ANTIBIOTIC USE AND ANTIMICROBIAL RESISTANCE IN A MULTI-LEVEL MODEL

COMPARING DAYS OF THERAPY (DOT) AND DEFINED DAILY DOSES (DDD) AS RISK FACTORS FOR ANTIMICROBIAL RESISTANCE IN A MULTI-LEVEL MODEL

By

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ABSTRACT

Antibiotic use is generally regarded as the major driver for resistance. Many studies reporting an association between antibiotic use and the emergence of resistance have been published. However, most studies have significant limitations such as single center data with comparably low number of cases, using retrospective designs with limited data availability, ecological studies with lack of assessing the individual level and risk for ecological fallacy, and inappropriate selection of controls in case-control studies.

A cohort study in adult patients hospitalized in 15 participating acute care hospital sites in Ontario, Canada, was conducted from April 1 2005 to June 30 2006. Antibiotic use on the unit level in defined daily doses (DDD) was only available for 3 sites. In order to assess antibiotic use on both the individual as well as on the unit level as a risk factor for resistance, days of therapy (DOT) could be calculated. However, it was unclear whether this approach would results in similar findings as when using DDD. Thus, the impact of using either DDD or DOT on the risk estimates for resistance was assessed for three antimicrobial-bacteria combinations, i.e. fluoroquinolone use and fluoroquinolone resistance in enterobacteriaceae an in *Pseudomonas aeruginosa*, and the use of betalactams and resistance to third generation cephalosporins in enterobacteriaceae.

The risk estimates for resistance were very similar for all three antimicrobial-bacteria combinations on acute care units, there were some discrepancies on the unit level on intensive care units, and discrepancies on both levels for step down and rehabilitation units.

In conclusion, the approach to use DOT instead of DDD to measure antibiotic utilization revealed similar results. However, the lack of comprehensive information on patient transfers when calculating DOT may bias the findings on units with frequent patient transfers such as intensive care units and step down and rehabilitation units.

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PART I: INTRODUCTION

Clinical relevance of resistant pathogens

Resistance to antibiotic drugs among clinically relevant pathogens continues to emerge worldwide. Methicillin-resistant *Staphyloccocus aureus* (MRSA), first described by Jevons in the 1960's (Jevons, et al. 1963), has since emerged as the most important and most common multiresistant pathogen globally (Klevens, et al. 2007; Tiemersma, et al. 2004). In Canada, an increase in both the incidence of infection and colonization over the last 12 years has been reported (Canadian Nosocomial Infection Surveillance Program). Furthermore, other clinically important multiresistant and difficult to treat pathogens such as quinolone resistant (Blaettler, et al. 2009), extended-spectrum beta-lactamase producing enterobacteriaceae (ESBL) (Freeman, et al. 2009), and vancomycin-resistant enterococci (VRE) (van den Braak, et al. 2000) are also spreading worldwide (Arias and Murray 2009), threatening to reduce the efficacy of current antimicrobials (Spellberg, et al. 2008). The most recently reported emerging resistance mechanism is a transmissible genetic element resulting in multidrug resistance among gram-negative bacteria, the New Delhi metallobetalactamase 1 (NDM-1) (Kumarasamy, et al. 2010), which has also been detected in Ontario (Tijet, et al. 2011).

Compared to their susceptible counterparts infections caused by resistant pathogens are associated with worse outcomes, such as longer length of stay, higher mortality rates, and greater costs (Allegranzi, et al. 2010; Cosgrove, et al. 2005; Cosgrove, et al. 2003; de Kraker, et al. 2011; Falagas and Rafailidis 2007; Lambert, et al. 2011; Lautenbach, et al. 2001; Lautenbach, et al. 2006; Lee, et al. 2006; Mauldin, et al. 2010; Schwaber, et al. 2008). Health-care associated infections by these resistant pathogens are estimated to be responsible for about 100,000 deaths and costs of $23 billion in the United States (US) annually (Roberts, et al. 2009). The reasons for the worse outcomes are probably multi-factorial (Vincent 2011), but include a tendency for greater underlying diseases, delayed antimicrobial coverage, and lastly, but potentially becoming increasingly important, the lack of effective antimicrobials. As a consequence of waning alternatives for the treatment of multi-resistant pathogens, the Infectious Diseases Society of America (IDSA) has recently founded the 10 x ’20 initiative, a global commitment to develop 10 new antibiotics by 2020 (Infectious Diseases Society of America 2010).

Antibiotic exposure as a risk factor for infections by resistant pathogens

The first antibiotic, penicillin, was introduced in 1943. Only 6 years later, about 60% of *Staphylococcus aureus* isolates were penicillin resistant in British hospitals (Barber and Whitehead 1949). Since, antibiotic utilization is generally regarded as the

major driver for emergence of resistant pathogens (Dellit, et al. 2007). In the light of still increasing use of antimicrobials and continuing emergence of resistant pathogens (Ansari, et al. 2009; Edwards, et al. 2008; Pakyz, et al. 2008), antimicrobial stewardship programs were developed as an essential field of work for infectious diseases specialists (Dellit, et al. 2007; Petrak, et al. 2003). The appropriate use of antimicrobial agents is crucial for patients' safety and quality assurance (Burke 2003; Davey, et al. 2006; Gould 1999; Shlaes, et al. 1997), particularly in view of increasing drug resistance and diminishing antibiotic pipelines (Spellberg, et al. 2008). In addition to the emergence of resistance, antibiotic use was estimated to be responsible for about 142,505 visits to emergency rooms in the United States for drug-related adverse events attributable to systemic antibiotics (Shehab, et al. 2008). Therefore, the major goals of stewardship programs are to optimize clinical outcomes and to minimize the unintended consequences of antimicrobial use, i.e., toxicity, high costs, and the selection of pathogenic organisms and emergence of resistance (Dellit, et al. 2007).

Many studies reporting an association between antibiotic use and the emergence of resistance have been published in the past. Most are ecological in design, evaluating an association between the aggregated antibiotic use and the incidence or prevalence of resistance either cross-sectionally or over time. The fact that there are important differences in the prevalence of resistant pathogens between specific hospital units is of importance for studies using an ecological design (Binkley, et al. 2006). Another design commonly in use for these types of studies is the case-control design using individual

level data. Both approaches have potential major limitations when concluding that exposure to a specific antimicrobial drug results in a specific resistance pattern. For aggregate data, the major limitation is the potential for an 'ecological bias', i.e. they fail to reflect the effect on the individual level, because individual exposure is not being linked to the individual outcome (Greenland and Morgenstern 1989). On the other hand, the effect of individual patient antibiotic exposure can either be decreased or amplified by an interaction between the individual and the group effect, i.e. the antibiotic utilization on the unit level (Lipsitch and Samore 2002). Therefore, an analysis of antimicrobial use as a risk factor for resistance should incorporate both, the antibiotic use on the unit as well as on the individual level. Such studies may shed light on the importance of the individual exposure and of the ecological environment influenced by the antibiotic utilization on the unit level.

The failure to take both levels of antimicrobial exposure into account is one major limitation of the published literature. To our knowledge, only two studies have analyzed both levels of exposure in the same population in acute care hospitals. In one study, almost no effect of antibiotic use on the unit-level on resistance of gram-negative pathogens was found, while the individual patient exposure was a strong risk factor for resistance (Harbarth, et al. 2001). One of the conclusions of this study was that individual-patient-level data should be included in multi-center studies to elucidate the relation between antibiotic exposure and resistance. In another study, a multi-level model incorporating both, individual antibiotic exposure and antibiotic use on the unit level, was

used to analyze risk factors for MRSA (Muller, et al. 2006a). They found that individual exposure to fluoroquinolones and collective exposure to penicillin was associated with MRSA isolation (Muller, et al. 2006a).

To provide an overview of the evidence, some of the most recent studies evaluating antibiotic use as a risk factor for resistance will be mentioned here despite the limitation of either evaluating the individual or aggregate level antibiotic exposure, only.

Ecological design:

Over time, an increase in the use of ciprofloxacin and amoxicillin/clavulanate on the level of individual medical specialty correlated with an increase in resistance rates of *Escherichia coli* (*E. coli*) (Willemsen, et al. 2009), and the resistance to fluoroquinolones was found to be associated with an increase in the use of fluoroquinolones (Blaettler, et al. 2009; Neuhauser, et al. 2003; Vernaz, et al. 2011). An increase in carbapenem use was shown to be associated with an increase in carbapenem-resistance (Meyer, et al. 2010), which was not true for ertapenem in another study (Eagye and Nicolau 2011). Extended-spectrum beta-lactamase (ESBL) resistance was reported to be associated with an increase in the use of fluoroquinolones and third-generation cephalosporins on the unit-level over time (Kaier, et al. 2009; Vernaz, et al. 2011). The use of imipenem and ciprofloxacin was shown to be associated with a variety of resistance mechanisms (Miliani, et al. 2011). Among gram positives, the incidence of MRSA was reported to be associated with fluoroquinolone use (Parienti, et al. 2011). However, there are very recent

studies showing no correlation between the unit-specific use of a specific antibiotic and the resistance rates against the same antibiotic drug (Bosso, et al. 2010; Jankovic, et al. 2011).

Case-control design

Case-control studies have shown associations between carbapenem resistance and individual exposure to antipseudomonal betalactams (Falagas, et al. 2007), carbapenems (Hussein, et al. 2009; Jeon, et al. 2008; Kwak, et al. 2005; Patel, et al. 2008), but not ertapenem, cephalosporins (Gasink, et al. 2009; Kwak, et al. 2005; Patel, et al. 2008), metronidazole (Jeon, et al. 2008), and fluoroquinolones (Falagas, et al. 2007; Gasink, et al. 2009; Hussein, et al. 2009; Lautenbach, et al. 2006; Schwaber, et al. 2008). In contrast, a negative correlation between the use of fluoroquinolones and carbapenem resistance was found in another study (Kwak, et al. 2005). In another study, previous exposure to any antibiotic, and in particular carbapenems, was found to be a major individual risk factor for ertapenem-resistance (Hyle, et al. 2010). Similarly to carbapenem resistance, isolation of ESBL was associated with exposure to betalactams (Rodriguez-Bano, et al. 2008), third-generation cephalosporins (Graffunder, et al. 2005), trimethoprim/ sulfamethoxazole (Graffunder, et al. 2005), aminoglycosides (Graffunder, et al. 2005), and fluoroquinolones (Rodriguez-Bano, et al. 2008). Carbapenem resistant ESBL was associated with increasing duration of prior treatment with betalactams and fluoroquinolones (Kritsotakis, et al. 2011). Aztreonam resistance was shown to be associated with exposure to fluoroquinolones (Gasink, et al. 2007) and antibiotics with

coverage of anaerobes (Gasink, et al. 2007). Furthermore, an association between the use of aminoglycosides (Muller, et al. 2003), betalactams (Muller, et al. 2003), fluoroquinolones (Muller, et al. 2003; Weber, et al. 2003), and macrolides (Muller, et al. 2003) with MRSA was shown. Antibiotics associated with co-colonization by VRE and MRSA were linezolid and clindamycin (Roberts, et al. 2009).

Limitations of previous research

In addition to not including both levels of antibiotic exposure in the same model, the generalisability and validity of previous research is further limited by the fact that most of these were single-center studies having a comparably low number of cases. They also tended to be retrospective with limited data availability and were focused on specific patient populations. Moreover, no study has analyzed risk factors in the same population for all of the most important drug resistant pathogens of potential interest.

Another methodological limitation is the selection of controls in case-control studies (Harris, et al. 2002; Kaye, et al. 2005). In general, a control group should represent the population from which the cases were derived (Wacholder, et al. 1992). Typically, either patients without any positive clinical culture of a specific pathogen or patients with a susceptible isolate of the specific pathogen are selected for the control group. Importantly, risk factors found in studies using patients with no positive clinical

isolate of a specific pathogen as controls consist of two types of risk factors: risk factors putting a patient at risk for infection by a specific pathogen in general and risk factors associated with infection by the specific *resistant* pathogen. Therefore, one cannot know from the results of this type of studies whether a specific risk factor is associated with hospital-acquired infections with the pathogen of interest in general, or whether it is a true risk factor for infections by resistant but not by the susceptible pathogen of interest. Although the selection of controls among patients with susceptible clinical isolates helps to identify risk factors associated with resistance, this approach has one major limitation: these controls may not be representative for the patients at risk, because the patients at risk for an infection are not primarily to be found among the patients with detection of a susceptible strain and thus, patients that have no clinical isolates should be used as the control group (Kaye, et al. 2005). The latter approach tends to inflate effect measures and exposure to antimicrobials can be falsely identified as a risk factor (Harris, et al. 2002; Kaye, et al. 2005).

Because none of these two approaches to define controls is entirely appropriate to identify patients at increased risk to harbour a particular resistant organism or to identify modifiable risk factors, a case-case-control design has been suggested (Kaye, et al. 2005). In this design, one single control group consisting of patients without any clinical isolates of a particular pathogen of interest are compared to two case groups: a first case group consisting of patients with a resistant pathogen and a second case group consisting of patients with a susceptible pathogen of interest. By comparing and contrasting the two

models, risk factors specifically associated with the isolation of the resistant pathogen can be identified. Three types of risk factors can be identified: a) risk factors exclusively associated with resistant isolates, b) risk factors exclusively associated with susceptible isolates, and c) risk factors for both, susceptible and resistant isolates.

Although the use of the identical control group for both models allows to compare the two risk models, this approach has one major disadvantage, namely, that the control group cannot be matched to the cases due to the use of two different case populations. However, matching is not a *conditio sine qua non* in case-control designs, and matching has limitations, too, such as introduction of bias, loss of statistical efficiency, and complication in selection of controls (Rothman and Sander 1998).

Measurement of antibiotic utilization

In order to analyze antibiotic utilization, e.g. as a risk factor for resistance on a unit level, a standardized measure of antibiotic utilization has to be used. The most commonly used approach is the *defined daily dose* (DDD) as suggested by the World Health Organization: one DDD is defined as the 'assumed average maintenance dose per day for a drug used for its main indication in adults' (http://www.whocc.no/ddd/definition_and_general_considera/). Usually, antibiotic use is reported in DDD/100 or

1000 patient days in order to standardize the results and to allow comparison over time and across different hospital units or hospitals.

