Summary and General Discussion
SUMMARY

Background and aim

The aim of this thesis is to contribute to efforts against resistant Staphylococcus aureus (S. aureus), particularly in primary care, and to reduce the impact of antimicrobial resistance (AMR) on society. The multifactorial public health problem of AMR crosses national borders [1] and calls for combined efforts in several settings, for example, the hospital setting, primary care and veterinary sectors [2-5]. Since antibiotic usage is the main risk factor for the development of AMR [6-7], the primary care setting, where the majority of antibiotics for human use is being prescribed, is a critical area of attention [8]. Next to this, an increasing prevalence of community-associated resistance has been observed in the last decade, whilst attention has mainly been on the hospital setting [9-11].

The thesis aims to fill a knowledge gap and assesses community-associated AMR in the commensal microbiota, an important reservoir of AMR [12,13]. In particular, we have studied the major pathogen S. aureus which is associated with diagnoses ranging from relatively mild skin and soft tissue (SSTI) infections to severe bacteraemia, e.g. caused by methicillin-resistant Staphylococcus aureus (MRSA) [9,14,15]. Based on the large variation in antibiotic prescription volume and patterns across Europe [16], we might be able to increase the appropriateness of antibiotic treatment by learning from best practices and changing prescription habits. In order to combat the development of AMR, national patterns of AMR should ideally be taken into account in the development of antibiotic treatment guidelines and in the treatment of bacterial infections [17-20]. Studies in the thesis therefore encompass AMR, antibiotic use and treatment guidelines in nine European countries, from Sweden in the North, Hungary in the East to Spain in the South.

The thesis collected valuable new data on AMR in commensal S. aureus, and combined with treatment guidelines and antibiotic treatment patterns of SSTIs in Europe several aspects of AMR have been assessed. The first part of the thesis concerns with AMR levels in commensal S. aureus in the community, and the risk factors for carrying such resistant bacteria. In the second part, we have applied this knowledge to daily practice by relating the AMR patterns to treatment guidelines and antibiotic prescriptions for skin infections.
Main findings

Commensal Staphylococcus aureus resistance in Europe

The first part of the thesis addressed AMR patterns of commensal S. aureus, using data collected in nine European countries. In Chapter 3 the design of the APRES study ‘The appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance’ is described, in which data were collected on AMR, antibiotic prescription patterns and treatment guidelines in primary care.

AMR was studied in a convenience sample from the community in the nine European countries by taking nose swabs: participants were eligible for inclusion when they visited their GP for a non-infectious condition and had not been prescribed antimicrobial agents nor had been hospitalized in the previous three months. Data on antibiotic prescriptions over the previous 5 years were extracted from the Electronic Medical Records (EMR) of General Practitioners (GPs). In all participating countries, the prevailing SSTIs treatment guidelines were collected.

The prevalence of S. aureus was described in Chapter 4. The overall prevalence of nasal S. aureus carriage was found to be 21.6%. After standardization for gender and age, a wide range in nasal S. aureus carriage rates was found between countries: prevalence in Swedish participants was more than twice as high compared with Hungarian participants (29% versus 12%).

In general, antibiotic resistance levels among the isolated S. aureus were low. Exceptions were the relatively high levels of macrolide resistance, which exceeded 15% in France and Belgium, and the (well-established) high levels of resistance to penicillin in all countries: ranging from 65% (Austria) to 87% (Spain). The prevalence of MRSA did not exceed 2.1% in any of the participating countries.

In Chapter 5 we assessed the relationship between potential risk factors other than antibiotic use (such as age and profession of the patient) and resistance in commensal S. aureus. Of the 6,906 S. aureus isolates, 77% showed resistance to at least one antibiotic, 7.1% demonstrated multidrug resistance (3 or more
antibiotic classes), 78 (1.3%) cases of MRSA were found. Based on the EMR data, a large variation in antibiotic exposure was found between and within countries. While the level of exposure to antibiotics had no significant effect on carriage of resistant bacteria, younger age and a higher proportion of penicillin prescriptions in a general practice were associated with higher odds for any resistance at all in commensal \textit{S. aureus}. Next to this, we found higher overall multidrug resistance rates in participants working in healthcare or a nursery. Finally, the variation in AMR was mostly explained at a country level (and not on a general practice level).

