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Screening Public Surfaces for Methicillin-Resistant Staphylococci

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Abstract

Illnesses resulting from antibiotic-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), are increasing. Recent evidence shows that pathogens once associated with clinical settings are now contaminating non-clinical environments, including public facilities. While methods of isolation and detection of MRSA in clinical settings are established, methods to detect MRSA in non-clinical environments have not been fully evaluated. Therefore, the aims of this study were to (i) determine the prevalence of methicillin-resistant bacteria on surfaces in the non-clinical environment and (ii) compare the efficacy of two specialized media to identify putative MRSA from fomites. Swabs collected from various surfaces were inoculated in Mueller-Hinton broth supplemented with oxacillin (6 mg l^{-1}). Over 50% of the surfaces were contaminated with antibiotic-resistant bacteria. Six bacteria were isolated and purified from each of the cultures that represented each fomite. These isolates were transferred to CHROMagar™ and oxacillin resistant screening agar base (ORSAB) media, which are commonly used to identify MRSA. While none of the isolates growing on ORSAB exhibited features diagnostic of MRSA, 47% of the isolates exhibited MRSA-specific characteristics on CHROMagar™. To verify the accuracy of the media, the isolates were subjected to multiplex PCR analysis diagnostic for MRSA. All colonies growing on both media were identified as methicillin-resistant *Staphylococcus spp.*, but none were MRSA. Our results indicated that over one-half of the community fomites sampled harbored methicillin-resistant bacteria, which might be of public health importance. However, the characterization of MRSA from environmental fomites using CHROMagar™ media might not be accurate and must be performed cautiously.

Keywords: MRSA, Pathogen reservoir, Chromogenic media

1. Introduction:

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important nosocomial and community pathogen that causes serious infections in patients[1]. It is estimated that MRSA causes approximately 19,000 deaths and 300,000 debilitating infections yearly in the US. Furthermore, MRSA has posed a financial burden on the health care system, claiming millions of dollars in hospital decontamination efforts, medication and prolonged inpatient care[2]. Historically, MRSA infections were prevalent primarily in hospitals and other health care facilities (health care-associated MRSA or HA-MRSA)[1]. However, the number of MRSA infections is currently increasing in the community (non-clinical environment)[3] and are caused by variant strains that are designated as community-associated MRSA (CA-MRSA). Although the origin of CA-MRSA is not clear, it is known to be more virulent than HA-MRSA, infecting otherwise healthy individuals[3]. CA-MRSA has been isolated from hospitalized individuals, hospital personnel, shared items and domesticated animals[4 5 6].

The timely detection and identification of MRSA is crucial for (i) prescribing proper treatment and (ii) facilitating environmental decontamination efforts[7]. Putative MRSA is usually identified by culturing on selective and differential growth medium, which is then followed by biochemical or genetic confirmation[8]. Two widely used media in clinical studies are CHROMagar™ MRSA (BD Diagnostic) and ORSAB (oxacillin resistance screening agar base) (Remel)[9]. CHROMagar™ is a relatively new chromogenic medium on which MRSA typically grows with a distinctive pink/mauve color as a result of the formation of an insoluble pigment, while other organisms are either inhibited or grow exhibiting different pigmentation[10]. CHROMagar™ contains colistin, nalidixic acid and an antifungal compounds as selective agents[10] and is reported to have 99.5% sensitivity and 99.2% specificity for MRSA[11]. ORSAB includes aniline blue to detect mannitol fermentation in

staphylococci. It also includes a dual antibiotic supplement (Polymyxin B and oxacillin) and 5.5% sodium chloride, which combine to reduce the growth of non-staphylococcal organisms and select for MRSA[12]. MRSA grows on ORSAB as an intense dark blue colony. ORSAB exhibits 100% sensitivity and 92.1% specificity for MRSA[12]. While ORSAB and CHROMagar™ were designed and used for MRSA screening in clinical samples, little is known about their efficiency and accuracy in the non-clinical environment, where community strains originate. The non-clinical environment harbors bacteria communities that are usually more diverse than those found in the clinical setting, posing a particular challenge to media specificity and selectivity. Therefore, CHROMagar™ and ORSAB were tested for their ability to detect and diagnose MRSA from environmental samples. Colonies exhibiting diagnostic MRSA phenotypes on CHROMagar™ and ORSAB were then subjected to polymerase chain reaction (PCR) analysis to confirm the detection of MRSA.

