Assessment of Microbial Load and Drug Resistant Staphylococcus Aureus Contamination on the Train Handles of Western Railways in Mumbai

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ABSTRACT

We aimed to study the bacterial load on handles of local trains' of western railwaysin Mumbai city. We mainly focused on the recovery of skin opportunistic pathogen Staphylococcus aureus from 20 swab samples, to study their virulence factors and antibiotic sensitivity profile. Besides the high bacterial load on studied samples, 17 gram positive and catalase positive Staphylococcus sp. were obtained. Among these, 8 isolates were identified as S. aureus by cultural, morphological and biochemical studies. All these isolates showed virulent characteristics. The Antibiotic Sensitivity Testing (AST) showed7 out of 8 S. aureus strains to be Multi Drug Resistant (MDR). Interestingly, a common resistance pattern was observed among these isolates where all 7 MDR S. aureus strains were resistant to penicillin G, 3rd generation cephalosporin (*i.e., cefoxitin and/ or cefotaxime*), ciprofloxacin and erythromycin. Overall, our study indicated poor hygienic conditions in local train compartments of western railways in Mumbai.

Keywords: AST, Fomites, S. aureus, Train handle, virulence.

INTRODUCTION

Micro-organisms are present everywhere. Although their presence does not generally concern human activities, sometimes the contamination of common objects by pathogens may turn into potential health risks. These objects are defined as fomites and typically transfer infectious agents by indirect contact from hand to common surfaces like door knobs, switches and handles, and thus from one person to another in a community [1]. The concealed and oblivious nature of pathogens combined with turning of fomites into reservoirs, thus leads to exponential spread of infections. To make matters worse, the infectious agent may have acquired antimicrobial resistance that may further aid in the spread of these resistance genes in the community.

Several studies have indicated smooth surfaces to be better reservoirs of infectious agents as compared to rough surfaces that absorb or trap the infectious agents, thereby limiting its spread through simple touch. The epidemiological studies have indicated high risk exposure to infectious agents in child-care, domestic environment, public transport and sports facilities, through fomites. However, they are particularly associated with hospital acquired infections through equipment like stethoscopes, IV drip tubes, catheters and life support systems [2].

The contamination of fomites is affected by several factors like moisture, frequency of use, unhygienic practices and frequency of exposure. The survival of pathogens on fomites is also affected by their specific virulent characteristic, bio film formation as well as its initial load (bio burden) during contamination. The presence of specific substrate components, acting as artificial niche, also favours microbial contact and growth [3]. The occurrence of infections in humans on contact with fomites under above mentioned ideal conditions ultimately depends on the immuno-competence, personal hygiene and overall health of the person in contact. Previous studies have reported the survival of viruses like HBV, HIV, CMV and HSV from few hours up to a week on fomites. Other viruses like astrovirus, polio and rotavirus can survive for over 2 months. Many infection- causing gram negative as well as gram positive bacteria may also survive for months and spore-forming bacteria may remain dormant for years, on fomites [4].

Some documented reports suggest ready transmission of gram positive bacteria, specifically S. aureus, through fomites as compared to viruses and gram negative bacteria. This is most probably due to its ubiquitous nature and occurrence as a part of normal flora of skin. Besides, they are also known to persist longer at low humidity levels and develop resistance to penicillin and other antibiotics. Although S. aureus is not always pathogenic, it is a common cause of skin infections (i.e., cellulitis, folliculitis, carbuncles, scalded skin syndrome, impetigo, boils), wound infections, abscesses and food poisoning. Less often they are also associated with respiratory infections (i.e., sinusitis, pneumonia), meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia, and sepsis [5]. It is commonly spread through infected towels, sheets, clothing, athletic and medical equipments. The above being said, the drug resistantStaphylococcus aureus infections, can spread easily in the healthcare environment, and become increasingly difficult to treat. More recently, they are also emerging as a community-associated infectious agent by means of transportation sources [5].