\textit{Treatment of SSTIs in primary care}

In Chapter 2, we reviewed primary care treatment guidelines for bacterial skin infections (often associated with \textit{S. aureus}) and assessed to what extent they are based on antibiotic resistance data. Thirteen primary care treatment guidelines were obtained from eight countries across Europe, regarding common skin infections in primary care: Impetigo, Cellulitis, Erysipelas, Folliculitis and Furuncle. Our results showed a high agreement across countries: all recommended antibiotics were of the beta-lactam class and mainly small spectrum. The advised treatment durations were consistent; the dosages however varied considerably, with the highest dosages recommended in Sweden. Seven guidelines (54%) were not based on scientific publications related to resistance, and in the guidelines that did include evidence on AMR, 75% of the references were from abroad.

In Chapter 6 the congruence between the AMR patterns in commensal \textit{S. aureus} and the actual treatment of SSTIs was presented in five countries. Beta-lactamase resistant penicillin, mainly represented by co-amoxiclav, was most often (43%) used for the treatment of SSTIs in primary care. France and the Netherlands stood out with a higher proportion (>20%) of tetracycline treatments (J01AA) and almost no lincosamides (ATC J01FF), while macrolides were more often prescribed for the treatment of SSTIs in Hungary. France and Belgium showed higher proportions of treatment of SSTIs with penicillins, mainly amoxicillin (both 11%), compared to the other three countries.

National congruence rates were calculated, based on the volume of each prescribed antibiotic and the resistance rate of commensal \textit{S. aureus} against that antibiotic. The rates ranged from 84% (France) to 93% (Croatia). We also showed that the use of more penicillins decreased the congruence rate:
excluding penicillins for the treatment of SSTIs resulted in a congruence rate of 94% or higher in all countries.

Finally, in Chapter 7 we assessed the treatment guidelines in the light of commensal *S. aureus* AMR patterns. Most of the first- and second-choice recommendations were congruent with the AMR patterns of commensal *S. aureus*, except for two recommendations for penicillin (a single dose aimed at pathogenic streptococci).

**DISCUSSION**

**Commensal *Staphylococcus aureus* resistance in Europe**

The prevalence of carriage of *S. aureus* in our study was on average 22%, with considerable inter-country variation (ranging from 12-29%). In previous studies, the average prevalence in healthy adults has been approximately 27%, although several recent studies have reported a prevalence of around 20% [21,22]. In our study, we have only used the anterior nares as a sampling site for *S. aureus*, which could lead to an underestimation of the true prevalence, since the pharynx, skin, and perineum are also common habitats of commensal *S. aureus* [23,24]. The inter-country variation in prevalence, with participants in Sweden having a prevalence more than double that of Hungary, could not be explained by the study design. Although *S. aureus* was isolated in eight different laboratories, we used standard protocols for the isolation and identification of *S. aureus* and carried out a validation study before the start of the investigation. It can be argued that genetic host factors play a role in this variation, as these have been found to contribute to *S. aureus* nasal carriage [25] and these factors were not taken into account in our study. Future studies on the prevalence of *S. aureus* carriage should further explore explanations for this inter-country variation [26].

The low resistance levels in commensal *S. aureus* found in our study cannot be directly compared to other investigations due to a lack of comparable studies. Few studies have focused on the commensal flora, and the ones that did investigate community-associated *S. aureus* often only focused on MRSA [10,27]. Nosocomial *S. aureus* pathogens have been studied more extensively and the European Centre for Disease Prevention and Control (ECDC) hosts a website on which up-to-date resistance rates of several pathogens are presented [28]. Several countries have also developed a national database in
which annual AMR data are presented [29].

