GENERAL DISCUSSION AND SUMMARY

Worldwide the colonization prevalence and incidence of infections with HRMOs is rising [1, 3]. Without adequate control measures, it is expected that this will further increase, as antibiotic (mis)use and poor infection control is associated with an increase in prevalence of HRMOs and antibiotic resistance [4-6]. Within the group of HRMOs, carbapenemase production among Enterobacteriaceae (CPE) is the most worrisome as usually only one antibiotic group (polymyxins like colistin) is available to treat CPE infections [7]. Alarming, plasmid mediated resistance is already detected to this ‘last’ antibiotic, also known as mobilized colistin resistance gene (mcr-1) [8]. It is on the other hand reassuring that both CPE and mcr-1 bacteria are sporadically found in the Netherlands, and when CPE is detected, it is mainly as colonizing micro-organism among travelers [9, 10]. When not properly controlled, these multi-resistant bacteria will become endemic, and we will return to the pre-antibiotic era with untreatable infections [11].

In order to study the regional rectal colonization prevalence with HR-GNR (Highly Resistant Gram Negative Rods) including CPE and mcr-1 among clinical patients within the region Kennemerland, we annually performed prevalence measurements (Chapter 2). In these prevalence studies we found no rectal colonization with mcr-1 bacteria, and only one with CPE (Chapter 8). Within these studies ESBL-producing and Q&A (fluoroquinolone and aminoglycoside) resistant Enterobacteriaceae were found most frequently as colonizing HR-GNR. Overall (over three years), a HR-GNR rectal colonization prevalence of 6.1% (including 5.0% ESBL) in clinical patients was found, which could be classified as low compared to other studies performed in Dutch health care institutions and community studies [12-14]. HR-GNR colonization prevalence rates can be stable at a national level, but performing such studies regionally can strengthen regional interventions and collaborations, as prevalences and underlying risk-factors can vary between regions and health care institutions. One of the recently identified drivers of antibiotic resistance is the flow of patients within a region [15]. For instance, from hospital to nursing home or the other way around. The study of Donker et al. stresses the need for more regional collaboration, data sharing and targeted regional interventions, involving all relevant actors including hospitals, general practitioners, nursing homes, pharmacies and (microbiological) laboratories.

From our prevalence studies not one ‘major risk factor’ for rectal colonization with HR-GNR could be detected. One factor, an early detection of (infection or colonization) was associated with colonization at the time of the measurement in our multivariable logistic regression model, implying prolonged carriage in some patients. In our study we found no evidence that a longer hospital stay was associated with increased prevalence of HR-GNR, suggesting that the main driver of HR-GNR acquisition was placed outside the hospital. Several other studies on possible risk factors identified: recent antibiotic use (1), earlier hospital admission (2), traveling (3), higher age (4) and treatment with antacids (5) [12, 16, 17]. This is still a relatively short list of identified determinants for rectal colonization. In theory, these risk factors can be used to construct a
clinical prediction rule in order to predict colonization or infection. However, studies that tried to construct such a prediction model showed low predictive values \([18-21]\). It is likely that the etiology of HR-GNR colonization is so divers and complex that such prediction rules are far from optimal. However, a prediction rule could be helpful to assess the colonization risk on admission to hospitals (e.g. risk factor based HR-GNR screening). For now, only known HR-GNR carriers, HR-GNR contacts and patients admitted in a foreign hospital need to be screened for colonization with HRMOs \([22]\). Based on our prevalence results and the microbiological history of these patients, it appears that a large part of HR-GNR carriers (almost 50% in our first prevalence study) are not known or screened (Chapter 2). This means that unidentified colonized patients are permanently present in hospitals with the risk of causing an outbreak as a result. Our data stress the need for adequate compliance to basic hygiene measures within health care institutions. In the ideal world, every patient is nursed with standard basic hygiene precautions in a single room or screened upon admission (with an immediate result) and isolated appropriately in order to prevent the spread of HRMOs.

