Methicillin-resistant *Staphylococcus aureus* Fomite Survival

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OBJECTIVE: To assess survival of methicillin-resistant *Staphylococcus aureus* (MRSA) on fomites encountered by health students.

DESIGN: Three suspensions of MRSA were made to mimic lab splashes: a 0.5 McFarland trypticase soy broth, whole blood with 50 colony forming units/mL and body fluid/serum with 2,000 colony forming units/mL. These were seeded onto three environmental surfaces (glass, vinyl floor tile, and countertop) and wet swabbed for 60 days. High touch areas of student stethoscopes were also wet swabbed. MRSA selective CHROMagar® was used to identify organism survival.

SETTING: Salisbury University, Salisbury MD

PARTICIPANTS: Salisbury University nursing and respiratory therapy students who volunteered to have their stethoscopes swabbed anonymously.

MAIN OUTCOME MEASURE: Detection of pink colonies on MRSA-selective CHROMagar®.

RESULTS: MRSA in 0.5 McFarland broth lived for ≥60 days on all surfaces. MRSA in blood was undetectable on any surface, and MRSA in serum survived 41 days on glass, 45 days on tile, and ≥60 days on countertop. Five of thirty-three stethoscopes (15%) tested were positive for MRSA.

CONCLUSIONS: Previous studies showed fomite survival of MRSA for about two weeks using contact plate sampling and MRSA on 7.4% of stethoscopes. We showed longer MRSA survival times by wet swab sampling and a higher stethoscope contamination rate. As expected, higher organism loads survived longer.

ABBREVIATIONS: ATCC = American Type Colony Collection; CFUs = colony forming units; MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-sensitive *Staphylococcus aureus*; TS = trypticase soy.

INDEX TERMS: fomite; methicillin-resistant *Staphylococcus aureus*; MRSA; survival.

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Multi-drug resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) are significant causes of infection in the healthcare environment and increasingly in the community.[1] The escalating antibiotic resistance of this virulent nosocomial pathogen makes MRSA infections especially difficult to treat.[2] Since MRSA can harbor on many surfaces in hospitals such as floors and door handles even after disinfection,[3] MRSA can be indirectly transmitted through fomites, or contaminated inanimate objects, causing infections.[1,3] One study showed that the rate of transfer of *Staphylococcus aureus* organisms from a contaminated object from a single hand contact was 100%, so the degree of risk from fomites is relatively high.[4] Accurate knowledge is therefore needed regarding how long the organism survives, and this can be accomplished by seeding various surfaces with a known isolate of MRSA and monitoring for a period of time. The growth medium CHROMagar® selectively inhibits many organisms and discriminates methicillin-sensitive staphylococci (MSSA) from MRSA.[5] This allows rapid identification of MRSA from non-living environmental samples.

There is extensive literature on survival of organisms on inanimate objects, but since the number of potential objects and

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organism loads is almost infinite, the literature is necessarily incomplete. Further, a comprehensive review in 2006 noted that the data from many of the studies is contradictory.[6] For example, one study using a 10^7 organism load of MRSA detected its survival on dry surfaces for several days to almost two weeks,[7] while another similar study concluded that 10^8 MRSA and other *S. aureus* strains can survive on dry surfaces for months.[8] The reasons for the contradictory information are many. Most of the studies reviewed used different types of surfaces, seeding protocols, organism loads, sampling techniques, growth media, means of assessing growth, and environmental conditions (temperature, humidity, etc.). For the study above in which survival was less than two weeks, organism suspensions were seeded and began drying immediately. In the second study with extended survival, 1 mL of organism suspension was placed in a bottle and the opening was plugged with cotton. Drying was not complete for 10 days, so the extended survival could easily be attributed lack of immediate dessication as well as the higher organism load. Further, within the species *Staphylococcus aureus* there are many strains, and the selection of a particular strain may influence survival time. Indeed, in one of the studies cited above,[8] the MRSA strain selected by the authors had a substantially longer survival time than the MSSA strain they selected for comparison. When the authors of this study did a follow-up study of five different MRSA strains,[9] they found that MRSA strains associated with outbreaks had better survival times than sporadic MRSA isolates. Therefore, there may be significant variation in survival times between MRSA strains that also have clinical relevance.

