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Duration of meticillin-resistant Staphylococcus aureus colonization after diagnosis - a four year experience from southern Sweden

Anna-Karin Larsson 1, Eva Gustafsson 2, Anna C.Nilsson 3, Inga Odenholt 3, Håkan Ringberg 2, Eva Melander 4
1 Department of Infectious Diseases, Helsingborg Hospital, Helsingborg, 2 Regional Centre for Communicable Disease Control, Skåne County, 3 Infectious Diseases Unit, Department of Clinical Sciences Malmö, Lund University, 4 Department of Infection Control, Laboratory Medicine, Skåne County, Sweden

Keywords: Antibiotic resistance, MRSA, community associated, risk factor, contact tracing

Running head: MRSA colonization

Corresponding author:
Anna-Karin Larsson
Infektionsmottagningen Helsingborgs lasarett
S Vallgatan 5
251 87 Helsingborg
Sweden
Telephone: +46424062950
+46709697996
Fax: +46424062362
E-mail: anna-karin.a.larsson@skane.se

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Background

The duration of colonization with meticillin-resistant Staphylococcus aureus (MRSA) is not well known and there is a debate whether a patient colonized with MRSA ever can be defined as “MRSA negative”.

Methods

Since 2003 all notified MRSA cases are systematically followed in Skåne County, southern Sweden. Cultures are taken from the nare, throat, perineum and possible skin lesions. Contact tracing is conducted. The screening program continues as long as cultures are positive and then until one year with consecutive negative cultures for MRSA.

Results

Of the 578 MRSA cases during 2003-2006, 535 were included in this retrospective study. The median duration of colonization of MRSA was 5.9 months. Having household contacts with MRSA, young age, spa-type t002 or colonization in 2 or more locations, was significantly associated with a longer duration of colonization. Having a clinical infection treated with antibiotics (compared to clinical infection with no antibiotic treatment or asymptomatic carriage) was significantly associated with shorter carriage time. Attempts to eradicate MRSA was associated with shorter carriage time.

Conclusion

These results may have implications for the management of patients with MRSA carriage. The study indicates that MRSA carriage can be defined as “negative” in a follow-up program and shows the importance of performing contact tracing among household members.
Introduction

Meticillin-resistant Staphylococcus aureus (MRSA) has become a global problem during the 1990s. In parts of the world, for example USA and southern Europe, MRSA now accounts for more than 50% of the invasive S. aureus isolates[1-3]. The percentage of MRSA among S. aureus in blood cultures in Sweden is below 1% but an increased proportion of cases in the community is noted [4]. Colonization or infection with MRSA used to be related to elderly people with healthcare contacts and healthcare associated risk factors. However, community associated cases have become more frequent in the 2000s and mostly children, young adults and previous healthy individuals without any apparent risk factors are affected [5]. Infections caused by MRSA are associated with a higher mortality and morbidity compared to meticillin-susceptible S. aureus (MSSA) [6]. MRSA is also associated with higher healthcare costs [7]. The duration of colonization with MRSA varies widely among studies [8-15]. Existing studies mostly deal with healthcare associated MRSA and the duration of carriage after hospital stay. The impact of household contacts carrying MRSA has not been studied in these reports. Some studies have shown that skin lesions are associated with prolonged carriage [8, 9].

In Sweden, MRSA is seen as a hazard to public health and is therefore regulated under the Swedish Communicable Diseases Act. In the year 2000 MRSA was made mandatory notifiable. This means that all detected cases are reported and registered and that contact tracing has to be performed. Every Swedish county may make their own interpretation on the extent of the contact tracing and how the follow up is to be conducted. There is an ongoing debate in Sweden whether an MRSA case ever can be defined as “MRSA negative” or not, and different counties have different rules for follow-up of MRSA cases and for the management of these patients during healthcare contacts. In Skåne County all notified MRSA
cases have been followed in one and the same way since 2003 enabling a valuable base for studying the duration of MRSA colonization [16].