The prevalence of MRSA we found in the commensal flora (maximum of 2.1%) is congruent with the reports of previous studies [30-33], and very low compared to prevalence in hospital settings [34,35]. Since several studies have shown a decrease in MRSA prevalence and/or infections [36], it can be argued that the low MRSA prevalence in our study could be the result of the increased public awareness of MRSA (and of AMR in general) and the subsequent measures taken to control MRSA over the recent years [37]. Several European countries have developed programmes aimed at reducing antibiotic prescriptions, and increasing public knowledge about MRSA and AMR [38]. Measures to control the spread of MRSA have focused on trying to break the chain of transmission (e.g. the isolation of colonized or infected patients or better hygiene) or trying to reduce the selective pressure for the emergence and persistence of MRSA by improving antibiotic prescribing) [39].

Regarding the risk factors we found for carrying resistant S. aureus, other studies have also reported multi-resistance in healthcare workers [40]. Higher proportional prescription of penicillins and younger age were also shown to be a risk factor in our study. While most other similar studies have not reported age as a risk factor [11,41,42], the ones that do report varying results [40,43]. For example, the elderly and children are often more exposed to antibiotic treatments and therefore more likely to carry a resistant S. aureus [43]. Other recent studies have confirmed that antibiotic prescriptions are a risk factor for AMR [41]. Although within-country variance in prescribed antibiotics was found, as has previously been described in other reports [16,44,45], we found the country level was the main factor which explained variations in AMR across Europe.

Treatment of SSTIs in primary care

The first part of the thesis described the resistance patterns of commensal S. aureus in primary care. Given that they are quite low across Europe, one might assume that the treatment of SSTIs, commonly associated with S. aureus [15], is straightforward. However, Chapter 4 showed national variation in resistance patterns, while at the same time local AMR data is often not available in empirical treatment in primary care. In the second part of the thesis, we have further investigated the national differences in the treatment of SSTIs.
Our analysis of treatment guidelines for skin infections showed quite a lot of consistency between countries. However, the Swedish guidelines stand out: the recommended dosage is higher, and a narrow-spectrum beta-lactamase susceptible penicillin is often the recommended antibiotic. Differences in national guidelines could, for example, be explained by the availability of AMR data, different interpretations of evidence, cultural factors such as differing evaluations of risks and benefits, local market authorization procedures or other characteristics of the health care systems [46]. Evidence-based data regarding AMR was relatively limited for the treatment guidelines: only 46% included data on AMR, mostly from other countries. Although similar studies have not been undertaken for *S. aureus* infections, a study on bacterial meningitis guidelines also showed many differences between countries and a low integration of relevant AMR data [47].

Although the recommendations in the treatment guidelines were consistent, wide variation in actual prescriptions was observed. The thesis did not consider the extent of guideline adherence in the different countries, since individual factors also need to be taken into account when prescribing an antibiotic. The decision to prescribe an antibiotic is complex, based not only on the diagnosis and an assessment of the likely risk of complications, but also, for example, on the doctor–patient relationship, the patient’s history and expectations, and the time constraints of the consultation [44,48]. The GP will, in choosing an antibiotic, also consider various characteristics of the patient, like allergies, or the likelihood of the diagnosis.

The variation in antibiotic prescription patterns that we found within and between countries has been confirmed by and is congruent with several other published studies [16,45,49]. Also regarding antibiotic prescriptions, Scandinavian countries (represented by Sweden in our study) stand out with a proportionally high use of narrow-spectrum penicillins, which is, for example, shown by the ESAC network [50]. A 2013 study which conducted qualitative interviews of 80 primary care clinicians in nine European countries, showed that Northern European respondents generally favoured narrow-spectrum agents, motivated by containing resistance whilst Southern/Eastern European respondents were more often motivated by maximizing treatment effectiveness and so justified the use of broad-spectrum antibiotics [51]. This approach might be more appropriate in southern and eastern European countries, where AMR rates are relatively high. Our study could not assess whether the higher AMR
rates in southern countries were the result of more diverse prescription patterns, or on the contrary the prescription patterns are the result of the national AMR patterns. Longitudinal studies could provide more insight into the exact nature of this relationship.

