Emergence of MRSA of unknown origin in the Netherlands

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ABSTRACT

The Netherlands is known for its low methicillin-resistant Staphylococcus aureus (MRSA) prevalence. Yet MRSA with no link to established Dutch risk factors for acquisition, MRSA of unknown origin (MUO), has now emerged and hampers early detection and control by active screening upon hospital admittance. We assessed the magnitude of the problem and determined the differences between MUO and MRSA of known origin (MKO) for CC398 and non-CC398. National MRSA Surveillance data (2008–2009) were analysed for epidemiological determinants and genotypic characteristics (Panton–Valentine leukocidin, spa). A quarter (24%) of the 5545 MRSA isolates registered were MUO, i.e. not from defined risk groups. There are two genotypic MUO groups: CC398 MUO (352; 26%) and non-CC398 MUO (998; 74%). CC398 MUO needs further investigation because it could suggest spread, not by direct contact with livestock (pigs, veal calves), but through the community. Non-CC398 MUO is less likely to be from a nursing home than non-CC398 MKO (relative risk 0.55; 95% CI 0.42–0.72) and Panton–Valentine leukocidin positivity was more frequent in non-CC398 MUO than MKO (relative risk 1.19; 95% CI 1.11–1.29). Exact transmission routes and risk factors for non-CC398 as CC398 MUO remain undefined.

INTRODUCTION

In the past 20 years, the Netherlands kept methicillin-resistant Staphylococcus aureus (MRSA) at bay through prudent use of antibiotics and a Search and Destroy policy. Part of Search and Destroy is active detection and isolation based on defined risk groups. For these reasons, MRSA prevalence in Dutch hospitals and community is still low [1,2]. The Dutch Working party on Infection Prevention developed a guideline on MRSA prevention. (Table 1) This guideline defines the national risk groups and the procedure of contact tracing around cases is described. The Dutch policy can therefore be seen as targeted surveillance on defined risk groups. However, MRSA was found in people who were not targeted by the Search and Destroy policy because they did not belong to the defined risk groups [3]. In the present study, these cases are defined as 'MRSA of unknown origin' (MUO). MUO can transmit, until detection, because preventive measures are not taken. To enable the targeting of control strategies for MUO, the magnitude of the problem was measured and the differences were determined between MUO and MRSA of known origin (MKO; comprising MRSA risk groups and contact tracing described in the targeted surveillance). Materials and Methods Data from the national MRSA surveillance database at the National Institute for Public Health and Environmental Protection (RIVM) between 1 January 2008 and 31 December 2009 were used. All MRSA strains sent to the RIVM by 68 Dutch laboratories, covering the whole country, are registered in this database. Of the cultures positive for MRSA taken from a single person, one, usually the first detected, strain is sent to the RIVM. A check on duplicates in the database further ensured one MRSA strain per person. At the RIVM the MRSA strains were confirmed by testing for the presence of the mecA gene and the coagulase gene. For all confirmed MRSA the *spa*-type, as described by Harmsen et al. [4], and the presence of the Panton–Valentine leucocidin gene (PVL-gene) were determined [5]. As there was no significant difference in the number and data of reported isolates and carriers between the 2 years, data were pooled to increase power. Based on *spa*-types we distinguished CC398 (livestock associated strains) and non-CC398 [6]. CC398 was checked by RIVM with multiple-locus variable number tandem repeat analysis (http://www.mlva.net/). CC398 was analysed as a separate group from non-CC398. Each strain was submitted with a form, with background information on hospital, demographic patient information, risk factors when applicable (Table 1), and other relevant epidemiological information. Laboratories were approached by the RIVM to complete their missing data as much as possible. Two defined groups, MUO and MKO, were classified based on the included information on defined risk factors requested. The absence of either defined risk factors or of risk factors found through contact tracing, led to a classification of MUO. Additional remarks were usually made on the form and/or the box for 'Unknown MRSA' was ticked. Isolates with no or incomplete additional epidemiological data (No data), which made classification impossible, were