In this study data from all known carriers in Skåne County during the four year period from 2003 through 2006 is presented. The duration of MRSA colonization was studied in relation to gender, age, household contacts, clinical infection/asymptomatic carriage, antibiotic treatment of a clinical infection, culture indication, risk factors, “eradication treatment”, spa-type (Single locus DNA-sequencing of the repeat region of the Staphylococcus protein A gene) and number of locations for carriage.

**Methods**

*Background*

Skåne County in southern Sweden consists of 1.2 million inhabitants with 13% of the population born outside the Nordic countries. It has both rural and urban areas. It is considered to be a low prevalence area for MRSA with an incidence of 10-20 MRSA-carriers per 100,000 inhabitants per year. The Regional Centre for Communicable Diseases Control in Skåne has registered all known cases of MRSA and contact tracing has been performed since 1999. Guidelines for management of carriers were set up in 2001 and from 2003 a long-term follow-up started [16]. All carriers were assigned a medical doctor at the Department of Infectious Diseases and an assigned nurse followed the patients with repeated monthly cultures from the nares, throat, perineum and possible skin lesions with good compliance. Examination for risk factors, contact tracing among household contacts and possible healthcare contacts was also performed. MRSA-carriers were followed monthly as long as cultures were positive for MRSA and further until one year with consecutive negative cultures with three to four cultures during the first two months and a final culture one year after the
first negative culture. Deregistration was decided on when an examination showed no sign of skin defect and when all household contacts were negative for MRSA.

Patients

All cases with an MRSA positive culture in Skåne county during the period 2003-2006 (n=578) were eligible for the study. The data were collected from the database at the Regional Centre for Communicable Diseases Control and the results of cultures were collected from the three clinical microbiology laboratories in the county. Clinical data were retrieved from the medical records of the patients at the Departments of Infectious Diseases.

Age, gender and spa-type were registered for each patient. Culture results, location/s for MRSA carriage, possible household or healthcare contacts and whether it was a clinical infection, or an asymptomatic carriage were recorded. In case of clinical infection, antibiotic treatment was recorded. Some of the patients had received treatment in an attempt to eradicate MRSA, either with topical treatment with intranasal Mupirocin and Chlorhexidine washings or combined with systemic treatment with 2 antibiotics (mainly rifampicin in combination with clindamycin or fucidic acid) for 2 weeks. It was also noted whether the patients had any risk factors in terms of a chronic skin lesion or any kind of a skin disease. One person at the Regional Centre for Communicable Disease Control evaluated if the cases were community or healthcare associated. It was recorded whether the MRSA was found by contact tracing (household- or health care contacts), screening (due to health care contacts abroad or in a Swedish institution with known spread of MRSA) or clinical investigation.

Exclusion criteria were transient carriers (defined as having only one positive culture from the nose and/or the throat and a following negative culture within one week) and the patients that had more than one year between the first positive culture and the first negative and no cultures performed in the lag time.
**Bacteriological methods and identification of MRSA**

Colonies were presumptively identified as *S. aureus* by colony morphology on blood agar and/or by giving a coloured reaction on *S. aureus* selective plates [17]. Coagulase positive colonies were tested for oxacillin susceptibility by the disk diffusion method according to instructions by the Swedish Reference group of Antibiotics (www.srga.org). Enrichment broths to detect staphylococci were used on all samples from patients in whom MRSA was actively searched for, i.e. patients designated ‘screening’ and ‘contact tracing’ but not on samples from patients where MRSA was not initially suspected, i.e. patients designated ‘clinical infection’ [17]. Tests for PBP2’ (MRSA-screen, Denka Seiken Co., Ltd., Japan) and/or PCR for the detection of nuc and mecA genes were used for verification of MRSA. PCR was performed essentially as described elsewhere [17]. Molecular characterization was performed on one of the MRSA isolates collected from a specific patient. Sequence analysis of the polymorphic X-region of the protein A gene (*spa* typing) was performed as described elsewhere [18].

