holm et al they found clinical cure to be inferior with the short penicillin V treatments. The included studies used lower and less frequent doses than we did. None of these meta-analyses discussed the importance of dosages and frequencies of penicillin V in relation to treatment efficacy. Thus, our study is the first to conclude that it is possible to maintain clinical effectiveness with a shorter penicillin V treatment for GAS pharyngotonsillitis: and one crucial difference to the earlier studies is the more frequent intake and higher daily dosage of penicillin V.
A more recent study made a comparison of adult patients treated with penicillin V for 5, 7 and 10 days and found no statistical differences in clinical outcome. However, this was a prospective observational study, and the patients were not randomized to respective treatment duration [223].

Our study was not dimensioned for subgroup analysis of treatment efficacy in patients with differing severity of disease or age. An earlier double blind and placebo controlled RCT found no effect from penicillin V treatment for pharyngotonsillitis in children [224]. The higher GAS carriage rate in children may lead to inclusion of GAS carriers with symptoms from viral disease, and thus any antibiotic treatment is expected to show less effectivity. Penicillin V treatment in children with pharyngotonsillitis is a relevant question to address in future research. Since there is a lack of evidence for effectiveness of penicillin V in GAS-positive children with pharyngotonsillitis a randomized controlled trial including a placebo treatment arm seems motivated.

SIDE-EFFECTS FROM PENICILLIN V TREATMENT

We found adverse events to be fewer and shorter with five-day penicillin V treatment, compared to ten-day. A few studies of penicillin V treatment for pharyngotonsillitis mention the existence of gastro-intestinal side-effects but have not evaluated the incidence. One study of penicillin V 1000 mg twice daily for ten days reported that 16.5% of patients had some type of adverse event as recorded by physicians. Mild gastro-intestinal tract disturbances were most frequently reported [240]. We found that 16% respective 21% of the patients had diarrhoea in the 5- and 10-day treatment groups as recorded by physicians. However, when reported by the patients themselves in the diaries, the incidence of diarrhoea was 26% and 35% in the 5- respective 10-day treatment arms. Also, the events had longer duration when reported by patients.

Despite penicillin V being recognised as a cause of vulvovaginal candidiasis [111] earlier trials on treatment for pharyngotonsillitis have not evaluated the incidence. We found 15.4% of female patients in the 5-day, and 25% in the 10-day treatment arm, to have symptoms of vaginal discharge or itching from the penicillin V treatment. Our finding indicates that this is a very common side-effect from penicillin V treatment among women.

Side-effects from penicillin V treatment are poorly reported in earlier studies, thus a comparison of side-effects is difficult. In our study the five-day treatment meant a decrease in the total penicillin V exposure from 30 g to 16 g, and thus it seems reasonable to find a substantial decrease also in side-effects with the shorter treatment. Further, we found an elevated proportion of Enterobacterales with reduced susceptibility to ampicillin and third generation cephalosporins directly after penicillin V treatment. We also found an increased number of ampicillin-resistant Gram-negative species within each patient that remained significantly increased during the follow-up period. The low number of patients included in the study hinders a comparison between the two treatment arms. Thus, we have not confirmed that the differences in gastrointestinal side-effects from the 5- and 10-day treatments are reflected in different effects on antimicrobial resistance in the microbiota.

We identified four earlier studies on penicillin V and the faecal microbiota. Three of these studies used culture methods and examined effects from low daily dosages of penicillin V, and one study constituted a case-report of observed ecological disturbances in one patient. Two of them used 800 mg penicillin V twice a day for 7 days, in 10 and 6 adults, [104, 105] and a third study used 1 g penicillin V twice a day for 10 days in 10 adults [39]. The fourth, and more recent study, investigated oral and faecal samples in a one-year-old infant treated with penicillin V for acute otitis media. This study used metagenomic sequencing methods and the child received penicillin V for 5 days. The dosage of penicillin V was not reported in the article [106]. All studies had a short follow-up period, the longest being 30 days.