Table 1 – Dutch defined risk groups

Risk groups (Patients)	Numbers Patients (n=2538) [a]
Contact with roommates or carrier	
Single room shared with MRSA carrier	89 (4%)
Contact tracing	485 (19%)
Foreign	
Cared for in a foreign hospital	342 (13%)
Foreign patients at a Dutch dialysis department	1 (0.04%)
Adopted children: hospitalized or frequently visit the outdoor department	62 (2%)
Dutch dialysis patients dialyzed abroad	1 (0.04%)
Livestock	
Work related contact with alive pigs or veal calves	1120 (44%)
Outbreak	
Patients from another Dutch hospital or nursing home, from a department	128 (5%)
or unit where there is a MRSA outbreak, which is not under control	
MRSA Carrier	
Proven carrier	119 (5%)
Risk groups (Healthcare workers (HCW))	**HCW (n=255) [a]**
Contact with roommates or carrier	
Unprotected contact without infection precautions	165 (64%)
Protected contact with infection precautions	19 (7%)
Contact tracing	33 (13%)
Foreign	
Cared for in a foreign hospital	2 (0.8%)
Worked < 2 months ago, but longer than 24 hours in a foreign	10 (4%)
hospital or nursing home	
Worked (regularly) in an abroad hospital or escort patients from a foreign	7 (3%)
to a Dutch hospital	
Livestock	
Work related contact with alive pigs or veal calves	1 (0.4%)
MRSA Carrier	
Proven carrier	5 (2%)

MRSA, methicillin-resistant Staphylococcus aureus

[a] MRSA in the Netherlands in 2008-2009. A single carrier can have more than one risk.

not included in further descriptive and multivariate analysis. Finally, additional remarks on the form were categorized to gain insight into new sources and risk factors. The most prevalent *spa*-types were determined for the total amount of MRSA, CC398, non-CC398 MRSA, MUO and MKO. The *spa*-types were ranked with rank 1 being the most prevalent *spa*-type within the (sub)group; rank 2 the second most prevalent, etc. SAS statistical software (ENTERPRISE GUIDE version 4.2) was used for descriptive analysis, univariate

analysis (Fisher's exact test) and multiple regression analysis (log-binomial regression model, proc GENMOD). A p value of <0.05 was considered significant. Goodness of fit was determined with the area under the curve of a receiver operating characteristic-curve (ROCR software). Relative risks (RR) with 95% CI were calculated.

RESULTS

General results

In 2 years, 5545 MRSA strains were sent to the reference laboratory and so were available for analysis: 2671 reported in 2008 and 2874 in 2009. From the 5545 MRSA, 3233 (58%) were non-CC398 and 2312 (42%) were CC398 (livestock-associated MRSA). The MUO and MKO proportions of these groups were determined (Table 2).

Table 2 – MUO and MKO proportions among MRSA

	MUO (%)	MKO (%)	No Data* (%)	Total MRSA
Non-CC398	998 (30.9)	1407 (43.5)	828 (25.6)	3233
CC398	352 (15.2)	1386 (59.9)	574 (24.8)	2312
Total MRSA	1350 (24.3)	2793 (50.3)	1402 (25.3)	5545

MUO, Methicillin-resistant Staphylococcus aureus (MRSA) of unknown origin;
MKO, MRSA of Known Origin; CC398, Livestock associated MRSA (LA-MRSA);
Data are from The Netherlands over a two year period (2008-2009).
* Excluded from analysis.

