Survival of epidemic strains of healthcare (HA-MRSA) and community-associated (CA-MRSA) meticillin-resistant *Staphylococcus aureus* (MRSA) in river-, sea- and swimming pool water

Ola Tolba, Anne Loughrey, Colin E. Goldsmith, B. Cherie Millar, Paul J. Rooney, John E. Moore*

Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Lisburn Road, Belfast, Northern Ireland BT9 7AD, UK

Received 19 June 2006; received in revised form 14 June 2007; accepted 28 June 2007

Abstract

The aim of this study was to examine the survival dynamics of several epidemic healthcare (HA) and community-associated (CA) meticillin-resistant *Staphylococcus aureus* (MRSA) in river, sea and swimming pool waters. Six different phage-types of HA-MRSA (Irish 1, Irish 2, EMRSA 15, EMRSA 16, distinct type and non-typable), as well as a community-associated MRSA (CA-MRSA), were examined in this study. Two strains of each type were examined resulting in a total of 14 organisms being examined. Cells were harvested from overnight cultures of Columbia blood agar (Oxoid) supplemented with 5% [v/v] defibrinated blood to make a 0.5 McFarland inoculum standard. An inoculum of each MRSA isolate was added individually to each water microcosm to give log\(_{10}\) 5 (10\(^5\)) colony forming units (cfu/ml water) and water was stored in the dark at ambient temperature for up to 14 days. Recovery experiments were unable to isolate any of HA- or CA-MRSA in the swimming pool water after 24 h storage. This study demonstrates that all 14 epidemic HA and CA MRSA studied can survive in sea and river water environments up to at least 14 days post inoculation. There was no significant differences in the survival dynamics between CA-MRSA and HA-MRSA in any water environment, but all MRSA died off more quickly in river water, compared to sea water, with decimal (\(D_{10}\)) reduction values of 3.53 and 7.4 days, for river- and sea water, respectively. This study indicates that contaminated sea and river water may serve as potential reservoirs of HA- and CA-MRSA, if such water sources become contaminated with these organisms.

© 2007 Elsevier GmbH. All rights reserved.

Keywords: Methicillin-resistant *Staphylococcus aureus*; MRSA; meca; PCR; Water; Environment; Survival; Persistence; Infection control

Introduction

Hospital outbreaks due to meticillin-resistant *Staphylococcus aureus* (MRSA) have become a major problem in nosocomial infections, warranting programmes to control its dissemination, given the potential of MRSA to
produce invasive infections, particularly in vulnerable patients and its multiple resistance to antibiotics which limits the therapeutic options available (Cunha, 2005). More recently, community-acquired (CA) MRSA has been documented among healthy individuals without any predisposing risk factors (Kowalski et al., 2005), unlike its nosocomial acquired (NA) relative. The appearance and spread of CA-MRSA represent a new challenge in medicine and have important clinical implications for therapy of infections caused by Staphylococcus aureus.

Although the epidemiology of healthcare (HA)-MRSA is relatively well understood in HA settings, there is less information available with regard to the sources, routes of transmission of CA-MRSA, outside the HA environment. Recently, contaminated whirlpool water has been implicated as the route of transmission of Panton-Valentine leukocidin (PVL)-positive MRSA in the community in a report of MRSA infection in a college football team (Begier et al., 2004). Furthermore, methicillin-sensitive Staphylococcus aureus (MSSA) was isolated in communal whirlpool water associated with a professional football team in the US (Kazakova et al., 2005), where there was an outbreak of skin abscesses due to MRSA infection. In the former outbreak, Begier et al. (2004) reported that athletics-associated MRSA infections have become a high-profile national problem with substantial morbidity. In this study, these workers described that in a college football team, of the 100 players questioned, 10 were positive for MRSA, of which three of four players with infection at a covered site (hip or thigh) had shaved the affected area, and these infections were also associated with sharing the whirlpool greater than or equal to two times per week (RR, 12.2; 95% confidence interval, 1.4–109.2) (Begier et al., 2004). Although these workers did suggest that MRSA-contaminated pool water may have contributed to infections at covered sites, the small numbers examined in the study did not allow any firm conclusions to be taken of the clinical significance of the MRSA-contaminated pool water. However, this study did conclude that appropriate whirlpool disinfection methods should be promoted among athletic trainers (Begier et al., 2004).

A more recent report of MRSA infection in a professional football team in St. Louis, Missouri, USA (Kazakova et al., 2005), suggested that the skipping of showers by players prior to use of communal whirlpools may have promoted MRSA cross-infection between players.

