General introduction and outline of the thesis
For the past 70 years, following up on the discovery of penicillin [1], antibiotics have been mass-produced and used for the treatment of bacterial infections, thereby prolonging the lives of many patients [2]. Several accomplishments of modern-day medicine such as transplantations and chemotherapy are only possible because of this discovery [3]. In the mid-twentieth century penicillin was even called the ‘miracle drug’, since deaths because of bacterial infections decreased dramatically [4]. Intrinsically linked to antibiotics is the development of antimicrobial resistance (AMR) in bacteria, i.e. resistance to a clinically relevant concentration of an antimicrobial drug that was originally effective for treatment of infections caused by this pathogen. This natural process has become a growing public health problem during the last decades. Across the globe, rising levels of AMR are seen in both hospital and community settings [5-7], hampering the successful treatment of bacterial infections. Several studies have demonstrated that AMR frequently leads to a delay in the administration of an effective therapy, which is associated with increased costs, morbidity or even mortality. It may even force clinicians to prescribe agents that are more expensive, more broad-spectrum, or more toxic [8,9].

The problem of how to contain the development of AMR has received increasing attention from doctors, policy makers and scientists. The current problems health care is facing, such as methicillin-resistant Staphylococcus aureus (MRSA) [10,11] or extended spectrum beta-lactamase (ESBL) producing Escherichia coli have led to urging reports from international stakeholders. For example the World Health Organization (WHO), the European Centre for Disease Prevention and Control (ECDC) and the European Academies Science Advisory Council (EASAC) have advocated more efficient use of antibiotics, and promote the development of new antibacterial drugs [6,12,13]. However, the number of new antibiotics produced by the pharmaceutical industry is decreasing [14,15] as new agents are expensive to develop and there are only a finite number of molecular targets for antibiotics available. Several stakeholders have launched initiatives aimed at developing new and effective antibiotics, e.g. WHO Europe, the Infectious Diseases Society of America (IDSA) and the European Medicines Agency (EMA) [8,16,17]. Recently a study reported the discovery of such a new antibiotic, based on soil samples [18]. However, as Summers stated: ‘Discovering new antibiotics will buy us time, but the same ancient molecular mechanisms will ensure their eventual loss of efficacy as well’
Overall, the options to treat bacterial infections might decrease in the future, narrowing the possibilities of health care by increasing the risks of procedures such as transplantations [3]. At the same time, globalization and increased travelling of the human population lead to an easier spread of AMR across the globe [20]. This alarming situation requires combined international efforts, in order to counteract the emergence of worldwide antibiotic resistance [3]. The first step in this process is to really understand the mechanisms behind AMR.

**ANTIBIOTIC RESISTANCE: MECHANISMS**

Two types of resistance mechanisms can be distinguished: 1) bacteria are considered to be resistant when not susceptible to a clinically relevant concentration of an antibiotic; 2) and/or the bacteria possess a property which will render the antibiotic ineffective [21]. Usually the resistance originates from being able to make a specific protein that inactivates the antibiotic agent or otherwise circumvents the agent’s damaging effect on the bacteria. The possibility of producing such a specific protein is an expression of a resistance gene [22].

Resistance genes can either be an intrinsic property of an organism, or be acquired via other bacteria. Intrinsic resistance is the innate ability of a bacterial species to resist activity of a particular antibiotic. This applies to the whole species, for example, enterococci are by default resistant to cephalosporins. The inherent structural or functional characteristics of the bacteria allow tolerance of a particular drug or antimicrobial class (also called insensitivity since it occurs in organisms that have never been susceptible to that particular drug). Intrinsic resistance can e.g. be due to lack of affinity of the drug for the bacterial target, inaccessibility of the drug into the bacterial cell, extrusion of the drug by chromosomally encoded active exporters or innate production of enzymes that inactivate the drug [21].

Acquired resistance on the other hand occurs when a particular microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible. This can result from mutations in existing chromosomal or plasmid coded genes (resulting in a small part of the population either way acquiring resistance), or from the acquisition of resistance genes from another bacteria. Acquiring genes takes place via several
ways of horizontal gene transfer (transferring genetic material to neighbouring bacteria by cell-to-cell contact, instead of transferring genes in the reproduction process) [23]. This can be achieved via conjugation, transformation or transduction, see figure 1 for an overview.