Molecular results

A total of 403 different *spa*-types were identified and 13 strains were not typeable. Five *spa*-types constituted 51% of the total MRSA, i.e. t011, t108, t008, t002 and t064. Among non-CC398, 364 different *spa*-types were identified, of which 210 were MUO and 209 were MKO. For CC398, there were 40 different *spa*-types, of which 17 *spa*-types were MUO and 26 were MKO (see also Tables 3 and 4). The *spa*-types t008 (ST8), t019 (ST30) and t044 (ST80) were more often found among non-CC398 MUO than among MKO (p <0.01) and type t034 (CC398) was more often found among CC398 MKO than CC398 MUO (p <0.01). Of all MRSA, 684 (12%) were PVL-positive. For non- CC398 MUO this was 461 (46%), for non-CC398 MKO it was 144 (10%) and for CC398 MKO it was 3 (0.2%) (see also Table 5). There were significantly more PVL-positive t008 (USA300) among non-CC398 MUO (106 events, 10.6% of total MUO), than among non-CC398 MKO (38 events, 1.7% of total MKO) (p <0.01). Comparing CC398 MUO with non-CC398 MUO Of the 998 non-CC398 MUO, 745 (75%) had added remarks on the form. Of the remarks, 101 (14%) were related to (health) care, 104 (14%) to foreigners (contact with a foreigner or being one), 95 (13%) to contact with a positive family member and no indica-

Table 3 – Most prevalent *spa*-types in The Netherlands

			Non-CC398			CC398	
	MRSA (n = 5545)	Non-CC398 (n = 3233)	MUO (n = 998)	MKO (n = 1407)	CC398 (n = 2312)	MUO (n = 352)	MKO (n = 1386)
Rank	Spa %	Spa %	Spa %	Spa %	Spa %	Spa %	Spa %
1	t011 24	t008 14	t008 17	t008 10	t011 59	t011 59	t011 59
2	t108 11	t002 8	t002 8	t002 8	t108 26	t108 27	t108 26
3	t008 8	t064 5	t019 6	t064 6	t034 4	t567 2	t034 4
4	t002 5	t032 4	t044 5	t179 5	t567 2	t571 2	t899 2
5	t064 3	t044 4	t064 3	t032 4	t899 2	t899 2	t567 2

MUO, Methicillin resistant Staphylococcus aureus (MRSA) of unknown origin; MKO, MRSA of known origin; CC-398, Livestock associated MRSA. Data are from The Netherlands over a two year period (2008-2009). The five most prevalent *spa*-types are shown for the total amount of MRSA, Non-CC398 and CC-398 distribution. The latter two have a subdivision in MUO and MKO. Rank 1 means first most prevalent *spa*-type, rank 2 means second most prevalent *spa*-type, etc. Percentages are of group totals (mentioned with no.). In total, 403 different *spa*-types were typed (out of 5565 MRSA).

Table 4 – Comparison of most prevalent *spa*-types in The Netherlands

			Non-CC398			CC398	
	MRSA (n = 5545)	Non-CC398 (n = 3233)	MUO* (n = 998)	MKO* (n = 1407)	CC398 (n = 2312)	MUO* (n = 352)	MKO* (n = 1386)
Spa-type	Rank %	Rank %	Rank %	Rank %	Rank %	Rank %	Rank %
t032	6 2	4 4	9 2	5 4	- -	- -	- -
t044	7 2	5 4	4 5	16 2	- -	- -	- -
t019	8 2	6 3	3 6	14 2	- -	- -	- -
t179	10 2	8 3	16 1	4 5	- -	- -	- -
t034	11 1	- -	- -	- -	3 4	6 1	3 4
t571	28 0.5	- -	- -	- -	7 1	4 2	8 1

MUO, methicillin-resistant Staphylococcus aureus of unknown origin; MKO MRSA of known origin; CC-398: Livestock associated MRSA. *Spa*-types mentioned in table 2 as most prevalent for one group, but not found in a top 5 for one of the other groups in table 2, can be compared in this table for its prevalence in other groups. Rank 1 means first most prevalent *spa*-type, rank 2 means second most prevalent *spa*-type, etc. A dash means the *spa*-type was not present within the specific group. Data is from The Netherlands over a two year period (2008-2009).

tion for a possible source was obtained from 253 (34%). Of the 352 CC398 MUO, 300 had added remarks (85%). Fifty (17%) were attributed to a link with animals in general, of which 16 were through a positive relative; 28 (9%) were linked to a positive family member not involved with any animals, and 197 (66%) had no indication for a possible source. Pigs were the specifically mentioned animals for half of the animal related events (26; 52%), followed by bovids (9; 14%, seven cattle, one goat, one sheep), horses (6; 12%) and chickens (3; 6%).