**METHODOLOGICAL CONSIDERATIONS**

The data that have been presented in the first part of the thesis form a unique database of commensal *S. aureus* AMR patterns across nine European countries. The results we have shown are valuable in two ways: 1) on an ecological level they provide insight into the selective pressure on a population level, and 2) the commensal AMR data can be used in daily practice to improve the treatment of individual patients with *S. aureus* infections, in particular SSTIs.

The most important assumptions we made concern the extrapolation of the resistance rates and patterns we found in the nasal commensal flora, to those in pathogenic *S. aureus* (related to skin infections). It has been demonstrated that commensal *S. aureus* can be isolated from several body sites. Although the nares are the most common site [23,24], by swabbing only the nares we potentially missed up to 50% of all carriers. Despite this, a study by Miller compared isolates from several body sites and classified both the infection strains and the colonizing strains regarding their Spa-type, resulting in a high congruency between the sites [24]. Nonetheless, we are not aware of any study comparing the actual AMR patterns of infection strains versus colonizing strains. We choose to study the commensal flora because of its importance as a reservoir of resistance, in which resistance can easily be transferred to other bacteria. This results in antibiotic resistance often being present in commensal bacteria first, before disseminating to their pathogenic counterparts [13,52], which often originate from one’s own flora [24,53]. For this reason, the commensal flora is the most important source of information regarding the current ecological status of AMR. Also, by including patients without current bacterial infection, our sample size is powered to assess differences on an international level, providing valuable information about a possible approach to combat AMR.

Another important assumption referred to the relationship between *S. aureus* and SSTIs: we assumed that the five assessed skin infections are always caused by *S. aureus*. While this has been reported for most of these skin infections [15,54], this is an extrapolation for all cases of skin infection. Other pathogens
often causing SSTIs are streptococci, with presumably different AMR patterns. For example, recent studies indicate that penicillin resistance in streptococci remains low [55], implying that penicillin, which is mostly ineffective against S. aureus, could be used for streptococci. This thesis only focused on skin infections, since the other infections related to S. aureus (e.g. bacteraemia) have a low prevalence in primary care and are more often found in a hospital setting, where different AMR levels are found [34]. Finally, we were only able to analyse diagnosed SSTIs which were treated with systemic antibiotics. Future studies should investigate not or otherwise treated diagnosed SSTIs.

The third major methodological assumption made for the thesis is related to the collection of prescription data. In our study, GP networks participated in each of the nine countries. For each network, approximately 20 practices took part in the data collection: the swabbing of the patients and the provision of EMRs. The participating practices were representative for the network, which in turn is assumed to be representative for the country as a whole (e.g. regarding age and gender). Nonetheless, we know that in some countries (e.g. Spain and Belgium) the GP network was located in one region, and therefore the participating practices were only representative for that particular region (respectively Catalonia and Flanders). This might influence the representativeness of the data for the whole country; however no data for the other regions was available.

Next to this point, the collection of prescription data was complex. Different registration and software systems are used, between and also within countries. Different systems for coding diagnostic information are also used (e.g. ICPC versus ICD10), and in some countries patients are required to be registered (listed) at a certain practice whilst GPs in other countries can only provide information on the patients who visited the practice in the last year. Diagnostic codes can be re-coded, and a different patient system does not affect the proportional prescription rates presented in Chapter 5, but this will influence our measurement of the total amount of antibiotics prescribed per 100 patients.

Not all practices that participated in the swabbing process were able to provide complete and valid prescription data in a comparable format. We assumed that missing data display the same patterns as the available data. This is supported by our validation of the data, as the prescription data we collected was
compared with the national ESAC data [16, 56]. Our initial goal was to also include data on the volume of prescribed antibiotics, using Defined Daily Doses (DDDs) [57], however, for most of the countries it was not possible to calculate valid DDD values for the prescribed antibiotic packages. Our study shows the drawbacks of different registration systems, within and between countries. This provides an opportunity for policy makers in Europe to encourage homogeneity and congruence in data collection procedures in the primary care setting.