Figure 1: Mechanisms of resistance acquisition. DNA containing an antibiotic resistance gene (pink) can be transferred by horizontal gene transfer into a recipient by several paths: cell-to-cell conjugation; transformation by naked DNA (on plasmids or as linear DNA) that is released by dead cells; or phage-mediated transduction. Resistance can also arise in the process of mutation (indicated by a red cross) [23]
The most common mechanisms resulting from mutations include alterations in the antibiotic target and increases in drug efflux, but resistance is also associated with gene amplification, reduced expression of the target and alteration of drug modification enzymes [23]. On the other hand, mechanisms originating from horizontal gene transfer mostly include production of beta-lactamases, reduced permeability of the bacterial cell, or actively pumping out the antibiotic drug. Transfer of resistance is possible to bacteria of the same but also to other species, however, transfer of chromosomal coded genes is less frequent. Also, resistance by gene transfer is preceded by the prior existence of a resistance gene in a reservoir [21,24].

Various laboratory studies have shown that most antibiotic resistance mechanisms are associated with a fitness cost that is typically observed as a reduced bacterial growth rate [23]. Unfortunately, resistant bacteria can decrease the costs of resistance by acquiring additional mutations to compensate. Together with the amount of horizontal gene transfer, the magnitude of this fitness cost is a main biological parameter that influences the rate of development of resistance [23].

**SELECTIVE PRESSURE**

Resistance to antibiotics becomes a clinical problem when the AMR rates hamper effective treatment of bacterial infections. This process is influenced by exposure to antibiotics: susceptible bacteria in the human microbiota are eradicated; thereby indirectly promoting the proliferation, prevalence and dissemination of resistant potential pathogens (the process is often called selective pressure) [21,22,25]. This mechanism implies that a discontinuation of antibiotic exposure could allow susceptible bacteria to outcompete resistant bacteria if the selective pressure from antibiotics is reduced. Unfortunately, the available data suggest that the rate of reversibility will be slow at the community level [23]: although declines in resistant bacteria have been noted when exposure to antibiotics is discontinued, a return to the preterm level of antimicrobial resistance does not usually occur because of the compensation mechanisms and cross resistance (resistance to a particular antibiotic resulting from exposure to another similarly working antibiotic).

It has become clear that exposure to antibiotics is a multi-faceted ecological problem, with different contexts in a globalized world [3]. Figure 2 depicts...
several sources from which antibiotic exposure stems: for example direct antibiotic use, the veterinary sector [26], or antibiotics present in soil or the water.

Figure 2: Important sources of exposure to antibiotics or resistant bacteria

Several studies have assessed the indirect forms of exposure, e.g. working in the veterinary sector is a risk factor for AMR [27]. It is difficult to distinguish the effects of the different sources [28-32]; however laboratorial spa-typing of the bacteria can provide insight into the mechanisms of transfer [33]. Next to these main sources, other risk factors influence the extent of exposure to antibiotics or resistant bacteria; e.g. transfer of (resistant) bacteria is facilitated in enclosed environments with high rates of physical contact or sharing equipment, such as day care centres or contact sports facilities [34,35]. Another example is found in hospitalized patients with a catheter running a higher risk of infections with resistant S. aureus [36].

The major source of exposure however is direct intake of antibiotics, which is therefore considered the most important risk factor for AMR [37-39]. Until now, most studies have focussed on antibiotic intake in the hospital setting, assessing AMR in this enclosed environment. Nonetheless, of all prescriptions for human use, 90% of all antibiotics is being prescribed in primary care [37], exerting selective pressure on the community. Next to this, an increase has been seen of AMR in healthy community-based patients, without healthcare-associated risk factors [40-42]. This community-associated (CA) resistance is related to the commensal flora: combining selective pressure in primary care with gene transfer and a pool of available bacteria, the human microbiota constitutes an enormous reservoir of resistance genes, serving as an early indicator of resistance development in a population [22].
HUMAN MICROBIOTA

The most important reservoir of resistance genes is the human microbiota [43]. Only at birth we are sterile; the average adult human body hosts approximately 100 trillion microbial symbionts, outnumbering our own cells with a factor 10 to 1 [44].