Epidemiological characteristics

The following determinants were positively associated with non-CC398 MUO after univariate analysis: age (≤20 years), being a male hospital patient, household (the location where the MRSA carrier resided at the time of detection), clinical isolates, three *spa*-types (t008, t019 and t044) and four Dutch provinces, (Table 5) whereas for CC398 MUO, these were age (≥65 years), patient, household, clinical isolates (but not blood) and three Dutch provinces (Table 5).

The log-binomial regression model, comprised four determinants (PVL, person, healthcare centre and source of specimen; Table 5), with an area under the curve of 0.81 (figure not shown) for non-CC398 and three determinants (source of specimen, age and provinces), with an area under the curve of 0.66 (figure not shown). There was no further significant effect when adding other determinants to the model. The strongest determinant associated with non-CC398 MUO was PVL positivity (RR 1.19; 95% CI 1.11–1.29). For CC398 MUO, this was age (20–65 years: RR 0.73; 95% CI 0.59–0.90). In the healthcare centre group, the nursing home had a lower risk for MUO in comparison with the other group (comprising revalidation centres and various other healthcare institutions) with an RR of 0.55 (95% CI 0.42–0.72). For nose, throat and perineum samples, there was a lower risk associated with MUO (RR 0.45; 95% CI 0.0–0.74). The risk for a healthcare worker to be associated with non-CC398 MUO rather than with non-CC398 MKO was greater in comparison with the risk for patients (Table 5).

DISCUSSION

Of the 5545 MRSA isolates registered during 2008 and 2009, 24% were not found by targeted surveillance. The Netherlands has a CC398 MUO group (352; 26%) and a non-CC398 MUO group (998; 74%). The primary conclusion from the regression model was that PVL-positive MRSA was more frequent in non-CC398 MUO than MKO (RR 1.19; 95% CI 1.11–1.29) and that non-CC398 MUO was less likely to come from a nursing home than MKO (RR 0.55; 95% CI 0.42–0.72). Only a small portion of the CC398 MUO had a described link to animals and was not defined in the risk groups for MKO (50; 17%). Animals mentioned were bovids, horses and chickens. It remains unclear whether there was any relation of these MUO to livestock-related work. It is known that livestock-associated MRSA CC398 is not only found in pigs, but also in cattle, calf farmers, horses, horse personnel, poultry, slaughterhouse personnel and rats [7–12]. Remarks on the forms indicated that a specific link was not always found. CC398 MUO needs further investigation as it could suggest spread through the community not by direct contact with livestock. In 2009 Cuny et al. [13] concluded that the dissemination of MRSA CC398 (CC398) to non-exposed humans was infrequent and probably did not reach beyond

Table 5 – Epidemiological data on Non-CC398 and CC398 MRSA in The Netherlands

	Non-CC398					CC398		
	MUO	MKO[a]	Univariate analysis	Multiple regression		MUO	MKO[a]	Univariate analysis
Characteristics	(n=998)	(n=1407)	p-value	RR (95% CI)*	p-value	(n=352)	(n=1386)	p-value
Sex								
Male	530 (53%)	594 (42%)	< 0.01	-	-	219 (62%)	917 (66%)	0.16
Female	452 (45%)	768 (55%)	< 0.01	-	-	133 (38%)	469 (34%)	0.16
Age								
≤ 20 years	161 (16%)	173 (12%)	< 0.01	-	-	50 (14%)	163 (12%)	0.21
20 – 65 years	543 (54%)	769 (54%)	0.94	-	-	227 (64%)	1086 (78%)	< 0.01[e]
≥ 65 years	294 (29%)	465 (33%)	0.07	-	-	75 (21%)	137 (10%)	< 0.01
Person								
Patient	981 (98%)	1188 (84%)	< 0.01	-	-	351 (99%)	1350 (97%)	< 0.01
Healthcare worker	17 (2%)	219 (16%)	< 0.01	3.21 (2.09-5.33)	< 0.01	1 (0.3%)	36 (3%)	< 0.01
Healthcare center								
General hospital	523 (52%)	662 (47%)	0.01	0.96 (0.86-1.12)	0.58	218 (62%)	1011 (73%)	< 0.01
Academic hospital	102 (10%)	155 (11%)	0.55	0.95 (0.81-1.13)	0.58	23 (7%)	59 (4%)	0.07
Categorical hospital	6 (1%)	4 (0.3%)	0.34	0.98 (0.54-1.26)	0.91	0 (0%)	2 (0.1%)	1.00
Nursing home	52 (5%)	259 (18%)	< 0.01	0.55 (0.42-0.72)	< 0.01	2 (0.6%)	17 (1%)	0.29
Unknown	20 (2%)	32 (2%)	0.67	0.92 (0.66-1.17)	0.56	8 (2%)	14 (1%)	0.06
Household	226 (23%)	200 (14%)	< 0.01	1.08 (0.97-1.25)	0.21	78 (22%)	223 (16%)	0.01
Other[b]	69 (7%)	95 (7%)	0.87	-	-	23 (7%)	60 (4%)	0.08