To date, no data has been presented describing the survival dynamics of MRSA in aquatic, marine or swimming pool environments and it was therefore the aim of this investigation to examine the survival of HA-MRSA and HA-MRSA in these environments.

Materials and methods

Description of community- and NA MRSA strains employed in this study

Fourteen strains of CA-MRSA (n = 2) and HA-MRSA (n = 12) were examined in this study, including representative MRSA phage-types, EMRSA 15 (n = 2), EMRSA 16 (n = 2), Irish 1 (n = 2), Irish 2 (n = 2), unique type (n = 2) and untypeable (n = 2), as well as CA-MRSA (n = 2). All isolates were taken from the MRSA culture collection archived in the Department of Bacteriology, Belfast City Hospital. All HA-MRSA isolates were obtained from clinical cases of MRSA-associated infection presenting at Belfast City Hospital over the period July 2003–December 2004. The CA-MRSA isolates were supplied by Dr. Trevor Winstanley, Royal Hallamshire Hospital, Sheffield, England. Initially, the phenotypic identity checks of the isolates were confirmed on revival of each isolate from the archive, including colonial morphology, Gram-stain (+ve), coagulase-tube test (+ve), latex slide agglutination assay (+ve) and detection of the presence of the mecA gene locus by PCR (mecA PCR +ve), as previously described (Moore et al., 2003). All isolates were previously phage-typed by the Staphylococcus aureus Reference Laboratory, Health Protection Agency (HPA), Colindale, London.

Source and preparation of river, sea and swimming pool water microcosms

Sea and river water (5 l) were obtained from local sources and transported to the laboratory, where they were filter-sterilized by membrane-filtration (0.45 m) (Whatmann Ltd., England). Swimming pool water (5 l) was obtained from a local public baths in the Greater Belfast area, and had a free chlorine concentration of 2.90 parts per million (ppm) and a combined chlorine concentration of 1.00 ppm, giving an overall chlorine concentration of 3.90 ppm. The pH of the pool water was 7.80 and the water had a clear and sparkling appearance. The swimming pool water was sterilized by filter-sterilization, as described above. Following this, sterility checks were performed on all three water specimens, by culturing 5 ml water in double-strength nutrient broth no. 2 (NB2) for 48 h at 37 °C, before plating 20 μl of broth onto Columbia Blood Agar (Oxoid CM0331) supplemented with 5% (v/v) defibrinated horse blood at 37 °C for 24 h.

Inoculation and storage of MRSA in water microcosms

CA-MRSA and HA-MRSA (n = 14 strains) were prepared overnight (t = 17 h) by growing each strain
individually at 37°C (24 h) on Columbia Blood Agar (Oxoid CM0331) supplemented with 5% (v/v) defibri-
nated horse blood. Cells were harvested separately into each water milieu (25 ml) to give approximately 10⁵
t₄ colon forming units (cfu)/ml water. Each water containing culturable MRSA was then stored in the
dark at ambient temperature for up to 14 days. Sampling of duplicate water specimens for each MRSA
strain at each timepoint was performed at t = 0, day 1,
and day 2 and on alternate daily intervals up to and
including 14 days duration. Sampling of the waters
involved aseptically plating each water microcosm,
where MRSA were enumerated at each timepoint on
NB2 supplemented with Agar A (15 g/l) by employing
the Whitley automated spiral plater (WASP) (Don
Whitley Scientific Ltd., Shipley, UK) in combination
with the protocol digital colony counter (Don Whitley
Scientific Ltd.), in accordance with the manufacturers’
instructions. Bacterial cell counts were expressed as the
mean log₁₀cfu MRSA/ml water of duplicate counts.

Statistical analyses

Statistical analyses were performed where appropriate
employing the Student’s t-test, where a probability value
of p<0.05 (5%) was considered significant.

Results

On retrieval from the culture archive, all isolates gave
phenotypic results that were consistent with MRSA and
all isolates were mecA PCR positive (data not shown).

Each water microcosm was inoculated with HA-
MRSA and CA-MRSA to give approximately log 5 cfu/
ml water at the commencement of the experiment. Quantitative enumerative counts in swimming pool
water were not able to detect any culturable organisms
after storage with any CA- or HA-MRSA examined.
Additionally, qualitative enrichment was not able to
detect any culturable MRSA in this milieu.

Survival investigations showed that MRSA survived
better in sea water, than in river water, which was
statistically significant (p = 0.037) (Fig. 1), where there
was an overall mean reduction of log 2.20 and
3.64 log cfu/ml, in seawater and river water, respectively,
over the 14-day period.