The previous culture studies found none or small changes in the faecal microbiota, except from an increasing number of *Clostridoides* species found with the higher penicillin V dosage, 1000 mg twice for ten days [39, 104, 105]. The fourth study, using metagenomics to investigate faecal samples from one

child, found large differences in the faecal microbiota before and after 5 days of penicillin V treatment, with the last follow-up made 30 days after the start of treatment. *Proteobacteria* went from 1% to >76% of the total reads, mostly due to a massive increase of *E. coli*. Furthermore, they found a substantial increase of antimicrobial resistance genes. At baseline they identified seven antimicrobial resistance genes conferring resistance to four different classes of antibiotics, in the last sample there were 21 antimicrobial resistance genes conferring resistance to seven classes of antibiotics, including resistance towards vancomycin [106].

The most apparent differences are the lower and less frequent doses of penicillin V used in the previous culture studies, the choice of culture screening methods and, most importantly, that we added new screening methods for β-lactam resistance. The fourth, and more recent study, investigated faecal samples from one infant with metagenomic sequencing methods and very large disturbances after treatment. Obviously, the young patient had an unstable intestinal microbiota. In our study we collected faecal samples from patients aged between 10–63 years with a median age of 38. The younger patients, known for highly unstable microbiotas and larger risk of ecological disturbances from treatment were excluded. Larger studies of ecological effects on the microbiota from penicillin V treatment in children would be of great interest in the future. In addition, metagenomic methods differ from the culture methods we used, but the substantial increase in antimicrobial resistance from penicillin V treatment is consistent across these studies. Adding metagenomic analyses to our investigation had probably revealed additional ecological effects of interest. Among the bacteria with reduced susceptibility to third generation cephalosporins it is likely that some may produce ESBL (extended spectrum beta-lactamases). Primary care physicians are already familiar with ESBL, since it sometimes presents a practical problem in treating urinary tract infections. Using molecular biological analysis to identify types of resistance well known to clinicians, could enhance the comprehensibility and the impact of future studies.

A previous interview study with GPs showed awareness of increasing antimicrobial resistance and of the urgent need to reduce selection pressure from antibiotics. However, the GPs were sceptical that penicillin V for pharyngotonsillitis would contribute to the emergence of antimicrobial

resistance: *"looking at our prescribing of penicillin for sore throat is nonsense to me"* [241]. The primary care physicians referred to the use of antimicrobials in the animal industry and of broad-spectrum agents in hospitals as a bigger problem. Our finding that penicillin V selects for resistance within the individual patient's microbiota puts penicillin V prescriptions in a new light. The point of avoiding penicillin V may primarily not be to stop the global emergence of AMR, but to protect the microbial ecology of the individual patient from potential hazardous effects of the treatment.
Notably, our study was conducted in healthy, largely middle-aged people living in an area with a relatively low abundance of antimicrobial resistance. In younger patients or in an area with high abundance of AMR in the microbial ecosystems, the ecological side-effects from penicillin V treatment could be different. In addition, changes in AMR may appear and be preserved on an *undetectable* level in the individual microbiota during treatment. These preserved effects may be announced first after additional antibiotic exposure [84].

For a treatment that aims to reduce discomfort in a self-limiting disease exploring the side-effects from treatment is warranted. Evaluating the degree and duration of side-effects has been largely neglected in previous studies. The significant increase of β-lactam resistance after penicillin V treatment is indeed an undesirable side-effect. We found diarrhoea and vaginal symptoms to be very common side-effects, and to have higher frequency and longer duration after 10 days of penicillin V treatment. Roughly, one third of the patients receiving the recommended ten days of penicillin V for pharyngotonsillitis had diarrhoea, and one fourth of the women had suspected vulvovaginal candidiasis. These widespread side-effects must be taken into account when considering treatment with penicillin V.

TESTS FOR GROUP A STREPTOCOCCI AFTER RECENT TREATMENT FOR GAS PHARYNGOTONSILLITIS

We found 21% (66/316) of the RADTs, and 13% (42/316) of the throat cultures to be positive in persons recently treated with penicillin V for GAS pharyngotonsillitis. Log rank test showed no significant difference between test results at follow-up. There were 27 patients with negative culture having

positive RADT for GAS and three patients with negative RADT having positive throat culture for GAS. 91% of the test results were in agreement at follow-up.