Looking at the sequence types of HR-GNR colonizing strains, we found that the diversity is mainly polyclonal. Only \(E. coli\) ST 131 was found more than once every year, which is comparable with other studies \([23, 24]\). This sequence type is a worldwide successful clone among infection isolates and could be marked as highly pathogenic \([25]\). Comparing our identified ESBL genes, we found mainly CTX-M ESBL genes, which can be subdivided into the predominant CTX-M-15 and CTX-M-1 genes. Also, these ESBL genes are found frequently in other regions and countries, showing that the ESBL epidemiology in the region Kennemerland does not differ from other regions \([13, 24]\).

A rapid test (result on the same day) that could be used to screen patients for ESBL colonization is Real-Time PCR (qPCR). Therefore, we evaluated a direct ESBL qPCR for the BD MAX platform (Chapter 3). This ESBL qPCR showed good sensitivity and specificity with 95.2% and 97.6% respectively. When a cycle threshold cut-off value of 37 was used, the ESBL qPCR displayed a sensitivity and increased specificity of 95.2% and 98.8% respectively. The only great omission is that TEM ESBLs were not incorporated as target in the panel. Several studies have shown that TEM ESBLs are still prevalent among ESBL-producers and they will be missed with this qPCR \([13, 24]\). Since the ESBL prevalence in our setting was rather low, some patients were marked as false positive (qPCR positive and culture negative), resulting in a relatively low positive predictive value (PPV) of 71.4%. In a screening setting this will mean unnecessary isolation of patients. One can question if these results are truly false positive or that this is a limitation of less sensitive culture methods. Some studies have shown that direct sequencing confirmed the qPCR result. However, an important question is how these results must be inter-
interpreted when this test is not performed side-by-side with culture. For now, all clinical decisions are made based on culturing bacteria and genotyping of these bacteria (when transmission is suspected). When no culturing in addition to ESBL qPCR is performed, should positive patients be followed-up? Is it necessary to have multiple qPCR negative tests to flag ESBL positive patients as negative?

In a screening setting, such as upon admission, tests for all HRMOs are performed (not only ESBLs), meaning that diagnostics for other HRMOs must be performed in order to mark the patient as HRMO negative. This means performing several qPCRs side-by-side, on multiple patient materials. These materials are the rectum (HR-GNR, MRSA and VRE), throat (MRSA and HR-GNR in some institutions) and nose (MRSA). In short, eight qPCRs must be performed with the limitation that not all HRMOs as mentioned in the general introduction are incorporated in these tests, limiting the detection of all clinical relevant HRMOs. Other fast screening options are also available and described, such as performing qPCRs on pooled samples. However, few to none studies are performed that determined the diagnostic value of this diagnostic option for all HRMOs. More studies must be performed in order to assess the ‘best screenings scenario’ and should include (diagnostic and isolation) costs. Independent of the results of such a study, application of a screening scenario is dependent on local isolation strategies and availability of isolation rooms.

In rectal samples, ESBL producing bacteria can be present in low numbers resulting in a (false) negative culture result. A method to increase the detection sensitivity of ESBLs and other HR-GNRs is the use of an enrichment step (selective or non-selective) before culturing. We could not confirm or deny this claim in our setting (Chapter 4). There are several limitations in our study that need to be further discussed. Our study design was far from optimal as we performed this study on rectal samples after freezing. Combined with a low prevalence of ESBLs, our statistical power was low. However, we found a non-significant increase of 21.1% in sensitivity when a non-selective enrichment was used which is in line with the results of other studies. We recommend that based on local epidemiology (e.g. prevalence) and screening setting a decision should be made if enrichment is beneficial or not.