Although DDDs roughly represent days of therapy (DOT), there are major discrepancies. This applies in particular to institutions with a significant number of patients with renal or hepatic failure and paediatric patients, because the daily dose actually prescribed is typically lower than the average dose defining the DDD. Furthermore, the DDDs may be misleading when measuring the utilization of antibiotics for which the definition of the DDD does not reflect the average daily dose administered to an average adult patient at one specific institution (Kern, et al. 2005; Mandy, et al. 2004; Muller, et al. 2006b; Polk, et al. 2007; Shetka, et al. 2005; Zagorski, et al. 2002).

Due to these concerns, there is a trend to indicate antibiotic utilization in DOT rather than DDD, whenever this information is available (Polk, et al. 2007). But as the calculation of DOT needs individual patient data (unless the same dose is being used all the time), while the calculation of DDD can be based on the pharmacy's sales figures, the latter is still the most common approach for reporting antibiotic use (Dellit, et al. 2007; MacKenzie 2005; 2004).

PART II: THE MAIN PROJECT

The dataset

The database available consists of data from a Canadian Institutes of Health Research (CIHR) funded project following hospitalized patients of 15 hospital sites in Ontario for up to 16 months (March 2005 to June 2006). The major goal of the study was to analyse institutional risk factor for infection by multiresistant pathogens. Detailed and comprehensive data on antibiotic use was available for 9 of these 15 hospital sites.

The database contained 3 sub-databases providing admission data with patient characteristics, data on individual prescriptions of systematic antibiotics, and information on positive clinical samples including susceptibility testing. In addition, a separate database from the hospital pharmacy provided the aggregated antibiotic use data in DDD/100 patient days of all hospital units for the three acute care sites of Hamilton Health Sciences.

Aims of the actual project

The final goal of analyzing this database was to assess antibiotic use on both the individual and the aggregate level, i.e. at the level of the hospital unit, as a risk factor for infection or colonization by multiresistant pathogens at all nine hospital sites with data on antimicrobial utilization available. Because aggregate antibiotic utilization in DDD was not available for all hospital sites but DOT could be calculated based on the individual prescriptions available at all sites, we first identified the best approach to calculate antibiotic utilization in DOT on the unit level and then compared risk factors identified using DOT as a measure of antibiotic utilization to a model using DDD instead. DDDs were only available for the three sites of Hamilton Health Sciences, therefore the analysis in this thesis was limited to these three hospital sites.

Of note, the individual patient dataset available to calculate DOT, i.e. the prescription database, has two limitations: potential of missing data and lack of information on patient transfers (see below). Although these data initially stemmed from the hospital pharmacy, too, the reports of DDD by the hospital pharmacy are taking all patients transfers into account and presumably do not have any missing data.

In order to evaluate the impact of measuring antibiotic utilization either in DDD or DOT, we analyzed the following antimicrobial-bacteria combinations for which an association was likely: enterobacteriaceae resistant to fluoroquinolones and use of

fluoroquinolones, enterobacteriaceae resistant to third generation cephalosporins and use of beta-lactams. and *Pseudomonas aeruginosa* (*P. aeruginosa*) resistant to fluoroquinolones and use of fluoroquinolones.

The official minimal inhibitory concentration (MIC) breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) were used (Clinical Laboratory Institute 2005). A clinical isolate of enterobacteriaceae and *P. aeruginosa* was defined as fluoroquinolone resistant, if ciprofloxacin resistance was found. An isolate of enterobacteriaceae was defined as resistant to third generation cephalosporins, if resistance to one of the following cephalosporins was found: cefotaxime, cefpodoxime, ceftazidime or ceftriaxone. Intermediate susceptibility was considered as resistance.

Research question

Does measuring antibiotic use on the unit level in DOT instead of DDD change the findings of associations between specific antimicrobial-bacteria combinations in a multi-level model?

Implications for the understanding of health care

An understanding of the implication of the use of different independent variables, i.e. DOT and DDD, respectively, will help interpret findings of the next study based on these data, which will examine antibiotic use as a risk factor at all nine hospital sites, using DOT as the only available risk factor on the unit-level in the same multilevel model as used in this thesis.

From the findings of the final analysis of the data of all hospital sites, we will be able to assess antibiotic use as a risk factor for infections by resistant pathogens in a more complex manner than done previously. The approach to include both levels of exposure in one model allows us to control each level of exposure for the other level. This may either corroborate the findings of previous studies, or it may contradict those findings and show that they were biased either due to ecological fallacy, or that the individual exposure per se was wrongly regarded as a risk factor, because it only indirectly represented antibiotic use on the unit level. Better knowledge about risk factors for infection by resistant pathogens may help to focus interventions to optimize antibiotic use and eventually to optimize empiric antimicrobial in individual patients based on a risk profile incorporating previous antimicrobial exposure.

PART III: PRELIMINARY PROJECT: AGGREGATION OF ANTIBIOTIC UTILIZATION DATA

Objectives of the preliminary project

In order to evaluate antibiotic utilization as an institutional risk factor, aggregate utilization data on the unit level was needed. These data in DDD were available for the three hospital sites of Hamilton Health Sciences, only. Because the doses of antibiotics applied were not available for all hospital sites, calculation of antibiotic utilization in DDD on the unit level was not feasible. Therefore, calculation of the DOT based on the individual data would be the best approach to measure antibiotic utilization on the unit level.

The aim of this subproject was to identify the best method to aggregate DOT by comparing the results to DDD/100 patient days on the three hospital units where DDD data were available. Therefore, the unit specific DOT/100 patient days were calculated based on two different assumptions.

Background: Disparities between DOT and DDD

There are three main explanations for discrepancies between DDD/100 patient days as reported by the pharmacy and the aggregated DOT/100 patient days from the individual patient data. The first is the discrepancy between the definition of the DDD and the actually prescribed doses, the second is the lack of information on patient transfers, and the third is the potential of missing data in the databases. The first reason is a well-known limitation of the definition of DDDs and will explain part of the discrepancies as outlined in the introduction. The latter two reasons are limitations of the individual patient dataset and apply only to the calculation of DOT, as the pharmacy dataset used to calculate the DDD took patient transfers into account and the database did presumably not have any missing data.

Methods of the preliminary project

Assumptions to calculate DDD/100 patient days

Information on patient transfers was missing entirely in the prescription database. Therefore, no information about the hospital unit the patient was hospitalized at the time of a specific antibiotic course was available. However, the admission database provided the date of admission and discharge and the hospital unit a patient has been initially admitted to. Therefore, the aggregated number of DOT of each patient was accounted to

the unit the patient was admitted to. However, this approach would be accurate only, if patient transfers were rare and therefore negligible. While this might be a reasonable assumption for most hospital acute care units, it would ultimately result in less reliable aggregate data in hospital units with a high rate of transfers, in particular critical and intensive care units (CCU/ICU). Aiming to minimize this potential bias, the information available in the data from the microbiological laboratories was used: this database comprises all bacterial pathogens detected during the study period and provides information on the source unit from which a specific sample from a specific patient has been sent. Therefore, antibiotic courses of patients found to be transferred to another unit based on the data available from a positive culture, were accounted not only to the unit the patient has been initially admitted to, but also to the unit the patient has been transferred to. This resulted in an overestimation of antibiotic utilization overall, because these courses were accounted to two units. However, as the date of transfer is unknown, this is the most reasonable approach to remedy the lack of information of patient transfers based on the data available.

Lastly, the total number of DOT needed to be divided by a denominator, the number of patient days. The denominator had to take the potential of missing data into account to remedy an underestimation of antibiotic utilization due to missing patient entries in either the admission-, or microbiology database. Therefore, the sum of patient days in the individual patient dataset was used as the denominator, instead of the complete number of patient days as used for the denominator for the calculation of

DDD/100 patient days. For the second approach to calculate DOT taking the information of transfers based on the microbiological samples into account, the patient days of all patients admitted to a specific hospital units plus the patient days of all patients known to be transferred to another unit with antibiotic prescriptions not covered by the admission data were added up to calculate the denominator.

In order to aggregate the DOT/100 patient days on a unit level, first the total number of days of antibiotic therapy and the total length of hospital stay were calculated for each month and unit, i.e. April 2005 to June 2006. Data collection started in March 2005, but no information on patients hospitalized on a specific hospital unit but admitted earlier than March 1st was available. Therefore, an accurate calculation of antibiotic use for March was not feasible. As the length of stay of individual patients exceeded 30 days only in less than 4% of admissions, an exclusion of a longer period of time than April was deemed unnecessary.

Data sample

For each site of the three acute care sites of Hamilton Health Science, the five non-ICU units with the highest antibiotic use in DDDs were evaluated for this preliminary project. In order to define an appropriate sample among the hospital units, those with the highest utilization were selected, as the highest discrepancies between the two approaches could be expected. As we expected different and probably less reliable data from the CCU/ICU due to frequent patient transfers, the ICU and cardiac care unit of

Henderson and MUMC site, and the three ICU at the General Hospital were evaluated separately. In total, we assessed 22 hospital units.

The individual patient data available consisted of the prescription database with data on all antibiotic courses, e.g. the specific drug, and the start- and end-dates of every antibiotic course. The aggregate pharmacy data consisted of the monthly antibiotic utilization in DDD for each hospital unit.

Statistical methods

In order to compare the aggregated DOT/100 patient days with the DDD/100 patient days, we calculated the monthly antibiotic utilization for each of the 22 hospital units. The association was estimated using linear regression. Under the assumption that the definition of DDD is equal to the average prescribed dose, DDD/100 patient days should approach a 1:1 correlation with DOT/100 patient days, and therefore a linear correlation with a r^2 of 1.0. Of note, the r^2 overestimated the real correlation between the two variables due to repeat monthly data within the same unit, i.e. the assumption of non-dependence of the data points was being violated. However, as the correlation of the monthly data within each single unit was the association of interest, and because we expected that the correlation was similarly overestimated in both models compared, we believe that the violation of this assumption is not a major concern in this context.

Two approaches were compared to the pharmacy data: DOT admin/100 patient days and DOT/100 patient days. *DOT admin* refers to accounting the individual prescriptions to the unit of admission, only, while *DOT* refers to the approach which takes the information on patient transfers into account. We expect that DOT/100 patient days better correlates with the DDD/100 patient days than does DOT admin/100 patient days.

The correlation coefficients will be compared by the use of a "single-sample" z-test:

Single sample *z*-test of H_0: $\rho_{12} = \rho_{13}$

$$Z = \frac{(r_{12} - r_{13})/\sqrt{n}}{\sqrt{(1 - r_{12}^2)^2 + (1 - r_{13}^2)^2 - 2r_{23}^3 - (2r_{23} - r_{12}r_{13})(1 - r_{12}^2 - r_{13}^2 - r_{23}^2)}}$$

(r_{12}/ρ_{12} refers to the correlation coefficient between DOT/100 patient days and DDD/100 patient days, r_{13}/ρ_{13} between DOT adm/100 patient days and DDD/100 patient days, r_{23}/ρ_{23} between *DOT/100 patient days and DOT adm/100 patient days*, *n* the number of observations).

Results of the preliminary project

Individual data of 24,251 patients admitted to the selected 22 hospital units from April 2005 to June 2006 was available. They represented 65% of a total of 37,075 adult patients admitted during this time period. The mean length of stay was 8.4 days and the total number of patient days was 208,122. Compared to the number of patient days used as the denominator in the pharmacy data to calculate the DDD/100 patient days

(222,138), a total of 14,016 (6.3%) patient days were missing in the individual patient database (Table 1). A total of 124,464 DOT could be linked to a specific hospital unit by the use of the admission database. An additional 16,243 DOT (13.1% of the total of 124,464) were linked using the data from the microbiology data, adding another 20,661 patient days to the denominator. The total number of DDD for the same time period was 113,651.

In the first step, we compared the correlations between the two approaches to measure DOT/100 patient days and the DDD/100 patient days (Table 2). For a final analysis of risk factors, groups of antibiotics would be used. Therefore, the mean of all r^2 across all 22 hospital units and six groups of antibiotics was probably the most representative determination coefficient. The two approaches to calculate the DOT/100 patient days were found to correlate equally with the DDD/100 patient days for non-CCU/ICU (mean r^2 of 0.59), while there was a non-significant difference of the r^2 in CCU/ICU in favour of the approach including the information on patient transfers (mean r^2 0.29 vs. 0.23; ($z = 0.64$).

In the next step, we calculated the standardized residuals for the non-CCU/ICU from the regression analysis between DDD/100 patient days and DOT/100 patient days taking the information on patient transfers into account (Figure 1). 67 of 1980 (3.4%) entries were found to have standardized residuals larger than +/- 1.96. Of interest, all 17 entries in which the DOT/100 patient days were overestimating the antibiotic utilization

compared to the DDD/100 patient days, had their origin in two single hospital units, and the group of cephalosporins was involved in 14 of 17 (82%) of entries. The other three entries stem from beta-lactams. In contrast, the entries in which the DOT/100 patient days underestimated the antibiotic utilization compared to the DDD/100 patient days, were more heterogeneous. They stemmed from 11 different units, with one unit being the source of 14 of 50 (28%) entries. While the months were equally distributed, there was again a predominance of beta-lactams (n=33, 66% of all entries) and cephalosporins (n=11, 22%). The remaining 6 entries (12%) were fluoroquinolones. Because of the clustering of outliers in three units, we have redone the calculation of the DDD, DOT, and the patient days for these units. However, the initial results were found to be correct.

In the last step, we assessed the correlations of the two "workhorse" beta-lactams (piperacillin +/- tazobactam and cloxacillin) on the five non-CCU/ICU hospital units with the worst correlation because the overall correlation was found to be much lower for beta-lactams compared to other groups of antibiotics. The best correlation was found for piperacillin (+/- tazobactam; r^2=0.978), while the correlation for cloxacillin (r^2=0.492) and the sum of both (r^2=0.403) was the worst (Figure 2).

Discussion of the findings in the preliminary project

Antibiotic utilization in DOT and DDD correlate well on non-CCU/ICU (mean r^2=0.59, r=0.77), even in the absence of knowledge on patient transfers for the calculation of DOT. In contrast, overall correlation was only moderate (mean r^2=0.29, r=0.54) on CCU/ICU, most likely due to the higher frequency of patient transfers. The comparably low correlation for beta-lactams (r^2=0.26, r=0.51) can be explained by discrepancies between the definition of DDD and the actually used average dose and range of doses at the three hospital sites.