With the increase in sophisticated transportation system, we have witnessed global pandemics of communicable diseases caused by familiar or completely new strain of microbes. This is a reflection of the faster movement of pathogenic microbes and the scenario may become more severe if the pathogens are drug resistant. With this understanding, the much contemplated convenience of Mumbai suburban railways helping over 7.5 million passengers [6] travel throughout the city per day may not be so convenient after all. Instead, the unhygienic and humid conditions inside trains provide the perfect environment for incubation of pathogenic microbes. Propelled by popularity of local train services as well as poor hygiene conditions, in Mumbai city, the current study was carried out with an objective to assess the bacterial load on local train handles, identify pathogenic S. aureus strains and characterize them on the basis of virulent factors, bio film forming ability and Antimicrobial Susceptible Testing (AST).

MATERIALS AND METHODS

Media and chemicals

All the nutrient media and chemicals used in the current study were procured from Himedia, and discs for AST were purchased Pathoteq Biological Laboratories.

Sample collection

Local train handles of the western railways in Mumbai city were selected as sampling sites. Approximately 10cm^2 area of a train handle was swabbed using a sterile cotton swab moistened with phosphate buffered saline (PBS). These samples were transported to the laboratory in sterile tubes and processed immediately for determination of bacterial load.

Determination of bacterial load

The samples collected above were grown on common nutrient media (Nutrient Agar, NA), and a selective and differential media (Salt Mannitol Agar, SMA) to determine the bacterial load and identify pathogenic S. aureus strains respectively. Initially, the samples were serially diluted using PBS up to 10^{-6} dilutions and 0.1ml volume of the last three dilutions (i.e., 10^{-4}, 10^{-5} and 10^{-6}) was spread on sterile NA using a glass spreader. Same volume of undiluted sample was spread on sterile SMA. These plates were incubated at 37°C for 48h and results were reported as cfu/cm^2. All the obtained isolates were maintained on NA slants and refrigerated until further use.

Identification of the isolates

Well isolated colonies from the selective and differential medium (i.e., SMA) were selected and their colony characteristics were studied. The isolates were identified on the basis of cultural, morphological and biochemical characteristics [7].

Detection of bio film formation

All the pathogenic isolates detected on SMA were screened for bio film production by tube method. It is a quantitative method for bio film detection. To carry out this process, the cultures were inoculated in 4ml Brain and Heart Infusion (BHI) broth containing 1% glucose and incubated at 37°C for 24h. After incubation the tubes were decanted, washed with PBS (pH7.2) and dried. The tubes were then stained with 4mL of 0.1% crystal violet for 20min. After staining, the tubes were decanted, washed with saline and 4 mL of ethanol was added to each tube. The intensity of blue colour in tubes is directly proportional to the amount of biofilm formed on the sides of the tubes. Hence it was determined spectrophotometrically at 570nm [8].

Determination of antimicrobial resistance profile of the isolates

The obtained isolates were subjected to AST by using Kirby Bauer method following CLSI guidelines [9]. To carry out AST, the cultures were spread on sterile Mueller Hinton agar plates and four antibiotic disks were placed at suitable distance. After overnight incubation, the plates were checked for zone of inhibition and the resistance pattern was recorded. The antibiotics used in our study were Ciprofloxacin (Ci, 5 µg), Chloramphenicol (Ch, 30 µg), Cefotaxime (Ce, 30 µg), Ampicillin/Sulbactam (AS, 20 µg), Tetracycline (T, 30 µg), Ceftizoxime (Cz, 30 µg), Amikacin (Am, 30 µg), Gentamicin (Ge, 10 µg), Ofloxacin(O, 5µg), Gatifloxacin (Ga, 10 µg), Cotrimoxazole (Co, 25 µg), Cefoxitin (Cf, 30 µg), Penicillin G (P, 10 units), Nitrofurantoin (N, 300 µg) and Erythromycin (E, 15 µg).