Negative RADT with positive culture for GAS

In previous studies, comparing RADT and throat culture for GAS after antibiotic treatment for pharyngotonsillitis, the main interest was to evaluate whether throat swabs became negative in children within 24 hours from the first dose [189, 190, 242]. One of the studies used both RADT and culture, but reported only the results from the cultures [242]. One was small (29 children) [190] and one used an older type of rapid antigen detection test, the latex agglutination [189]. These studies did not determine whether a modern RADT for GAS is reliable after recent penicillin V treatment for pharyngotonsillitis.

Another study compared results from RADT and throat culture for GAS in patients with relapse of pharyngotonsillitis within 28 days from prior treatment [188] to test results in a control group of patients having a new episode of pharyngotonsillitis without recent diagnosis. In the relapse population they found 10% (10/104) negative RADTs with positive culture, and in the control group with new pharyngotonsillitis they found 30% (19/63). These divergent and unexpected results may be related to the different managements of these study populations: control patients were assessed by nurses, and relapse patients were assessed by physicians. They suggested the use of a back-up culture in case of negative RADT for GAS.

We found negative RADT with positive culture for GAS to be rare at follow-up and conclude that back-up culture is unnecessary. None of the three persons with negative RADT and a positive culture had abundant growth of GAS in the culture. This finding is in agreement with earlier studies, finding the RADT sensitivity to be lower when the growth of GAS in throat culture is sparse [243-245].

Positive RADT with negative culture for GAS

Our aim was to evaluate possible false positive RADTs due to non-viable streptococci after penicillin V treatment for pharyngotonsillitis. The throat

swabs were taken at a planned follow-up visit and the studied population was mostly free from symptoms, though a few patients reported some level of remaining symptoms from earlier disease. The low rate of positive cultures for GAS in this mainly asymptomatic population left room for detection of possible false positive RADTs. We found 27 of 66 patients with positive RADT for GAS to have a negative culture at follow-up.

Possible explanations are: i) contamination of *Staphylococcus aureus*, that may inhibit growth of GAS in cultures [33], and ii) non-haemolytic strains of group A streptococci. Group A β-haemolytic streptococci induces complete haemolysis of the red blood cells in the agar and degradation of haemoglobin which occurs as a transparent, yellowish window in the red agar plate [246]. Some strains of GAS are non-haemolytic and will not be detected in conventional throat cultures [247]. In addition, there is a case report of false positive RADT for GAS due to cross-reaction from carriage of a α-haemolytic *Streptococcus milleri* [248]. However, these special circumstances are not likely to explain all the 27 positive RADTs with negative culture. We cannot rule out the possibility of RADTs being positive due to remaining antigens from dead bacteria. Not to be forgotten, RADT may also be a more sensitive test than throat culture for identification of GAS [249]. Due to the reported practice to prescribe antibiotics pending throat culture results [186], we conclude that even with occasional false positive RADTs after recent treatment for GAS, using RADT will protect patients from unnecessary antibiotics. Since there were only a few negative RADTs with positive cultures a negative RADT for GAS is reliable, also after recent treatment.

Carriage rate of GAS after recent treatment

Interestingly, we found 21% (66/316) of the RADTs, and 13% (42/316) of the throat cultures to be positive in mainly asymptomatic individuals after treatment. Thus, both RADT and culture indicated a rather high carriage rate after recent GAS pharyngotonsillitis, though the duration of this carriage was not made clear. In this thesis, we did not address the usefulness of throat swabs in patients with relapsing disease. The usefulness of RADT for GAS in patients with recurrent symptoms is not only related to the correctness of the rapid test but also to the rate of GAS carriage after recent infection. The benefit from antibiotic treatment for GAS pharyngotonsillitis has been questioned when the asymptomatic carriage rate exceeds 6-11% [196].

Interestingly, the reconsultation rate for pharyngotonsillitis within 30 days from first episode is higher in GAS positive patients [250]. The reasons may be several, but the clinical management of pharyngotonsillitis is reported to affect the patients' behaviour of reconsulting. An antibiotic prescription means an obvious risk of medicalising a self-limiting condition [251].

