Discussion and conclusions
The discovery of many new antibiotics in the 1940s and 1950s heralded a new era, promising humanity's final triumph over infectious diseases. However, natural selection in the form of emerging and expanding antimicrobial resistance soon thwarted this optimistic view. Faced with an unknown scale of antimicrobial resistance as a threat to human health, surveillance networks were put in place at the local, national as well as international level. For Europe, the most comprehensive resistance data were collected by the EARSS (1999-2009). This effort is now managed by the ECDC and renamed EARS-net. The on-going surveillance of antimicrobial susceptibility data of invasive isolates for seven major pathogens has substantially contributed to knowledge about the geographical distribution and temporal dynamics of antimicrobial resistance in Europe. For *S. aureus* and *E. coli*, the two most common pathogens causing BSIs, surveillance data showed high proportions of methicillin resistance and rapidly increasing resistance to third-generation cephalosporins. However, a lack of clinical outcome data precluded the appraisal of the associated human costs in terms of morbidity and mortality at continental scales. At the same time, most observational studies carried out to determine the impact of infections caused by multi-resistant bacteria were hampered by scope, design and statistical approach, curtailing their general validity. The studies included in the present thesis were designed to provide better estimates of the health and economic consequences associated with BSIs caused by MRSA and G3CREC in Europe.

**Main findings**

In order to generate more accurate and more precise estimates about the impact of antimicrobial resistance for Europe, multi-centre, prospective cohort studies were conducted in 13 tertiary care centres in as many different European countries. These data showed that *S. aureus* and *E. coli* BSIs had a considerable impact on mortality and duration of treatment among hospitalized patients. Additionally, we found that methicillin resistance significantly increased mortality, while, third-generation cephalosporin resistance not only increased mortality but also prolonged hospital stay. Part of the additional impact of antimicrobial resistance could be explained by a difference in clinical effectiveness of treatment. While most patients suffering from susceptible *S. aureus* and *E. coli* BSIs received effective treatment in a timely manner, administration of *in vitro* active antibiotic therapy was often delayed for patients with MRSA and G3CREC BSIs. Yet, administration of the appropriate treatment after 48 hours did not improve survival among these patients.
To assess the burden of disease associated with antimicrobial resistance at European level, patient based risk estimates from the cohort studies were applied to prevalence data extrapolated from the EARSS. In 2007, it was estimated that 27,711 episodes of MRSA BSIs (4.8/100,000 inhabitants) and 15,183 episodes of G3CREC BSIs (2.6/100,000) resulted in 8,215 excess deaths (1.4/100,000), and incurred 62.0 million excess hospital costs by prolongation of hospital stay. At the same time, forecasts for the next five years indicate that - despite anticipated gains in the control of MRSA - the rapid increase in G3CREC is likely to double the burden of disease in the European region. When examining surveillance data for *S. aureus*, *E. coli*, *S. pneumoniae*, *E. faecium* and *E. faecalis*, it could be shown that the occurrence of BSIs, irrespective of species and susceptibility, has substantially increased between 2002 and 2008. The most significant increase was found for *E. faecium* and *E. coli*. The trend for the latter was mainly due to a rise in antibiotic-resistant isolates and probably associated with the expansion of successful and antimicrobial resistant clones. Since trends for BSIs caused by resistant and susceptible bacteria are dissociated (implying independence), expansion of antibiotic resistance seems to cause additional cases, rather than replace infections of susceptible strains. As a consequence, we expect that antimicrobial resistance *per se* creates extra strain on limited health care resources.

**Novel methodological approaches introduced in the presented investigations**

For ethical reasons, differences in clinical outcomes of patients infected with resistant or susceptible agents cannot be assessed by clinical trials. Thus, any impact of antimicrobial resistance can only be inferred from epidemiological observation. In order to obtain valid estimates it is essential to minimise bias and to control for confounding. For the investigations of clinical outcomes associated with antimicrobial resistance presented in this thesis, we applied a novel study design and state-of-the-art analytical approaches to improve the validity of our estimates compared to previous studies.