There are some common themes in the literature, however. Organism recovery is highly dependent on initial organism load[10, 11] and sampling conditions,[10] with higher initial organism loads showing extended survival. Variables that contributed to increased survival include lower temperatures,[6] humidity >70%[6] and surfaces that offered "pores" into which organisms could embed.[3] One study verified the presence of organisms on seeded surfaces not only with colony counts but also with scanning electron microscopy. Microscopic physical features of inanimate object surfaces that allow organisms to penetrate the surface and perhaps escape dehydration were associated with increased organism visually and by colony count.[3]

Data on survival of MRSA on fomites directly associated with clinical laboratory practice was not found in a comprehensive literature search. In clinical laboratories, there are three MRSA sources of major concern: blood specimens, body fluid specimens, and microbiological cultures. A 0.5 McFarland suspension is a standard laboratory organism load for microbiological manipulation, and it will contain millions of CFUs. Patients with clinically important sepsis have more than 30 CFUs/mL of bacteria in their blood, and acquisition of MRSA into wounds from skin is associated with >200 CFUs.[12] Therefore, a serum suspension containing approximately 2,000 CFUs/ml and a whole blood suspension with approximately 50 CFUs/mL could be used to mimic body fluid and blood splashes, respectively.

Also in the clinical environment there is concern about the transmission of MRSA from "high touch" fomites such as stethoscopes.[13] Sampling these types of objects will help to determine if strains of MRSA can survive on them and if MRSA is capable of being transferred from the hospital into the environment. Clearly if MRSA is being transferred out of the hospital by objects, it poses a threat to society because it can be spread into the community.

MATERIALS AND METHODS

Two standard organisms were used from the American Type Colony Collection (ATCC): methicillin-resistant *Staphylococcus aureus* (MRSA) strain #43300 and methicillin-sensitive *Staphylococcus aureus* (MSSA) strain #25923. BBL® CHROMagar® for MRSA was used to select for and identify methicillin-resistant *Staphylococcus aureus*. BBL® trypticase soy (TS) broth and agar were used to dilute and quantify organisms.

Human serum and whole blood anticoagulated in acid/citrate/dextrose were collected by standard phlebotomy from a healthy volunteer. Squares of glass and floor vinyl and a melamine countertop scrap were purchased from a home improvement store.

The nature of the study was explained to Salisbury University respiratory therapy and nursing students, and they were told participation was voluntary and anonymous. Faculty collected student stethoscopes in class on a cart without identifying them in any way, and the stethoscopes were returned to class after swabbing.

Sterile TS broth was used to make a standardized inoculum (0.5 McFarland) from stock broth solution of MRSA. Serial dilutions were made of the 0.5 McFarland standard (1:10, 1:100, 1:1,000, and 1:10,000) using broth. With a 0.001 inoculating loop, each dilution was streaked onto a separate TSA plate and incubated overnight at 37°C. The plate with

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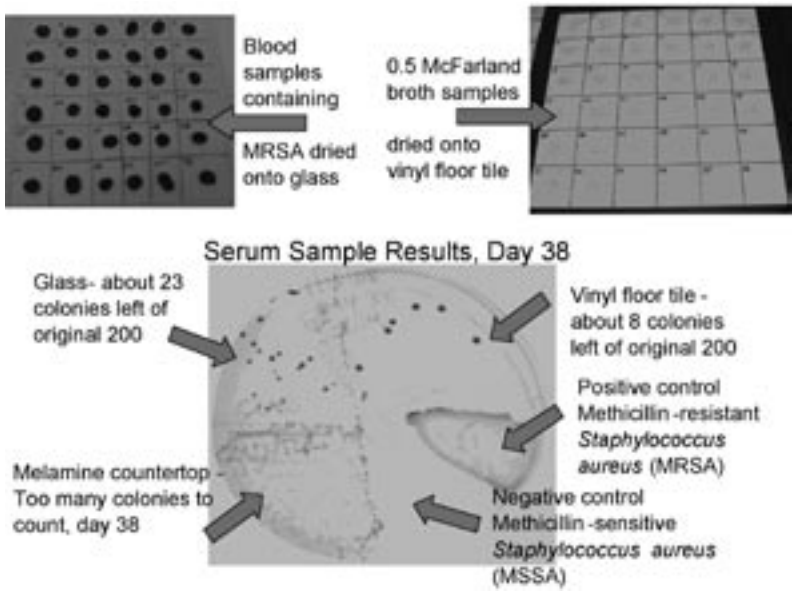
the best countable colonies was used to determine the total colony forming units (CFUs)/mL in the 0.5 McFarland standard. The 0.5 McFarland standard was then diluted with whole blood to obtain a suspension with 50 CFUs of MRSA/mL and with serum to obtain a suspension with 2,000 CFUs of MRSA/mL.