Our microbiota plays an important role: it supports us by producing or breaking down nutrients, and by priming our immune system. The bacteria in our flora (up to 1,000 different species) are mostly harmless, but at the same time potentially pathogenic, with the well-known examples of e.g. *Staphylococcus aureus* and *Escherichia coli* [43,45]. When the normal flora is disturbed in one way or the other (e.g. by means of a viral infection, open wounds or antibiotics aimed at treating an infection in another part of the body), bacteria from our microbiota can cause opportunistic infections. This reservoir of antibiotic resistant microorganisms includes a pool of resistance genes: via horizontal gene transfer resistance can spread throughout the microbiota [21]. Resistant strains of bacteria in multiple sites like the skin or the respiratory tract can persist in the human microbiota for up to one year in the absence of selection by any antimicrobial agent [38].

*Staphylococcus aureus*

One of the geni of bacteria in our human microbiota is *S. aureus*, a potentially pathogenic, gram positive commensal with a high impact on public health. It can cause several mild to severe infections, for example skin and soft tissue infections (SSTIs) or bacteraemia [46-48]. Carriership of *S. aureus* is dynamic and occurs on multiple body sites, with the nares being the most common location [44,49]. Approximately 20% of the overall population is a persistent carrier, whilst 50-60% is an intermittent carrier, which means that 20-30% is never a carrier [47]. Carriership is demonstrated to be a risk factor for infection (i.e. odds for infection are 4 times higher in carriers than non-carriers) [40] and most bacterial infections are caused by our own microbiota [49-51]. Since the start of antibiotic use in the previous century, resistance of *S. aureus* has gradually developed, however in the last decades a steep increase occurred in the prevalence of a variant of *S. aureus*: methicillin resistant *S. aureus* (MRSA) [41].

Methicillin resistance was first reported in hospitals [46], and MRSAs are typically resistant to a number of other agents, including beta-lactam antibiotics, erythromycin and clindamycin, aminoglycosides, tetracyclines, and
The presence of this multi-drug resistant MRSA previously seemed to be confined to the hospital or veterinary settings. During the last decades its prevalence has increased in European hospitals and long-term care facilities, forming one of the major causes of bacterial nosocomial infections [51,53]. Within Europe, large differences in MRSA prevalence can be found, from <1% in Sweden to over 50% in Portugal [53-57]. More recent reports describe colonization and transmission of MRSA in different populations: younger people and persons without health-care associated risk factors such as hospitalization or antibiotic use [40].

The prevalence of community-associated MRSA (CA-MRSA) has risen substantially across Europe, and is associated with substantial morbidity and mortality, resulting in a challenging public health problem [46]. This CA AMR is of a different nature than the nosocomial resistance previously receiving most attention [41,58]. Spa-typing has indicated that strains of MRSA found in the community differ from health care-associated MRSA strains in several aspects, unfortunately not less pathogenic [59,60]. CA-MRSA cause different infections in different populations, they differ in AMR patterns and they spread rapidly among healthy people in the community [41]. The presence of CA S. aureus is often linked to SSTIs [46,61,62], e.g. impetigo, folliculitis, furuncle or cellulitis. SSTIs are frequently treated in primary care and are one of the main indications for antibiotic treatment in primary care [48,63,64].

**ANTIBIOTIC TREATMENT IN PRIMARY CARE**

Given the high antibiotic prescription rates in primary care, many stakeholders have advocated cautious and appropriate prescribing of antibiotics to control the emergence of AMR: empirical treatment with antibiotics should only take place if necessary and should ideally include appropriate agents which are effective against the most common causative pathogen for the infection [65,66]. The actual effectiveness of the prescribed antibiotic is dependent on many other factors, such as way of administration, dosage pattern or compliance of the patient [67], but one of the first steps is to choose an appropriate antibiotic in the light of AMR. An inappropriate antibiotic treatment
will have several effects, in the first place for the patient: the effectiveness of the treatment will be limited. Secondly, unnecessary costs will occur for the health care system; and finally, the exposure to antibiotics could lead to a further increase of AMR [68].