The Netherlands are known for the relatively low prevalence of MRSA compared to other countries. However, much is unknown about the contributing factors of this low prevalence and its associated costs and benefits. The Dutch MRSA Search and Destroy (S&D) policy is considered as contributing factor, which was introduced in Dutch health care institutions in 1988. This policy focusses on the screening and pre-emptive isolation of high risk groups, and treatment and follow-up of known carriers. Several Dutch studies have shown that
In line with these studies, we showed that application of this policy is cost-beneficial compared to a situation where no measures were performed (Chapter 5). The costs in this study were based on all additional measures (e.g., screening, isolating, and treating) that were applied when S&D was performed. The estimated benefits were calculated based on the number (and associated costs) of prevented MRSA bloodstream infections (BSI), calculated for different MRSA prevalence rates (up to 50%). As the benefits were calculated on a hypothetical situation (with no S&D policy), uncertainty exists in the benefits aspect. Important questions are which infections besides MRSA BSI are also preventable by application of S&D and how these infections are prevented. In other words, will S&D reduce the absolute number of infections (MRSA infections are additional to MSSA) or are MRSA infections replaced by MSSA infections (infection numbers stays the same). Also, the exact costs to treat a MRSA-infected patient in the Dutch situation is largely unknown. Namely, the MRSA S&D policy does not only prevent MRSA BSI but also other infections such as surgical site infections (SSI) and skin infections which were not incorporated in our study. Eventually, these data will lead to a much more accurate calculation of the preventive benefits of the MRSA S&D policy. Preventative measures are difficult to explain to policy makers. Therefore, continuous reporting about these benefits is important as preventative measures are frequently seen as costly and labor intensive.

When not properly controlled or identified, HR-GNRs can cause outbreaks (infections and colonization’s). In Chapter 6 we described the epidemiology of ESBL-producing Klebsiella spp. (with additional gentamicin resistance) in the Dutch region Kennemerland in 2012. All consecutive clinical isolates that were routinely collected were retrospectively genotyped with AFLP and HiMLST. Our results showed that much of the regionally collected isolates were polyclonal. From our typing results, four clusters (with epidemiologically linked patients) could be identified of which two were unknown. The other two clusters were known and controlled successfully. Three of the four clusters were at different locations showing that not one institution was responsible for transmission. Overall, we calculated a transmission index of 0.27, indicating a considerable transmission capacity. Given the nature of the isolate collection (with some isolates collected as part of contact tracing), bias towards an increased transmission rate had probably occurred. However, the study of Willemsen et al., which could be considered as the best comparable study, showed an overall transmission rate of 0.07 for HR-GNRs in total (mainly ESBL E. coli) [34]. A comparison showed that the transmission capacity of Klebsiella spp. is probably higher than that of E. coli. Regional surveillance is therefore important, as patients are treated by several health care providers. Fortunately, no transmission between institutions had occurred. Regional insight into routinely collected micro-organisms and hospital admission data can help to control these highly resistant bacteria.
Numerous patients are found unexpectedly HR-GNR positive in clinical cultures that were performed to diagnose a possible infection, meaning that these patients had a certain time of unprotected exposure to other patients. Dutch directives advise to perform contact tracing in such a situation as transmission to other patients is possible. However, the added value of routine contact tracing in case of a solely HR-GNR positive patient is unknown (i.e. what is the nosocomial transmission rate from unexpected HR-GNR positive patients to contact patients?). In order to study this objective we performed a regional prospective cohort study where we screened all admitted contact patients with a minimal contact time of 12 hours (Chapter 7). Before detection, all patients were nursed under standard hygiene precautions, including wearing gloves and performing hand hygiene according to the five moments of the WHO. After detection, all HR-GNR positive patients were nursed in contact isolation in a single room. We identified 35 index and 69 contact patients, with a median time between start of contact and sampling of three days. None of these contact patients were found HR-GNR positive as a result of nosocomial transmission indicating that the nosocomial transmission rate in a local setting under standard hygiene precautions (before installing contact isolation) is low. Our results are in line with several other studies that studied the transmission rate of ESBL \textit{E. coli} to contacts \cite{36-37}. A study performed in a Swiss hospital found a transmission rate of 2.2%. Another study performed in an university hospital in Germany was based on clinical isolates only and found a transmission rate of 2.3% (with isolation precautions after detection) and 5.0% (without isolation precautions after detection) for multi-drug resistant gram negative bacteria (MDR-GNB), including ESBL \textit{E. coli} in a before and after study design \cite{38}. The reported increase in transmission rate (after lifting isolation precautions) was non-significant and decreased counterintuitive on high risk wards. A huge omission in this study is that only clinical isolates were reported, limiting the generalizability to patients that are colonized (but not infected) with a MDR-GNB.