2. Materials and Methods:

2.1 sample collection and processing

A total of 64 surfaces were screened for MRSA. These included: 14 door handles located in five different buildings; 16 shopping cart handles from four convenience stores; four coins obtained during a transaction in a restaurant; eight computer keyboards from three internet cafés and restaurants; five ATM machine keyboards; nine gas pump handles from three gas stations; three drinking fountains; two pieces of equipment from a fitness center; four elevator buttons from two buildings; one sink handle and one urinal handle. Surface samples were collected using culture collection and transport swabs (Fisher Scientific, USA) and processed within one hour following collection.

2.2 culture conditions and MRSA screening

The swabs were placed in tubes containing Mueller-Hinton broth supplemented with oxacillin (6 mg l^{-1}), which has replaced methicillin as the antibiotic of choice for selecting bacteria resistant to ß lactam antibiotics[4]. The tubes were incubated at 35 ºC and shaken at 200 rpm for 24 h. Samples that exhibited growth were inoculated (50 µl) onto ORSAB and CHROMagar™ and incubated at 35 ºC for 24 h to select for MRSA. Six colonies (including those that did and did not exhibit diagnostic MRSA phenotype) representing each fomite were selected for genetic analysis. A pre-identified isolate of MRSA was used as a positive control during testing of the agar media.

2.3 DNA isolation and PCR analysis

DNA was isolated from each isolate using bead beating and phenol/chloroform extraction[13], quantified with spectrophotometry (A_{260}) and stored at -20 ºC. To identify MRSA, the DNA was subjected to a multiplex PCR analysis[4] that targeted the following genes: 16S rRNA gene specific for staphylococci, *femB* (involved in peptidoglycan synthesis) specific for *S. aureus*, and *mecA*, which confers resistance to methicillin. The PCR product was visualized on 1.5 % agarose gels containing ethidium bromide. Two controls were used in every PCR reaction, a positive control containing MRSA DNA and a negative control containing no DNA template.

3. Results:

3.1 surfaces harboring methicillin-resistant bacteria

Approximately one-half of the surfaces harbored bacteria that grew in Mueller-Hinton broth supplemented with oxacillin (Table 1). The number of samples harboring methicillin-resistant bacteria from each type of swabbed surfaces varied, ranging from 100% (computer keyboards, sink handle) to 0% (coins, urinal) (Table 1).

Table 1: Surfaces sampled to assay for contamination with methicillin-resistant bacteria.

Surface type	Number of surfaces swabbed	Number of samples that exhibited bacterial growth in Muller-Hinton broth with oxacillin
Door Handles	14	6 (43%)
Shopping Carts	16	6 (38%)
Coins	4	0 (0%)
Computer Keyboards	8	8 (100%)
ATM Keyboards	5	3 (60%)
Gas Pumps	9	7 (78%)
Drinking Fountains	3	1 (33%)
Exercise Equipment	2	1 (50%)
Elevator Buttons	4	1 (25%)
Sink Handles	1	1 (100%)
Urinals	1	0 (0%)
Total	67	34 (51%)

3.2 isolation of putative MRSA using CHROMagar and ORSAB

Surface swabs enriched in Mueller-Hinton broth were inoculated onto ORSAB and CHROMagar™ media to identify fomites contaminated with MRSA and to compare the MRSA screening ability of the two media. While several white colonies grew on ORSAB, no blue colonies were observed, indicating the absence of MRSA. To confirm that the white colonies were not MRSA, 30 randomly selected colonies (representing several fomites) were subjected to multiplex PCR analysis. While the 16S rRNA gene of staphylococci and *mecA* were consistently detected, *femB* was never detected, indicating that methcillin-resistant, non-*aureus*-staphylococci were present on the fomites.

In contrast, 17 mauve colonies (putative MRSA) representing 3 surfaces were isolated from CHROMagar™ media. However, PCR analysis revealed that these isolates lacked the *femB* gene, indicating the presence of methicillin-resistant, non-*aureus*-staphylococci, but not MRSA. Additionally, multiplex PCR analysis of 30 randomly-selected, non-mauve colonies resulted in the detection of the 16S rRNA gene and *mecA*, confirming the presence of methicillin-resistant, non-*aureus*-staphylococci, but the absence of MRSA.