Comparison between DOT and DDD

The calculation of DOT overestimated antibiotic utilization in comparison to DDD by 17%. This is in contrast to a recently published study in Germany, in which DDD were reported to overestimate antibiotic use by 32% (de With, et al. 2009). This discrepancy may be easily explained by the mix of antibiotics used in each institution: if one institution tends to use more frequently antibiotic drugs, for which the DDD is smaller than the actual prescribed dose –as in the study by de With et al.-, DDD will overestimate antibiotic utilization compared to DOT and vice-versa (Polk, et al. 2007). Due to the high correlation between the antibiotic use in DDD and DOT in non-CCU/ICU, the approach to calculate the DOT based on the individual data available without comprehensive information on patient transfers seems to be accurate and accounting for patient transfers seems negligible. In a large study of 130 hospitals in the

US, the overall correlation coefficient between DDDs and DOTs per 1000 patient days was only 0.6 (Polk, et al. 2007), compared to a correlation coefficient of 0.77 on non-CCU/ICU and 0.54 in CCU/ICU, respectively, in our study. Therefore, one can assume that the remaining 41% of the variance not explained by the model on non-CCU/ICU is more likely due to the methodological differences between DDD and DOT than due to the lack of information on patient transfers. In addition to potential differences in the mix of antibiotics evaluated in our study and the study mentioned above, the better correlation in our study may be partly explained by the use of monthly data and therefore lack of non-independence of data in our study, which may results in an overestimation of the correlation coefficients.

Of note, the worst correlation in our study was found for beta-lactams (mean r^2=0.27, r=0.52), which is in line with previous studies, and could be explained by differences in individual daily dosing and the mix of antibiotics on the level of antibiotic groups (de With, et al. 2009; Polk, et al. 2007). Cloxacillin was the most commonly used beta-lactam (16,286 (34%) of 47,563 DDD) at Hamilton Health Sciences, followed by Piperacillin/Tazobactam (10,297 (22%) of 47,563 DDD). While the prescribed dose of cloxacillin (typically 4-12 grams per day) was very different from the definition used to calculate the DDDs (2 grams per day), the definition of 1 DDD of Piperacillin/ Tazobactam (15g a day) very well represented the typically prescribed dose. Therefore, the correlation between DDD and DOT of beta-lactams is highly influenced by the mix of beta-lactams used. To this end, we hypothesized that the comparably low r^2 for beta-

lactams was probably a problem of the (inaccurate) definition of the DDD, rather than a lack of appropriateness of the approach to calculate the DOT/100 patient days. In order to assess this hypothesis, we selected the five non- CCU/ICU hospital units with the lowest r^2 for betalactams, and estimated the r^2 for piperacillin (+/- tazobactam), cloxacillin, and the sum of both, respectively (Figure 2), which supported this hypothesis (r^2=0.98 for piperacillin +/- tazobactam versus r^2=0.49 for cloxacillin versus 0.40 for the composite).

Impact of potentially missing data

Unfortunately, there was no way to assess whether there are entire patient entries missing in our databases. We could only assume that the data was complete based on the number of entries per month. For example, we observed a 30% reduction of patient admissions in April 2006 compared to all other months at two of three hospital sites. Importantly, this month is not overrepresented in the outliers (only 4 (6%) of 67 outliers), which corroborates that by dividing the DOT by the number of known patient days using the available data appropriately corrects for missing admission data.

However, we observed that missing prescription data can have some impact on our calculation while comparing the individual patient data available in our database to the individual patient prescriptions available in the pharmacy database for one far outlier (standardized residual of 6.5 for beta-lactams in February 2006 on one specific unit): the DOT/100 patient days was 16.7 compared to 44.3 DDD/100 patient days. The reason for the discrepancy can be explained as follows; firstly, the discharge date of one patient who

received a long-term and high-dose therapy with cloxacillin (8 to 12g per day during the entire month) was missing in the admission database. Therefore, we assumed that the patient has been discharged on the same day as admitted while data cleaning, and therefore, the prescription could not be linked to this patient. But even if this patient had been included, it would have resulted in a large discrepancy: the patient received 28 days of cloxacillin in this month, i.e. 28 DOT, but as one DDD of cloxacillin equals by definition only 2g per day but the patient received much higher daily doses, a total of 120 DDD of cloxacillin were administered to this patient.

Limitations of the study

The major limitation of this study is the fact that the discrepancy between DOT and DDD cannot be ultimately accounted to one single explanation among the three reasons for discrepancies outlined above, i.e. discrepancy between the definition of the DDD and the actually prescribed doses, second, the lack of information on patient transfers, and third, missing data in the databases.

However, among the three potential reasons explaining these discrepancies, the differences in definitions of DDD compared to the actual daily doses and, therefore, the methodological differences between DDD and DOT seem to be the key, in particular when putting our findings into the context of published evidence. If we had monthly reports in DOT/100 patient days provided by the pharmacy using a complete dataset and taking all patient transfers into account, we would have been able to estimate the amount

of variability explained by the methodological differences between DOT and DDD compared to the amount due to the limitations of the database, i.e. potential missing data and the lack of information on patient transfers.

Summary

Antibiotic utilization in DOT and DDD are highly correlated in non- CCU/ICU, even in the absence of information on patient transfers for the calculation of DOT. However, since the approach of taking patient transfers into account might be more accurate, we would prefer to use this approach to calculate DOT on the unit level. As antibiotic utilization in DOT and DDD may differ, we have addressed the question of whether the use of either of these may influence the associations with resistance in the main project for this thesis.

PART IV: METHODS

Patient selection

Adult patients (≥18 years of age at date of admission) admitted to one of three acute care sites of Hamilton Health Sciences from April 1 2005 to June 30 2006 and known to be hospitalized on one of the 41 *a priori* selected hospital units were included (21 acute care units, 9 CCU/ICU, 11 rehab and step-down units; emergency, gynaecology/obstetric, and paediatric units excluded). Patients staying in the hospital for less than 48 hours (i.e. a length of stay of 3 or less days when counting the day of admission as day 1) were excluded, because they would by definition not be at risk for an infection by a hospital-acquired bacterial pathogen, which is in accordance with the Centers for Disease Control and Prevention (CDC) (Horan and Emori 1997). Only the first hospital admission of each patient meeting the above mentioned criterion within the time period of the study was included to avoid non-independence in the data analysis.

Outcomes

Any positive microbiological isolate sampled at least 48 hours after admission was regarded as hospital-acquired (Horan and Emori 1997). Because only the dates and

not the time of admission and collection were available, samples collected on the third day of hospitalization or earlier were defined as community-acquired (the day of admission is counted as day 1) and thus excluded from the analysis. Only the first specific isolate of interest meeting the inclusion criteria was included in the analysis.

Adapting the case-case-control concept (Kaye, et al. 2005) for this cohort study, two main analyses were conducted. In the first model, we assessed time from admission to detection of a specific, resistant pathogen of interest. In a second model, we assessed the time to detection of a specific susceptible pathogen. For each model, patients with the other outcome were excluded from the analysis, e.g. patients with detection of the susceptible pathogen of interest were excluded from the analysis of resistant pathogens.

Antimicrobial utilization in DDD and DOT/100 patient days at the level of the hospital unit either at the time of detection of a pathogen of interest or at admission for patients with no clinical isolates of interest, respectively, as well as exposure to specific antibiotics prior to detection of a pathogen of interest were regarded as potential risk factors.

Statistical analyses

We conducted a two-level multivariate Cox proportional hazards analysis with and without adjustment for clustering on the unit-level. In order to adjust for patient mix, age and sex were included in the final multivariate model.

PART V: RESULTS

Descriptive results

From April 1 2005 to June 30 2006, a total of 24,394 admissions of 19,452 adult patients met the inclusion criteria. 3,487 (17.9%) patients had more than one hospital admission meeting the inclusion criteria and a total of 4,942 non-first admissions (20.3% of all admissions) were excluded from the analysis. The mean age was 65.4 years and 49.5% were females. The total length of stay of these 19,452 first admissions was 224,279 days and the mean length of stay was 11.5 days (day of admission counted as day 1). The majority of admissions (8.733, 44.9%) took place at the Hamilton General Hospital, 6,857 (35.2%) at the Henderson General Hospital, and 3,862 (19.8%) patients were admitted to the McMaster University Medical Clinic.

At least one hospital-acquired pathogen was detected in 1,810 of 19,452 (9.3%) first admissions. In total, 6,732 bacterial pathogens were found in 6,264 samples in these patients. The most frequently detected pathogen was *P. aeruginosa* with 1,629 (24.2%) isolates (Table 3). The urine was the most common source of samples (n=2,832, 42.1%; Table 3). A total of 101,443 entries of antimicrobial susceptibility information were available. Only the first isolate of each respective pathogen of interest from each patient was taken into account for the analysis.

There were a total of 43,345 prescriptions recorded on the 41 hospital units of interest relevant to the calculation of DOTs. A total of 171,317 days of systemic antibiotic therapy were administered to patients. Of these, 25,840 (15.1%) patient days were linked to the relevant unit based on the location of the microbiological sample rather than the unit of admission.

For the 19,452 first admissions included in this study, 30,578 prescriptions were recorded. In 11,797 (60.6%) of these first admissions, antibiotics were administered. A total of 123,712 days of therapy were administered to these patients with a mean duration of each prescription of 4 days. The highest use in days of therapy was recorded for ciprofloxacin (22,987 DOT, 18.6%) and the group of fluoroquinolones (37,553 DOT, 30.4%), respectively (Table 4).

Comparison of the two prescription databases

There appeared to be a reduction in the number of days of therapy in February and April 2006 in the available dataset (Figure 3). This decrease is mirrored by a decrease in number of prescriptions and also in the known patient days (Figure 3). While a similar decrease in February 2006 could be observed in the administrative database run by the hospital pharmacy, the decrease in April may indicate that the data of some patients had not been collected during this time period. Of note, this potential lack of data is

compensated by the fact that there might be a lack of information in both, admission data (denominator) and prescription data (nominator); the DOT/100 patient days are in line with DDD/100 patient days in April 2006 (Figure 5).

The overall r^2 for the monthly data for 12 groups of antibiotics between DDD and DOT per 100 patient days was 0.38. When stratifying the data into three types of hospital units, i.e. acute care units, CCU/ICU, and rehabilitation and step down units, the r^2 were 0.74, 0.52, and 0.07, respectively (Figure 5). The correlations tended to be higher in certain groups of antibiotics in comparison to the preliminary project (data not shown). In particular, we found an increase for beta-lactams (r^2=0.46 versus 0.27) and the fluoroquinolones (r^2=0.73 versus 0.59).

Fluoroquinolone use and fluoroquinolones resistance in enterobacteriaceae

Among the 19,452 first admissions, hospital-acquired enterobacteriaceae were detected in 998 patients (5.1%). Both a susceptible and a resistant isolate of enterobacteriaceae were detected in 28 patients (0.1% of patients, 2.8% of patients with detection of enterobacteriaceae). In 19 of these (67.9%), the resistant isolate was detected later in the hospitalization than the susceptible isolate, in 4 patients (14.3%), a susceptible and resistant enterobacteriaceae were detected on the same collection date, and in 5 patients (17.9%), the resistant isolate preceded the susceptible isolate. The identical

species in its susceptible and resistant form, respectively, was isolated only from 10 patients.

Of the 1026 isolates (among 998 patients, 28 patients with either outcome), 145 (14.1%) were fluoroquinolones resistant (MIC for ciprofloxacin >=2), and 881 (85.9%) were susceptible. The majority, 531 of 1,026 (51.7%) isolates, were *E. coli* followed by *K. pneumoniae* (n=152, 14.8%). Three quarters (766 of 1,026, 74.7%) of isolates were sampled from the urine. Fluoroquinolones were prescribed to 5,788 of 19,452 patients (29.8%).

Analysis of risk factors for fluoroquinolone resistant enterobacteriaceae

For the analysis comparing patients with resistant enterobacteriaceae and patients without any detection of enterobacteriaceae during their first hospitalization, 18,599 patients met the inclusion criteria: 145 (0.8%) patients with and 18,454 (99.2%) without an event. The mean time at risk was 10.7 and the last observation at risk was 144 days. The total time at risk was 199,455 days. Among patients with the event, 86 of 145 patients (59.3%) were prescribed fluoroquinolones before the event. 5,141 of 18,454 (27.8%) patients were prescribed fluoroquinolones and had no event. Individual exposure to fluoroquinolones was an independent risk factor for detection of fluoroquinolones resistant enterobacteriaceae in all models (Table 6, Figure 6). Antibiotic utilization on the unit level was found to be an independent risk factor in model 3 only, which did not take the non-independence of observations on the same hospital unit into account. With

adjustment of this, only individual pre-exposure remained statistical significant. Of note, the hazard ratios in the models with DDD were similar to those observed in the models with DOT with no qualitative differences. Among the co-variates, female sex was found to be an independent risk factor (HR 1.54, 95% CI 1.13-2.11, p=0.007) in the final model while age was not associated with an increase in risk.

When subdividing the analyses by type of unit (Table 7), the hazard ratios were very similar. No statistically significant independent risk factors were found on CCU/ICU. Antibiotic utilization on the unit level was found to be an independent risk factor on rehab/step down units, only. Of note, there was a discrepancy in the findings on rehab/step down units: individual exposure was a significant risk factor in the model using DDD but not in the model using DOT.

Analysis of risk factors for fluoroquinolone susceptible enterobacteriaceae

Among the 19,335 patients meeting the inclusion criteria for the analysis of risk factors for susceptible enterobacteriaceae, 881 (4.6%) of patients had an event. Only 82 (9.3%) of these were pre-exposed to fluoroquinolones. In all the models, exposure to fluoroquinolones reduced the hazard to detect fluoroquinolone susceptible enterobacteriaceae (Table 6). In contrast, utilization of fluoroquinolones on the unit level was an independent risk factor for detection of hospital-acquired fluoroquinolone susceptible enterobacteriaceae. Again, the risk estimates were very similar between models using DDD versus DOT as a measure for antibiotic utilization on the unit level,

and no qualitative differences were observed.

In the analyses subdivided by type of unit, individual exposure to fluoroquinolones was found to be an independent protective factor in all models (Table 7). While fluoroquinolone utilization on the unit level was no longer a significant risk factor on acute care units ($p>0.05$), it was still independently associated with detection of hospital-acquired susceptible enterobacteriaceae in patients on CCU/ICU, and in patients on rehab and step down units. Whether DDD or DOT was used as a measure of antibiotic consumption had a small impact on the association with fluoroquinolone use on the unit level on CCU/ICU ($p<0.001$ and 0.058, respectively), while this difference has not affected the risk estimates of the individual exposure.

