

# **Summary of main findings Conclusions, recommendations and future perspectives**



## SUMMARY OF MAIN FINDINGS

In this thesis we studied MRSA of Unknown Origin (MUO). MUO are MRSA without known risk factors as defined in the risk categories of the Dutch Workinggroup for Infection Prevention (WIP) guideline for MRSA.<sup>1</sup> As a consequence, MUO carriers are not recognized by the so called triage taken at hospital admission. MUO carriers remain undetected and thus, during admission, they can spread MRSA in the hospital. We studied MUO strains and carriers to find new risk factors and transmission routes, so the MRSA guideline can be updated and future MRSA transmission reduced. Below a summary of the main findings is given, followed by its conclusions and the recommendations to reduce the number of MUO.

In **chapter 1** we assessed the magnitude of the MUO problem and determined the differences between MUO and MRSA of known origin (MKO). We found that a quarter (24%) of 5545 registered MRSA isolates (2009-2010) known at the national reference laboratory (RIVM) were MUO. Based on typing, MUO isolates could be divided in two main groups: CC398 MUO (26%; 352/1350) and non-CC398 MUO (74%; 998/1350).

We hypothesised that MUO could have been acquired in the community setting. Therefore, we checked all MUO isolates for the presence of Panton-Valentine Leukocidin (PVL), as the presence of this virulence factor promotes the spread of MRSA in a community setting.<sup>2</sup> Of all MRSA isolates, 12% (684/5554) were PVL positive. Of all MUO isolates 46% (461/998) were PVL positive. Within the group of non-CC398 types, PVL positivity was more frequently encountered in MUO compared to MKO (relative risk 1.19; 95% CI 1.11–1.29). Moreover, community associated *spa*-types such as *spa*-types t008 (ST8), t019 (ST30) and t044 (ST80) were significantly ( $p < 0.01$ ) more present among MUO compared to MKO. PVL-positive *spa*-type t008 was found significantly more often among MUO than MKO (10.6% vs. 1.7%,  $p < 0.01$ ). We hypothesised that the presence of PVL in these *spa*-types gave an evolutionary advantage in the community but the found difference could also be explained by overrepresentation: MUO are often clinical isolates detected by sampling clinical sites although exact figures on this are not known, as medical microbiological laboratories (MML) send the MRSA as soon as the MRSA carrier is detected. If a MRSA infection occurs later, than this is not known at the RIVM. (**Chapter 1**).

CC398 MRSA positive individuals are known to acquire their CC398 MRSA due to exposure to livestock (pigs, veal calves, broilers). However, we identified persons with CC398 MUO, who lacked direct contact with livestock. Based on literature, it is assumed that human-to-human transmission of CC398 MRSA is less likely, because CC398 MRSA is 72% less likely and 6 times less transmittable than non-CC398 MRSA<sup>3,4</sup>, although sporadic nosocomial transmission events and outbreaks were described.<sup>5,6</sup> In regards to the origin (animal or human) and transmission routes (direct or indirect) of MUO CC398,

all options are open. Therefore, CC398 MUO was further explored in **chapter 2** to study whether the CC398 MUO strains were different from the CC398 MKO strains, and as such could more easily transmit from human-to-human than CC398 MKO strains, directly transmitted from livestock to humans. For now, there are two CC398 variants: one of animal and one of human origin. Besides their differences in transmission, they also differ in presence or absence of certain mobile genetic elements (MGEs).<sup>7</sup>

By polymerase chain reaction (PCR) we determined whether MGEs - *scn*, *chp*,  $\phi 3$  *int*,  $\phi 6$  *int*,  $\phi 7$  *int*, *rep7*, *rep27*, and *cadDX* - were present in MUO isolates and by comparing them with a set of CC398 isolates of various known origin (humans, pigs, horses, chickens, and veal calves), we studied the origin of CC398 MUO. We found that the distinct human CC398 MSSA *spa*-type, t571, was not present among the Dutch CC398 MRSA strains from humans and pigs that we studied. Furthermore, the CC398 MUO were tetracycline resistant and carried no  $\phi 3$  bacteriophage with *scn* and *chp*.

In short, the CC398 MRSA in this study, MUO or MKO, were found to resemble CC398 MRSA as found in pigs and not CC398 MSSA as described as human pathogens. We concluded that human CC398 MUO carriers carried MRSA of livestock origin, despite MUO carriers not having an epidemiological link with pigs and pig farming. This finding is worrisome, as it suggests other transmission routes than direct exposition to livestock.