RESULT AND DISCUSSION

Determination of bacterial load

Figure 1 represents the bacterial load on train handle samples collected in our study. From the observations on NA plates and as per our expectations, it was clearly noticed that the train handles were heavily contaminated and harboured upto 8.2×10^5 cfu/ cm^2 bacteria.This can be attributed to the popularity and over-crowdedness of trains, frequent skin contact with train handles, absence of routine cleaning practices and lack of consciousness among passengers. Apparently, the poor hygienic conditions of trains, thus, can be concluded without further assessments.

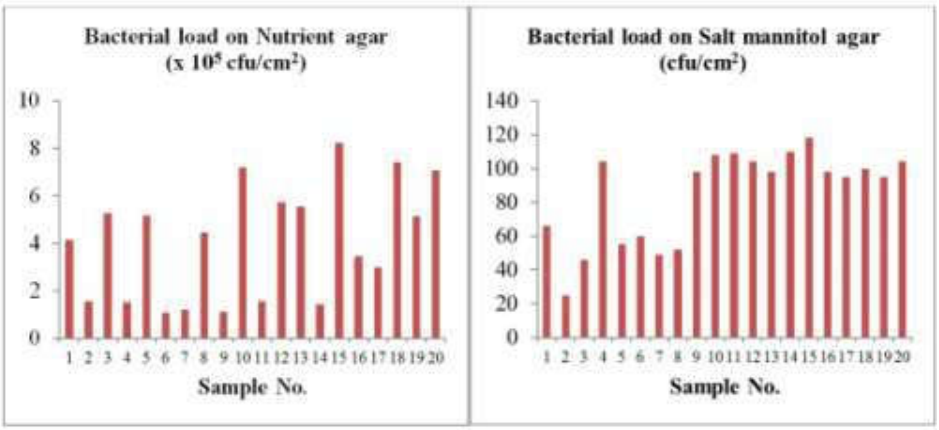

Figure 1: Bacterial load on train handle samples

Previously, considerable number of studies has been published describing hand-touch surfaces in buses, trains, mobile phones, door knobs, and computer keyboards as potential sources of spread of infectious agents [10, 11]. These studies reported presence of Escherichia coli, Vibrio cholerae, multi drug resistant Staphylococcus aureus and even Mycobacterium tuberculosison these surfaces. However, to the best of our knowledge, this is the first systematic study to report the occurrence of Staphylococcus strains on local train handles of western railways in Mumbai city.Although, the transportation system is indispensable to fulfil the needs of every individual, recently, it has drawn great attention from public health researchers as a means of spread of infectious agents at a community level. The advantages of efficient transportation including speed and connectivity, ironically puts people at risk for highly contagious and infectious diseases. More frequently than realised, it can lead to epidemics of communicable diseases, and as observed recently with the spread of Covid-19, it can lead to a serious global pandemic as well.

A similar study was carried out in Guangzhou metro system, China that reported extremely high bacterial load in all 320 samples collected in their study. In their study, 75.6% strains were identified as Staphylococcussp. [12]. The surfaces of public transport vehicles in Kathmandu showed bacterial load up to $2.5x10^5$ cfu/cm^2 [13] whereasthe handles of trams, metro and buses in Turkey showeda bacterial load of upto $6.2x10^6$ cfu/ ml [14].Among other sources, a recent study reported heavy contamination of S. aureus strains (from 50% up to 62.5%) on weight ball, cable driven curl bar, weight plates and treadmill handles, in gym [15].

Identification and characterization of the isolate

Table 1 represents the morphological and biochemical characteristics of test cultures isolated in our study. Given the increased skin contact with fomites in public transport, we mainly focused on the identification of S.aureus, since it is one of the main causes of skin infections. Out of the 20 samples collected in our study, 17 distinct isolates were obtained and confirmed to be Staphylococcus species. The catalase test was used to differentiate between morphologically similar, but catalase negative, Enterococcus and Streptococcus from catalase positive Staphylococcus species [16]. The ability to ferment mannitol was further tested to identify cultures that may most probably be S.aureus

strains.On the basis of the cultural characteristics on SMA, together with the observed biochemical tests, 8 isolates were confirmed to be S.aureus strains.All these isolates showed positive coagulase, haemolysis and lecithinase test confirming their pathogenic nature.