Fluoroquinolone use and fluoroquinolones resistance in *P. aeruginosa*

P. aeruginosa was detected in 369 (1.9%) of 19,452 patients. Both, susceptible and a resistant *P. aeruginosa* isolate were detected in 27 patients (0.1% of patients, 7.3% of patients with detection of *P. aeruginosa*). In 15 of these (55.6%), the resistant was detected after the susceptible form, in 3 patients (11.1%), both isolates were sampled on the same collection date, and in 9 patients (33.3%), the resistant isolate preceded the susceptible isolate. These patients were included in both analyses.

Of the 396 isolates (among 369 patients, 27 patients with either outcome), 157 (39.6%) were fluoroquinolone resistant (MIC for ciprofloxacin >=2), and 239 (60.4%) were susceptible. The majority of specimens were obtained from tissue, aspirates or swabs (n=181, 45.7%), followed by urine samples (n=119, 30.1%).

Analysis of risk factors for fluoroquinolone resistant P. aeruginosa

For the analysis for fluoroquinolone resistant *P. aeruginosa*, 19,240 patients met the inclusion criteria: 157 (0.8%) patients with and 19,083 (99.2%) without an event. The mean time at risk was 11.1 days and the last observation was at risk for 195 days. The total time at risk was 214,328 days. Among patients with the event, 113 of 157 patients (72.0%) were prescribed fluoroquinolones before the event, while 5,534 of 19,083 (29.0%) were prescribed fluoroquinolones and had no event.

Individual exposure to fluoroquinolones was an independent risk factor for detection of fluoroquinolones resistant *P. aeruginosa* in all models (Table 8, Figure 7). Antibiotic utilization on the unit level was found to be an independent risk factor in model 3 irrespective of whether DDD or DOT was used as the independent variable. In model 4 with adjustment for the cluster effect, utilization on the unit level was a significant risk factor only for DDD, but not for DOT. Among the co-variates, female sex was found to be an independent protective factor (HR 0.73, 95% CI 0.55-0.98, p=0.034) while an increase in age by one year was associated with a lower risk (HR 0.98, 95% CI 0.97-0.99, p<0.001).

In the analysis subdivided by type of unit (Table 9), individual pre-exposure to fluoroquinolones was a significant independent risk factor on acute care units, only. Unit-level fluoroquinolone utilization was an independent risk factor on rehab and step down units, only, but not when using DOT instead of DDD as an independent variable.

Analysis of risk factors for fluoroquinolone susceptible P. aeruginosa

A total of 19,322 patients met the inclusion criteria for the analysis of risk factors for susceptible *P. aeruginosa*. Among those, 239 (1.2%) of patients had an event. Pre-exposure to fluoroquinolones was observed in 55 (23.0%) of patients with an event compared to 29.1% in patients with no event.

In all the models, exposure to fluoroquinolones was found to be an independent protective factor for detection of fluoroquinolones susceptible *P. aeruginosa* (Table 8). Utilization of fluoroquinolones on the unit level was an independent risk factor for detection of hospital-acquired fluoroquinolone susceptible *P. aeruginosa* across all models.

In the analyses subdivided by type of unit, individual exposure to fluoroquinolones was found to be an independent protective factor, except when using DDD as the independent variable on rehab and step down units. Fluoroquinolone utilization on the unit level was no longer a risk factor on acute care units. However, it

was still independently associated with detection of hospital-acquired susceptible *P. aeruginosa* in patients on CCU/ICU when using DDD as the independent variable, and in patients on rehab and step down units. Whether DDD or DOT was used as a measure of antibiotic utilization on the unit level had some impact on the association on CCU/ICU, while this difference has not affected the risk estimates of the individual exposure on these units. However, it had animpact on the risk estimate for individual pre-exposure on rehab and step down units.

Use of betalactams and resistance to third generation cephalosporins in enterobacteriaceae

Among the 19,452 patients' first admissions, enterobacteriaceae were detected in 998 patients (5.1%). Both, a susceptible and a resistant isolate to third generation cephalosporins, were detected in 27 patients (0.1% of patients, 2.7% of patients with detection of enterobacteriaceae). In 17 of these (63.0%), the resistant isolate was detected later during the same hospitalization than the susceptible isolate, in 4 patients (14.8%), a susceptible and resistant enterobacteriaceae were sampled on the same date, and in 6 patients (22.2%), the resistant isolate preceded the susceptible isolate. The identical pathogen in its susceptible and resistant form, respectively, was isolated from 13 patients.

Of the 1025 isolates (among 998 patients, 27 with either outcome), 146 (14.2%) were resistant to at least one third generation cephalosporin, and 879 (85.8%) were susceptible. The majority, 521 of 1,025 (50.8%) isolates, were *E. coli* followed by *K. pneumoniae* (n=150, 14.6%). Three quarters (764 of 1,025, 74.5%) of isolates were sampled from the urine.

Among the total of 7,079 patients prescribed beta-lactam during the hospitalization or before the event for patients with detection of enterobacteriaceae, respectively, 5,952 (84.1%) patients were receiving cephalosporins, 84 (1.2%) carbapenems, and 1822 (25.7%) any other beta-lactam antibiotics (patients may have received beta-lactam antibiotics from more than one of the subgroups mentioned above).

Analysis of risk factors for enterobacteriaceae resistant to third generation cephalosporins

For the analysis comparing patients with resistant enterobacteriaceae and patients without any detection of enterobacteriaceae during their first hospitalization, 18,600 met the inclusion criteria: 146 (0.8%) patients with and 18,454 (99.2%) without an event. The mean time at risk was 10.7 days and the last observation was at risk for 144 days. The total time at risk was 199,678 days. Among patients with detection of enterobacteriaceae resistant to third generation cephalosporins, 94 of 146 patients (64.4%) were prescribed any betalactam antibiotic before the event, while 6,985 of 18,454 (37.9%) had a prescription and no event.

Exposure to any beta-lactam antibiotic during the hospitalization was associated with an increased risk for detection of enterobacteriaceae resistant to third generation cephalosporins in all models but the models using DOT as the independent variable to measure antibiotic utilization on the unit level (Table 10, Figure 8). In contrast, a higher utilization of beta-lactam antibiotics on the unit level was an independent risk factor for resistant enterobacteriaceae. Among the co-variates, age was not a significant risk factor, while male sex was associated with detection of resistant enterobacteriaceae (HR 1.63, 95% CI 1.13-2.34, p=0.008).

In the analyses subdivided by type of unit (Table 11), pre-exposure to betalactams on the individual level was only a significant independent risk factor on rehab and step down units when measuring antibiotic utilization in DDD. Whether antibiotic utilization was an independent risk factor depended on how antibiotic utilization was measured on both, CCU/ICU and on rehab and step down units. When subdividing betalactam pre-exposure and utilization into the subgroups cephalosporins, carbapenems, and other betalactams, the following was found: individual cephalosporin prescription on the individual level was a significant risk factor when using DDD (HR 1.39, 95% CI 1.00-1.93, p=0.047) but not when using DOT (HR 1.30, 95% CI 0.97-1.83, p=0.11). Individual prescription of a carbapenem was a risk factor in both models (DDD: HR 4.18, 95% CI 1.71-10.20, p=0.002, DOT: HR 3.72, 95% CI 1.37-10.10, p=0.01). The other betalactams were not associated, neither on the individual, nor on the unit level. When entering all these subgroups of beta-lactams into a multivariate model, only carbapenem prescriptions

on the individual level was found to be an independent risk factor (Table 12). Of interest, there was a large discrepancy in the findings of carbapenems as a risk factor: carbapenem utilization was a significant protective factor in the model with DDDs, but a –non-significant– risk factor in the model with DOTs. Of note, this model comprised 8 variables and the adjustment for non-independence with only 86 events, therefore, this model might be underpowered and potentially misleading.

Analysis of risk factors for enterobacteriaceae susceptible to third generation cephalosporins

For this analysis 19,333 patients met the inclusion criteria: 879 (4.5%) patients with and 18,454 (99.2%) without an event (Table 10). As for resistant enterobacteriaceae, the mean time at risk was 10.7 days and the last observation was at risk for 144 days. The total time at risk was 199,333 days.

Among patients with detection of susceptible enterobacteriaceae, 336 of 879 patients (38.2%) were prescribed any betalactam antibiotic before the event. Exposure to any beta-lactam antibiotic was a protective factor for detection of susceptible enterobacteriaceae in all models (Table 10). As for enterobacteriaceae resistant to third generation cephalosporins, a higher utilization of beta-lactam antibiotics on the unit level was an independent risk factor. Both covariates were significant risk factors: per one year of age, the hazard increased slightly by 1% (HR 1.01, 95% CI 1.003-1.017, p=0.006) and males were at a significant higher risk (HR 1.92, 95% CI 1.61-2.28, p<0.001).

When looking at the different types of units separately (Table 11), pre-exposure to beta-lactams on the individual level was a protective factor in all analyses except on the rehab and step down units. Of interest, in the model with DDD to determine antibiotic utilization on the unit level in rehab and step down units, individual beta-lactam pre-exposure was even a risk factor (HR 1.59, 95% CI 1.17-2.17, p=0.003), which was not true in the model using DOT (HR 0.86, 95% CI 0.63-1.18, p=0.345) and for the other types of units. Antibiotic utilization on the unit level was associated with a higher risk for detection of enterobacteriaceae resistant to third generation cephalosporins on rehab and step down units, and on CCU/ICU when using DDD.

As a total of 625 events were observed on acute care units, we were confident that a model using the subgroups cephalosporins, carbapenems, and other betalactams as independent variables instead of the composite could be used. When entering the subgroups of beta-lactams into the model instead of the composite variable, very similar risk estimates could be found. Exposure to non-cephalosporin non-carbapenem beta-lactams was a protective factor in either model (Table 12). All the other factors were not independently associated with the outcome of interest.

PART VI: DISCUSSION

Summary of findings

We found that discrepancies in antibiotic utilization on the unit level between defined daily doses (DDD) reported by the hospital pharmacy and days of therapy (DOT) calculated based on individual data available from a cohort of hospitalized patient can be explained by a) differences between the typically prescribed dose and the DDD, and b) by the lack of detailed information on patient transfers.

When comparing DDD and DOT in statistical models to calculate risk estimates for resistance, we have found that DDD and DOT reveal similar results on acute care units with a presumably limited number of patient transfers. In contrast, there were significant discrepancies on CCU/ICU and most importantly on rehab and step down units, which can most likely be explained by the lack of detailed information on patient transfers for the calculation of DOT.

This study confirms that the approach to calculate DOT based on the individual patient information available in this cohort study can be used to estimate the risk of resistance on acute care units for hospital sites for which pharmacy data in DDD is not

available. The methodological differences between DDD and DOT seem to only marginally influence the risk estimates for resistance.

DDD and DOT to measure antimicrobial use on the unit level

When correlating the monthly antibiotic utilization of 12 groups of antibiotics on the 41 hospital units included in this study, we have found mean r^2 of 0.38. This was a weaker association than previously found in the preliminary project on a subgroup of hospital units and antimicrobial groups ($r^2 = 0.57$). This discrepancy can be explained by the fact that there were no rehabilitation and step down units included in the preliminary project. We have found that DOT and DDD are only marginally associated when data are collected from these types of hospital units ($r^2=0.07$). When subdividing the analysis, we found that the correlation on acute care units was very high ($r^2=0.74$) and comparable to the findings in the preliminary project ($r^2=0.67$), as it was on CCU/ICU ($r^2=0.52$ compared to $r^2=0.47$ in the preliminary project). On acute care units, where patient transfers are not very frequent, the largest amount of variability not explained in the regression analysis can be explained by methodological differences between calculation of DDD and DOT. This is corroborated by previous studies that have shown a correlation coefficient between DDD and DOT of 0.6 or an r^2 of 0.36 (Polk, et al. 2007), even when lack of information on patient transfers was not an issue. This lack of information may

potentially bias the results on CCU/ICU, and is very likely to bias the results on rehab and step down units.

As previously found in the preliminary project, missing data does not seem to affect the correlation between DOT and DDD. In April 2006, where we can assume that 20-30% of patient admission and prescription data was missing, the difference between DDD/100 patient days and DOT/100 patients days was even smaller than expected.

These findings corroborate both of our findings in the preliminary project as well as the hypothesis that DOT as calculated on the dataset available is a good surrogate for the DDD on acute care sites, but may bias results on CCU/ICU. Missing data does not seem to bias the results when calculating antibiotic use in DOT divided by the denominator of known patient days. On rehab and step down units, DOT should not be used due to the high discrepancies as compared to DDD, which is most like due to the very high number of internal patient transfers to this type of hospital units.

Impact of the method (DDD versus DOT) to measure antibiotic utilization on risk estimates for resistance on acute care units

The main goal of this project was to evaluate whether the use of DOT calculated on the basis of the data available in this cohort of patients, with all its limitations, results

in a similar risk pattern for resistance as when using the commonly used DDD, i.e. we expected the risk estimates to be similar with no qualitative differences for acute care units. This was corroborated in our results: the risk estimates in the models using DDD and DOT, respectively, for detection of either susceptible or resistant pathogens for all three single antimicrobial-bacteria combinations and for both levels of exposure were similar and with no qualitative differences (a total of 6 analyses and 12 independent variables). The same was true for the more detailed analysis for resistance to third generation cephalosporins when using the subgroups of beta-lactams for detection of susceptible enterobacteriaceae.

We found one single important discrepancy between DDD and DOT, namely with respect to enterobacteriaceae resistant to third generation cephalosporins and use of carbapenems on the unit level. Carbapenem use on the unit level was a protective factor for detection of resistant enterobacteriaceae when using DDD (HR 0.15, 95% CI 0.02-0.94, p=0.042), but a non-significant risk factor when using DOT (HR 1.32, 95% CI 0.24-7.37, p=0.753). Notably, this discrepancy had no important effect on the risk estimates of the other independent risk factors. Furthermore, the same discrepancy has not been found for susceptible enterobacteriaceae, where, in fact, all risk estimates in particular for carbapenem utilization on the unit level were very similar (HR 0.74, 95% CI 0.27-2.02, p=0.555 for DDD and HR 0.86, 95% CI 0.28-2.70, p=0.801 for DOT, respectively). Therefore, a likely explanation for the discrepancy in the model for resistant enterobacteriaceae is probably not bias due to the calculation of DOT, but the

comparable low number of events for the analysis on resistant enterobacteriaceae of 86. A total of 6 independent variables of interest, 2 co-variates (age and sex), and the adjustment for clustering were entered into this model, violating the rule of thumb of at least 10 events for each variable in the model (Concato, et al. 1995; Peduzzi, et al. 1995). Of note, there were no qualitative differences in a simple exploratory model not violating the rule of 10 (non-significant factor in both analyses, data not shown). When correlating carbapenem utilization in DDD/100 patient days and DOT/100 patient days on acute care units in linear regression analysis, the correlation was very good (r^2=0.67). Nonetheless, due to the low number of events and the relatively low utilization of carbapenems, single outliers may have had a higher impact on these findings than for other, more frequent events and antibiotics with a higher monthly usage.