Overall, the methodological context of the entire thesis is an ecological one, particularly regarding Chapters 5 and 7. In Chapter 5 we compare the individual characteristics of commensal AMR with antibiotic exposure on a (higher) practice level: the amount of antibiotic prescriptions in the practice of the participant. In Chapter 7 we compare aggregated AMR data on a national level with antibiotic treatment recommendations in national primary care treatment guidelines. We have assumed that these ecological measures are the best approximation of the same variable on an individual level. A study design with antibiotic exposure and treatment data on an individual level would have provided other information; however, given that empirical treatment in primary care is often carried out with no individual data (e.g. from culture), we believe our design contributes to daily practice in primary care.

**RECOMMENDATIONS**

The international and ecological approach of the thesis has filled a knowledge gap and provides valuable insights into the current situation regarding AMR in commensal *S. aureus*. These insights can be used to further control the development of AMR and improve the treatment of *S. aureus* infections in primary care. This section will provide recommendations for several stakeholders: the scientific field, general practitioners and policy makers.

**Recommendations for future research**

The ecological study design applied in this thesis has provided some valuable new insights and was a first step in collecting information on commensal AMR across Europe. The comparison between countries has shown differences in the treatment of SSTIs in primary care. A study conducted on the individual patient level, however, assessing the direct link between diagnosis, resistance and outcomes of the treatment would provide more detailed information on the effectiveness of the antibiotic treatment of SSTIs or other *S. aureus* infections.
Secondly, related to one of the assumptions made in this thesis, we recommend the scientific community to further investigate the congruence between *S. aureus* in the nasal flora and on other bodily sites, and in particular the relationship between resistance in the commensal flora and in pathogenic bacteria. This information would be a valuable contribution to making AMR data more easily interpretable.

Next to this, studies regarding commensal AMR patterns of other pathogens would also be very useful. Since infections are typically caused by a range of different pathogens, more AMR data could be integrated into the guidelines, along with information on how to distinguish between the clinical expressions of the different pathogens.

Finally, it would be interesting to further assess the adherence to treatment guidelines. Our study revealed, for example, that multiple guidelines may exist in one country. What are the reasons for GPs to choose a treatment, and if an antibiotic is prescribed, what influences their choice of antibiotic? A recent study in nursing homes showed that up to 25% of the antibiotic prescriptions in that setting were not appropriate [58]. This could, for example, be improved by interventions aimed at prescribers, like was shown by other studies [59,60].

**Recommendations for general practice**

Several interventions have been developed lately to decrease or improve antibiotic prescription behavior. Given the national variations in AMR patterns these incentives would preferably be fine-tuned to the national situation, but the overall aim of these approaches is usually to reduce unnecessary prescriptions, and to increase both patient and physician knowledge on AMR [44]. For example, a multicenter trial in six European countries, combining point-of-care testing with communication training led to a 62% reduction in antibiotic prescribing for acute respiratory tract infections in primary care [60]. Other interventions might include delayed prescribing: the patient receives a prescription but is urged to only obtain the actual drug once the situation remains unaltered after a certain amount of time. This has been shown to reduce antibiotic use by patients without negatively impacting on patient outcome and has the additional benefit that patients become less reliant on antibiotics [44]. Interventions to increase knowledge might include posters.
aimed at patients, or initiatives such as the “Antibiotic Awareness Day” which is initiated by the EC each year in November. Its aim is to inform the public and general practice about AMR, change social norms about prescribing, and influence attitudes towards antibiotic resistance and use [61].