As mentioned before, the majority of all antibiotics for human use are prescribed in primary care [37]. In Europe a variation in antibiotic consumption is observed from approximately 9 Defined Daily Doses (DDD) per 1,000 inhabitants a day in Switzerland to 38 DDD per 1,000 inhabitants a day in Greece [69]. The variation in antibiotic prescription rates across Europe, as depicted in Figure 3, suggests a possibility to decrease the prescription volume for several countries [69].

Also, prescription patterns vary per country: e.g. in Scandinavian countries penicillins are prescribed more often than in southern countries [71]: physicians in Northern European countries more often prescribe small-spectrum beta-lactamase susceptible penicillins (J01CE), while physicians in Southern European countries more often opt for broader spectrum beta-lactamase resistant penicillins (J01CA). This variation might be explained by cultural differences, market access, reimbursement variations, or national policies in medical education [72]. For all prescriptions, the choice of antibiotic should ideally be congruent with the AMR pattern of the causative agent. However, for general practitioners (GPs), information about the resistance patterns of the causative pathogen is often not available as performing microbiological cultures is not common practice in primary care. Therefore, ecological data on AMR could be supportive in this setting [17,73].
Figure 3: Antimicrobial consumption of antibacterials for systemic use (ATC group J01) in primary care in Europe, 2013. Source: ECDC [70]
Next to general resistance levels in the population, a GP could also take into account more specific individual risk factors when prescribing an antibiotic. Several studies so far have assessed risk factors for carriage of resistant bacteria, for example age (children and elderly), admission to long-term care facilities and use of antibiotics [40,74]. However, GPs often only focus on eliminating pathogenic bacteria and disregard the impact of exposure to antibiotics in general [75].

Studies supporting the association between antibiotic use and antibiotic resistance often concern streptococci or *E. coli*, and use either data on a national level, or focus on local enclosed environments [76-79]. Treatment of bacterial infections, in the case of *S. aureus* particularly SSTIs, would benefit from improved knowledge on (risk factors for) resistance in commensal *S. aureus*.

**TREATMENT GUIDELINES**

The purpose of evidence-based practice guidelines is to bridge the gap between scientific research and practice [80]; they can serve as a powerful step towards effective health care and – in case of guidelines for treatment of infections - a decrease in the development of antibiotic resistance [80,81]. The development of antibiotic treatment recommendations is a complex process which involves many factors, such as pharmacoeconomics, side effects, quality of care and the effectiveness of the antibiotic [82,83]. In the light of AMR, the recommended antibiotic should be effective against the most likely causative pathogen. Therefore, several studies recommend including relevant AMR data when developing or revising primary care treatment guidelines for bacterial infections [17,65,73,84].

Local outpatient AMR patterns could be used as evidence base for treatment guidelines, in the case of *S. aureus* mainly for skin infections. The Infectious Diseases Society of America recently updated their guidelines for skin and soft infections [85], but given the variation in AMR, treatment guidelines on a national or regional level are recommended. However, well-documented information about resistance patterns of commensal *S. aureus* is currently lacking.
COMBATTING AMR IN EUROPE

The dynamics of AMR have created a public health problem of an ecological nature: both the intra- and extramural settings are involved, risk factors such as age, profession and hospitalization have been identified on the individual or the ecological level, and bacteria do not stop at customs to declare their resistance [3]. The costs of using antibiotics faced by society are weighed differently by the individual who receives them or the GP who prescribes them [86]. The possible individual profit of antibiotic treatment is counterbalanced by the ecological effect of spreading antimicrobial resistance: a bittersweet conundrum.

