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Major article

Risk factors for development of methicillin-resistant *Staphylococcus aureus*-positive clinical culture in nasal carriers after decolonization treatment

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Background: Active surveillance systems are effective in reducing health care-associated infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Nonetheless, some patients develop MRSA infection despite control measures. We tried to identify risk factors related to the appearance of MRSA at sites other than the nasal fossa in patients who were nasal carriers of MRSA.

Methods: A retrospective case-control study was conducted in an active surveillance program for MRSA between January 2009 and December 2010 at a Spanish teaching hospital. Cases were patients with MRSA in the anterior nares and a length of stay of at least 5 days who developed MRSA-positive clinical culture after decolonization treatment had started. Controls were patients with the same characteristics as the case group, except that they did not develop MRSA-positive clinical culture as verified by negative clinical cultures.

Results: After intrinsic and extrinsic risk factors were analyzed, the emergence of mupirocin-resistant MRSA clones after decolonization treatment, and residence in a nursing home were marginally significant in the univariate analysis. The detection of the emergence of mupirocin-resistant MRSA clones was independently associated with the detection of MRSA in other clinical locations.

Conclusions: In an active surveillance program for MRSA it is important to determine the mupirocin susceptibility of the isolates to determine appropriate treatment and to verify negativity after decolonizing treatment has been completed.

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Active surveillance systems, whether targeted or universal, have been shown to be effective and efficient in reducing health care-associated infections (HAI) caused by methicillin-resistant *Staphylococcus aureus* (MRSA).[1-4] Nonetheless, despite their success, these systems have various shortcomings such as delayed detection of cases when screening is not performed over the weekend; onset of infection in patients with previously negative screening results (because of colonization of another untested location or by hospital cross-transmission); and more remarkably, the development of infection in patients in whom nasal colonization had previously been detected[5,6] and in those for whom decolonization guidelines and contact isolation measures had been followed according to international recommendations.[7]

Since 2008 we have been performing a universal active surveillance system for MRSA at Hospital Universitario de Canarias, except in the pediatrics, obstetrics, and psychiatry departments. This system has achieved a 60% reduction in HAI and a 50% reduction in MRSA bacteremia. Nonetheless, 3% of cases of MRSA infection annually occur in carriers who had previously been decolonized. The aim of our study was to determine which risk factors are related to the acquisition of MRSA at clinical locations other than the nasal fossa in these patients, to detect new infections in a timely manner and thus improve our active surveillance system.

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Conflicts of interest: None to report.

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METHODS

Design

Our retrospective case-control study involved patients included in the universal surveillance system for MRSA at Hospital Universitario de Canarias, a tertiary teaching hospital in the Canary Islands, Spain, between January 2009 and December 2010. Our 647-bed hospital is the reference hospital for the northern area of the islands of Tenerife and La Palma, and provides care for a population of 425,814 inhabitants. The active surveillance system is a universal system in which nasal samples are taken from all patients admitted to the hospital, except in the pediatric, obstetrics, and psychiatry wards, where we have detected no cases of MRSA infection in recent years. Thus, our active monitoring system identifies nasal carriers of MRSA on admission and implements the following infection control measures in culture-positive patients: decolonizing treatment with 2% mupirocin ointment applied topically to the nares 3 times daily for 5 days, daily bath or shower with 4% chlorhexidine gel for 5 days, and contact precautions with the use of gloves and gowns, single rooms (or isolation in cohorts), and rigorous hand hygiene. According to our standard procedures, contact precautions remained in effect until 3 nasal swabs obtained 1 week apart were negative and a positive clinical culture for MRSA, if present, became negative. We also determined mupirocin susceptibility, and in case of resistance, decolonizing treatment was switched to fusidic acid 3 times daily for 7 days. Fusidic acid was used only when the patient had a new nasal MRSA colonization in the control sample from the nasal fossae after treatment with mupirocin and the initial strain was mupirocin resistant.

In addition we retrospectively reviewed all cultures of different clinical samples (eg, wound exudates, respiratory specimens, and blood culture), as requested by the clinician when infection was suspected, and MRSA isolates were classified as colonization, infection, or community or nosocomial acquisition, according to Centers for Disease Control and Prevention 2008[8] criteria.

A case was defined as a patient with MRSA nasal colonization admitted to the hospital for at least 5 days who developed MRSA-positive clinical culture at any location other than the nasal fossa after decolonization treatment had begun. Case status was verified by MRSA-positive cultures from clinical samples tested at the physician's request.