The point-of-care tests have an important role to target antibiotic treatments. Letting a negative RADT for GAS be the stopping rule for antibiotic treatment is advantageous in reducing antibiotic usage in pharyngotonsillitis patients. However, it is important to limit the testing to patients for whom antibiotic treatment can be justified. New methods for identification of GAS, such as real-time PCR, will retain the problem of mixing up the carrier state with disease, and an orderly identification of which patients to test is crucial.

Complications and early reattendance are very rare in pharyngotonsillitis patients not receiving antibiotics [251]. It is likely that a more restrictive use of antibiotics for pharyngotonsillitis means avoiding a substantial number of side-effects, and very few risks for the patients. The work to update the Swedish guideline for management of sore throat is scheduled to take place this year, 2023. Hopefully, our findings can contribute to the development of a new restrictive guideline for sore throat management.

C-REACTIVE PROTEIN IN PATIENTS WITH INFLUENZA-LIKE ILLNESS

We found the CRP concentrations in patients with influenza-like illness, (ILI) due to different aetiology, to have a great overlap. ILI patients having an influenza A infection could not be identified based on the CRP concentration. One previous study prospectively recruited outpatients with respiratory tract infection and, contrary to our findings, found CRP ≥5 mg/L to predict influenza A or B [164]. However, they included a wider range of respiratory tract infections, including milder disease not having fever, such as the common cold. We included patients with influenza-like illness, defined as *fever* and at least one *systemic symptom* (headache, muscle ache, sweats or chills or tiredness) and one *respiratory symptom* (cough, sore throat or runny or congested nose) during the annual influenza epidemic season. Thus, we studied

a more severely ill outpatient population. In addition, we used a higher cut-off for CRP ≥30 mg/L. These factors may explain the conflicting results.

High CRP is associated with severity of disease and risk of mortality in hospitalised patients with influenza A [158-161]. In the hospitalised patients the reported CRP concentrations have had a median of 140 mg/L [161], or a mean of 118 mg/L [252] in influenza A infections, but with wide ranges of CRP concentrations. When compared to patients with influenza like-illness diagnosed with bacterial pneumonia the average CRP in patients with bacterial diagnosis was significantly higher than in patients diagnosed with only influenza [252]. However, a common feature of these studies is the large range of CRP concentrations in patients with confirmed influenza and the large overlap with ILI of different aetiology. This is in accordance with our findings. We found the median CRP in patients with influenza A to be 13 mg/L and influenza B to be 5.0 mg/L. In our study an increasing CRP was associated with a significantly lower risk for the presence of influenza B. This finding has not been previously reported. Influenza B is known to circulate during seasonal influenza epidemics and sometimes cause severe illness, particularly in children and adolescents. Although the clinical picture of influenza B is indistinguishable from influenza A infections there are other differences reported. One is the induction of immune response, where influenza B and A induce different interferon and cytokine responses [253]. That may be an explanatory factor for the difference in median CRP for influenza A and B that was found.

The clinical implication of our findings is that CRP is not useful in identifying influenza infection among patients with influenza-like illness in primary care. The subset of colleagues in primary care who use CRP >50 mg/L as a sign of bacterial infection, or rely heavily on CRP in the assessment of respiratory tract infections [152], are at risk of misdiagnosing some patients with influenza A infection as bacterial. The use of CRP in patients with a viral diagnosis is known to increase the prescription of antibiotics [254]. Unless bacterial pneumonia is suspected CRP should be avoided in primary care patients with influenza-like illness.

6 CONCLUSION

We found it possible to maintain the clinical effectiveness with a shorter but more intense penicillin V treatment for GAS pharyngotonsillitis. The clinical implementation of this could be a new treatment recommendation for pharyngotonsillitis: patients with sore throat and no symptoms of cough or coryza, having at least 3 Centor criteria and a positive throat swab for GAS, can be offered penicillin V 800 mg 4 times daily for five days, instead of the currently recommended 10-day treatment.