**Statistical Analysis**

The data was collected and analyzed at the Regional Centre for Communicable Disease Control. All data were analyzed with SPSS software (version 15). The duration of MRSA colonization was analyzed by Kaplan-Meier estimates and since data were not normal distributed the median time was used. Determinants for the duration were analyzed by univariate and multivariate Cox regression analysis. It was modelled with the chance of becoming negative for MRSA as event, thus hazard ratios (HR)>1 indicate shorter carriage time. P <0.05 was considered significant. Since the patients started topical or systemic eradication treatment at different times after the detection of MRSA, these were included as
time dependent variables. We checked proportional hazard assumption by investigating the Kaplan-Meier curves and checking for intersection for the significant variables.

The study was approved by the Ethics Committee of the Faculty of Medicine at Lund University.

Results

Between January 2003 and December 2006, 578 MRSA cases were notified in Skåne County. Of these 43 cases were excluded, 23 cases were considered to be transient carriers and 20 cases had more than one year between the first positive culture and the first negative, and no cultures were performed in the lag time. The remaining 535 cases were included in the study, of these 150 cases as censored cases due to that they had not completed the follow-up schedule (29 died, 45 moved before follow-up, 30 had not yet completed the follow-up schedule and 46 patients were still carriers of MRSA). According to our evaluation 344 (64%) of the cases were community associated. 154 cases (29%) were associated with healthcare, 127 with care abroad and 27 with care in Sweden. In 37 (7%) of the cases it was unknown whether it was associated with healthcare or community. Of the 535 cases, 338 (63%) were healthy individuals without underlying diseases. Of the 197 patients with some kind of underlying disease 103 patients had a chronic skin lesion or chronic skin disease, 11 patients had asthma, 9 patients diabetes mellitus, 6 patients had a malignancy, 6 patients had some kind of allergy, 6 patients had an acute operation, 6 patients had some kind of trauma and the rest had a variety of different diseases. The median age was 28 (range 0-93) years (Figure 1). There were 83 different spa-types and the 5 most common were t044, t002, t008, t131 and t355. The median follow-up time was 422 (266-1942) days and the median time from the last positive culture for MRSA to the first negative culture, indicating a frequent sampling, was 41
(2-365) days. The median number of cultures (each culture date includes the nare, throat, perineum and possible skin lesions) were 8 (4-55) with a median of 3 (1-23) positive cultures. 230 (43%) of the patients were found by contact tracing, 127 (24%) by screening and 178 (33%) by investigation of clinical symptoms.

The overall median duration for colonization with MRSA in our study was 179 days (5.9 months) (Table 1). There was a wide spread with 230 (43%) of the patients being colonized for less than 2 months (Figure 2). The cases that remained colonized had at the end of the study period been followed for 940 (291-2030) median days. No statistical difference in duration of colonization was noted between men and women or between community and healthcare associated cases. The univariate analysis showed that the young patients (0-17 years) were colonized for a significantly ($p < 0.001$) longer time than the older. Having household contacts with MRSA led to significantly ($p < 0.001$) longer duration of colonization. 195 patients had a clinical infection and 340 were asymptomatic carriers. Of the 195 patients with clinical infection 125 were treated with systemic antibiotics. These patients were colonized for a significantly ($p < 0.001$) shorter time compared to patients with clinical infection without antibiotic treatment or patients with asymptomatic colonization. Having a chronic skin lesion or skin disease was significantly ($p = 0.004$) associated with longer duration of colonization.

Eradication treatment was given to 204 patients, of these 67 patients received topical treatment and 137 systemic treatment combined with topical treatment. The median time until start of systemic eradication treatment was 66 days after the detection of MRSA and it was successful in 70 % of the cases. The median time until start of topical treatment was 20 days and treatment was successful in 67 % of the cases. Patients who received topical eradication treatment had a shorter colonization time ($p = 0.039$) and patients receiving systemic treatment had an even shorter colonization time ($p < 0.001$). Of the five most common spa-
types t002 was significantly \( p < 0.001 \) associated with longer colonization. No statistical difference was noted for the other four most common spa-types. Colonization of MRSA in two or more locations conferred to a significantly \( p < 0.001 \) longer duration of colonization compared to one location.