The contamination of fomites with pathogenic strains of S.aureus raises considerable public health concern. This is mainly because it is a normal flora of skin and thus possesses naturally acquired ability to colonize the skin. The pathogenic nature of S.aureus can hence aid in increasing the spread and severity of infections.In the last 10 years, the drug resistant and clinically significant MRSA is increasingly being reported to occur in community settings from its initial hospital settings [2]. An earlier study carried out in Japan, reported isolation of 2.3% MRSA strains from public transport facilities in Tokyo and Niigata cities [17]. In another study, 25.9% isolates were found to be S. aureus and another 31.4% were identified as MRSA from samples isolated from public transport facilities in Kathmandu, Nepal [13].

Table 1: Biochemical characteristics of Staphylococcus sp.

Sample	Catalase	Mannitol Fermentation		Glucose Fermentation Reduction		Nitrate	Salt Tolerance	Urease	Coagulation	Haemolysis blood agar	Lecithinase and	Gram Nature morphology
		Anaerobic	Aerobic	Anaerobic	Aerobic							
1	+	-	-	-	-	+	+	+	-	-	-	Gram positive cocci in clusters
2	+	+	+	+	+	+	+	+	-	+	+	
3	+	+	+	+	+	+	+	+	-	+	+	
4	+	+	+	+	+	+	+	+	+	+	+	
5	+	+	+	+	+	+	+	+	+	+	+	
6	+	+	+	+	+	+	+	+	+	+	+	
7	+	-	-	-	-	+	+	-	-	+	-	
8	+	-	-	-	-	-	+	+	-	-	-	
9	+	+	+	+	+	+	+	+	+	+	+	
10	+	-	-	+	+	+	+	+	-	+	-	
11	+	-	-	+	+	-	+	-	-	+	-	
12	+	-	-	+	+	+	+	+	-	+	-	Gram positive cocci in tetrads
13	+	+	+	+	+	+	+	+	+	+	+	Gram positive cocci in clusters
14	+	-	-	+	+	-	+	-	-	+	-	
15	+	-	-	+	+	-	+	+	-	-	-	
16	+	-	-	+	+	-	+	-	-	+	-	
17	+	+	+	+	+	+	+	+	+	+	+	

Detection of bio film formation and determination of antimicrobial resistance profile of test isolates

Apart from the virulent factors like production of coagulase and lecithinase, and blood haemolysis, all pathogenic S. aureus strains tested positive for bio film formation by Tube method. These isolates showed a visible film lining the walls and the bottom of the test tube. Bio films are microbial communities characterized by close assembly of cells that are irreversibly attached to a substratum or to each other. The study of bio film forming ability of a pathogenic strain is extremely important because the environment in bio films offer several selective and proliferative advantages to cells allowing them to evolve notoriously. Few of these advantages include restricted penetration of antibiotics through bio films, availability of nutrients and decreased growth rate [18]. Although micro-titre plate is shown to be much more sensitive in detection of bio films, they require sophisticated instruments to read the plates and document the results [19]. The tube method, on the other hand, is a simple and sensitive technique that can be carried out without the help of expert technicians, making it a reliable method for detection of biofilm formation.

Table 2 represents the antibiotic resistance profile of pathogenic bio film forming S. aureus strains isolated in our study. The antimicrobial resistance profile was checked against 15 common antibiotics used for treatment of infections caused by S. aureus. Only one isolate showed sensitivity towards all these antibiotics. The remaining 7 isolates were found to be resistant to 3 or more antibiotics used in our study, thus they were characterised as MDR strains. Careful observation of AST results indicated an interesting finding of common resistance pattern among these isolates. All 7 MDR S.