We found diarrhoea and vulvovaginal symptoms to be very common side-effects from penicillin V treatment. The incidence and duration of these side-effects was less with shorter treatment. We also found different signs of ecological disturbances, including emergence of resistance to β-lactam antibiotics, in the faecal microbiota from penicillin V treatment. This is relevant information: the patients should be made aware not only of the beneficial effects, but also the common side-effects when deciding on penicillin V treatment for GAS pharyngotonsillitis. In addition, the magnitude of adverse effects from penicillin V therapy highlights the need for continued development of more restrictive treatment guidelines for GAS pharyngotonsillitis.

We found no significant difference between the results of RADT and throat culture for GAS after recent penicillin V treatment for pharyngotonsillitis. The conclusion of our findings is that RADT for GAS is reliable even after recent penicillin V treatment.

We found the CRP concentrations in patients with influenza-like illness due to different aetiology, to have a great overlap. ILI patients having an influenza A infection could not be identified based on the CRP concentration. The clinical implication is that CRP should be avoided in primary care patients with influenza-like illness, unless bacterial pneumonia is suspected.

7 FUTURE PERSPECTIVES

In the era of antimicrobial resistance, is it wise to use antibiotics in spontaneously resolving infections? Does the limited benefit from penicillin V for GAS pharyngotonsillitis justify treatment, considering the side-effects identified in this thesis? A recent report of elevated MIC for β-lactams in group A streptococci further highlights the importance of a restrictive penicillin V usage. The future direction of antibiotic use in primary care is inevitably to treat less.

This thesis presents a new treatment strategy with a shorter but more intensive course of penicillin V for GAS pharyngotonsillitis. However, additional restrictions will be necessary. An upcoming challenge in further limiting the use of antibiotics for pharyngotonsillitis is the possible early identification of a few patients with complicated or potentially complicated disease. How to predict and identify complicated throat disease from GAS and other etiological agents is an area for future research. Continuity in health care contact and evaluation of the course of the disease may become more important in infectious diseases in primary care. Pain relief from other drugs and measuring vital signs may take on a new function when abstaining from antibiotics for different types of self-limiting infections. To determine which pharyngotonsillitis patients benefits from throat spray treatment with a mix of α-streptococci could further limit the use of antibiotics in primary care. The use of rapid tests such as CRP and antigen detection test for GAS may be modified and supplemented with other near-patient tests.

GAS carriers with sore throat due to other causes than group A streptococci are at risk for unnecessary exposure to antibiotics. Given the level of GAS carriage in asymptomatic patients that we found early after penicillin V therapy for pharyngotonsillitis, the effectiveness of penicillin V therapy in GAS-positive patients with early recurrence of sore throat must be evaluated. Since the carriers are not expected to have any symptomatic relief from antibiotics this may impact treatment effectiveness in the relapse population. Similarly, the effectivity of penicillin V treatment in GAS-positive children with pharyngotonsillitis is relevant to determine. Even in children, there is a high abundance of asymptomatic GAS carriage which may impact the overall treatment effectivity in younger age groups.

In this thesis, the effects of penicillin V on the microbiota were evaluated in patients ≥ 10 years of age. At the same time, the intestinal microbiota of the youngest children is known to be the most unstable and sensitive. Going forward, the ecological side effects on the faecal microbiota from penicillin V therapy in children need to be identified. The possible long-term effects of penicillin V on the microbiota will be of particular interest to define in those children receiving repeated courses of penicillin V.

In patients of all ages future studies on effects of penicillin V on the faecal microbiota could have longer follow-up periods and combine culture methods with molecular biological analyses. With such methods the types of resistance, such as presence of ESBL in bacterial species with resistance to third generation cephalosporins, could be identified. Not least, this type of information can be of value to make the research comprehensible, clear, and relevant to people outside the research field: when communicating it to clinicians and patients. Adding metagenomics could measure possible changes in α- and β-diversity – changes in an individual microbiota as well as comparisons between microbiotas of different individuals.

From a global perspective, ending the use of antimicrobials in the meat industry is a top priority. However, each single course of antibiotics is important to the individual and her internal microbial ecology. Knowledge of possible hazardous side effects of antibiotics is necessary to achieve an ethically acceptable, patient-safe and sustainable prescribing practice. Adjustments in primary care treatment guidelines may have a strong impact over time, because it affects so many people. This is why infections and antimicrobial use is such an important area of future primary care research.

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