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Original Research Article

Public transport: a large scale fomite of methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Background: The role of public transport as reservoirs of antibiotic-resistant staphylococci was determined.
Methods: 200 swabs were collected from 50 public buses (urban and rural) circulating in Davangere, Karnataka. Swabs collected were inoculated on Blood agar, Mannitol salt agar and MacConkey agar plates. After incubation for 24-48 hours, plates were examined for the growth of *Staphylococcus aureus*. Anti-microbial susceptibility test was performed using oxacillin 1ug disc to detect methicillin resistance as per CSLI guidelines.
Results: Out of 40 *Staphylococcus aureus* isolated 35 isolates were resistant to more than two classes of antibiotics, hence multidrug resistant Staphylococcus aureus. Out of 35 MDR isolates, 18 were resistant to oxacillin and cefoxitin. Minimum inhibitory concentration test revealed that out of 35 MDR isolates, 18 isolates had MIC value of ≥ 4µg/ml.
Conclusions: The recovery methicillin-resistant *Staphylococcus aureus* from public transport system implies a potential risk for transmission of these bacteria in community.

Keywords: Antibiotic resistance, Fomite, MRSA, Pathogen reservoir, Public transport

INTRODUCTION

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are implicated in serious infections and nosocomial outbreaks. The choices of treatment are reduced, as they are resistant to various antibiotics. *Staphylococci* are the normal inhabitants of human skin and mucous membranes. *Staphylococci* play a role in bacteremia, endocarditis, urinary tract infections, surgical site infections, and so on. MRSA are prevalent worldwide and are an important cause of nosocomial infections, resulting in an increased morbidity and mortality in the hospital settings worldwide.[1] In order to treat the penicillin-resistant *S. aureus*, methicillin was first introduced in human medicine in the 1960s, but within a few years, MRSA emerged.[2] Several mechanisms for the methicillin resistance seen in *S. aureus* have been detected. The most important is the production of an altered penicillin-binding protein (PBP).[3]

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important public health threat in India, with significant disease burden.[4] Of particular concern are the growing number of cases that have origins in the community (CA-MRSA); these infections often affect individuals without underlying risk factors for disease. Because MRSA colonizes a large number of people, can be easily spread by fomites, and survives on surfaces for months, the environment is an important element in MRSA transmission.[5]

Staphylococcus aureus (S. aureus) first discovered in 1880s is transmitted either directly by skin-to-skin contact with an infected person or through indirect contact with fomites.[6] This antibiotic-resistant bacterium is a significant nosocomial infectious threat, causing infections characterized by painful skin and soft tissue conditions such as boils, scalded-skin syndrome, and impetigo.[7] If left untreated, *S. aureus* can lead to life threatening infections like pneumonia and bloodstream infections.[8] In 1968, the first case of MRSA was diagnosed in the United States.[4] Gradually MRSA has become resistant to penicillin, amoxicillin, oxacillin, methicillin, and many others beta-lactams.[4]

The increasing number of bacterial infections may be a result of the two way transmission of bacteria from the community to the hospital setting and vice versa.[5-8] This may be due to the increasing asymptomatic carriage of MRSA in humans along with the occurrence of community-associated MRSA infection.[1,2]

The reservoir of interest in our study is the public transport system where there is frequent dermal contact by a broad user base which facilitates the increased transmission and prevalence of MRSA throughout the community. The study was undertaken to access the incidence of MRSA among the public transport system, mainly the bus which caters the service to different locations at Davangere, Karnataka.

METHODS

Ethics statement

The study and the consent procedure were approved by the Institutional Ethics Review Board of S. S. Institute of Medical Sciences and Research Centre, Davangere, Karnataka.

Sampling of vehicles

In this study, we collected swabs from 50 public buses (urban and rural) circulating in Davangere, Karnataka**.** These buses catered services to different destinations (Figure 1).

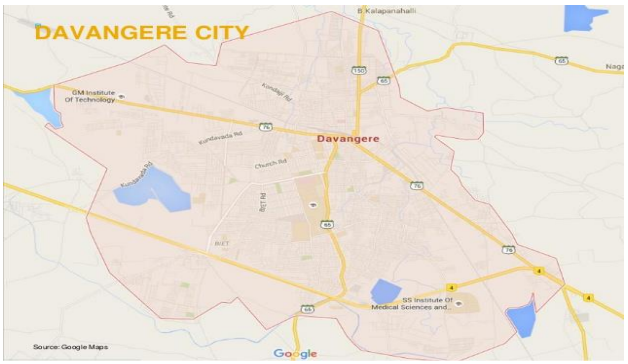

Figure 1: Map depicting the bus routes of Davangere.

