An outbreak of Methicillin-resistant *Staphylococcus aureus* cutaneous infection in a saturation diving facility.

J. WANG¹, S. BARTH², M. RICHARDSON², K. CORSON¹, J. MADER¹

¹Division of Hyperbaric Medicine and Wound Care, Department of Orthopaedics and Rehabilitation, University of Texas Medical Branch; ²Department of Internal Medicine University of Texas Medical Branch; ³Microbiological Investigation Section, Texas Department of Health.

Wang J, Barth S, Richardson M, Corson K, Mader J. An outbreak of Methicillin-resistant *Staphylococcus aureus* cutaneous infection in a saturation diving facility. Undersea Hyperb Med 2003; 30(4):277-284 - We present a molecular epidemiological investigation of an outbreak of cutaneous tissue infection, which involved six divers during a 45 day saturation exposure dive. The cutaneous infection manifested as boils, folliculitis and small abscesses involving different body sites, including nose, external ear canal, necks, back, extremities, and buttocks. *Staphylococcus aureus* was consistently isolated from the skin lesions of affected divers. A study of the antibiogram revealed that all *Staphylococcus aureus* isolates were uniformly resistant to penicillin, oxacillin and erythromycin, but sensitive to clindamycin, tetracycline, trimethoprim-sulfamethoxazole, rifampin and vancomycin. Molecular typing by pulse field gel electrophoresis (PFGE) demonstrated that all the Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates had an indistinguishable pulsed field gel electrophoresis pattern. The source of outbreak was identified as a colonized diver (diver D). Personal contact was most likely the mode of transmission among the six divers. Infection with MRSA should be suspected in outbreaks of boils that are not responding to standard antibiotic therapy among healthy divers and their close contacts. To our knowledge, this is the first report of Methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak in a saturation diving facility.

*Methicillin resistant Staphylococcus aureus, Cutaneous infection, Saturation diving, Pulsed-field gel electrophoresis (PFGE)*

INTRODUCTION

Saturation diving is used extensively for maintenance and inspection of off shore sub-sea oil production systems. The saturation period includes compression, bottom time (working period) and decompression. A typical saturation dive is between two weeks to seven weeks depending on working depth. During recompression, occupational saturation divers live in a closed, high humidity environment with close physical contact. Cutaneous infections are frequent health complications during saturation diving (1, 2), *Pseudomonas aeruginosa* is recognized frequently as a causative agent (3,4,5,6). Methicillin resistant *Staphylococcus aureus* outbreak in a saturation diving setting has not been previously reported.

Description of the outbreak

In March 2002, there was an outbreak of cutaneous infections involving all six members of a diving team during a 45 day saturation exposure dive at 927 fsw (29.09 ATA). The most
likely source was diver D who had a painful, erythematous nasal lesion at the beginning of the dive and, subsequently, the other five divers were found to have cutaneous infections involving different sites, including nose, external ear canal, neck, back, buttocks and extremities. They were treated with several courses of oral antibiotics (initially cephalixin, then amoxycillin/clavulanic acid, ciprofloxacin), as well as topical application of bacitracin ointment, but the skin lesions did not respond to the treatment. On day 35 (5th weeks) of diving, cultures were taken from the lesion drainage of each diver by the diving company physician, who later reported “Staphylococcus aureus.” After completing the dive, five of six divers still had active lesions and only one (diver A) reported near resolution of lesions on his ear and arm.

METHODS

Sample collection and antimicrobial susceptibility testing
Participants were interviewed and routine physical and neurological examinations were performed by a hyperbaric medicine physician. Blood samples for routine laboratory studies were obtained from each diver. The swab cultures from the drainage of skin lesion sites and the environment control samples (interior chamber, diver’s personal utilities and diving equipments) were collected. Cultures were also taken from the anterior nares of each diver to identify potential MRSA carriers. All microbiological sampling was performed by a certified nurse, and collecting and transporting procedures carried out according to the American Association of Infection Control guidelines (7). Nasal samples were collected by inserting a swab into either nares until the entire tip was inside. The tip was then rolled five times to expose the on all sides. Skin lesion samples were collected by dipping the swab directly into the drainage without touching other parts of skin surface. The environment sample was collected by first moistening the swab in a commercial transport system, breaking the ampule of the transport medium, and allowing it to saturate the swab tip before obtaining the sample and placing it into the transport tube. Cultivation of these samples, strain phenotyping and antibiotics sensitivity were performed according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (8).