**Time is of the essence**

The standard control group for determining excess mortality associated with antimicrobial-resistance in BSIs consists of patients infected with susceptible bacteria. This approach is chosen to address the etiologic fraction attributable
to antimicrobial resistance; i.e. what would be the (counterfactual) outcome if patients had been infected with resistant bacteria instead of susceptible ones. This selection of controls however ignores the fact, that patients with resistant infections have typically stayed longer in hospitals before their infection than patients infected with susceptible bacteria. This means that the risk profile of both patient groups is not the same: Patients with longer admission periods (before infection) have more indwelling devices, such as urinary catheters, venous or arterial drip lines and have more often experienced invasive therapeutic interventions than patients who witness shorter periods of hospitalisation (before infection). It is very likely, that both patient groups also differ in more fundamental aspects, such as co-morbidities, severity of illness, or other important clinical determinants that are difficult or even impossible to quantify. If - as one might expect - these underlying differences are associated with mortality, direct comparison of patients with resistant infections versus patients infected by susceptible bacteria introduces confounding as well as selection bias. To overcome these threats to the validity of our estimates, we applied a parallel matched-cohort design (Figure 1), whereby patients with MRSA or G3CREC BSIs (resistant cohort) and patients with MSSA or SEC BSIs (susceptible cohort) were matched to separate, uninfected controls based on equal hospital exposure periods before infection of the case. The study results showed that matching indeed improved comparability between patients and their controls, for both cohorts. Cases and controls drawn for the resistant cohort had more hospital exposure in the year prior to enrolment, frequently had elective admissions and surgical procedures and more often had received antibiotic therapy when compared to the cases and controls from the susceptible cohort. In order to determine the etiological fraction for antimicrobial resistance, risk measures from both cohorts could be compared.

Another advantage of the parallel design was the ability to determine the impact associated with MRSA and G3CREC BSIs for two different scenarios: addition, whereby infections with antimicrobial resistant bacteria add to the amount of BSIs caused by susceptible bacteria, or replacement, whereby resistant bacteria replace infections by susceptible strains. For the first scenario, the impact of MRSA and G3CREC was compared to uninfected controls. For the replacement scenario, the risk measures from the resistant and susceptible cohorts were contrasted. A disadvantage of this design is a reduction in precision of the clinical effect estimates for the replacement scenario. In this case, the effect is determined by comparing the outcomes from two independent cohorts, whereby the variance of this difference is the sum of the variances from the two cohort estimates, resulting
in a wider confidence interval. Consequently, for this scenario, not all associations studied in these investigations reached statistical significance at the conventional threshold of $p \leq 0.05$. However, all point estimates indicated that antimicrobial resistance increased mortality for patients with BSIs.

![Figure 1](image_url)

**Figure 1.** The parallel matched-cohort design, whereby infected cases are matched to uninfected controls based on LOS before infection of the case. This is done separately for patients with an infection caused by resistant bacteria (R; left) and for patients infected by susceptible bacteria (S; right). Through matching, cases and controls within each cohort have similar risk profiles and become better comparable. Thus, the risk of dying (from resistant and susceptible infection) can be determined independently and more accurately for both cohorts. The excess risk attributable to antimicrobial resistance is then estimated by subtracting the obtained (log) estimates in a second step.