We are not aware of any studies comparing DDD and DOT as risk factors for resistance. However, this is not the first study showing that the method to measure antibiotic utilization may have an impact on risk estimates. Hyle et al. (Hyle, et al. 2007) have previously reported that different methods for describing the extent of antibiotic exposure can result in contradictory results: in a systematic review, the use of third-generation cephalosporins as a continuous variable was a risk factor for infection by ESBL-producing *E. coli* and *Klebsiella species*, while it was not when antibiotic use was described as a categorical variable (Hyle, et al. 2007).

However, it remains unknown whether DDD or DOT better represents antibiotic utilization as a risk factor for resistance. In patients with renal impairment on lower doses of antibiotics, the serum concentration at lower DDD will be equivalent to full DDD in patient with a normal renal function, but this cannot be taken into account in the measurement by DDD, but will be taken care of in DOT. On the other hand, if the amount prescribed per day is important, then the DOT fails to take this into consideration.

Impact of the method (DDD versus DOT) to measure antibiotic use on risk estimates for resistance on CCU/ICU, and rehab and step down units

More discrepancies between the risk estimates when using DDD and DOT, respectively, were expected on CCU/ICU and on rehab and step down units due to the higher frequency of patient transfers, which may have had a significant impact on calculation of DOT/100 patient days.

In fact, one may have drawn diverging conclusions in the analyses on CCU/ICU depending on whether antibiotic utilization was measured in DDD or DOT. In the first analysis on fluoroquinolone utilization as a risk factor at the unit level for fluoroquinolone susceptible enterobacteriaceae, we have found a HR of 1.28 (95% CI 0.99-1.66, p=0.058) for DDD and a HR 1.97 (95% CI 1.49-2.60, p<0.001) for DOT, respectively. The risk estimates at the individual level were similar (HR 0.15 and 0.13, respectively). The same was true for fluoroquinolone utilization and detection of

fluoroquinolone susceptible *P. aeruginosa* (HR 1.24, 95% CI 0.91-1.69, p=0.165 for DDD and HR 1.67, 95% CI 1.15-2.44, p=0.008 for DOT, respectively). Finally, in the analysis for enterobacteriaceae resistant to third generation cephalosporins, one may again have drawn divergent conclusions based on the p-values. First, utilization of beta-lactams on the unit-level was a significant risk factor when measured in DDD (HR 1.16, 95% CI 1.04-1.28, p=0.006), but not when measured in DOT (HR 1.03, 95% CI 0.78-1.35, p-value=0.847). Second, it was a significant risk factor for detection of susceptible enterobacteriaceae when measured in DDD (HR 1.23, 95% CI 1.09-1.37, p<0.001), but again not when measured in DOT, although the risk estimate was very similar (HR 1.21, 95% CI 0.97-1.52, p=0.087). Even more discrepancies were found on rehab and step down units. And in addition to discrepancies of the risk estimates on the unit-level, discrepancies were also found on the individual level risk factor on rehab and step down units, but importantly not on ICU/CCU.

Apart from the different methods to measure antibiotic utilization, low number of events may again have biased the findings, in particular on rehab and step down units. Therefore, one cannot definitely conclude that the approach to calculate and measure antibiotic utilization in DOT would necessarily bias the risk estimates. However, we could not prove the contrary, either. Based on the low correlation between DOT and DDD on rehab and step down units and the discrepancies in the risk estimates, the use of DOT cannot be justified for these types of units. For CCU/ICU, it seems that the use of

DOT may have biased the risk estimates of the unit-level variables, but has not influenced the risk estimates of the individual-level variables.

Antibiotic utilization as a risk factor for resistance

Although the main focus of this project was not to interpret the risk estimates per se, we will summarize and discuss the findings here. We will focus on the *a priori* defined main analyses, i.e. the risk estimates in the final models using DOT to measure antibiotic exposure subdivided into the type of units. Due to the limitations on rehab and step down units outlined above, we will limit the discussion of the results to acute care units and CCU/ICU.

Fluoroquinolone use and fluoroquinolone resistant enterobacteriaceae

This antimicrobial-bacteria combination is an excellent example to show that individual exposure to antibiotics is a risk factor for resistance. While individual exposure to fluoroquinolones is a significant risk factor for colonization or infection by fluoroquinolone resistant enterobacteriaceae (HR 1.59, 95% CI 1.18-2.16, p=0.003), it was also found to be a protective factor for detection of fluoroquinolone susceptible enterobacteriaceae (HR 0.11, 95% CI 0.08-0.14, p<0.001) on acute care units. In this context, the approach adapted from the case-case-control design (Kaye, et al. 2005) can show that individual fluoroquinolone exposure is uniquely associated with

fluoroquinolone resistance but not with detection of hospital-acquired enterobacteriaceae in general. A simple Cox regression analysis of resistance cases among all patients at risk or a simple case-control model using logistic regression analysis comparing cases with detection of resistant enterobacteriaceae to patients with no clinical isolates would not have proven this due to the above explanation, however, one would have also concluded that fluoroquinolone exposure was a risk factor as well (OR of 3.4, antimicrobial pre-exposure as the unique risk factor; data not shown). If another common analysis had been used, i.e. patients with susceptible pathogens as the control group, we would have concluded the same, but the risk estimate would have been much higher (OR of 14.8, antimicrobial pre-exposure as the unique risk factor; data not shown). This is in line with previous evidence showing that this approach to select the control groups may actually inflate the risk estimates (Harris, et al. 2002; Kaye, et al. 2005). An alternative approach would have been to run a multinomial logistic regression analysis, which would have corroborated our findings (relative risk for fluoroquinolone exposure and detection of susceptible enterobacteriaceae of 0.23 and of resistant enterobacteriaceae of 3.4; antimicrobial pre-exposure as the unique risk factor; data not shown). The comparison of these findings underline the importance to select the appropriate population at risk in observational studies, and that the risk for detection of resistant pathogens needs to be analysed separately from the risk of detection of susceptible pathogens (Kaye, et al. 2005). On CCU/ICU, there was a trend to similar findings as on acute care units, although the risk estimates for detection of resistant enterobacteriaceae did not reach statistical significance.

While antibiotic utilization on the unit-level measured in DOT/100 patient days was not a significant risk factor on acute care sites, a higher use was associated with a higher risk of detection of both, fluoroquinolone resistant and susceptible enterobacteriaceae on CCU/ICU. Although one would expect that the direction of the risk for fluoroquinolone exposure on the individual level and utilization on the unit level, respectively, would be into the same direction, this finding can be explained by the potential of confounding: CCU/ICU patients hospitalized with complex medical problems resulting in a higher risk for hospital-acquired infections tend to be hospitalized on CCU/ICU with a higher use of antibiotics, while patients with more limited medical problems like otherwise healthy patients with e.g. coronary heart disease, would be hospitalized on cardiac critical care units which have a lower antimicrobial use. It is possible that when an adjustment for main diagnoses and co-morbidities were feasible, this divergent finding on CCU/ICU would be corrected.

Fluoroquinolone use and fluoroquinolone resistant P. aeruginosa

Essentially, the same associations for fluoroquinolone resistance in enterobacteriaceae were found for *P. aeruginosa*: individual exposure to fluoroquinolones was a risk factor for infection or colonization by fluoroquinolone resistant (HR 1.96, 95% CI 1.01-3.77, p=0.045), but a protective factor for susceptible *P. aeruginosa* (HR 0.23, 95% CI 0.14-0.37, p<0.001) on acute care sites. Again, the combination of the results shows that exposure was a unique risk factor for resistance, but

not for detection of *P. aeruginosa* infection or colonization in general. And again, these findings would have been qualitatively similar when modelling it as a case-control study using logistic regression analysis including only cases with detection of resistant enterobacteriaceae versus patients with no clinical isolates (OR of 6.28, antimicrobial pre-exposure as the unique risk factor; data not shown), or in a model using the resistant pathogens as events and patients with susceptible pathogens as non-events (OR of 8.59, antimicrobial pre-exposure as the unique risk factor; data not shown). This was again corroborated by the other approach, a multinomial logistic regression analysis (relative risk for fluoroquinolone exposure and detection of susceptible *P. aeruginosa* of 0.73 and for resistant enterobacteriaceae of 6.2; antimicrobial pre-exposure as the unique risk factor; data not shown). For CCU/ICU, individual exposure to fluoroquinolones as a risk factor for fluoroquinolone resistance among *P. aeruginosa* did not reach statistical significance, but was shown to be a protective factor for fluoroquinolone susceptible *P. aeruginosa*.

Fluoroquinolone use on the unit level was not significantly associated with either outcome on acute care sites. On CCU/ICU, an increase in the utilization of fluoroquinolones was associated with a higher hazard for detection of fluoroquinolone susceptible *P. aeruginosa*. This can be explained by confounding as previously discussed for fluoroquinolone utilization and fluoroquinolone resistant enterobacteriaceae.

Beta-lactam use and enterobacteriaceae resistant to third generation cephalosporins

For this antimicrobial-bacteria combination, an association for individual exposure to beta-lactams was found only for enterobacteriaceae susceptible to third generation cephalosporins on both, acute care sites and CCU/ICU. In the more detailed analysis breaking up the independent variable into three subgroups of beta-lactams, exposure to carbapenems became an independent risk factor for detection of resistant enterobacteriaceae while exposure to 'other' beta-lactams was found to be an independent protective factor for detection of susceptible enterobacteriaceae. Based on these findings, it can be assumed that individual exposure to carbapenems puts patients at higher risk for colonization or infection by third generation cephalosporins than any other type of beta-lactam antibiotics. However, one needs to be careful when interpreting this data: the number of events was comparably low (n=84) to model 6 risk factors plus adjustment for clustering. Furthermore, these associations may be due to confounding rather than due to an effect of carbapenems per se. In fact, carbapenems are the group of beta-lactams typically used for treatment of enterobacteriaceae resistant to third generation cephalosporins. Therefore, patients empirically treated with carbapenems are typically multi-morbid patients with previous detection of multi-resistant gram negative bacteria, a factor for which the analysis could not be adjusted for, although the time of the event in comparison to the time of exposure would argue for a real association.

When looking at the results of the unit level utilization of beta-lactams or subgroups of beta-lactams measured in DOT, no effect could be shown on either acute care units or CCU/ICU.

Summary of the associations in antimicrobial-bacteria combinations

Antimicrobial use was shown to be a risk factor for resistance as previously shown for the antimicrobial-bacteria combinations analyzed for this study (Blaettler, et al. 2009; Graffunder, et al. 2005; Kaier, et al. 2009; Neuhauser, et al. 2003; Rodriguez-Bano, et al. 2008). The analysis of risk factors for both outcomes, either detection of resistant or susceptible bacteria, respectively, allowed us to identify risk factors which uniquely result in detection of resistant but not of susceptible bacteria of interest. Of importance, only the individual exposure was shown to be a significant risk factor for resistance, while utilization on the unit level only puts the patient at a higher risk of detection of any hospital-acquired pathogen, either susceptible or resistant to the antibiotic of interest. As discussed above, the association on the unit-level can rather represent confounding than a true effect of the antimicrobial utilization per se. This may explain the discrepancies among previous ecological studies and in particular the negative findings in some of the most recent ecological studies (Bosso, et al. 2010; Jankovic, et al. 2011) in contrast to the vast majority of studies which were showing these associations.

Limitations

The dataset

The data was collected in 2005/2006 and is therefore not very recent. However, there is no evidence that the association between antibiotic use and resistance may have changed over the last 5 years. Furthermore, no new commonly used antibiotics have become available since. Nevertheless, these data cannot be used to analyze risk factors for resistant pathogens not yet established as common nosocomial pathogens in 2005 and 2006 such as vancomycin-resistant enterococci. The major limitation of the dataset per se is the lack of comprehensive information on patient transfers, which has been discussed previously. Another limitation of the dataset is the lack of information on co-morbidities, accurate dosing information, and on antibiotic exposure prior to hospital admission. All of these factors are potential confounders we could not adjust for in our analysis of risk factors.

Statistical issues

Discrepancies in findings across the models can be explained in part by a lack of sufficiently large number of events. Based on two stimulation studies (Concato, et al. 1995; Peduzzi, et al. 1995), at least 10 events for each independent variable added to the model are recommended in Cox proportional hazard and logistic regression models as a rule of thumb. In the final model which comprised four independent variables, i.e. antibiotic utilization on the unit level, individual pre-exposure to specific antibiotics, age,

and sex, at least 40 events would be deemed necessary. Furthermore, the adjustment for the cluster effect on the unit-level would need even more events. The number of events was marginal for some of the analyses for resistant pathogens on CCU/ICUs and rehab and step down units, which may have resulted in an increased risk of bias and variability in these analyses (Concato, et al. 1995; Peduzzi, et al. 1995). This may explain some of the discrepancies found between models based on the DDD and DOT, respectively. However, Vittinghoff and McCulloch have suggested to relax this rule of ten events (Vittinghoff and McCulloch 2007). They concluded that even with 5 to 9 events, the risk of bias is only slightly increased, and that more than 5 events for each variable may be sufficient in observational studies (Vittinghoff and McCulloch 2007). However, even the rule of 5 prevailed to enter specific antimicrobials instead of groups or subgroups of antibiotics into the model.

We have performed a large number of statistical analyses for this study. Therefore, some associations between antibiotic use and resistance may have been found by chance alone. However, we have *a priori* defined the analysis of final interest, i.e. the final model incorporating antibiotic use on the individual and unit level, the two co-variates age and sex, and an adjustment for non-independence or clustering. All other models and results are exploratory in nature and should therefore be interpreted with caution.

Conclusions

There is only a minimal impact of how antimicrobial utilization (DDD or DOT) is being measured when assessing individual exposure and antimicrobial use on the unit-level as risk factors for resistance on acute care units with a presumably limited number of patient transfers. Due to limited data on patient transfers, we have found discrepancies on units with a higher frequency of patient transfers: whether DDD or DOT are used may qualitatively affect the risk estimates on the unit level on CCU/ICU, and can even influence the risk estimates on the individual level on rehab and step-down units.