Every day the exterior of the vehicles were washed and cleaning of the interior was limited to removal of trash. Cleaning of the hand touched surfaces like handrail, handgrip, seat rail and the seat with water is done once a week. For sampling we selected surfaces of handrails, seat rails, handgrips and seat as they have high levels of hand contact. 3-5 buses were arbitrarily chosen each day for sampling. The vehicle identifier number and the route of service were noted down at the time of sampling.

Bacterial isolation

Four samples were collected from each bus immediately post-service and before any cleaning and disinfection. All the swabs were inoculated on Blood agar, Mac-Conkey's agar and nutrient agar and incubated at 37ºC for 24 hours. The bacteria grown were identified by standard microbiological techniques.[9]

Antimicrobial susceptibility test was performed by disc diffusion method as per Clinical Laboratory Standards Institute (CLSI). Disk diffusion testing was performed according to the Kirby-Bauer method, as described in the guidelines of the CLSI with a 1µg oxacillin and Mueller-Hinton agar (Hi-Media, India).[10] The CLSI recommends the direct colony suspension method for testing *Staphylococci* for potential methicillin or oxacillin resistance. The plates were incubated in ambient air at 35°C, and inhibition zones around the disk were measured after 24h. In addition, all isolates were screened by the disk diffusion method for resistance to 30µg cefoxitin according to CLSI guidelines to ensure that they were methicillin resistant.[10]

Based on CLSI recommendation, a zone of =19mm for *S. aureus* and =14mm for CNS were reported as methicillin resistant. There is no intermediate category with the cefoxitin disk diffusion test.[10] Furthermore, to evaluate the susceptibility pattern of methicillin resistant *Staphylococci*, we used five antimicrobial agents including gentamicin (10µg), carbenicillin (100µg), ciprofloxacin (5µg), clindamycin, linezolid, vancomycin (30µg) and amikacin (30µ). *Enterococcus faecalis* ATCC 29212 and *S. aureus* ATCC 25923 were used as controls. The minimum inhibitory concentration for oxacillin was done by E test.

Epsilometer test technology is based on a combination of the concepts of dilution and diffusion principles for susceptibility testing. E test strips for oxacillin were provided by Hi-Media (India). MICs were performed according to the manufacturer's instructions. E test strips were placed on Mueller-Hinton agar plates containing 2% NaCl, which enhance the growth of micro-colonies and the expression of the resistance. Inoculum suspensions were adjusted to the turbidity of 0.5 McFarland standard and the plates were then incubated at 35°C for a full 24h. After the period of incubation, the E test MIC results were read where the edge of the inhibition ellipse intersects the MIC scale on the strip. According to the

manufacturer's instructions and CLSI, MIC breakpoints for defining interpretative MRSA was ≥4µg/ml.[11] In addition, the oxacillin-salt agar screening plate procedure may be used in order to detect and confirm the presence of MRSA. This test was performed as directed in CLSI guidelines.[10] For each isolate, 1µl or a swab of 0.5 McFarland suspensions was streaked on a Mueller-Hinton agar plate supplemented with 4% NaCl and 6mg of oxacillin per ml. The plates were then incubated in ambient air at 35°C for 24h. Any growth on the plate was recorded as indicating oxacillin resistance.

RESULTS

Table 1: Bacteria isolated from swab collected from different sites of the bus.

Organisms	Numbers
Bacillus species	112
Diphtheriods	66
Coagulase negative staphylococcus	52
Staphylococcus aureus	40
E. coli	12
Acinetobacter species	8
Pseudomonas species	3
Total	293

Total of two hundred swabs were collected from the bus catering service to different location at Davangere. On culture all the two hundred specimens showed mixed bacteria growth (Table 1).

Bacillus species was the predominant bacteria grown followed by diphtheriods, CoNS and *Staphylococcus aureus*.

Out of 40 *Staphylococcus aureus* isolated 35 isolates were resistant to more than two classes of antibiotics, hence multidrug resistant *Staphylococcus aureus.*

Out of 35 MDR isolates, 18 were resistant to oxacillin and cefoxitin. Minimum inhibitory concentration test revealed that out of 35 MDR isolates (Table 2), 18 isolates had MIC value of ≥ 4µg/ml.

All the MRSA strains were resistant to penicillin, chloramphenicol, carbenicillin and gentamicin. 22.7% of MRSA strains were resistant to Clindamycin and Linezolid. 44.4%were resistant to amixacin. 61.1% were resistant to netillmycin and none of the isolates were resistant to vancomycin.