Colonization of MRSA in the nares was seen in one or more occasion in 318 (59%) and in 41 (8%) cases MRSA was only found in the nare. Colonization of MRSA in the throat was seen in one or more occasion in 308 (56%) cases and 48 (9%) only in the throat. Colonization of MRSA in the perineum was seen in one or more occasion in 243 (45%) cases and 14 (3%) only in the perineum. In 273 (51%) cases MRSA was found in one or more occasion in “other locations” (the skin 258 cases, 11 in the urine, 2 in the ear canal, one in the blood and one in a joint) and in 96 (18%) cases only in “other locations”.

All factors that showed significance in the univariate analysis were further analysed by multivariate Cox regression analysis. Young age \( p = 0.023 \), having household contacts colonized with MRSA \( p = 0.011 \), spa-type t002 \( p = 0.014 \), and being colonized with MRSA in 2 or more locations \( p < 0.001 \) were significantly associated with a longer colonization time. Having a clinical infection treated with antibiotics \( p < 0.001 \) and receiving eradication treatment (topical \( p = 0.006 \), systemic \( p < 0.001 \)) were significantly associated with a shorter colonization time. The presence of risk factors did not remain significant \( p = 0.105 \) in the multivariate model.

**Discussion**

In this study cohort, the median duration of colonization with MRSA was 5.9 months. There was a great variation and 43 % cleared the MRSA colonization in less than 2 months. The duration of MRSA carriage varies between different studies [8-15]. Most studies show longer durations of carriage compared to our study and the existence of household contacts or
spa-type are not taken into account. In a study by Marshall et al. [8], comprising 116 patients followed intermittently after hospital discharge, the median duration for carriage was 7.4 months. Scanvic et al. [9] made a prospective study of 78 patients who were readmitted to hospital and showed a median duration of MRSA carriage of 8.5 months. In one cohort of 135 patients that had been hospitalized Vriens et al. [10] found a median carriage time of 14 months. Sanford et al. [11] found a half-life of MRSA carriage of more than 40 months in a cohort of 102 hospitalized carriers. In a recent study by Robicsek et al. [12] including 1564 patients readmitted to hospital, 48.8% were still colonized after one year. However in the study of Robicsek a rapid reduction to a 50 % rate of colonization (in less than 1 month) was noted. In accordance to these latter results we noted that 43 % of our patients became MRSA-negative in less than 2 months. In a French study by Lucet et al. [13] an estimated time to clearance of MRSA of 9.4 months was found in a group that was screened for MRSA before being discharged from hospital to home health care.

Most studies regarding the duration of MRSA carriage included only patients with health care associated MRSA, where the patients were older, had more underlying conditions and had been hospitalized. In most of these studies there were no regular follow-up interval and the presence of household contacts with MRSA colonization was not evaluated. The difference between our results and other studies may be due to the fact that our study cohort includes all known MRSA cases in the county. The study population is a mix of ages with a median age of 28 years and an even gender distribution. The cases come from both rural and urban areas and are both of Swedish and foreign origin. It consists mostly of previously healthy individuals and 64% of the cases are community associated. Another difference is that our patients are followed at regular intervals with repeated cultures from at least three body sites and the presence of household contacts with MRSA colonization is evaluated. In other studies the patients were screened only at readmission to the hospital and a negative screening result
could maybe have been detected more rapidly in some patients. Also, the prevalence of MRSA in our county is low and thus the risk of getting recolonized is small. Most of the other studies are from high prevalence countries.

As in other studies, no difference was noted in the duration of carriage between men and women in our study cohort [8, 9, 12] The young MRSA cases (0-17 years) carried MRSA for a significantly longer time than the older. This is in accordance with studies on colonization with MSSA where a higher persistent carriage is seen in children compared to adults [19].

The pattern of carriage is changing between the age of 10 and 20 years in a majority of cases [20, 21]. In a study by Datta et al. [22] a high carriage rate of *S. aureus* in infants (57%) and children 8-13 years (45.1%-65.5%) was noted.

Johansson et al. [23] have earlier shown the importance of culturing household contacts for MRSA and Bogaert et al. found large households to be positively associated with *S. aureus* nasal carriage [24]. In the study of Lucet [13] transmission was seen to 20% of the household contacts. This is in agreement with our results where we found a strong correlation between long duration of colonization and other persons in the household carrying MRSA. This is probably due to recolonization from the household members.