Time also plays an important role in the association between administration of *in vitro* active antibiotic therapy and mortality. Adjustment of therapy informed by antibiotic susceptibility testing takes about two days after isolation of pathogens. Consequently, patients who survive for at least two days have a higher likelihood of receiving appropriate therapy, and naturally these patients are likely to be less ill than those who already died. Patients who receive appropriate therapy have therefore a higher *a priori* chance of survival even if therapy was ineffective. This type of selection bias is an analogy of the “healthy worker” effect, whereby only those who are healthy enough to still be at work are enrolled into the study. In our specific case, the effect is termed immortal time bias; patients have to survive for
a minimum period of time (two days) to be able to receive appropriately adjusted therapy. By including appropriate therapy as time-dependent variable in the Cox’s regression, bias due to this potential spurious association between longer pre-treatment survival, a higher likelihood of receiving appropriate therapy and longer post-treatment survival can be eliminated. The results from our study indicated the presence of immortal time bias (data not shown) and studies ignoring time until appropriate treatment are likely to overestimate the clinical effectiveness of treatment. Finally, time also plays an important role when comparing different mortality endpoints. In the case of hospital mortality, the length of follow-up is dependent on LOS, which is highly variable. Therefore, hospital mortality should be analysed by time-to-event methods. Inherent to this method is censoring, whereby patients are censored if they are lost-to-follow-up or still alive at the end of the study period. For these patients it is not known when or whether they will experience the event-of-interest: i.e. hospital mortality. However, patients who were discharged alive will never experience the event of interest and should not be regarded as censored. Therefore time-to-event analysis should be adjusted for the competing events of hospital discharge versus hospital mortality. Ignoring these competing events will result in an overestimation of the effect. So far, most studies on the impact of infections caused by resistant pathogens have not applied time-to-event analyses when measuring hospital mortality and to our knowledge competing events have been completely ignored. Mortality can also be measured at a predefined interval after infection. This is a more straightforward measure, which can be analysed by standard logistic regression, resulting in ORs that are easier to interpret than HRs. However, this mortality measure encompasses other risks to validity. Severely ill patients have a higher likelihood to die in hospital within the predefined period, while more healthy patients have a higher likelihood of being already discharged, making it more difficult to establish their life status post discharge. This could result in loss-to-follow-up specifically for the more healthy group of patients, and thus to differential loss-to-follow-up. Fortunately, this type of information bias can easily be prevented by active collection of life status data, for example through contact with the family doctor, which was done in the studies described in this thesis. Both types of mortality endpoints were analysed in the studies presented in chapter 2 and 3: hospital mortality and 30-day mortality. Both indicated an increased risk of dying for patients with BSIs caused by MRSA and G3CREC, although not all estimates were statistically significant. Future studies on the impact of antimicrobial resistance should acknowledge these different time-related issues at the design and analyses stage. As demonstrated, this
can be accomplished by applying a parallel matched-cohort design and appropriate
time-to-event analyses. Another method that takes into account time-related bias in
the association between infections and hospital mortality are the multi-state models,
which represent an extension to the competing events analysis.7,8 Instead of having
one initial exposure state, for example MRSA infection, a multi-state model can take
into account the timing of several intermediate events, like admission to hospital,
colonization and subsequent infection, while timing of the endpoint is considered
as well. Thereby, the multi-state model can reduce time-related bias even further.
A major disadvantage of multi-state models is that they require clinical data to be
collected for all hospitalized patients at risk of infection over the entire study period.

**Burden of antimicrobial resistance in perspective**

The emergence and expansion of antimicrobial resistance has considerable
health and economic consequences at the level of the individual patient, the local
hospital as well as at the national, continental and global level. But how does the
burden of disease of antimicrobial resistance compare to other health threats?
When the cause-specific mortality rates for G3REC and MRSA BSIs are compared
to that reported for two of the most prominent causes of death, cardiovascular
disease and cancer, the impact seems rather modest.9 (Table 1) Compared with
other infectious diseases, however, the estimates are more similar and in the same
range as tuberculosis and HIV in Europe.9 In the following paragraphs, the mortality
estimates presented in his thesis will be put into perspective, by discussing the
impact of antimicrobial resistance for other species, comparing our data using
other burden of disease measures and considering possible future scenarios.
Although antimicrobial resistance among obligate pathogens such as *M. tuberculosis*, *N. gonorrhoea*, HIV, and *Plasmodium spp.* may have dire consequences
for human health especially in low resource communities, the present work
focused on cosmopolitan opportunistic bacteria. They are part of the human
residential flora and emergence of resistance among these species is a reflection
of the selective forces imposed on human microbiota by antibiotic use for any
indication. Infections caused by these resistant species, mainly occurring in
health care institutions, are thus a sensitive marker for the degree with which
antimicrobial resistance has already encroached on human populations. However,
little attention has been paid to the burden of disease caused by these infections,
while their public health impact may be substantial. *S. aureus* and *E. coli* are two of
the most important examples of this genre. These bacteria are a common cause of hospital-acquired infections, and account for almost half of all BSIs identified in hospitals.\textsuperscript{10,11}