Several countries have established initiatives combatting AMR on a national level, e.g. Sweden [87], Denmark [88] and The Netherlands [89]. Given the ecological nature of the public health problem of AMR, it should be viewed in an international context [6]. However, coordinated action is largely absent, especially at the political level, both nationally and internationally [3, 15]. Nowadays, with open borders and a high travel-intensity across the globe, transfer of AMR and exposure to antibiotics is not only limited to regions. Global mobility of populations and food products create new possibilities for transmission of antimicrobial resistance [66,90]. However, due to specific exposure patterns in certain regions (e.g. different prescription behaviour in northern and southern Europe) [37,69] different AMR patterns can emerge. Given national or regional resistance patterns another choice of antibiotic might be more appropriate and a European policy of harmonization might not always be the best approach, but rather adjustment to and taking into account national resistance and prescription patterns. The strategic action plan of WHO Europe encompasses several of these solutions, e.g. promoting national coordination, promoting rational use of antibiotics, and strengthening surveillance of antibiotic resistance [16]. It is important for improvement of prescribing quality to monitor antimicrobial resistance and usage data on national levels and benchmark these by comparisons with other countries.

SCOPE AND AIM OF THIS THESIS

In summary, the multifactorial public health problem regarding AMR crosses borders and requires combined efforts in several settings. Considering the main risk factor for the development of AMR, antibiotic use, the primary care setting where the majority of antibiotics for human are being prescribed is an
important area of attention. Combined with the increasing prevalence of community-associated resistance, this thesis will fill a gap in knowledge and focus on assessing AMR in the commensal microbiota. In particular we will study the major pathogen *S. aureus* which is associated with mild SSTI infections but also with severe diagnoses (e.g. caused by MRSA). Provided the large variation in prescription volume and patterns across Europe, we might be able to combat AMR by increasing the appropriateness of antibiotic treatment by learning from best practices and changing prescribing habits. In order to combat the development of AMR, national variations in AMR should ideally be integrated in antibiotic treatment guidelines and actual treatment patterns. Therefore, the studies in this thesis encompass AMR, antibiotic use and treatment guidelines in nine European countries, from North to South.

The aim of this thesis is to contribute to the efforts in dealing with resistant *S. aureus* and in decreasing its impact on society. This will be done by collecting valuable new data regarding the antimicrobial resistance of *S. aureus* in the commensal flora, combined with treatment patterns of SSTIs. Crossing the bridge from science to implementation by including treatment guidelines also allows us to further a practical implementation of our results: we will be able to assess and contribute to the improvement of daily practice. To reach this goal several questions will be answered throughout this thesis:

1) What are AMR levels for commensal *S. aureus* in the community across Europe?
2) What are risk factors associated with AMR in commensal *S. aureus*?
3) Are national primary care treatment guidelines for skin infections in Europe congruent with commensal *S. aureus* AMR levels in the community?
4) Are antibiotic treatments of skin infections in primary care congruent with commensal *S. aureus* AMR levels in the community?

**APRES STUDY**

The data for this thesis is collected within the EC (FP7 DG Research) funded APRES study: ‘The appropriateness of prescribing antibiotics in primary care in Europe with respect to antibiotic resistance’ [91; chapter 3] This 4.5 year study was led by The Netherlands Institute of Health Services Research (NIVEL), together with three other main partners (Maastricht University, University of Nottingham and University of Antwerp), and collaborating with fifteen partners.
in nine countries (coordinators of General Practice Networks and laboratories). For more information and a complete list of partners see www.nivel.eu/apres.

OUTLINE OF THE THESIS

Chapter 2 presents an overview of treatment guidelines for skin and soft tissue infections in 9 European countries and the evidence base for the treatment recommendations. In Chapter 3, the design of the study ‘The Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance’ (APRES) is described. Nine European countries were involved in the APRES study, selected on different levels of antibiotic use in the community. The data collected in the different Work Packages of the APRES study are used for the analyses in this thesis. The results regarding AMR of commensal S. aureus are presented in Chapter 4, including the MRSA prevalence.

In Chapter 5 we take a closer look at the risk factors for carriage of resistant S. aureus bacteria, focussing on the exposure to antibiotics based on treatment data. Following this, in Chapter 6 we investigate daily practice by relating AMR and antibiotic treatments further: to what extent is antibiotic treatment of SSTIs in primary care congruent with national resistance patterns of S. aureus? A similar question will be answered in Chapter 7: collating all information gathered in the APRES study, are the treatment guidelines congruent with the AMR data? Finally in Chapter 8 we discuss and summarize the main findings along with recommendations and implications for future research.
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