Analysis of DNA restriction pattern by pulse field gel electrophoresis PFGE
The MRSA isolates were submitted to the Microbiology Laboratory, Department of Health, Austin, Texas. Molecular typing of all isolates was performed by pulse field gel electrophoresis (PFGE). Briefly, chromosomal DNA was digested with Smal I restriction endonuclease (New England Biolabs), and analyzed by gel electrophoresis in CHEF-DR III (contour clamped homogeneous electric field) apparatus (Bio-RAD) for 20 hours in 0.5X TBE buffer. Electrophoresis parameters were as follow: 6 volts/cm, 120 degree included angle, linear ramp, switching times 2.0 to 50 seconds. Gels were stained with ethidium bromide, visualized under UV illumination, and photographed. NCTC 8325 Staphylococcus aureus was included as controls in PFGE analysis. The analysis of the Smal I high-molecular-weight DNA restriction pattern was carried out by using Molecular Analyst Fingerprinting Plus software (version 1.12, Bio-Rad laboratories) by aligning standard NCTC 8325 Staphylococcus aureus isolates located in lanes 1, 5, and 10 of each gel with the global standard for the database (also NCTC 8325). The software creates a dendrogram to estimate the similarity among strains on the basis of the relative genetic distances (9,10).
RESULTS

The divers in this study were young Caucasian, or Hispanic males, between 28 and 38 years old. There were no significant past medical histories or prior hospitalizations. No evidence of decompression sickness was found during interviews and physical examinations. All routine laboratory studies for each diver were within normal limits.

Five out of the six divers still had active cutaneous infection at the time of diving completion. Diver A was the only one with near resolution of cutaneous infection, and a sample was not collected from him because there was no drainage at the lesion site. The characteristics of the cutaneous lesions and their culture results are listed in Table 1.

Table 1. Characterization of Methicillin resistant Staphylococcus aureus isolated from divers.

<table>
<thead>
<tr>
<th>Nasal culture</th>
<th>Cutaneous infection sites</th>
<th>Source/Strain (35th days)</th>
<th>Source/Strain (45th days)</th>
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<tbody>
<tr>
<td>Diver A</td>
<td>Respiratory flora</td>
<td>Ear/Arm</td>
<td>Ear/MRSA</td>
</tr>
<tr>
<td>Diver B</td>
<td>MRSA</td>
<td>Post neck/Palm</td>
<td>Neck/MRSA</td>
</tr>
<tr>
<td>Diver C</td>
<td>MRSA</td>
<td>Arm/Buttock/Ear</td>
<td>Arm/MRSA</td>
</tr>
<tr>
<td>Diver D</td>
<td>MRSA</td>
<td>Right hand/Lt nare</td>
<td>Hand/MRSA</td>
</tr>
<tr>
<td>Diver E</td>
<td>Respiratory flora</td>
<td>Right thigh</td>
<td>Thigh/MRSA</td>
</tr>
<tr>
<td>Diver F</td>
<td>Respiratory flora</td>
<td>Left great toe</td>
<td>Toe/MRSA</td>
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All six cultures of skin lesion sites at 5th week of saturation diving by the diving company physician grew *S. aureus*; of the total fourteen samples collected from skin lesion sites at end of diving, seven grew *S. aureus*. Three out of six divers (50%) had positive nasal culture for *S. aureus* at end of diving. The staphylococcus strains isolated during and after the dive shared the same antibiotics sensitivity pattern, which was uniformly resistant to penicillin, oxacillin, erythromycin, but susceptible to clindamycin, tetracyclin, trimethoprim-sulfamethoxazole, rifampin, and vancomycin. The MICs of each tested antibiotic are listed in Table 2.

The *S. aureus* isolates were sent to the Texas State Health Department for genotyping by pulsed-field gel electrophoresis (PFGE). The results of PFGE showed all the isolates exhibited an indistinguishable PFGE pattern, which designated SM-Star-035. One example of the PFGE DNA restriction pattern is shown in Figure 1. Examples of cutaneous infection in divers are shown in Figure 2.
Table 2. Antibiogram type of isolated Staphylococcus obtained from dives at the time of completion of forty-five days diving.

<table>
<thead>
<tr>
<th>MIC(µg/ml)</th>
<th>Susceptibility</th>
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<tr>
<td>Penicillin</td>
<td>&gt;=16 R</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>&gt;=8 R</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>&gt;=0.5 R</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>&gt;=8 R</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>&lt;=1 S</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>&lt;=10 S</td>
</tr>
<tr>
<td>Rifampin</td>
<td>&lt;=1 S</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>&lt;=0.5 S</td>
</tr>
</tbody>
</table>

Figure 1. Example of PFGE pattern of the Smal I-digested genomic DNA of Staphylococcus aureus (MRSA) strains isolated from divers. Lane 1, 7, 8. NCTC 8325 Staphylococcus aureus standard. Lane 2-6, 9-10. Methicillin resistant Staphylococcus aureus isolates from divers. 1. Diver B nasal culture; 2. Diver B lesion culture; 3. Diver C nasal culture; 4. Diver C lesion culture; 5. Diver D nasal culture; 6. Diver E lesion culture; 7. Diver D lesion culture.