Patients with a clinical infection treated with antibiotics, compared to a clinical infection without treatment or asymptomatic carriers found by screening or tracing, carried MRSA for a shorter time. A part of the explanation for this may be that many of these patients got a primary skin infection and received treatment before becoming colonized in the other locations. These successful results may indicate that for a clinical infection with MRSA antibiotic treatment should be considered.

In contrary to other studies [8, 9], patients with chronic skin lesions and skin disease surprisingly did not have a longer duration of colonization compared to patients without skin lesions and skin disease.
In the present study patients who received topical or systemic eradication treatment were colonized for a shorter time indicating that decolonization of MRSA carriers is possible to achieve.

To our knowledge carriage time for MRSA has not previously been studied in relation to spa-type. The MRSA isolates in our study belonged to 83 different spa-types. The five most common types represented 47 % of all spa-types (t044, t002, t008, t131 and t355). We found that t002 was associated with longer duration of colonization.

A previous study by Harbarth et al. [25] showed that carriage at ≥2 locations was associated with persistent MRSA carriage. This is in accordance with our results which showed that if MRSA was found in ≥2 locations the patients carried MRSA for a longer time. Carriage of MRSA in >1 location probably indicates a higher MRSA load and hence it is more difficult to clear the colonization.

This study has some limitations. It is a retrospective study and even though we have a standardized program for follow-up, all patients were not cultured as often as others. We also do not know the true starting time for colonization. The patients may have been colonized with MRSA long before the first positive culture. Of our cases 292 were clustered within families and should therefore have been analyzed with more sophisticated statistical models, unfortunately we lacked data for this. We tend to underestimate the statistical uncertainty leading to p-values that could be too low. However, since we had highly significant values it is not probable that this would have changed the results.

In conclusion, our results may have implications for the management of MRSA carriage. It indicates that MRSA carriage can be defined as “negative” in a follow-up program and shows the importance of performing contact tracing among household members.
Acknowledgements

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References


18. Petersson AC, Olsson-Liljequist B, Miormer H, Haeggman S. Evaluating the usefulness of spa typing, in comparison with pulsed-field gel electrophoresis, for