In our study, HR-GNR \textit{E. coli} was found in 86% of the index patients limiting the generalizability to other important species (such as \textit{Klebsiella} spp. or \textit{Acinetobacter} spp.). Also, our results are not sufficient to conclude that isolation is not necessary for these patients, as our data was collected in the period before appropriate isolation. Traditionally, methods to identify outbreaks are built on the identification of bacteria of the same species group (e.g. ESBL \textit{E. coli} vs. ESBL \textit{E. coli}) followed by genotyping of these isolates. However, as resistance genes are frequently located on plasmids, which are known to be interchangeable between bacteria of different species \cite{39}. In practice, this means that a ESBL \textit{E. coli} and ESBL \textit{K. pneumoniae} positive patient could be related, based on resistance gene and plasmid type. Some studies have described outbreaks by horizontal (plasmid) gene transmission. As it is obvious that horizontal gene transfer (HGT) can happen, no evidence is available of this contribution in daily practice in a local setting. If HGT (for CPE or ESBL) is an important source of transmission within institutions, it
would result in a higher incidence of (hospital acquired) clinical cultures with ESBLs or CPEs that could not be explained by clonal transmission. Future studies must clarify this complex epidemiology, as it has important consequences for infection control in terms of detection and interruption of transmission.

For many infections, such as urinary tract infection (UTI) and bloodstream infection (BSI), it is believed that they originate from an intestinal (endogenous) source \cite{40, 41}. Although not all colonized patients develop an infection they could be a source for other patients. From this point of view, the identification of colonized patients is of major importance in infection control. However, what are the individual patient risks for an infection with a HR-GNR? Can rectal HR-GNR colonization be considered as an independent risk factor for infections with HR-GNR? When an association is found, this information could be helpful in the choice of the most appropriate antibiotic treatment when an infection is suspected. In order to study this objective, we followed 1133 clinical patients during one year in a historic cohort design (Chapter 8). The baseline measurement consisted of a rectal swab cultured for HR-GNRs. Infection follow-up (up to one year) was performed by analyzing all clinical culture results that were known in the laboratory information system (LIS) of the Regional Public Health Laboratory Kennemerland (RPHLK). For the association between rectal HR-GNR colonization and subsequent infection with a HR-GNR, we found a significant odds ratio (95%CI) of 5.16 (1.83-14.54) in our multivariable logistic regression model. In conclusion, colonization with HR-GNRs is independently associated with HR-GNR infections in colonized patients. How these results must be incorporated in a clinical prediction rule should be studied further. Some researchers incorporated only historical culture results in a clinical prediction rule to predict BSI with ESBLs and described that screening patients not at risk for infections may decrease predictive values \cite{18}. In our study we found that 73.7% of the HR-GNR infections were UTIs. Because UTIs are an important focus for BSIs, initial appropriate antibiotic therapy within patients with an UTI is important.

Our regional approach to act against the increasing problem of antibiotic resistance is not only based on the in this thesis reported studies. But also, on systematic reporting on HRMO incidence and prevalence within clinical cultures, systematic analysis of surgical site infections, hospital acquired infections and central line infections. All these results are presented in informative reports with interpretation of the data. Furthermore, questions that were raised in daily infection control practice were first analyzed and discussed in a regional infection control meeting with all stake holders and implemented afterwards. This region wide evidence based (decision making) approach helps to keep the fight against antibiotic resistance manageable and feasible.
REFERENCES