The divers with active infection were subsequently treated with a two week course of oral clindamycin. The colonized divers were also treated with administration of topical mupirocin to the nostrils and given the recommendation to use daily antimicrobial soap body wash. All six divers were excluded from further saturation diving until their lesions healed and they were advised to not return to work until three nasal samples were MRSA-negative. The five divers with active MRSA cutaneous infection subsequently responded well to treatment with clindamycin with complete resolution of all lesions.
Figure 2. Examples of cutaneous staphylococcal infection in divers.

DISCUSSION

First reported in the 1960’s (11), Methicillin resistant *Staphylococcus aureus* (MRSA) strains, have increased in incidence and are frequently associated with nosocomial outbreaks in hospitals and nursing homes (12, 13). MRSA is usually highly transmissible and very difficult to eradicate. Recently, concern has increased regarding the spread of MRSA throughout a community (14,15) and involving a person without known risk factors. However, infection with Methicillin resistant *Staphylococcus aureus* associated with hyperbaric oxygen therapy or a saturation diving chamber has not been previously reported.

We describe a molecular epidemiology investigation of an outbreak of Methicillin resistant *Staphylococcus aureus* (MRSA) cutaneous infection, which involved all six members of a diving team during a 45 day saturation dive. In our study, all MRSA isolates shared the same antibiotic sensitivity pattern, suggesting a common source of infection. Molecular typing by pulse field gel electrophoresis (PFGE) confirmed that all MRSA isolates belonged to the same clone and shared an indistinguishable pattern, which was designated as SM-STAR-035. The pattern of SM-STAR-035 is commonly seen in communities in Texas (S. Barth, personal communication).
Reports from various geographic regions indicate that the prevalence of community-acquired Methicillin-resistant (MRSA) infection is increasing. Nasal carriage of Staphylococcus aureus is the primary reservoir and plays a key role in the epidemiology and pathogenesis of infection (16, 17). Those with close contact to index patient are 7.7 times more likely to be colonized (18). The nasal colonization rate in the general diver population is unknown. In our small group, MRSA nasal carriage was present in 3 out of 6 divers at the end of the dive.

PFGE in this study demonstrated that the Staphylococcus aureus obtained from the anterior nares of the three divers and the cutaneous lesion sites were of the same strain. This is the first report of MRSA transmission in a group of healthy divers in a saturation diving chamber in which the divers lived in close body contact for forty-five days. The MRSA strain that caused the outbreak was most likely introduced by diver D, who had an infected nasal lesion at beginning of the diving. Outbreaks of skin infections among the team members in close contact sports are well documented (19,20, 21). Mattresses, equipment, bathing facilities and even room air exhaust have been implicated in MRSA transmission (22, 23). It is tempting to speculate that shared towels, diving equipment, and facilities might have contributed to MRSA transmission among the divers, however, none of the samples or cultures from chamber and equipment in our study were positive for MRSA. We cannot exclude the possibility the negative culture results might be due to low sensitivity of the methodology used for environment samples (7).

No specific risk factors for acquisition of MRSA were identified among the six divers. Skin infection is still the most frequent health problem associated with occupational saturation diving, micro-trauma from diving, and a closed, high humidity environment in which organisms can thrive are apparent contributing factors. The potential effects of saturation diving on human immunity are also of interest. Saturation diving induces lymphocyte subset changes, including a decrease in CD4/CD8 ratio, fraction of CD4 T cells and an increase in NK cells (24, 25). It also has been postulated that decreased resistance of skin to other infections encountered by deep-sea divers may be due to a decreased functional killing capacity of granulocytes (26).

Rapid and reliable typing methods have been developed to obtain information about the relatedness of MRSA isolates and to allow faster implementation of appropriate infection control measures after outbreaks occur. These typing methods can be characterized based on phenotypic and genotypic analyses. Phenotypic analyses include assessing hemolytic activity, antimicrobial susceptibility testing and phage typing. Genotyping techniques are increasingly used as valuable tools for the epidemiologic study of Staphylococcus aureus. Pulsed-field gel electrophoresis (PFGE) has proven an excellent method with high reproducibility and resolving power for epidemiological differentiation of MRSA isolates (10).

SUMMARY AND RECOMMENDATIONS

The study demonstrates that a distinct clone of MRSA was responsible for an infectious outbreak in six divers in close physical contact during a 45 day saturation dive. A nasal colonized and single infected diver (Diver D) is postulated as the source of infection. Understanding the significance and spread of MRSA in the chamber in this study was assisted by the application of molecular typing. Based on our study, we recommend prevention of MRSA outbreaks in saturation diving facilities using the following steps: 1) Identify early cutaneous infection before diving by pre-dive physical examination; 2) Good hygiene practice, including frequent hand washing and daily shower with antimicrobial soap; 3) Prompt and aggressive treatment of early skin lesions; 4) Judicious use of antibiotics to reduce resistant organisms; 5)
Obtain cultures from lesions to guide therapy when the lesions are slow to respond to antibiotics, or if MRSA is suspected; 6) Eradication of MRSA nasal carriage by topical application of mupirocin; 7) Thorough cleaning of environment and equipment after each saturation dive.

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REFERENCES