	Non-CC398					CC398		
	MUO	MKO[a]	Univariate analysis	Multiple regression		MUO	MKO[a]	Univariate analysis
Characteristics	(n=998)	(n=1407)	p-value	RR (95% CI)*	p-value	(n=352)	(n=1386)	p-value
Source of Specimen[c]								
Nose, throat, perineum	278 (28%)	1083 (77%)	< 0.01	0.45 (0.0-0.74)	< 0.01	233 (66%)	1229 (89%)	< 0.01
Urine	66 (7%)	29 (2%)	< 0.01	1.31 (0.92-2.15)	0.20	6 (2%)	6 (0.4%)	0.01
Respiratory	54 (5%)	21 (1%)	< 0.01	1.32 (0.92-2.16)	0.20	19 (5%)	6 (0.4%)	< 0.01
Skin and soft tissue	392 (39%)	144 (10%)	< 0.01	1.31 (0.95-2.13)	0.18	42 (12%)	27 (2%)	< 0.01
Blood	18 (2%)	4 (0.3%)	< 0.01	1.44 (0.96-2.38)	0.10	2 (0.6%)	1 (0.1%)	0.05
Indwelling device[d]	12 (1%)	12 (1%)	0.41	-	-	2 (0.6%)	1 (0.1%)	0.05
Unknown	59 (6%)	85 (6%)	0.93	0.8 (0.54-1.34)	0.32	24 (7%)	97 (7%)	0.91
Other	119 (12%)	29 (2%)	< 0.01	1.35 (0.97-2.20)	0.14	24 (7%)	19 (1%)	< 0.01
Typing								
PVL-positive	317 (68%)	144 (10%)	< 0.01	1.19 (1.11-1.29)	< 0.01	0 (0%)	3 (0.2%)	1.00

MUO, methicillin-resistant *Staphylococcus aureus* (MRSA) of unknown origin; MKO, MRSA of known origin; PVL, Panton–Valentine leukocidin; RR, relative risk. These data are from the Netherlands over a 2-year period (2008–2009). CC398 is the livestock-associated cluster in the Netherlands.

[a]As defined by the Dutch Working group of Infection Prevention. See also Table 1.

[b]Revalidation centre, prison, correctional facility, etc.

[c]Only one strain is sent to the reference laboratory. These are the counts of the sources of the strains sent. No information is available for whether other sources were positive as well. Therefore these numbers reflect the minimum.

[d]Catheters etc.