In **chapter 3** we studied the necessary number of follow-up culture-sets in a retrospective MRSA carrier cohort (2005-2010) to declare a successful MRSA eradication therapy and a non-carriage status. We compared the then standard procedure of three MRSA follow-up culture-sets to six culture-sets, to determine when to be cultured and how many culture-sets are necessary during a time-period of one year. We found that between the third and sixth follow-up culture-set 54% (35/65) of total recurrences occurred. Over 88% of all recurrences were detected within two months. Combined nose and throat carriage OR 25.5 (1.6-419.1)) and intravascular lines (OR 13.6 (1.2-156.2)) were found to be risk factors for early recurrence. An early recurrence is defined as a recurrence during the first three follow-up culture-sets as opposed to the last three culture-sets.

Many seafarers visit the port city of Rotterdam. They are a difficult to contact group due to language and cultural barriers and their short stay before shipping off again. Because of above reasons, we could not include them in our MUO case control study. Therefore, we studied the seafarers separately from the MUO case control study (as described in **chapter 5**) and determined the MRSA prevalence of seafarers in the Netherlands upon hospital admission in (**chapter 4**). We detected a 5.8% (7/124) MRSA prevalence. This was a MRSA prevalence 52-times higher than the normal prevalence in the Netherlands (0.11% at hospital admission).<sup>8</sup> Furthermore, we found that the presence of wounds or abscesses gave seafarers a 40-times higher risk of being MRSA positive. This finding resulted in addition of seafarers as new risk group for MRSA in the Harbour hospital and the Erasmus University Medical Center in Rotterdam.

In **chapter 5** we described a prospective case control study to determine risk factors for MUO acquisition/carriage with the aim to find new risk factors for MRSA, and add these to the risk categories of the Dutch MRSA guideline by the (successor of) the Dutch Workinggroup for Infectionprevention.

Between September 1<sup>st</sup> of 2011 and September 1<sup>st</sup> of 2013, MUO cases were included in a case control study. Cases were all MUO carriers reported to the RIVM in this period, with randomly selected controls from the community during the same period. Cases and controls were approached by mail and were asked to fill in a questionnaire which included the known risk factors as described in the WIP guideline, to be able to filter the MKO misclassified as MUO. The questionnaire also contained questions on health, behaviour, profession and (sport) activities that could be possible MUO risk factors. These questions were chosen based on a literature study and an earlier pilot study (trawing study).

One of the case control study outcomes was that 38% (144/376) of MUO carriers were misclassified and actually MKO. These misclassified carriers were excluded from further analyses. We found the following significant risk factors for MUO in logistic multivariate analysis: antibiotic use in the last twelve months, aOR 8.1 (5.6-11.7), screened as contact in a contact tracing but not detected as a MRSA carrier at the time, aOR 4.3 (2.1-8.8), having at least one foreign parent, aOR 2.4 (1.4-3.9) and receiving ambulatory care, aOR 2.3 (1.4-3.7). These risk factors explained 83% of the MUO cases.

We hypothesised that part of the MUO carriers were initially missed as MRSA carrier, only to be later detected and subsequently categorized as MUO. This meant that some MUO carriers had unidentified links to other MRSA carriers. Therefore, in **chapter 6**, we studied genetic links between MUO strains and other MRSA (MUO or MKO) strains, together with some crude epidemiology data of the carriers.

Therefore, we included MUO carriers from the case control. We then selected well-defined hospital and household clusters, and compared them with randomly selected, well-defined regional and national isolates. All isolates had the same genetic background (ST8) and were clustered with core genome multi-locus sequence typing (cgMLST). Our goal was to discern or link MUO isolates to known MRSA clusters (MKO) or single MRSA isolates (MUO or MKO). In this study, we elucidated 28% (15/54) of MUO by linking them to known MRSA clusters or isolates through cgMLST and epidemiology. Moreover, we were able to link isolates from the same region but different MML to each other. This resulted in the formation of new regional clusters that previously had gone unnoticed. These isolates were considered MUO and not part of a cluster. Also, the use of cgMLST resulted in reclassification of old clusters previously based on epidemiology and *spa*-typing.

Different typing methods, such as cgMLST, will generate different outcomes and thus change the presence and/or the extent of outbreaks and therefore how many people need to be screened. Those not screened at the time of the outbreak, and detected later in time, will be defined as MUO. CgMLST has a higher discriminatory power, so we were able to

discern within and between t008 MRSA clusters as based on *spa*-typing. Furthermore, we successfully discerned between Dutch MRSA t008 clusters and import-MRSA t008 clusters from Aruba and Taiwan, which was previously not possible with *spa*-typing.