Next to this, GPs could benefit from knowledge on local AMR patterns. These could be obtained in cooperation with local laboratories, where samples are sent for culture. While treatment guidelines in primary care are based on empirical treatment, a suggestion might be that each GP practice regularly sends a culture to the laboratory (e.g. 1 in every 20 patients), focusing on the most common pathogens in primary care such as streptococci, staphylococci and/or *Escherichia coli*. After a while, this would result in an overview of AMR in the commensal flora from the practice population, providing valuable information to be discussed in GP practice and with interdisciplinary medical teams. This surveillance information, alongside the incorporation of risk factors regarding carriage of a resistant pathogen, could increase treatment effectiveness for individual patients [62]. Finally, feedback on their own prescription behavior could increase awareness regarding AMR in GPs. To achieve such a feedback system the first step is to maintain EMRs correctly and consistently [63].

**Recommendations for policy makers**

Although the public health problem of AMR at the moment might be smaller in primary care compared with the hospital setting [ears], it is certainly important to keep the problem at bay and try to improve the current situation. Our results show between-country variation for both commensal AMR levels and antibiotic prescription patterns; therefore an important approach towards AMR will be on the national policy level. International efforts such as learning from best practices might be useful to decrease the impact of AMR on society [64]. This is, for example, one of the activities of WHO-Euro, which connects several countries, regions and settings in order to approach AMR with interdisciplinary expertise [65]. Several differences on a country level can be identified as influencing the development of AMR, for which tools can be developed.

For example, one of these characteristics is over-the-counter-sales of antibiotics, which used to be common practice in part of the European Union, mostly in the southern countries [66]. Nowadays, this is prohibited and a prescription is mandatory to receive an antibiotic. However, in the era of digital
purchases this might be difficult to control. The contribution of this former policy to the higher overall AMR levels in the South is unknown, but rational and prudent use of antibiotics will certainly improve the situation [17].

Another important factor that sets apart certain countries is the existence of a surveillance network. It is widely argued that this type of surveillance systems contributes to controlling AMR, e.g. by increasing awareness of GPs and policy makers and resulting in more evidence-based guidelines and possibly higher adherence [67-69]. The first countries to have developed such a network in the mid-nineties were Denmark, Sweden and The Netherlands (respectively DANMAP, STRAMA and SWAB [29,70,71]. These countries are known internationally for their low AMR rates, both in hospitals and the community. The activities deployed by these groups focus on matters regarding antibiotic use and containment of antibiotic resistance, and facilitating an interdisciplinary working model, involving all relevant stakeholders, including national and local authorities and non-profit organizations. This has, for example, resulted in slightly different policies in Sweden, regarding an overall lower prescription volume, high dosage of antibiotics and a smaller variation in antibiotic treatments [56], and it is not clear yet whether this policy might also be beneficial for other European countries [72].

These interdisciplinary and multi-faceted approaches are needed to tackle this ecological public health problem. All across Europe, national and international stakeholders have urged action on the area of diagnostics [2], the development of new antibiotics or exploring non-antibiotic approaches [73], and initiatives in the husbandry and food sector [74,75]. This has, for example, led to a decrease of antibiotic prescriptions in the veterinary sector in the Netherlands, previously one of the highest prescribers for animal use, of 50% in only a few years’ time [76,77].

These aforementioned initiatives can be grouped under the often used term ‘antimicrobial stewardship’. Several programmes have been initiated across Europe, mostly in hospitals [78-82]. Evaluations of these programs have reported lower antibiotic use and resistance in the hospitals concerned, which have translated into lower costs and shorter hospital stays [83]. The Infectious Diseases Society of America (IDSA) has identified several important factors on how to set up a successful antimicrobial stewardship programme and has created guidelines [84]. These have already been extrapolated to other settings,
such as the education of GPs or providing the public with information on AMR [60,85]. Actions on multiple levels involving stakeholders from several settings will increase our chances in the battle against the natural evolutionary force of bacteria, and will help maintain all of the current tools to combat infectious diseases at our disposal. Finally, in order to not only stabilize but also improve the current situation, we will need to open up our minds: “Progress is impossible without change, and those who cannot change their minds cannot change anything”.
— George Bernard Shaw
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