Table 2: Incidence of MDR *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* in bus.

Swabs collected from different location from the bus	Number of swabs	Number of Staphylococcus aureus isolated	Number of MDR among Staphylococcus aureus	Number of MRSA among total Staphylococcus aureus isolated
Hand rails	50	12	10	06
Seat rails	50	06	06	03
Hand Grips	50	18	17	09
Seat	50	02	02	-

DISCUSSION

Staphylococcus aureus is a versatile and precarious human pathogen armed with various virulence factors and is the foremost cause of important infections in hospital settings and community.[1] *S. aureus* is responsible for infections ranging from folliculitis, food poisoning, osteomyelitis, endocarditis, septic arthritis, pneumonia, and skin and deep tissue infections to life-threatening invasive diseases.[2] It is well-established that there is increasing in the prevalence of methicillin resistant *S. aureus* infections in hospitals and health care institutions. MRSA are usually resistant to aminoglycoside, lincosamides, macrolides and all available beta-lactam antimicrobial agents, beta-lactamase inhibitor combinations and carbapenems.[1,3]

MRSA is a major cause of morbidity and mortality both in healthcare settings and in healthy individuals in the last two decades. The global emergence and spread of MRSA harbouring multi-resistance genes limits the effectiveness of therapeutic options for staphylococcal infections and worsens their clinical outcomes.[5] The difference between hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) is becoming hazy as transmission of *S. aureus* from the community to hospitals and vice versa easily occurs.[11]

Studies have identified MRSA contamination in public facilities, probably because of the higher prevalence of CA-MRSA in the developed countries.[12,13] Kaseem et al., identified MRSA on two (8%) of 24 keyboards in open access student computer rooms.[13] These studies suggest widespread contamination with CA-MRSA. Furthermore, contaminated fomites have been implicated in community outbreaks of CA-MRSA, and a recent review proposes CA-MRSA infection pathogenesis relies much more on acquisition from environmental sources and less on nasal

colonization.[14] If this is the case, we can expect to see more environmental contamination with CA-MRSA as prevalence increases, particularly in community settings prone to outbreaks such as gymnasium, facilities used by sports teams and public transport. In the present study we could not classify the MRSA isolates as community-associated MRSA clones (SCCmec type IV) and health care-associated MRSA clones (SCCmec type II) due to lack of funding. Further work is required to assess whether CA-MRSA environmental contamination can be identified frequently in community settings and whether such contamination contributes to transmission. In our study 45% of *Staphylococcus aureus* were methicillin resistant and the incidence of the MRSA was more in numbers among the buses catering service to the location which had more number of hospitals. By definition, methicillin-resistant Staphylococcus aureus strains have an oxacillin MIC of 4g/ml or harbour the mecA gene, which encodes the low-affinity penicillin-binding protein (PBP) designated PBP2a.[1,2] Among the MRSA isolates, only a few express homogeneous oxacillin resistances (i.e., 1in 10^2 express high-level resistance) while the majority show heterogeneous drug resistance (heteroresistance).[3] In our study 18 Staphylococcus aureus showed the MIC of 4 g/ml suggesting high incidence of MRSA from non-clinical specimens. Of the total Staphylococcus aureus isolated, 87.5% were resistant to multiple class of drugs.

In a systematic review, Kramer et al reported that many bacterial, fungal, and viral pathogens could survive on the inanimate objects for several months, and such pathogens could cause epidemic infections as a result of direct or indirect transmission in "hand-object susceptible patient" cycle. Specifically, high rates of microbial accumulation were found on the mobile phones and computers' keypads which had similar features with ATMs according to their physical and operational aspects. Tekerekoglu et al reported that cell phones of patients, visitors and health care workers carried multidrug-resistant hospital pathogens including *Acinetobacter spp*., *S. aureus*, and extended-spectrum β lactamase ESBL-positive Enterobacteriaceae, hence, they suggested frequent disinfection of mobile phones to reduce bacterial reservoir on these devices. Similarly, Dogan et al found many types of pathogens on the computers' mice and key-pads which were used in hospitals and in education institutes.

The MRSA transmission is facilitated through the crowded vehicles where there is significant hand to fomite contact.

CONCLUSION

Our study highlights the need for the public awareness to hygiene following the use of public facilities as there is no possibility for hand hygiene during or immediately after the journey.

Therefore, this study can form a ground work for epidemiologists and health care workers to chalk out decontamination strategies.

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