aureus strains were resistant to penicillin G, 3rd generation cephalosporin (i.e., cefoxitin and/ or cefotaxime), ciprofloxacin and erythromycin. Moreover, six isolates showed resistance to ampicillin sulbactam. Resistance to

ciprofloxacin was observed in 3 isolates. All isolates showed sensitivity towards chloramphenicol and nitrofurantoin antibiotics that target the protein synthesis and ribosomal proteins respectively. Relatively higher sensitivity towards other antibiotics like gentamycin (5/8), gatifloxacin (5/8), amikacin (4/5) and cotrimoxazole (6/8) was also observed.A similar study reported isolation of 242 Staphylococcus sp. from metro system in Guangzhou, China. Among these isolates, 79.75% MDR strains were identified. These strains showed resistance towards penicillin (94.21%), erythromycin (88.84%), rifampicin (64.46%), trimethoprim (45.04%), clindamycin (40.91%), gentamicin (31.40%), moxifloxicin (13.64%), tobramycin (12.40%), cefoxitin (10.74%), linezolid (2.89%) and teicoplanin (2.48%) [12]. In another study, 35 MDR strains were identified out of the 40 S. aureus cultures isolated from public buses in Kerala, India. Eighteen of the MDR strains were resistant to oxacillin and cefoxitin and had MIC value of≥4µg/ml. Among the MRSA strains resistance was observed towards clindamycin and linezolid (22.7%), amikacin (44%) and netilmycin (61.1%) [20].

Table 2: Antibiotic resistance profile of S. aureus strains isolated from train handles

Isolate No.	Sensitive	Intermediate	Resistant
2	Ch, AS, T, Cz, Am, Ge, O, Ga, Co, N		Ce, Ci, Cf, P, E
3	Ci, Ch, T, Cz, Am, Ge, O, Ga, Co	N	Ce, Cf, AS, P, E
4	Ci, Ch, T, Am, Ge, O, Ga, Co, N		Ce, Cf, AS, Cz, P, E
5	Ch, Ge, Co, N	Ga	Ce, Ci, Cf, AS, T, Cz, Am, O, P, E
6	Ch, N		Ce, Ci, Cf, AS, T, Cz, Am, Ge, O, Ga, Co, P, E
9	Ch, N	Ci	Ce, Cf, AS, T, Cz, Am, Ge, O, Ga, Co, P, E
13	Ce, Ci, Ch, Cf, AS, T, Cz, Am, Ge, O, Ga, Co, P, E, N		
17	Ci, Ch, O, Ga, Co, N	T	Ce, Cf, AS, Cz, Am, Ge, P, E

Key: Ciprofloxacin (Ci, 5mcg), Chloramphenicol (Ch, 30mcg), Cefotaxime (Ce, 30mcg), Ampicillin/Sulbactam (AS, 20mcg), Tetracycline (T, 30mcg), Ceftizoxime (Cz, 30mcg), Amikacin (Am, 30mcg), Gentamycin (Ge, 10mcg), Ofloxacin(O, 5mcg), Gatifloxacin (Ga, 10mcg), Cotrimoxazole (Co, 25mcg), Cefoxitin (Cf, 30mcg), Penicillin G (P, 10 units), Nitrofurantoin (N, 300mcg) and Erythromycin (E, 15mcg).

CONCLUSION

The number of MDR pathogenic microbes is increasing globally. However, till now it was believed that the infections caused by these microbes are hospital acquired, and maintenance of hygienic practices at personal and home level may suitably prevent serious infections. In the current study, the observed potential of all pathogenic S. aureus strains to form bio films is epidemiologically concerning. The high moisture levels, continuous contact, sweating and unhygienic practices may collectively contribute to the formation of rich microbial bio films on train handles, similar to those observed in bathrooms and kitchens. Our study reports important findings relevant to the ineffective hygienic conditions prevalent in the local trains of western railways of Mumbai, clearly suggesting otherwise. The common day to day objects may be potential sources of infectious agents, and hence we may unknowingly we may get exposed to pathogens causing serious infections. In order to prevent possible health hazards an interdisciplinary research approach integrating public health, microbiology as well as architectural understanding, needs to be undertaken to prevent colonization of pathogens on common surfaces.

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