## CONCLUSIONS, RECOMMENDATIONS AND FUTURE PERSPECTIVES

In Nethmap 2018, the RIVM reported that 38% (1,066/2,792) of the reported MRSA isolates to the MRSA surveillance in 2017 were MRSA of Unknown Origin (MUO).<sup>9</sup> Based on our findings in the case control study, 38% (144/376 returned questionnaires) of the MUO cases in the MRSA surveillance were actually misclassified MKO. Assuming a stable amount of misclassification every year this would mean that, in 2017, 23% (661/2792) of the reported MRSA to the surveillance were MUO. (In absolute numbers, this is 405 “MUO” less than originally reported) If we calculate the same for 2009, we had 786 MUO from 2874 reported MRSA originally, which becomes 17% (487/2874) after correction for misclassification. Which is an estimated increase of 35.7% of MUO over a period of 8 years (2009-2017). Despite misclassification we are still confronted with a large group of MUO. It remains important to understand MUO's origin. In this thesis we studied MUO and learned that they are diverse and fall apart in several subgroups, both genetically as epidemiologically.

### MUO CC398

An important subgroup are the MUO CC398 isolates (352/2312 MRSA CC398; 15% as described in **Chapter 1**). These are MUO isolates from the livestock associated cluster 3398, whose carriers had no direct occupational contact to livestock. (As discussed in **Chapter 2**.) It is important because The Netherlands, like Denmark, is a low MRSA prevalence country with a relative large livestock-associated MRSA reservoir. Also, it is currently assumed that the tetracyclin-resistant livestock-associated MRSA (LA-MRSA) does not significantly transmit from human to human and acquisition is based on exposure through (occupational) contact.<sup>3</sup> The limited human-to-human transmission fortunately limits the impact of these LA-MRSA strains. The existence of MUO CC398 however implies acquisition despite no direct contact. Thus we hypothesised if MUO CC398 isolates belong to a subgroup of this cluster that is solely isolated from humans. McCarthy et al. showed that CC398 strains from humans in contact with animals differed from strains isolated from humans without contact with animals in mobile genetic element (MGE) content.<sup>7</sup> However, we found MUO CC398 to be similar to MKO CC398 and not part of the subgroup of the CC398 solely isolated from humans. (**Chapter 2**) This means that the human host adapted MRSA CC398 is less likely the source of MUO CC398. A more likely hypothesis for MUO CC398 existence could be indirect transmission, for example by air, rodents, or

indirect by exposure of persons in the community to livestock exposed CC398.<sup>10-14</sup> Further studies on MUO CC398 are necessary to determine if indirect exposure indeed plays an important role in the transmission of MUO CC398. We also recommend to analyse the reported number of MUO CC398, and report the aggregated data nationally and to the MML. Presence of MGEs like  $\phi 3$  ( $\phi Sa3$ ) in MUO CC398 can be easily monitored, as was shown by a study by van Rijen et al. who screened for MUO CC398 with  $\phi 3$  and *tetM* allowing the quick distinction between CC398 of animal-, and human origin.<sup>15</sup>

### Import-MRSA and seafarers

A proportion of MUO is directly related to (an exposure to) higher MRSA prevalence abroad: so called import-MRSA. For example, seafarers (**Chapter 4**), having a foreign parent (**Chapter 5**) or import-MRSA clusters through travellers visiting friends and relatives or through immigration (Aruba, Taiwan) (**Chapter 6**) are all factors explaining part of MUO carriers. Interestingly, abroad visits, travelling abroad, and abroad hospital or healthcare visits longer than two months ago were not significant risk factors in the case control (**Chapter 5**). The question remains whether the arbitrary cut-off of two months for hospital visit abroad is not too strict and not too general, as the arbitrary cut-off could potentially label some MRSA as MUO who would otherwise be classified and treated as MKO. (**Chapter 5**)

Furthermore, we recommend to add seafarers as risk factor for MRSA carriage. Given the high prevalence rate of carriage among seafarers, we recommend that all seafarers should be classified as WIP category 2, and thus screened for MRSA when they are present in the Netherlands, regardless of wounds or underlying disease; and to apply pre-emptive isolation while awaiting test results. Since 2010, seafarers have been included as such in the triage for admission to the Erasmus MC (and the former Harbour hospital). The global impact on transmission of MRSA by seafarers is currently unknown. (**Chapter 4**)