There was no significant difference (p>0.05) in the
survival dynamics between any of the HA-MRSA strains
examined with any water milieux examined, nor was there
any significant difference in the survival dynamics between
HA-MRSA and CA-MRSA (Figs. 2 and 3). Log₁₀
survivors/ml water versus time plots for seawater and river
water were linear, indicative of first-order death kinetics, as
shown in Figs. 2 and 3. D₁₀ values were calculated from the
slope of each exponential curve, namely the time required
to reduce the culturable count by 1 log unit in each
microcosm, employing the equation of the line (y = mx + c).
D₁₀ values for seawater and river water were 7.4
and 3.53 days, respectively, under the conditions tested.

Discussion

HA-MRSA and more recently CA-MRSA have
become important pathogens, infecting both susceptible
and immunocompetent hosts, respectively. Several studies have reported on the contamination of various items of hospital clinical equipment in the nosocomial setting, as well as air in rooms which MRSA positive patients have occupied (Cunha, 2005; Kowalski et al., 2005).

To date, there have been no reports in the literature on the survival of these in aquatic environments, including seawater, river water and swimming pool water. Recreational use of water, particularly for swimming, is a common summer activity, especially for children and young people, worldwide. Asymptomatic carriage of MRSA in individuals using such waters to swim in, would allow the contamination of such waters with MRSA organisms. It may be hypothesized that such contaminated water may subsequently act as a transient environmental reservoir for MRSA, if not properly chlorinated, thus allowing the

![Fig. 2. Comparison of the mean survival in seawater of nosocomial-MRSA (EMRSA 15 [n = 2], EMRSA 16 [n = 2], Irish 1 [n = 2], Irish 2 [n = 2], unique type [n = 2], non-typable [n = 2]) and two strains of community-acquired MRSA.](image1)

![Fig. 3. Comparison of the mean survival in riverwater of nosocomial-MRSA (EMRSA 15 [n = 2], EMRSA 16 [n = 2], Irish 1 [n = 2], Irish 2 [n = 2], unique type [n = 2], non-typable [n = 2]) and two strains of community-acquired MRSA.](image2)
colonization of new hosts, particularly those with open wounds or other skin trauma, who use such sources for recreational purposes. A variety of additional factors may also influence the potential of these organisms to colonize new hosts, including volume of water (dilution factor), frequency of replenishment, number of persons using the recreational site, nature of the wound and level of contamination of the water with MRSA.

Overall, our findings are in broad agreement with the seminal work of Kloos and Schleifer (1975a, b), who also demonstrated the higher concentration of salt in the environment is much more selective for culturing staphylococci and micrococci. The DIN19643 regulation entitled “Treatment and disinfection of water used in bathing facilities – General requirements” allows for the eradication of contaminating pathogens through several control strategies. Therefore any examination of contaminating pathogens should take into account factors, other than free chlorine for their bacteriocidal activity.

Normal infection control precautions in the setting of municipal swimming pools include: (i) monitoring of total chlorine content, total viable count and pH; (ii) regular maintenance of pool complex including replacement of cracked tiles and regular changing of filters; (iii) foot baths and showering of swimmers prior to entering the pool; and (iv) wearing of swim hats. In conclusion, this study demonstrates that all the epidemic NA and CA MRSA strains examined could not survive in swimming pool water, with a free chlorine concentration of 2.90 ppm, but these organisms did survive in both marine (sea) water and fresh river water, for at least 14 days.

Although such water may remain contaminated with these pathogens for several days, given the dilution factor associated with the large volumes of water involved, although the risk of infection remains, it is improbable that such exposure would normally result in human infection, as recent epidemiological studies in recreational water have shown that staphylococci are not usually associated with infectious diseases with bathers. Even if there is a high frequency of visitors in natural pools, Staphylococcus aureus was seldom provable (Anon, 2003).

Additional studies are now required to help mathematically model the survival dynamics of MRSA in water systems, in order to help assess the risk to public health.

Acknowledgements

This work was supported financially by a kind charitable donation given by the wife and family of and in memory of the late Mr. John (Jackie) McCaul-ehern, for the purposes of pursuing microbiological investigations into the sources and transmission of bacteriological aetiologic agents of infection. The authors wish to thank the MRSA Reference Laboratory, Health Protection Agency (HPA), Colindale, London, for provision of phage-typing of the MRSA isolates, as well as Dr. Trevor Winstanley, Royal Hallersham Hospital, Sheffield, for provision of the CA-MRSA isolates and Environmental Health Officers, Belfast City Council, for collection of specimens of swimming pool water.

References