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Appendix B: Tables

Table 1: Antibiotic utilization in days of therapy (DOT) and defined daily doses (DDD), and the number of patient days April 2005 to June 2006 in 22 selected hospital units

	DOT	DOTadm	DDD
Antibiotic utilization (numerator)	140,707	124,464	113,651
Number of patient days (denominator)	228,783	208,122	222,138
Antibiotic utilization / 100 patient days	61.5	59.8	51.2

Abbreviations. DDD defined daily doses, DOT days of therapy, DOTadm DOT based on admission data only

Table 2: Correlation between days of therapy (DOT) and defined daily doses (DDD) per 100 patient days, overall, for non-CCU/ICU and CCU/ICU

	DOT adm/100 patient days	**DOT / 100 patient days**
r^2 overall	0.52	0.57
Non- CCU/ICU		
- r^2	0.67	0.68
- r^2 by antibiotic group: mean	0.62	0.62
- Anti-anaerobes (clindamycin and metronidazole)	0.76	0.77
- Beta-lactams (w/o cephalosporins and carbapenems)	0.25	0.27
- Carbapenems	0.66	0.63
- Cephalosporins	0.62	0.62
- Fluoroquinolones	0.59	0.60
- Vancomycin	0.85	0.83
- r^2 by antibiotic group and unit, mean*	0.59	0.59

	DOT adm/100 patient days	**DOT / 100 patient days**
CCU/ICU		
- r^2	0.39	0.47
- r^2 by antibiotic group: mean	0.34	0.42
- Anti-anaerobes (clindamycin and metronidazole)	0.23	0.37
- Beta-lactams (w/o cephalosporins and carbapenems)	0.22	0.22
- Carbapenems	0.60	0.67
- Cephalosporins	0.28	0.43
- Fluoroquinolones	0.32	0.34
- Vancomycin	0.42	0.23
r^2 by antibiotic group and unit, mean, mean*	0.23	0.29

Abbreviations. r^2 determination coefficient, CCU/ICU critical and intensive care units, DOT days of therapy, DOTadm based on admission data only

* mean of r^2 of each unit for each group of antibiotics

Table 3: Most frequently isolated pathogens and sample sites (n=6,732 isolates)

Pathogen	**n**	**%**
Pseudomonas aeruginosa	1,629	24.2
Escherichia coli	1,316	19.5
Staphylococcus aureus	1,005	14.9
Enterococcus, non-faecalis non-faecium	696	10.3
Klebsiella pneumoniae	400	5.9
Enterobacter species	305	4.5
Staphylococcus, non-aureus	273	4.1
Enterococcus faecalis	207	3.1
Proteus mirabilis	166	2.5
Serratia marcescens	125	1.9
Klebsiella oxytoca	99	1.5
Citrobacter freundii	83	1.2
Enterococcus faecium	68	1.0
Others (<1% of isolates)	360	5.3

Sample site	**N**	**%**
Urine	2,832	42.1
Tissue / Aspirate / Swab	1,995	29.6
Respiratory specimen	1,139	16.9
Blood	690	10.2
Cerebrospinal fluid	57	0.8
Catheter tips (venous/arterial)	19	0.3

Table 4: Days of therapy (DOT) of antibiotic groups and agents prescribed (n=123,712 DOT)

Antibiotic group	**DOT**	**%**
Fluoroquinolone	37,553	30.4
Cephalosporin	26,793	21.7
Beta-lactam	18,157	14.7
Anti-anaerob	17,508	14.2
Glycopeptide	9,354	7.6
Other groups (<5% of DOT)	14,347	11.6
Antibiotic agents	**DOT**	**%**
Ciprofloxacin	22,987	18.6
Levofloxacin	14,390	11.6
Metronidazole	13,988	11.3
Cefazolin	12,697	10.3
Vancomycin	9,354	7.6
Piiperacillin-Tazobactam	8,931	7.2
Cefotaxime	4,981	4.0
Ceftazidime	3,863	3.1
Co-Trimoxazole	3,590	2.9
Clindamycin	3,520	2.8
Cephalexin	3,376	2.6
Ampicillin	2,926	2.4
Cloxacillin	2,751	2.2
Gentamicin	2,582	2.1
Other agents (<2% of DOT):	12,061	9.7

Table 5: r^2 of monthly data between days of therapy (DOT) and defined daily doses (DDD) per 100 patient days stratified by group of antibiotics

Antibiotic group	**r^2 all units**	**r^2 subproject**
Aminoglycoside	.92	
Glycopeptide	.85	.85
Tetracycline Group	.76	
Anti-anaerob	.75	.77
Fluoroquionolone	.73	.59
Carbapenem	.67	.66
Macrolide	.65	
Cephalosporin	.58	.62
Antimycobacterial	.49	
Beta-lactam	.46	.25
Sulfamethoxazole and trimethoprim	.23	
Other	.06	

Table 6: Summary of risk estimates for fluoroquinolones use and time to event of detection of susceptible and resistant enterobacteriaceae, respectively

	FQ resistant enterobacteriaceae				**FQ susceptible enterobacteriaceae**			
number of patients	**18599**				**19335**			
days at risk	199455				207776			
mean days at risk	10.7				10.7			
	event		**no event**		**event**		**no event**	
number of events (%)	145 (0.8)		18454 (99.2)		881 (4.6)		18454 (95.4)	
FQ prescriptions n (%)	86 (59.3)		5141 (27.8)		82 (9.3)		5141 (27.8)	
	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value
Model 1:								
FQ preexposure	1.86	0.32	1.32-2.62	<0.001	0.14	0.017	0.11-0.18	<0.001
Model 2: (adjusted for age and sex)								
FQ preexposure	1.83	0.32	1.30-2.58	0.001	0.14	0.017	0.11-0.18	<0.001
Model 3: (adjusted for age and sex)								
FQ preexposure	1.65	0.29	1.17-2.34	0.005	0.13	0.015	0.10-0.16	<0.001
per 10 DDD/100 patient days	1.41	0.16	1.13-1.76	0.002	1.46	0.066	1.34-1.60	<0.001
FQ preexposure	1.64	0.30	1.15-2.34	0.006	0.12	0.015	0.10-0.15	<0.001
per 10 DOT/100 patient days	1.33	0.16	1.05-1.70	0.019	1.53	0.073	1.39-1.68	<0.001
Model 4: (adjusted for age, sex, and cluster)								
FQ preexposure	1.65	0.23	1.25-2.17	<0.001	0.13	0.014	0.10-0.16	<0.001
per 10 DDD/100 patient days	1.41	0.25	0.99-2.00	0.056	1.46	0.22	1.09-1.95	0.010
FQ preexposure	1.64	0.25	1.22-2.21	0.001	0.12	0.014	0.10-0.15	<0.001
per 10 DOT/100 patient days	1.33	0.28	0.88-2.02	0.172	1.53	0.25	1.11-2.11	0.010

Abbreviations. FQ fluoroquinolones, DDD defined daily doses, DOT days of therapy, HR hazard ratio, SE standard error, CI confidence interval

Table 7: Summary of risk estimates for fluoroquinolones use and time to event of detection of susceptible and resistant enterobacteriaceae, respectively, stratified by type of unit

FQ resistant enterobacteriaceae

	Acute care units				CCU/ICU				Rehab/step down units			
number of patients (% of all units)	16301 (87.6)				1117 (6.0)				1181 (6.4)			
days at risk (% overall days at risk)	169138 (84.8)				11199 (5.6)				19118 (9.6)			
mean days at risk	10.4				10.0				16.2			
number of events (% of patients)	88 (0.5)				32 (2.9)				25 (2.1)			
	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value
FQ preexposure	1.59	0.24	1.18-2.14	0.003	1.95	0.73	0.94-4.05	0.074	2.02	0.50	1.24-3.29	0.005
per 10 DDD/100 patient days	1.08	0.17	0.80-1.47	0.620	1.34	0.25	0.93-1.96	0.123	1.96	0.58	1.10-3.51	0.023
FQ preexposure	1.59	0.25	1.18-2.16	0.003	1.86	0.65	0.94-3.67	0.074	1.32	0.31	0.77-2.28	0.311
per 10 DOT/100 patient days	1.04	0.17	0.76-1.44	0.792	1.62	0.42	0.97-2.70	0.066	2.13	0.66	1.16-3.91	0.015

FQ susceptible enterobacteriaceae

	Acute care units				CCU/ICU				Rehab/step down units			
number of patients (% of all units)	16836 (87.1)				1252 (6.5)				1247 (6.4)			
days at risk	175704 (84.6)				12389 (6.0)				19683 (9.5)			
mean days at risk	10.4				9.9				15.8			
number of events (% of patients)	623 (3.7)				167 (13.3)				91 (7.3)			
	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value
FQ preexposure	0.11	0.015	0.08-0.14	<0.001	0.15	0.019	0.12-0.19	<0.001	0.19	0.08	0.08-0.42	<0.001
per 10 DDD/100 patient days	0.97	0.17	0.70-1.36	0.877	1.28	0.17	0.99-1.66	0.058	2.18	0.38	1.55-3.07	<0.001
FQ preexposure	0.11	0.015	0.08-0.14	<0.001	0.13	0.017	0.09-0.17	<0.001	0.13	0.73	0.04-0.39	<0.001
per 10 DOT/100 patient days	0.93	0.18	0.62-1.36	0.688	1.97	0.28	1.49-2.60	<0.001	2.54	0.47	1.76-3.67	<0.001

Abbreviations. FQ fluoroquinolones, CCU/ICU critical or intensive care units, DDD defined daily doses, DOT days of therapy, HR hazard ratio, SE standard error, CI confidence interval

Table 8: Summary of risk estimates for fluoroquinolones use and time to event of detection of susceptible and resistant *P. aeruginosa*, respectively

	FQ resistant *P. aeruginosa*				**FQ susceptible *P. aeruginosa***			
number of patients	**19240**				**19322**			
days at risk	214328				214665			
mean days at risk	11.1				11.1			
	event		**no event**		**event**		**no event**	
number of events (%)	157 (0.8)		19083 (99.2)		239 (1.2)		19038 (99.2)	
FQ prescriptions n (%)	113 (72.0)		5534 (29.0)		55 (23.0)		5534 (29.1)	
	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value
Model 1:								
FQ preexposure	2.62	0.48	1.83-3.75	<0.001	0.35	0.055	0.25-0.47	<0.001
Model 2: (adjusted for age and sex)								
FQ preexposure	2.65	0.49	1.85-3.80	<0.001	0.35	0.055	0.25-0.47	<0.001
Model 3: (adjusted for age and sex)								
FQ preexposure	2.34	0.43	1.62-3.36	<0.001	0.30	0.049	0.22-0.42	<0.001
per 10 DDD/100 patient days	1.55	0.15	1.28-1.88	<0.001	1.54	0.13	1.31-1.82	<0.001
FQ preexposure	2.36	0.44	1.63-3.41	<0.001	0.30	0.048	0.22-0.41	<0.001
per 10 DOT/100 patient days	1.41	0.17	1.11-1.79	0.004	1.57	0.14	1.32-1.88	<0.001
Model 4: (adjusted for age, sex, and cluster)								
FQ preexposure	2.34	0.58	1.44-3.79	0.001	0.30	0.052	0.22-0.43	<0.001
per 10 DDD/100 patient days	1.55	0.30	1.07-2.26	0.021	1.54	0.33	1.01-2.36	0.046
FQ preexposure	2.36	0.57	1.47-3.80	<0.001	0.30	0.052	0.21-0.42	<0.001
per 10 DOT/100 patient days	1.41	0.30	0.93-2.14	0.103	1.57	0.36	1.01-2.46	0.046

Abbreviations. FQ fluoroquinolones, DDD defined daily doses, DOT days of therapy, HR hazard ratio, SE standard error, CI confidence interval

Table 9: Summary of risk estimates for fluoroquinolones use and time to event of detection of susceptible and resistant *P. aeruginosa*, respectively, stratified by type of unit

FQ resistant *P. aeruginosa*

	Acute care units	**CCU/ICU**	**Rehab/step down units**
number of patients (% of all units)	16802 (87.3)	1175 (6.1)	1263 (6.6)
days at risk (% overall days at risk)	180846 (84.4)	12176 (5.7)	21306 (9.9)
mean days at risk	10.8	10.4	16.9
number of events (% of patients)	61 (0.4)	84 (7.1)	12 (1.0)

	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value
FQ preexposure	2.02	0.68	1.04-3.91	0.036	1.69	0.93	0.57-4.98	0.342	3.55	2.36	0.97-13.03	0.056
per 10 DDD/100 patient days	0.78	0.17	0.51-1.21	0.266	0.90	0.10	0.72-1.12	0.347	2.44	0.69	1.41-4.24	0.001
FQ preexposure	1.96	0.65	1.01-3.77	0.045	1.68	0.94	0.56-5.05	0.358	4.24	3.64	0.79-22.77	0.092
per 10 DOT/100 patient days	0.98	0.29	0.55-1.75	0.949	0.91	0.17	0.64-1.30	0.610	1.65	0.55	0.86-3.18	0.135

FQ susceptible *P. aeruginosa*

	Acute care units	**CCU/ICU**	**Rehab/step down units**
number of patients (% of all units)	16876 (87.3)	1168 (6.0)	1278 (6.6)
days at risk	181653 (84.6)	11525 (5.4)	21487 (10.0)
mean days at risk	10.8	9.9	16.8
number of events (% of patients)	135 (0.8)	77 (6.6)	27 (2.1)

	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value
FQ preexposure	0.22	0.056	0.14-0.36	<0.001	0.31	0.10	0.17-0.59	<0.001	0.51	0.27	0.18-1.43	0.202
per 10 DDD/100 patient days	0.74	0.13	0.53-1.05	0.095	1.24	0.19	0.91-1.69	0.165	2.01	0.41	1.35-3.01	0.001
FQ preexposure	0.23	0.057	0.14-0.37	<0.001	0.29	0.089	0.16-0.53	<0.001	0.32	0.16	0.12-0.88	0.027
per 10 DOT/100 patient days	0.73	0.16	0.47-1.13	0.156	1.67	0.32	1.15-2.44	0.008	2.43	0.59	1.51-3.92	<0.001

Abbreviations. FQ fluoroquinolones, CCU/ICU critical or intensive care units, DDD defined daily doses, DOT days of therapy, HR hazard ratio, SE standard error, CI confidence interval

Table 10: Summary of risk estimates for use of beta-lactams and time to event of detection of enterobacteriaceae resistant to third generation cephalosporins, respectively