**Table 1.** Mortality rates (per 100,000 population) and disability adjusted life years (DALYs), for those causes ranking highest in each category, and for HIV, tuberculosis, gonorrhea and BSIs in Europe\textsuperscript{*} in 2004.\textsuperscript{9,13}

<table>
<thead>
<tr>
<th>Cause</th>
<th>Deaths per 100,000 population</th>
<th>Total DALYs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-communicable diseases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>373</td>
<td>7,915,000</td>
</tr>
<tr>
<td>Cancer</td>
<td>256</td>
<td>8,273,000</td>
</tr>
<tr>
<td>Lung</td>
<td>50.9</td>
<td>1,627,000</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>33.7</td>
<td>994,000</td>
</tr>
<tr>
<td>Breast</td>
<td>22.1</td>
<td>908,000</td>
</tr>
<tr>
<td><strong>Communicable diseases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower respiratory tract infections</td>
<td>29.5</td>
<td>424,000</td>
</tr>
<tr>
<td>HIV</td>
<td>1.5</td>
<td>40,000</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1.0</td>
<td>196,000</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>0.0</td>
<td>14,000</td>
</tr>
<tr>
<td>Bloodstream infections\textsuperscript{^}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA and SEC</td>
<td>3.3</td>
<td>66,000</td>
</tr>
<tr>
<td>MRSA and G3CREC</td>
<td>1.5</td>
<td>28,000</td>
</tr>
<tr>
<td><strong>Injuries</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Road traffic accidents</td>
<td>9.3</td>
<td>1,017,000</td>
</tr>
</tbody>
</table>

\* Austria, Belgium, Cyprus, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Israel, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovenia, Spain, Sweden, Switzerland, United Kingdom.

\^ These estimates apply to 2007, DALYs were approximated\textsuperscript{14} based on the average age of affected patients\textsuperscript{15,16} and the average life expectancy\textsuperscript{17}

A recent report from the ECDC provided a rough estimate for the number of deaths attributable to six different multi-resistant bacterial pathogens causing infections of the bloodstream, respiratory tract, skin and soft tissue and urinary tract.\textsuperscript{12} These figures suggest that the number of deaths associated with MRSA and G3CREC BSIs encompass about two thirds of all those related to BSIs caused by resistant pathogens, and about 20% for all infections included in that analysis. Overall, it can be expected that the mortality estimates for MRSA and G3CREC BSIs are a sensitive marker that captures a large proportion of the excess number of deaths associated with antimicrobial resistance encountered in European hospitals. Based
on these estimates, antimicrobial resistance contributes to mortality in a order, comparable to that reported for HIV, tuberculosis, and potentially similar to road traffic accidents (if deaths from infections of all bacterial causes at all anatomical sites were included).\(^9\)

The conventional currency in which burden of disease is measured has become the DALY, which stands for disability adjusted life year. It combines disease associated mortality and morbidity into a single value, adding up the number of life years lost due to premature death as well as life years lived with disability.\(^9\) Thereby, loss of healthy life in younger people will contribute proportionally more DALYs than adverse health events that strike at older age. As a result, burden of disease measured in DALYs will rank diseases differently than cause-specific mortality rates. In high income countries, DALYs associated with BSIs and indeed infections caused by antimicrobial resistance bacteria will yield relatively low figures, as the average age of affected patients (65+) is close to the average life expectancy (76 for men, 82 for women)\(^{17}\). This contrasts with fatal road traffic accidents, which represent the single most important cause of death in the age group 15-30 years in Europe. Similarly, tuberculosis and HIV will result in a higher number of life years lost, although the difference is less extreme. This explains why DALYs for MRSA and G3CREC infections are significantly smaller than for tuberculosis, HIV and fatal road traffic accidents, even though mortality rates are similar. (Table 1, Figure 2)