4. Discussion:

The aim of this study was to first determine the prevalence of methicillin-resistant bacteria on public surfaces, and second, to compare the suitability of two growth media used primarily in the clinical setting, to isolate MRSA from non-clinical samples. The prevalence of antibiotic resistant staphylococci in the non-clinical environment is increasing[3]. This trend is of particular importance, as several staphylococci represent human pathogens, capable of causing life threatening infections in compromised (e.g. *S. epidermidis, S. hominis, S. hemolyticus*) as well as healthy individuals (e.g. MRSA). A prevailing concern that surrounds the emergence of CA-MRSA is the identification of reservoirs in which it survives. Currently, the environmental reservoirs of CA-MRSA are not well defined, partly due to the absence of proper detection and surveillance methods. The rapid identification of potential environmental reservoirs is a crucial step in the control of community-associated antibiotic-resistant staphylococci. Our results showed that oxacillin/methicillin-resistant staphylococci were prevalent on several public surfaces (Table 1). The number of contaminated surfaces was high but not surprising. Antibiotic-resistant staphylococci have been isolated from the nasal cavities and other regions of the human body and domesticated animals[14 15 16]. Therefore, constant dermal shedding from human and animal hosts likely drives the occurrence of antibiotic-resistant staphylococci in the environment, including inanimate surfaces[4]. The spread of antibiotic-resistant bacteria in the environment is also facilitated by the lack of proper hygiene practices and the misuse of antibiotics[4]. Community pathogens can pose a serious public health concern. For example, a review of several published reports of severe cases of CA-MRSA infections revealed a fatality rate of 64%, while 40% of the surviving individuals suffered from “significant disabilities” following treatment[17].

In contrast to clinical samples, which might contain a relatively simple assemblage of bacteria, environmental samples often harbor diverse bacteria communities. Virtually all growth media formulated to select for the staphylococci were designed for clinical samples and the suitability of these media to detect MRSA from

complex environmental samples is not clear. Although clinical studies have reported CHROMagar™ to be nearly 100% effective (sensitive and specific) in identifying MRSA[18 19], our results showed that the use of CHROMagar™ for environmental samples might result in false positive identification. Specifically, colonies that displayed a diagnostic MRSA phenotype on CHROMagar™ (mauve color) did not harbor the *femB* gene, indicating that they were not *S. aureus*, and therefore not MRSA. However, CHROMagar™ did not appear to produce false negative identification, as PCR analysis of randomly-selected, non-mauve colonies (n=30) did not identify any colonies as MRSA. Our results indicated that staphylococci other than MRSA grew as mauve colonies during our analyses. On a previous formulation of CHROMagar™, *S. epidermidis*, *S. caprae* and *S. schleiferi* were shown to exhibit a colony pigment similar to that of *S. aureus*[1]. It is possible that environmental variants of these staphylococci remain an obstacle preventing the use of CHROMagar™ for environmental samples.

ORSAB was reported to outperform other media, such as mannitol salt agar, in the isolation of MRSA in clinical samples. Specifically, ORSAB recovered 98% of the MRSA known to occur in clinical samples [20] and exhibited 100% sensitivity and 92.1% specificity[12]. Since none of our samples appeared to contain MRSA, we cannot report on the sensitivity of either media. However, our results did show that CHROMagar™ suffered from a lack of specificity when challenged with environmental samples. Mauve colonies on CHROMagar™ (putative MRSA) isolated from three fomites were found, in fact, not to be MRSA following multiplex PCR analysis. In contrast, no MRSA were putatively identified following growth on ORSAB. This result was further confirmed following PCR analysis.

5. Conclusion:

Our results suggest that ORSAB might be more effective in determining the prevalence of MRSA in the community when compared to CHROMagar™, as the use of CHROMagar™ as a primary detection medium might lead to false positive identification of MRSA. Although our study used a relatively small number of samples per surface type, it is clear that the effectiveness of CHROMagar™ in detecting MRSA in the non-clinical environment is not as accurate as in the clinical setting. While ORSAB was more specific than CHROMagar™ (no false positives), further analysis is needed to confirm the suitability of ORSAB for MRSA detection from non-clinical samples.

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