### Case control risk factors: intersection between healthcare and community

The results of the MUO case control suggest that a large portion of MUO cases are still healthcare or disease related as we found risk factors such as antibiotic use and ambulatory care. We also found that MUO carriers had been screened before in contact tracing but had not been detected as MRSA carrier at that time. It seems MUO carriers have regular contact with healthcare, but not necessarily the hospital. Most likely they acquired their MRSA somewhere in the “fringes of healthcare”, outside the hospital where the community and healthcare world overlap. Examples can be ambulatory care or the use of antibiotics in nursing homes or at general practitioners. (**Chapter 5**) Adding these more general risk factors to the risk categories of the Dutch MRSA guideline of the WIP

will possibly decrease the number of MUO, but will also generate high costs of screening and also a higher burden if we also choose to pre-emptive isolate the additionally screened patients as well, because of the larger number of people who qualify for having one of these risk factors. The total number of people needed to be screened to find one MUO with new, general risk factors such as ambulatory care use or use of antibiotics, could become quite high and therefore be too expensive and too labour intensive. Still, our general risk factors explained 83% of the MUO cases in the case control. A different approach using a risk algorithm is therefore desirable, (*vide infra*). For the remaining 17% of MUO cases no explanation was found.

For case control studies, the choice for the controls is difficult but important. As stated above, we found general risk factors that explained 83% of MUO. However, the outcome of a case control study depends highly on the choice of the control; matched or not. Our study of MUO's characteristics (**chapter 1**) and the initial trawling questionnaire (**chapter 5**) revealed a very diverse group of carriers, a diversity better paralleled by choosing a community control group. Cases, i.e. MUO carriers were not just MRSA carriers reported by hospitals, but also those reported by general practitioners (GP) or long-term care facilities (LTCF). If we would select controls only from patients admitted or treated at the hospital or also from the GP or LTCF, then each option would result in a different control population. We also considered matching on having an (suspected) infection, as MUO are often discovered by accident – for example from clinical isolates. However, this was not possible as it was not certain whether reported cases had an infection or not. The registration of the presence of infection was not reliable. If the submitted case isolate was not an isolate of an infectious site, then infection elsewhere or later could not be ruled out. Furthermore, our case control was set up to find unknown risk factors without a-prior bias. Matching would introduce bias and decrease the change on finding risk factors. Therefore, we chose unmatched controls selected from the community, reducing bias as much as possible while maximizing the chance to find new risk factors within the MUO population. Given the known prevalence of MRSA in the community (between 0 and 0.11%<sup>8</sup>) we assumed the chance of including a MRSA carrier as a control too low to screen for.

To identify patients at risk for MRSA carriage, an algorithm is necessary that computes the a-prior risk for MRSA carriage using the current WIP risk factors and our newly defined case control risk factors. Unfortunately, some of the new risk factors are too general and/or too frequent, to use them as a yes/no answer in normal triage for admission. In future, an algorithm should be developed in which factors are weighted in a specified population, so a weighted risk can be calculated from more than one factor. The outcome of this algorithm should then lead to measures to prevent transmission of MRSA. Ideally, a national uniformity in this algorithm and performance should be aimed for.



Finally, to enhance feasibility and completeness, an interactive chatbot could be programmed with the questions to be used by patients to be admitted or treated. The inputted answers can be loaded into an algorithm to calculate the a-prior risk of MRSA carriage. Chatbot technology is already in use today and used by companies with many clients, such as KLM.<sup>16</sup>

### **MRSA recurrence after MRSA eradication therapy**

MRSA recurrence is missed when using only three follow-up culture-sets after MRSA eradication therapy. These MRSA recurrences can later be detected as MUO. We showed that MRSA recurrences are missed in 54% (35/65 recurrences) when using three follow-up culture-sets in comparison to six follow-up culture-sets. Using our results, the then WIP guideline was changed on the timing of culture-sets after MRSA eradication therapy (**Chapter 3**). We recommend five culture-sets within a period of one year after eradication therapy. In the 6 months after eradication therapy, we recommend to frequently culture carriers whose MRSA was eradicated and who are at risk for early recurrence. Persons at risk for early recurrence are those with combined nose and throat carriage (OR 25.5 (1.6–419.1)) or those who have intravascular lines (OR 13.6 (1.2–156.2)). Persons with late recurrence, could have their follow-up culture-sets with more time in between each set, with a final culture-set one year after their eradication therapy. Persons at risk for early recurrence are detected sooner, and their recurrence happens within six months. These persons would then not have to wait a year for their final follow-up culture-set. This recommendation is a balance between the need for swift detection of MRSA recurrence, the patients' burden, and lowering the number of missed MRSA recurrences after successful MRSA eradication therapy.