A control was defined as a patient with MRSA nasal colonization admitted to the hospital for at least 5 days, and who, after decolonization treatment had begun, did not have any positive clinical cultures at any other location different from the nasal fossa. Control status was verified by MRSA-negative cultures from clinical samples tested at the physician's request.

No randomization or matching procedure was used for the case or control group; patients were included if they met the inclusion criteria. All case and control patients were given decolonizing treatment with 2% mupirocin ointment applied topically to the nares 3 times daily for 5 days, in addition to a daily bath or shower with 4% chlorhexidine gel for 5 days.

The following variables were recorded to verify comparability between the case and control groups: length of stay (days), follow-up time (days), delay in decolonizing treatment as the interval between MRSA diagnosis and the start of treatment (days), reason for admission, and mortality during admission.

The following variables were recorded for each patient: sex, age, admission department (ie, medical, surgical, or intensive care), use of a central line, mechanical ventilation, urinary catheter, surgery during admission, skin lesion (ie, decubitus ulcers, vascular ulcers, burns, or psoriasis), length of stay less than or more than 2 weeks, underlying diseases (ie, diabetes mellitus, respiratory disease, chronic kidney disease, and chronic liver disease), and morbidities (ie, more than or less than 2, including the reason for admission). The number of previous hospitalizations or surgeries during the previous year, other MRSA colonization during the previous last year, and residence in a nursing home were also recorded.

The mupirocin susceptibility of the MRSA strains isolated by nasal screening was determined. If MRSA was detected again in a patient who had completed decolonizing treatment, the onset of mupirocin resistance in initially susceptible clones was analyzed to track the emergence of mupirocin resistance.

Microbiologic methods

Nasal smears were seeded on ChromID MRSA chromogenic medium (bioMerieux, La Balme Les Grottes, France) and in brain-heart infusion broth (Oxoid, Ltd, Basingstoke, Hampshire, UK); they were subsequently moved to chromogenic medium after 24 hours of incubation.[9] Characteristic colonies were identified with the Vitek2 system (bioMerieux, Inc, Hazelwood, Mo) and resistance to methicillin was confirmed with the PBP2a latex agglutination test (MRSA-Screen; Denka Seiken Co, Ltd, Tokyo, Japan). Mupirocin susceptibility was determined with the E-test (AB Biodisk, Solna, Sweden) and classified as either low-level resistance with minimum inhibitory concentration 8-256 μg/mL or high-level resistance (HLR) with minimum inhibitory concentration $\geq$ 512 μg/mL.[10] Clinical samples were processed according to standardized protocols issued by the Spanish Microbiology Society.[11] MRSA isolates in the nasal fossa and clinical samples were classified by chromosomal DNA analysis using macrorestriction and pulsed-field gel electrophoresis,[12] DNA sequencing, and identification of the *Staphylococcus* chromosomal cassette. Band patterns obtained by pulsed-field gel electrophoresis were assessed according to the Tenover criteria[13]; the degree of similarity between patterns was established by a dendrogram constructed with InfoQuest FPv. 4.5 software (Bio-Rad Laboratories Inc, Hercules, Calif).

Statistical analysis

To determine the association between putative risk factors and subsequent MRSA infection or colonization, the 2 groups were compared with the Pearson χ^2 test for nominal variables and the Mann-Whitney *U* test for variables that were not normally distributed. For multivariate analysis, the factors that yielded marginal statistical significance ($P \leq .15$) in the initial analysis were selected to search for potential interactions among these variables. The model chosen for these multivariate analyses was logistic regression for binary outcomes, with infection or colonization as a dependent variable; a backward stepwise strategy with the Wald statistic; and a bilateral statistical significance of $P \leq .05$. The minimum sample size necessary for this model with 2 explanatory factors was 30 cases according to Hosmer-Lemeshov criteria. For this analysis we used all data from all the patients included during the study period: 43 cases and 42 controls. All calculations were done with the IBM SPSS 21.0 statistical software package (IBM-SPSS Inc, Armonk, NY).

RESULTS

During the study period a total of 830 colonization episodes were diagnosed in 730 patients; from this population we selected 43 patients for the case group and 42 for the control group, all of whom met the inclusion criteria.

Comparisons between cases and controls of factors that were not independent risk factors but that might reveal differences in the likelihood of development of a MRSA-positive clinical culture were done for length of stay (median was 43 days in case group [range,

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