Vinyl floor tiles, glass plates, and melamine countertop material were divided into squares and each square was labeled with sequential numbers. To mimic splashes on each of the three surfaces, the following were pipetted and allowed to air dry:

- Into each of 36 squares (5 cm x 5 cm), 0.5 ml of the MRSA 0.5 McFarland standard with 110,000,000 CFUs/mL(55 million CFUs/square)
- Into each of 36 squares (2.5 cm x 2.5 cm), 0.1 mL of blood with 50 CFUs/mL MRSA (5 CFUs/square)
- Into each of 31 squares (2.5 cm x 2.5 cm), 0.1 mL of serum with 2,000 CFUs/mL MRSA (200 CFUs/square)

To standardize sampling of detectable MRSA remaining in the squares, 100 µl of sterile water were pipetted onto the square to be sampled. A sterile swab was used to absorb the water and swab the surface. Swabs were then inoculated onto CHROMagar® along with MRSA (a positive control) and MSSA (a negative control). Agar plates were incubated at 37ºC and read after 24-48 hours. Positive growth was detected as visible pink colonies. This process was repeated on one square at a time for remaining squares until no MRSA was detectable or until all squares were sampled. Initially, samples were taken daily, but later samples were spaced with several days in between. Examples of seeded fomites and an inoculated plate are shown in Figure 1.

Figure 1. Examples of seeded fomite surfaces and an inoculated CHROM-agar® plate



To sample stethoscopes, a sterile swab was dipped into an uninoculated TS broth tube and the stethoscopes were swabbed in the areas which are most likely to frequently come into contact with human skin (near the earpiece, front and back of the diaphragm). CHROMagar® plates were divided into separate quadrants and a different stethoscope sample was swabbed onto each quadrant. A positive and negative control were included on each plate. Agar plates were incubated at 37ºC and read after 24-48 hours. Positive growth was detected as visible pink colonies.

RESULTS

As shown in Table 1, live MRSA from the 0.5 McFarland Standard (110 million CFUs/ml) was demonstrated for 60 days, after which all samples had been taken. No live MRSA was recovered from whole blood (50 CFUs/ml), while MRSA in serum (2,000 CFUs/ml) survived at different rates on the different surfaces: 41 days on glass, 45 days on tile, and at least 60 days on countertop.

Table 2 shows that MRSA was present on 3 out of 15 stethoscopes from respiratory therapy students and 2 out of 18 from nursing students for an overall positive rate of 5 out of 33 or 15%.

DISCUSSION

Survival of a 10^7 organism load of dried MRSA for up to about two weeks was shown in a previous study using a curtain, a plastic patient chart, and a laminated table as fomites[7] when organism was seeded and dried immediately. The exact strains used in this study were not given. Our maximum organism load was 5.5 x 10^7 and we also allowed samples to dry immediately, so based on the survival times

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quoted, our samples were set up for about a month of daily sampling. However, because of the unexpected duration of survival, the later samples were spaced with several days in between to allow the surfaces to be sampled for a total of 60 days.

Survival of MRSA in whole blood on environmental surfaces was not demonstrated. Since these samples had the smallest organism load, this result is not unexpected. Further, the nature of the dried blood made sampling difficult. It is likely, then, that transmission of MRSA from dried blood on objects would be poor.