### **Misclassification of MKO as MUO**

We encountered a high number of misclassified MKO isolates that were listed in the national MRSA database as MUO. Analysis of the case control study showed 38% of all MUO isolates in the MRSA surveillance database were actually MKO. Misclassification can be attributed to early uploading accompanying epidemiological data to the RIVM together with sending the MRSA strain by medical microbiological laboratories (MML) to the RIVM. This early uploaded data, especially before the typing results are known, will lead to less details on possible MRSA risk factors (**Chapter 5**). It is not always possible to ascertain the source of acquisition before the typing result is available. Typing results should be used to elucidate sources and transmission routes. When sources happen to be the healthcare centre, then the MRSA carrier can be defined as a MKO. The solution to this problem is a two-step procedure: first, the strain is sent and then, after getting back and analysing the typing results, the MML is asked for the final epidemiological decision whether we are dealing with a MKO or a MUO. This decision will depend on the

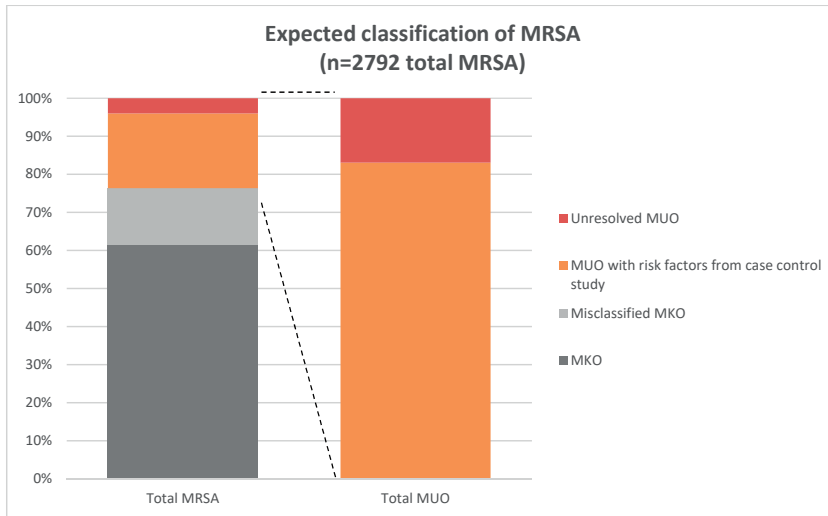
(assumed) sources and risk factors of the carrier. Ideally the typing results are returned to the medical professionals in the shortest turn-around-time to support contact tracing efforts and help them define MRSA sources. The latter should then be reported back to the MRSA surveillance, further improving the reliability of the epidemiological data.

Misclassification is due to the quality of the epidemiological data. This quality is based on the time, effort and possibility to acquire the data. Currently, there is no standard procedure when contacting and questioning MRSA carriers or contact tracing and who should do that. So the inputted effort will vary per person as will the quality. A procedure for questioning and contacting MRSA carriers is therefore recommended. Misclassification can also happen through limited contact tracing in MRSA outbreaks (**Chapter 5 and 6**). Too limited contact tracing will miss MRSA carriers (and thus MKO) who can be detected later or elsewhere as MUO. This could explain the found risk factor in the case control study: having been screened in the past before for MRSA carriage, but found to be MRSA negative at that time.