![Figure 2](image-url). The age distribution of cause-specific mortality for tuberculosis, HIV and fatal transport accidents for the 27 European Member States in 2007.\(^{17}\)
Notably, DALYs and mortality rates for BSIs caused by MSSA and SEC are more than two times larger than for those caused by resistant phenotypes (Table 1). Although the individual risks are larger for infections caused by multi-resistant *S. aureus* or *E. coli*, the higher incidence of MSSA and SEC BSIs (47.4/100,000 inhabitants versus 7.4/100,000) results in a higher attributable mortality. This indicates that prevention efforts should address BSIs as a whole and not focus on antimicrobial resistance alone. Although community onset infections will be difficult to prevent, it has been shown that a large part of the nosocomial events can be avoided. One of the major risk factors for infections of the bloodstream is the improper insertion and prolonged use of central lines. Instigation of multi-modal prevention bundles could greatly reduce the risk, as exemplified by the remarkable reductions of MRSA BSIs after the start of the national “Saving lives” strategy among Hospital Trusts in the UK. Although the burden of disease associated with BSIs is much lower than for chronic diseases, the prevention potential may be significant, making it a relevant target for public health interventions. A simple intervention like enhanced promotion of best practice among health care personnel could considerably reduce attributable mortality.

For the near future, trends indicate a rapid and dynamic change in public health relevance of antimicrobial resistance. We predict that the amount of infections caused by antimicrobial resistance pathogens may double within the next five years (chapter 5), providing that the unabated increase in number of G3CREC BSIs continues, and despite the fact that the number of MRSA BSIs may slowly decrease. These predictions were based on the assumption that observed dynamics remain unchanged and newly emerging clones would be equally successful as previous ones. However, recent observations suggest that this assumption may not hold. In Europe, ESBL-positive Enterobacteriaceae have emerged in the community: carriage was detected in 5% of healthy adults in the Netherlands in 2011 (unpublished data) and similar proportions were found in Spain and Switzerland. For these bacteria, a poultry reservoir has also recently been identified and these developments may further accelerate trends for G3CREC infections. For MRSA, live-stock associated clones (ST398) have been found to spread to humans as well, but the clinical impact of this specific clone seems limited as yet.

The largest threat associated with antimicrobial resistance is emergence of pan-resistant bacteria causing infections defined as untreatable with antibiotics presently in clinical use. In this perspective, the expansion of carbapenemase-producing Enterobacteriaceae is highly relevant. These strains are resistant...
to all beta-lactams and commonly harbour co-resistance to cephalosporins, aminoglycosides and quinolones. This limits treatment options to very few antibiotics, often with unfavourable pharmacokinetics and toxic side-effects. So far, the public health impact of these pan-resistant strains appears to be negligible; most outbreaks were self limiting or rapidly contained and affected only a small number of patients. However, the public health impact could grow rapidly when a successful clonal lineage emerges, as exemplified by the nationwide outbreak of KPC-3 positive *K. pneumoniae* in Israel between 2006 and 2008.

**Conclusions**

Estimating the number of excess deaths and the excess costs associated with MRSA and G3CREC BSIs represents a first step to assess the burden of disease attributable to antimicrobial resistance. Our estimates indicate that relative to other health threats the impact of antimicrobial resistance on mortality and DALYs appears to be modest. A serious, but as yet, unpredictable threat is associated with the emergence of pan-resistant strains causing untreatable infections, but so far the fear for a return to the pre-antibiotic era appears to be premature. In the end, the burden of disease related to BSIs caused by susceptible pathogens greatly exceeds that for resistant bacteria, emphasizing the need for improved prevention and management of infections by opportunistic bacteria in general.
Reference List