	Enterobacteriaceae resistant to 3GC				**Enterobacteriaceae susceptible to 3GC**			
number of patients	18600				19333			
days at risk	199678				207625			
mean days at risk	10.7				10.7			
	event	**no event**			**event**	**no event**		
number of events (%)	146 (0.8)	18454 (99.2)			879 (4.5)	18454 (99.2)		
Prescriptions of any betalactam antibiotic (%)	94 (64.4)	6985 (37.9)			336 (38.2)	6985 (37.9)		
	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value
Model 1:								
Beta-lactam pre-exposure	1.66	0.29	1.17-2.35	0.004	0.68	0.048	0.59-0.78	<0.001
Model 2: (adjusted for age and sex)								
Beta-lactam pre-exposure	1.65	0.30	1.16-2.35	0.005	0.74	0.052	0.64-0.85	<0.001
Model 3: (adjusted for age and sex)								
Beta-lactam pre-exposure	1.43	0.261	.00-2.049	0.050	0.68	0.050	0.59-0.79	<0.001
per 10 DDD/100 patient days	1.30	0.058	1.19-1.42	<0.001	1.20	0.027	1.15-1.26	<0.001
Beta-lactam pre-exposure	1.24	0.23	0.86-1.80	0.247	0.62	0.046	0.53-0.71	<0.001
per 10 DOT/100 patient days	1.53	0.10	1.35-1.75	<0.001	1.37	0.043	1.29-1.46	<0.001
Model 4: (adjusted for age, sex, and cluster)								
Beta-lactam pre-exposure	1.43	0.23	1.05-1.95	0.024	0.68	0.084	0.54-0.87	0.002
per 10 DDD/100 patient days	1.30	0.11	1.11-1.53	0.001	1.20	0.10	1.02-1.42	0.032
Beta-lactam pre-exposure	1.24	0.20	0.91-1.70	0.171	0.62	0.074	0.49-0.78	<0.001
per 10 DOT/100 patient days	1.54	0.14	1.29-1.84	<0.001	1.37	0.14	1.12-1.68	0.003

Abbreviations. 3GC third generation cephalosporin, DDD defined daily doses, DOT days of therapy, HR hazard ratio, SE standard error, CI confidence interval

Table 11: Summary of risk estimates for use of beta-lactams and time to event of detection of enterobacteriaceae resistant to third generation cephalosporins, respectively, stratified by type of unit

Enterobacteriaceae resistant to 3GC

	Acute care units				**CCU/ICU**				**Rehab/step down units**			
number of patients (% of all units)	16299 (87.6)				1132 (6.1)				1168 (6.3)			
days at risk (% overall days at risk)	169356 (84.8)				11484 (5.8)				18837 (9.4)			
mean days at risk	10.4				10.1				16.1			
number of events (% of patients)	86 (0.5)				47 (4.2)				13 (1.1)			
	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value
Beta-lactam pre-exposure	1.28	0.23	0.90-1.83	0.170	0.71	0.34	0.28-1.80	0.469	6.48	3.68	2.13-19.69	0.001
per 10 DDD/100 patient days	1.04	0.083	0.89-1.22	0.615	1.16	0.06	1.04-1.28	0.006	1.27	0.23	0.89-1.81	0.194
Beta-lactam pre-exposure	1.22	0.23	0.84-1.76	0.291	0.83	0.37	0.34-2.01	0.680	2.70	1.85	0.70-10.38	0.149
per 10 DOT/100 patient days	1.23	0.17	0.94-1.61	0.131	1.03	0.14	0.78-1.35	0.847	2.34	0.54	1.49-3.68	<0.001

Enterobacteriaceae susceptible to 3GC

	Acute care units				**CCU/ICU**				**Rehab/step down units**			
number of patients (% of all units)	16838 (87.1)				1239 (6.4)				1256 (6.5)			
days at risk	175574 (84.6)				12209 (5.9)				19842 (9.6)			
mean days at risk	10.4				9.9				15.8			
number of events (% of patients)	625 (3.7)				154 (12.4)				100 (8.0)			
	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value	HR	Robust SE	95% CI	p-value
Beta-lactam pre-exposure	0.67	0.11	0.48-0.92	0.014	0.33	0.06	0.23-0.47	<0.001	1.59	0.25	1.17-2.17	0.003
per 10 DDD/100 patient days	0.99	0.088	0.82-1.18	0.895	1.23	0.071	1.09-1.37	<0.001	1.31	0.13	1.08-1.59	0.007
Beta-lactam pre-exposure	0.66	0.99	0.49-0.88	0.005	0.35	0.08	0.22-0.56	<0.001	0.86	0.14	0.63-1.18	0.345
per 10 DOT/100 patient days	1.04	0.18	0.75-1.46	0.796	1.21	0.14	0.97-1.52	0.087	1.85	0.23	1.45-2.37	<0.001

Abbreviations. 3GC third generation cephalosporin, CCU/ICU critical or intensive care units, DDD defined daily doses, DOT days of therapy, HR hazard ratio, SE standard error, CI confidence interval

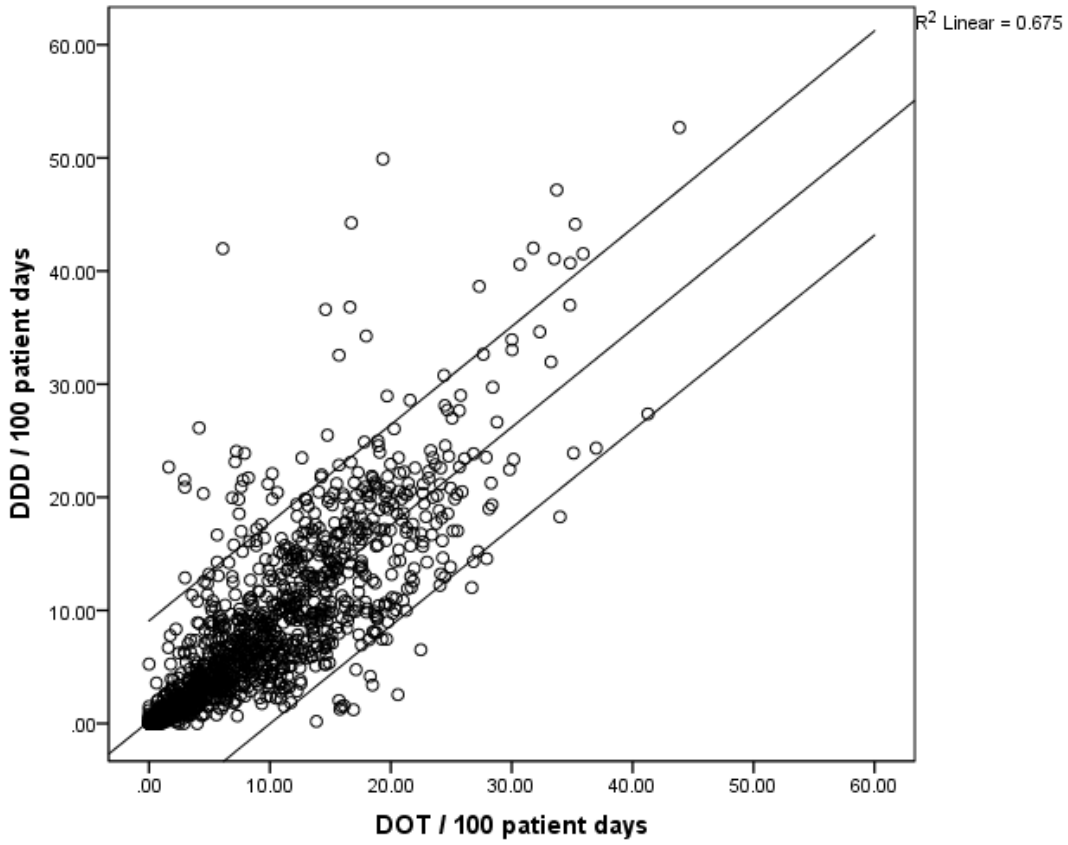
Table 12: Summary of risk estimates for use of subgroup of beta-lactams and time to event of detection of enterobacteriaceae resistant to third generation cephalosporins, respectively, on acute care units

	Enterobacteriaceae resistant to 3GC				**Enterobacteriaceae susceptible to 3GC**			
	HR	Robust SR	95% CI	p-value	HR	Robust SR	95% CI	p-value
Exposure to cephalosporins	1.35	0.24	0.96-1.90	0.087	0.77	0.14	0.53-1.10	0.151
Cephalosporin use per 10 DDD/100 patient days	1.05	0.11	0.85-1.30	0.640	0.86	0.12	0.65-1.14	0.293
Exposure to carbapenems	4.11	1.92	1.64-10.27	0.002	1.31	0.44	0.68-2.54	0.417
Carbapenem use per 10 DDD/100 patient days	0.15	0.14	0.02-0.94	0.042	0.74	0.38	0.27-2.02	0.555
Exposure to other beta-lactams	0.99	0.21	0.56-1.40	0.591	0.58	0.10	0.42-0.80	0.001
Other beta-lactam use per 10 DDD/100 PD	1.13	0.15	0.87-1.46	0.350	1.11	0.11	0.92-1.35	0.277
Exposure to cephalosporins	1.30	0.22	0.91-1.83	0.122	0.76	0.12	0.56-1.03	0.072
Cephalosporin use per 10 DOT/100 patient days	1.28	0.20	0.94-1.75	0.115	1.06	0.25	0.68-1.67	0.791
Exposure to carbapenems	3.51	1.83	1.27-9.73	0.016	1.24	0.41	0.64-2.37	0.524
Carbapenem use per 10 DOT/100 patient days	1.32	1.16	0.24-7.37	0.753	0.86	0.50	0.28-2.70	0.801
Exposure to other beta-lactams	0.85	0.22	0.51-1.39	0.508	0.57	0.09	0.42-0.78	<0.001
Other beta-lactam use per 10 DOT/100 PD	1.09	0.25	0.69-1.71	0.710	1.06	0.22	0.71-1.60	0.769

Abbreviations. 3GC third generation cephalosporin, DDD defined daily doses, DOT days of therapy, HR hazard ratio, SE standard error, CI confidence interval

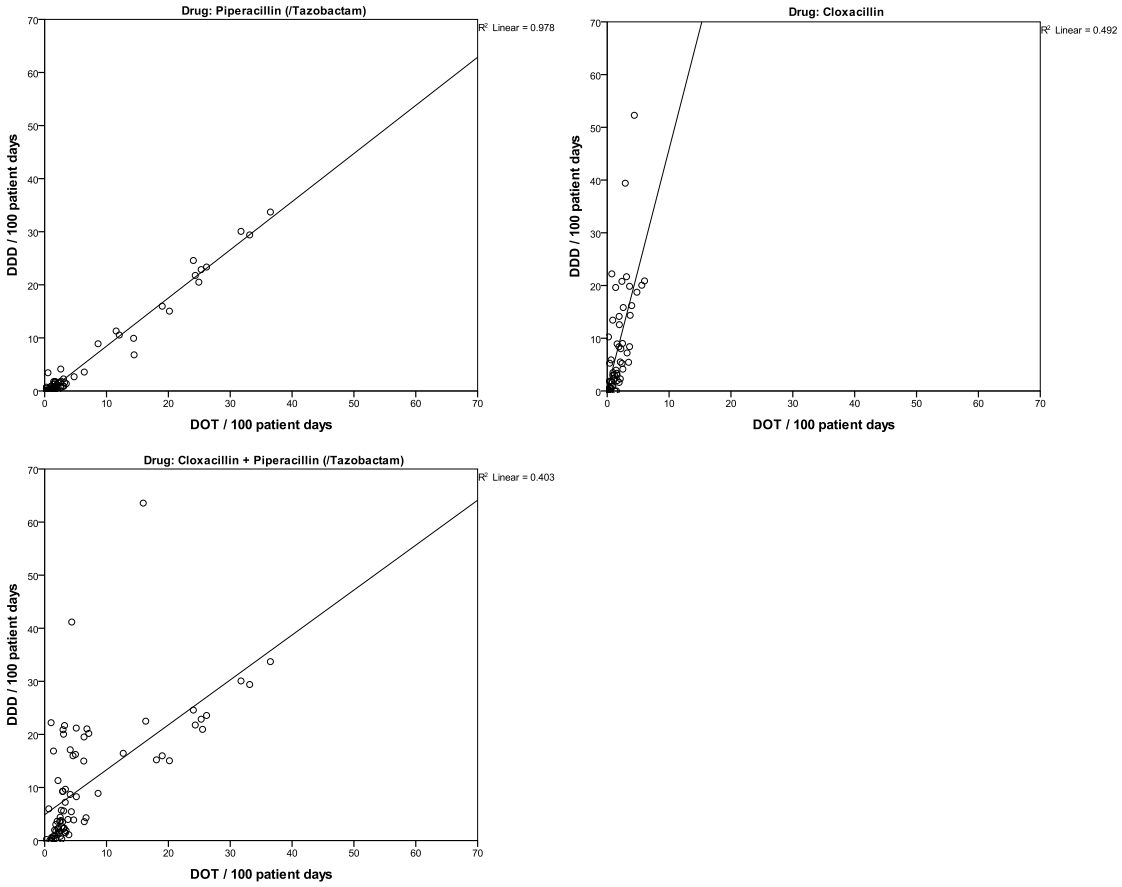
Appendix B: Figures

Figure 1: Correlation between days of therapy (DOT) and defined daily doses (DDD) per 100 patient days on non- CCU/ICU



Abbreviations. DDD defined daily doses, DOT days of therapy

Figure 2: Comparison of the correlation between days of therapy (DOT) and defined daily doses (DDD) per 100 patient days for piperacillin (+/- tazobactam) and cloxacillin on five selected non-CCU/ICU hospital units



Abbreviations. DDD defined daily doses, DOT days of therapy

Figure 3: Comparison of days of therapy (DOT), patient days and patient days according to the pharmacy data over time