Serum suspensions with a higher organism load than the whole blood did survive, but at different rates on the different surfaces. Although the countertop serum samples persisted until day 60, the colony count was very low, so additional survival is probably not more than a week or two. Still, this is well beyond the survival expected and indicates that dried body fluids contaminated with MRSA could be a significant source of MRSA transmission. We note, however, the heterogeneity of the term "body fluids". Sterile serum without immunocompetent cells and with low levels of immune mediators would be expected to overestimate MRSA survival in other types of fluids. However, while the dose of 200 CFUs is considered infectious,[12] it was shown to be the minimum infectious dose on compromised human skin, and many body fluids could have higher organism loads.

The 0.5 McFarland standard samples survived for the entire 60 days on all three surfaces with too many colonies to count remaining. Further studies will be needed to determine the total survival time, and it may significantly exceed 60 days. Since this was the highest organism load and it was in a nutrient medium, extended survival was to be expected. This sample has the fewest implications for the community as the high organism load would only be encountered in laboratories. It does, however, indicate that strict adherence to safety protocols in labs is vital.

Stethoscopes are not objects commonly used in the clinical laboratory, but they are considered "high touch" fomites that might provide surrogate data about the survivability of MRSA on high touch laboratory fomites composed of similar materials. We also had a ready source of stethoscopes that had been removed from the clinical environment. A previous study swabbed 55 stethoscopes from community pediatric clinics and showed that MRSA was present on 7.4%.[13] Since the stethoscopes in this study were from nursing and respiratory therapy students and only 33 were sampled, statistically significant comparisons cannot be made. However, a higher contamination rate may be expected in this research because of significant contact of nursing and respiratory therapy students with hospital inpatients. This information indicates that further study on "high touch" laboratory objects such as microscopes and pipettes might be useful.

Table 1. Survival time in days of MRSA on fomites

Fomite	0.5 McFarland Standard 110 million MRSA CFUs/ml in broth sample seeded for 55 million CFUs/square	Human serum 2,000 MRSA CFUs/ml seeded for 200 CFUs/square	Human whole blood 50 MRSA ml seeded for 5 CFUs/square
Melamine counter top	≥60*	≥60*	0
Vinyl floor tile	≥60*	45	0
Glass	≥60*	41	0

*Growth was positive at 60 days at which time all samples were taken.
CFU = colony forming unit

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This data suggests that different concentrations of MRSA can survive for a much longer time on inanimate objects than was expected prior to conducting this research study. To date, there is no universally accepted method for sampling fomites, and wide variation in organism recovery has been shown depending on sampling technique.[10] The study cited above[7] which showed about two weeks of survival on sampled surfaces used contact agar plates while we applied a standard amount of sterile water and swabbed the surfaces. It is possible that rehydrating the sample not only allowed better swab sampling but also improved growth of dried organism. This may have implications for identifying which fomites are more likely to transmit MRSA. Wet areas such as bathrooms, gyms, and showers may contain surfaces more likely to transmit MRSA. However, our study suggests that MRSA survival on stethoscopes is occurring, so inherently dry surfaces are not necessarily without risk.

The MRSA organism strain we selected was the strain cited in the BBL CHROMagar package insert[5] as the test organism used to verify agar performance. While this strain gave us confidence that use of this medium was justified in the project, at this time we do not know how much influence this particular strain had on the extended survival and if survival would be significantly different with other strains, as implied by other studies mentioned earlier.

This research supports the importance of decontamination to help reduce the spread of highly pathogenic infections, particularly since survival of MRSA was much longer than expected.

Table 2. MRSA recovery from respiratory therapy and nursing student stethoscopes

Respiratory therapy student stethoscopes
Number sampled: 15
Number harboring live MRSA: 3
Percentage harboring live MRSA: 20%

Nursing student stethoscopes
Number sampled: 18
Number harboring live MRSA: 2
Percentage harboring live MRSA: 11%

Total rate of stethoscopes positive for MRSA = 5 out of 33 or 15%

Further studies will be needed to determine the exact survival time of MRSA on the various fomites we selected, and survival studies could be done using other strains. In addition, since the physical nature of surfaces contributes to organism survival[3] there are many more potential fomites to survey. Given that there is no accepted standard for seeding inanimate objects or assessing organism survival, the contradictory data available in the literature is probably to be expected, and the topic will continue to have opportunities for further study.

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