[e]The only significant factor from the multivariate analysis, for CC398 MUO, was age (20–65 years old: RR 0.73; 95% CI 0.59–0.90).

familial communities and a low human-to-human transmission was confirmed in several studies [14–16]. Surveillance will remain necessary to monitor livestock-associated MRSA evolution, its spread in the surrounding (innate) environment and to detect new risk factors or transmission routes. The possibility of increased incidence of livestock-associated MRSA, and subsequently of livestock-associated MRSA infections in the future, cannot be ruled out. Overall, there was more MUO in Dutch provinces without areas dedicated to intensive cattle breeding. Three *spa*-types in the non-CC398 MUO group, t008 (ST-8; North America, Europe and Southeast Asia [17,18]), t019 (ST-30; North America and Southeast Asia [17]) and t044 (ST-80; mainly found in Europe [19–21]), were found more often among MUO than among MKO (Tables 3 and 4). Addition of these three *spa*-types to the regression model of non-CC398 gave no significant effect in the presence of PVL. By definition, we do not know where MUO come from. MUO could be community-associated MRSA or comprise one or several new risk groups or reservoirs. A possible explanation for the PVL correlation with non-CC398 MUO might be found in the association of young age (children and young adults) with non-CC398 MUO (Table 5, univariate). The literature reports that children and young adults were a risk factor for community-associated MRSA infections [22]. The CANWARD study described a trend toward younger patient age for community-associated MRSA genotypes [23]. At first the univariate analysis in this study revealed a positive association with young age (≤20 years) as well, but its significant effect or trend was lost in the regression model. Another difference between the two studies is that this study defined MRSA MUO and MKO epidemiologically. Surprisingly the regression model of non-CC398 showed that it was less likely for (non-CC398) MUO to come from a nursing home (Table 5) than MKO. Dutch MRSA prevalence in nursing homes is still low (<1%) [24], in contrast to nursing homes in other parts of Europe (20%) and North America (18.8–35.7%) [25]. For nursing homes, the Working party on Infection Prevention also applies guidelines for general precautions and in particular to prevent MRSA. It is likely that, up to now, the Dutch nursing homes have not served as a source for MRSA and, as far as we can conclude from our data, nursing homes are not the source of MUO. Previous research has shown that spread of MRSA within households (not a risk group in the Working party on Infection Prevention) was substantial [26]. Mollema et al. [26] showed that the transmission of MRSA from an index person to household contacts occurred in nearly half of the cases, and two-thirds of household contacts became MRSA positive. Yet in the regression model for non-CC398 the determinant household lost its significance. In our Search and Destroy policy, eradication is one of the cornerstones for keeping rates low [3,27]. If detected MRSA carriers were not offered eradication therapy, this would allow further spread, presumably in the household or through other close contacts. The early opportunity to eradicate MUO and to interrupt its transmission (according to the Search and Destroy policy) is missed, because MUO are not actively cultured for the presence of carriage [3,27]. Considering the amount of MUO, this would be at least

24% of the total MRSA in the Netherlands. It is important to realize that MUO are not targeted by the risk groups for active detection and isolation and go unnoticed until they are unexpectedly detected from a clinical sample. This explains the significantly higher MUO proportion found in clinical specimens, compared with MKO from persons who were actively screened. This gives a possible second explanation for the PVL correlation with non-CC398 MUO, but also suggests that the unexpected MUO found so far are the tip of an iceberg. Exact transmission routes and risk factors for MUO are, for now, obscure, although there is an indication that the community is a source of non-CC398 MUO. In addition, remarks on the forms for non-CC398 that are returned to the RIVM indicate having a foreign origin or having been abroad without having visited a hospital or having foreign relatives, which are all in line with studies reporting immigration as a risk factor [21,28]. Although cross-dissemination as a result of past foreign hospital visits, longer than 2 months before admission to a Dutch hospital, could also play a role [29]. The small proportion of CC398 MUO needs further research to see whether community spread indeed happens, despite the current dogma of no spread outside the risk population, because of person-to-person transmission or spread as a food-borne pathogen [30]. In conclusion, at least a quarter of the total Dutch MRSA is not from the defined risk groups. Studies on new sources and transmissions are urgently needed to possibly update the guidelines and to keep the MRSA prevalence low. Furthermore, Search and Destroy policy should be evaluated on their defined risk groups and the number of MUO. These are essential steps to take in order to cope with the dynamic nature of Staphylococcus aureus and its changing epidemiology.

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