Table 1. Duration and factors influencing the time of colonization with MRSA

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (censored)</th>
<th>Time (mediandays)</th>
<th>Univariate Cox regression HR(95% CI)</th>
<th>P</th>
<th>Multivariate Cox regression HR(95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>535 (150)</td>
<td>179 (143-215)</td>
<td>0.88 (0.72-1.08)</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>280 (80)</td>
<td>210 (154-266)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men (reference)</td>
<td>255 (70)</td>
<td>161 (108-214)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-17</td>
<td>190 (56)</td>
<td>344 (250-438)</td>
<td>0.65 (0.49-0.87)</td>
<td>&lt;0.001</td>
<td>0.66 (0.49-0.90)</td>
<td>0.023</td>
</tr>
<tr>
<td>18-50</td>
<td>227 (53)</td>
<td>124 (81-167)</td>
<td>1.14 (0.87-1.49)</td>
<td></td>
<td>0.85 (0.64-1.13)</td>
<td></td>
</tr>
<tr>
<td>&gt;50 (reference)</td>
<td>118 (41)</td>
<td>168 (74-262)</td>
<td>1.00</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Household contacts with MRSA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>292 (76)</td>
<td>258 (176-340)</td>
<td>0.61 (0.50-0.72)</td>
<td>&lt;0.001</td>
<td>0.75 (0.60-0.94)</td>
<td>0.011</td>
</tr>
<tr>
<td>No (reference)</td>
<td>243 (74)</td>
<td>94 (55-133)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes: antibiotic treatment</td>
<td>125 (19)</td>
<td>64 (37-91)</td>
<td>2.06 (1.63-2.59)</td>
<td>&lt;0.001</td>
<td>2.17 (1.66-2.83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes: no antibiotic treatment</td>
<td>70 (28)</td>
<td>221 (19-423)</td>
<td>0.94 (0.68-1.30)</td>
<td></td>
<td>1.07 (0.76-1.50)</td>
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<tr>
<td>No (reference)</td>
<td>340 (103)</td>
<td>258 (191-325)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of MRSA acquisition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Community associated</td>
<td>344 (84)</td>
<td>173 (136-210)</td>
<td>0.99 (0.65-1.49)</td>
<td>0.72</td>
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<td>Healthcare associated</td>
<td>154 (54)</td>
<td>210 (152-268)</td>
<td>0.72 (0.70-1.68)</td>
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<td>Unknown</td>
<td>37 (12)</td>
<td>224 (11-437)</td>
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<tr>
<td>Yes</td>
<td>103 (36)</td>
<td>172 (137-207)</td>
<td>0.68 (0.52-0.88)</td>
<td>0.004</td>
<td>1.27 (0.95-1.69)</td>
<td>0.105</td>
</tr>
<tr>
<td>No (reference)</td>
<td>432 (114)</td>
<td>239 (85-393)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spa-type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t044</td>
<td>79 (13)</td>
<td>160 (81-239)</td>
<td>1.24 (0.94-1.64)</td>
<td>&lt;0.001</td>
<td>1.10 (0.82-1.48)</td>
<td>0.014</td>
</tr>
<tr>
<td>t002</td>
<td>79 (35)</td>
<td>554 (0-1220)</td>
<td>0.54 (0.39-0.76)</td>
<td></td>
<td>0.57 (0.40-0.80)</td>
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<tr>
<td>t008</td>
<td>43 (12)</td>
<td>135 (53-217)</td>
<td>1.08 (0.74-1.57)</td>
<td></td>
<td>0.90 (0.61-1.33)</td>
<td></td>
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<tr>
<td>t131</td>
<td>20 (2)</td>
<td>183 (0-374)</td>
<td>1.47 (0.91-2.39)</td>
<td></td>
<td>1.12 (0.66-1.90)</td>
<td></td>
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<tr>
<td>t355</td>
<td>20 (3)</td>
<td>46 (30-62)</td>
<td>2.20 (1.34-3.61)</td>
<td></td>
<td>1.38 (0.82-2.33)</td>
<td></td>
</tr>
<tr>
<td>Other (reference)</td>
<td>294 (85)</td>
<td>198 (147-249)</td>
<td>1.00</td>
<td></td>
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</tr>
<tr>
<td>No. of locations</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>1 (reference)</td>
<td>198 (44)</td>
<td>52 (46-58)</td>
<td>1.00</td>
<td>&lt;0.001</td>
<td>1.00</td>
<td>&lt;0.001</td>
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<td>2</td>
<td>130 (32)</td>
<td>224 (130-318)</td>
<td>0.38 (0.29-0.49)</td>
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<td>0.35 (0.26-0.45)</td>
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<tr>
<td>3</td>
<td>142 (45)</td>
<td>404 (302-506)</td>
<td>0.29 (0.22-0.37)</td>
<td></td>
<td>0.28 (0.21-0.37)</td>
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<tr>
<td>4</td>
<td>65 (29)</td>
<td>521 (409-632)</td>
<td>0.25 (0.17-0.36)</td>
<td></td>
<td>0.18 (0.12-0.27)</td>
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</tr>
<tr>
<td>Eradication treatment a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Topical</td>
<td>67 (15)</td>
<td>1.45 (1.02-2.07)</td>
<td>0.039</td>
<td></td>
<td>1.70 (1.16-2.49)</td>
<td>0.006</td>
</tr>
<tr>
<td>Systemic</td>
<td>137 (26)</td>
<td>3.16 (2.51-3.98)</td>
<td>&lt;0.001</td>
<td></td>
<td>3.45 (2.69-4.43)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>331 (109)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The Cox regression analysis is modelled with the chance of becoming negative for MRSA as event, thus, hazard ratios (HR) > 1 indicates shorter carriage time.

* time dependant variable
Figure 1.
Age distribution

Figure 2.
The duration of colonization with MRSA