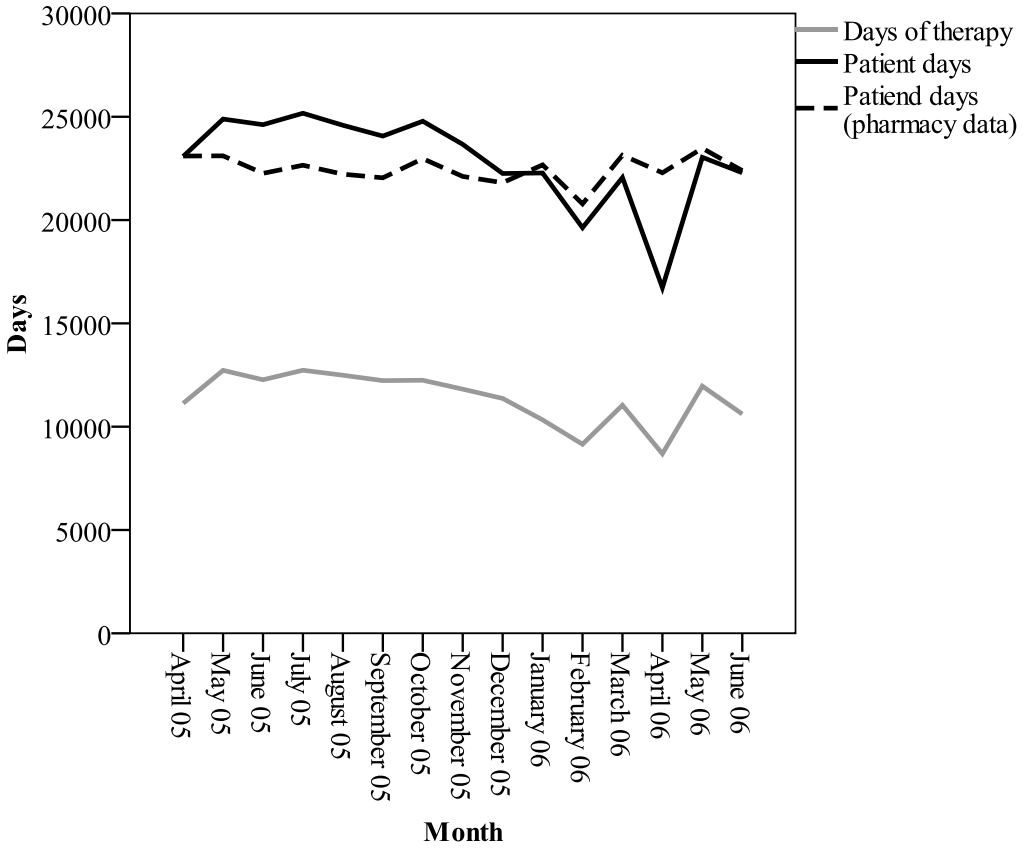


Figure 4: Comparison of days of therapy (DOT) and defined daily doses (DDD) per 100 patient days over time

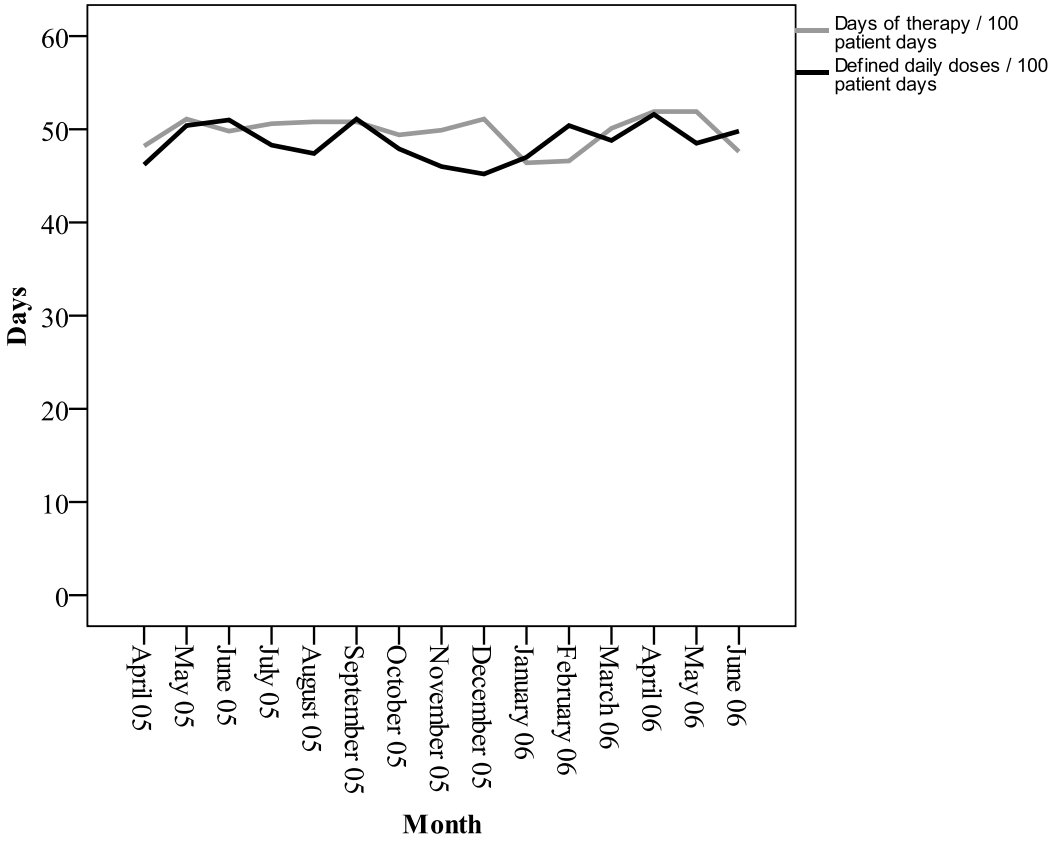
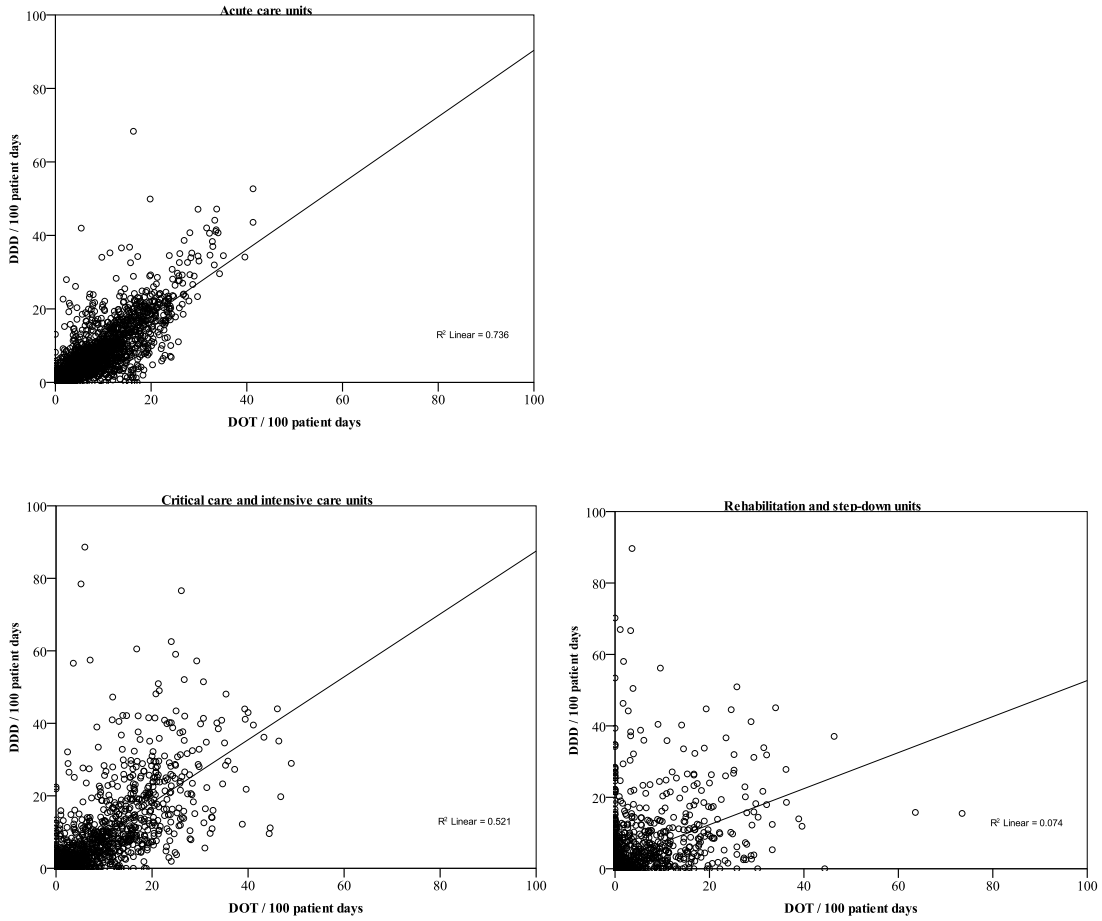
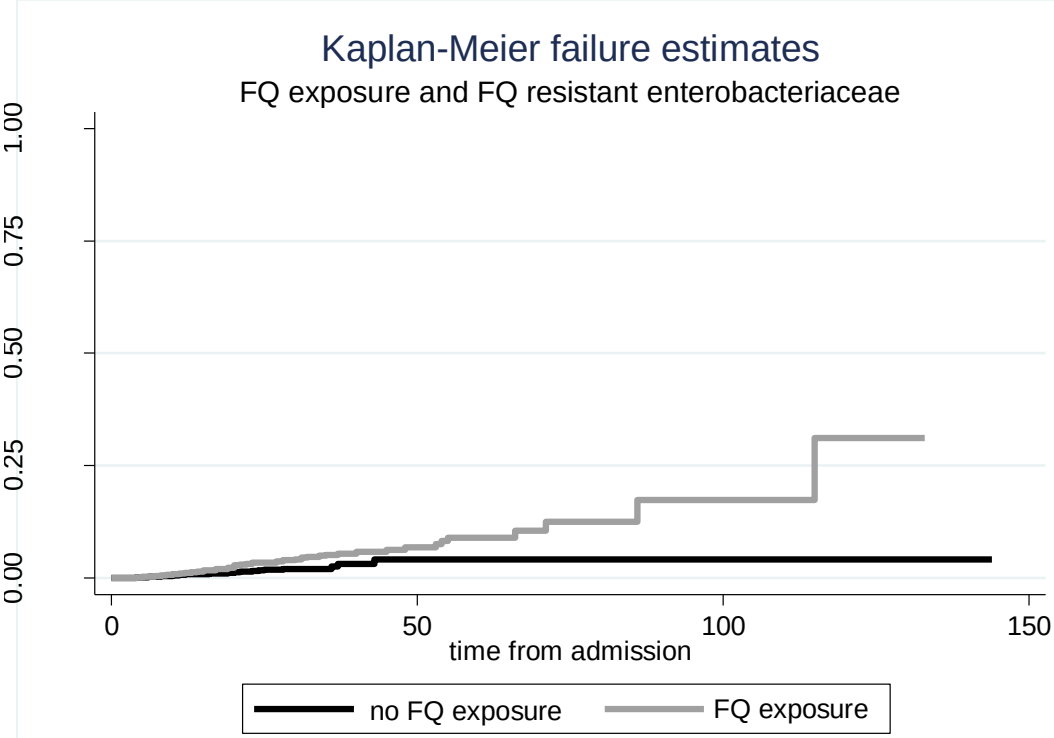


Figure 5: Comparison of monthly data for 12 groups of antibiotics between days of therapy (DOT) and defined daily doses (DDD) per 100 patient days for three types of hospital units



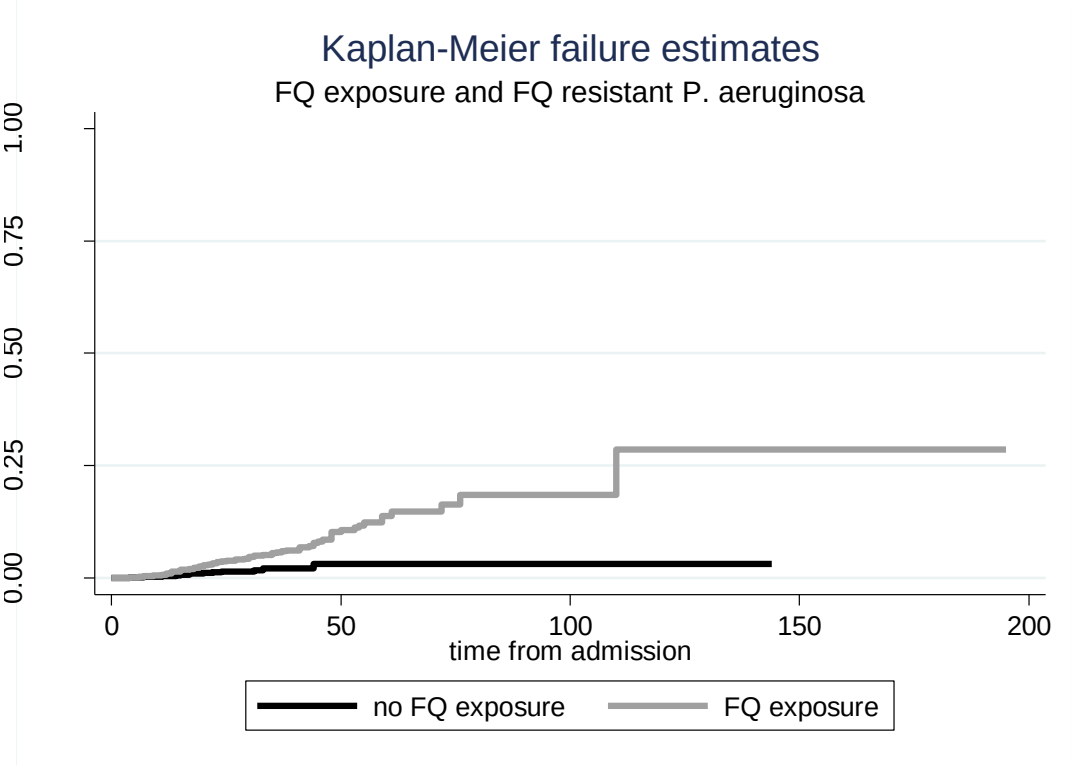
Abbreviations. DDD defined daily doses, DOT days of therapy

Figure 6: Kaplan-Meier failure estimates for fluoroquinolones resistant enterobacteriaceae stratified by fluoroquinolones pre-exposure



Abbreviations. FQ fluoroquinolone

Figure 7: Kaplan-Meier failure estimates for fluoroquinolones resistant *P. aeruginosa* stratified by fluoroquinolones pre-exposure



Abbreviations. FQ fluoroquinolone

Figure 8: Kaplan-Meier failure estimates for enterobacteriaceae resistant to third generation cephalosporins stratified by pre-exposure to any beta-lactam