### Remaining MUO and the necessity to cooperate and type

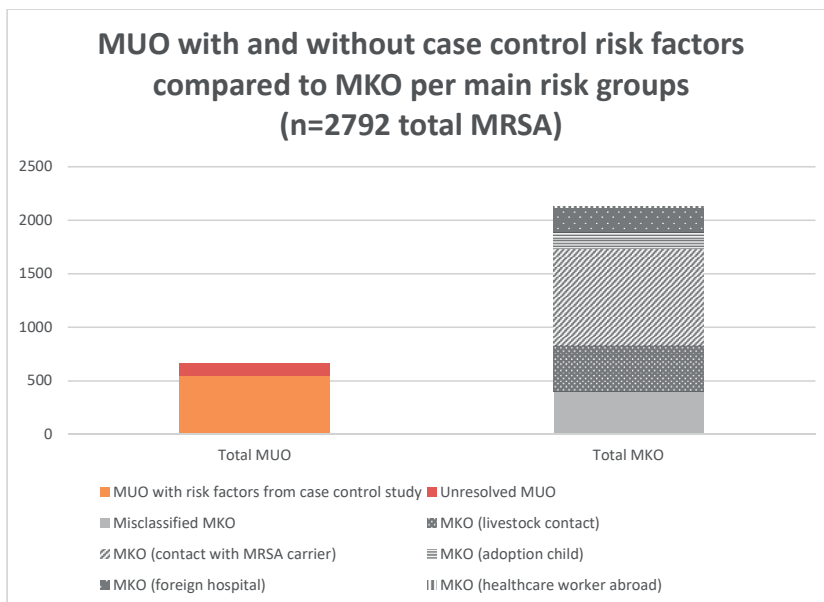
There is a remaining portion of MUO that cannot be explained. In our case control study, this was 17%. Taking the Nethmap 2018 data, this would mean that out of the 661 MUO in 2017 (23% of total reported MRSA to the surveillance), 17% (112/661) would remain without explanation. That would be 4% (112/2792) of total MRSA reported to the MRSA surveillance in 2017. (Figure 1) This remaining group consists of various MUO cases in which we could not identify risk factors in our study. A proportion of these MUO may still be elucidated if genomic information from MRSA isolates across healthcare institutions or within a ZorgRegio or even nationally are combined. We have shown that two MMLs in the Rotterdam region each discovered a MUO independent of each other, without knowing these two were genetically linked, until they were linked through our cgMLST data (**Chapter 6**). We also show in **chapter 6** that we can discern between clonal t008 MRSA clusters with the higher discriminatory power using cgMLST. Application of cgMLST data by MML in contact tracing could prove crucial to elucidate the last remaining 17% of MUO cases in The Netherlands.

Preferably these data should also be shared within the regional network of healthcare institutions to detect intraregional (outbreak) clusters (such as described in **Chapter 6**). The current Antibiotic Resistance Care Network or ZorgRegio ABR could take up this task and find a way to share data on MRSA strains, typing results and carriers. Ideally, healthcare institutions within the ZorgRegio should be informed on MRSA carriers in the region, and they could be asked to make agreements on sharing information and equalizing policies, taking into account the privacy laws. Sharing information on MRSA carriers and MRSA strains between institutes, especially if the patient is not directly transferred, is



**Figure 1 – Dutch MRSA and MUO, relative contributions**

Data for MUO were generated by using results of the MUO case control study applied to the Nethmap 2018 MRSA data (n=2792). Data for MKO were generated by using MRSA surveillance data of 2008-2011 and extrapolating the average percentage to the given numbers in the Nethmap 2018 data. Definition of misclassified: MRSA isolates indicated as MUO by the MMLs in the questionnaire sent to RIVM but defined in our case control study as MKO.



**Figure 2 – MUO compared to MKO in absolute numbers**

Data for MUO were generated by using results of the MUO case control study applied to the Nethmap 2018 MRSA data (n=2792). Data for MKO were generated by using MRSA surveillance data of 2008-2011 and extrapolating the average percentage to the given numbers in the Nethmap 2018 data. Definition of misclassified: MRSA isolates indicated as MUO by the MMLs in the questionnaire sent to RIVM but defined in our case control study as MKO.

highly desirable from an infection prevention point of view, but may right now be a bridge too far.

In summary, we make the following recommendations: to conduct further studies on MUO CC398, to monitor MUO CC398 in surveillance and feedback to national and MML level, to add seafarers as MRSA risk group to the Dutch MRSA guideline, as well as classify seafarers as a WIP category 2, to make a risk algorithm, in which a weighted a-prior risk is calculated based on multiple risk factors, to culture post-MRSA eradication therapy with five follow-up culture-sets, four of which in the first two months (the fourth in 2 months) after MRSA eradication therapy and the latter after half a year or year depending on the presence of risk factors or being a healthcare worker. Other recommendations are to reduce misclassification through more intensive collaboration between RIVM which provides typing, and the MML that provide the epidemiological data, to share at least the typing data within the ZorgRegio and if possible also on a supraregional level so that arbitrary boundaries between adherence areas of MML do not limit our ability to discover MRSA clusters and to continue to monitor the number of MUO as 17% of Dutch MUO are without risk factor or explanation.

Continuous control of MUO is essential to keep MRSA prevalence low in The Netherlands!

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