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Methicillin Resistant *Staphylococcus Aureus* (MRSA) in Public Transportation Systems: A Global Perspective

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As part of living, travelling is a basic need of people. Thousands of people can now travel from small to large distances through various transportation systems. With the advent of truly global travel, the last five centuries have seen more new diseases than ever before. With efficiency, speed and reach of modern transport networks, people are always at risk from the emergence of new strains of familiar diseases, or from completely new diseases (1).

Public transportation systems can facilitate transmission of pathogensto large number of people. Presence of such pathogens particularly drug resistant bacteria is critical public health concern. Many such pathogens are hospital associated infections (Nosocomial) are now frequently linked in community associated infections. One best studied example is Methicillin resistant *Staphylococcus aureus* MRSA (16).

MRSA has become a worldwide pathogen causing morbidity and mortality at rates higher than those caused by methicillin-susceptible *S. aureus* (MSSA). MRSA can be transmitted through direct contact from touch sites (fomites) or by aerial route (17).

Present review is compilation of reports of presence of Drug resistant *staphylococci* and MRSA in public transportation systems across globe.

Bangladesh:
Only study in Chittagong city in Bangladesh where bacterial contamination level on three hand touch surfaces of public buses was done with screening 45 swab samples from grab rail, armrest and vinyl seat of 15 buses. Phenotypic characterization revealed presence of 12 MRSA isolates with sensitivity to most of the antibiotics except ceftazidime (2).

Brazil:
One conference abstract explains prevalence of *S. aureus* and MRSA in the greater line of the public transportation system of the city of Goiania, and phenotypic and genotypic characterization. 852 swabs were collected from fixed bars of the doors of the 90 buses and were subjected to standard microbiological procedure for isolation of *Staphylococci*. The overall prevalence of *S. aureus* contamination was 18.4% (157/852 swabs). For the buses, the prevalence was 18.6% (134/720), for platforms 16.8% (17/101) and for terminals 19.3% (6/31). Four (2.5%) *S. aureus* was identified as MRSA. Nine isolates (5.7%) were positive for PVL, and one of these isolates was MRSA. The iMLSB phenotype was found in 40.8% of the isolates and only one (0.6%) presented cMLSB phenotype. Sixty-two isolates (39.5%) were considered multidrug-resistant (3).

China:
Guangzhou city metro system was screened for presence of drug resistance *staphylococci.* 320 surface samples analysed, prevalence of MRSA was reported to be 2.5% while 78.9% strains were identified as MDR. 8 MRSA isolates carried a range of staphylococcal cassette chromosome *mec* (SCC*mec*) types [I (1), II (3), III (2) and NT (2)]. Only one MSSA isolate showed presence of PVL gene (4). In another study airborne *staphylococci* isolated from different metro station were subjected to phenotypic and genotypic characterization to compare between hospital and other environmental samples. Airborne *Staphylococcus* samples in the metro were resistant to an average of 2.64 antibiotic types, and 58.0% of the strain samples were resistant to at least three antibiotics; this was a significantly higher rate than strains from the park, but was lower than those from hospitals. This is the only study in which drug resistant staphylococci from air in transport (5).

Ethiopia:
Handle surfaces of the six city buses were sampled through total of 300 swabs in Mekelle, Tigray, Ethiopia. *E. coli*, *Enterobacter*spp. and *S. aureus* were 8 (4%), 4 (1.3%) and 54 (18%) were prevalent respectively. Methicillin resistant *S. aureus* was seen in 17 (5.7%) of the total 300 swab samples collected and 17 (31.5%) of the *S. aureus* isolates (6).

France:
Lyon's metropolitan network (Metro) in France was under investigation for MRSA contamination. Hand-touched surfaces were sampled with sterile swabs (Transystem) during a 1-day transversal study by collecting 50 samples from stations and trains in different metro lines. In simultaneous longitudinal study 60 swabs were taken from busiest and crowded hub station. Of the 110 swabs tested, 24 presumptive MRSA colonies were isolated, of which 2 were confirmed as *S. aureus* by VITEK-MS. These two isolates tested negative using the PBP2a Culture Colony Test and PCR. Low levels of MRSA contamination was observed when compared other studies (7).

India:
50 public buses (urban and rural) circulating in Davangere city, Karnataka were screened using swabs for bacteriological analysis. Total 40 *Staphylococcus aureus* isolated 35 isolates were resistant to more than two classes of antibiotics, hence multidrug resistant *S. aureus*. Out of 35 MDR isolates, 18 were identified as MRSA based disk diffusion and MIC [Minimum inhibitory concentration] (8). Another study focuses on the estimating bacterial load characterizing drug resistant *staphylococci* on handles of local trains of western railways in Mumbai city. Out of 17 *staphylococci* isolates 7 were classified as MDR based disk diffusion testing. High level resistance to penicillin G, 3rd generation cephalosporin (*i.e., cefoxitin and/ or cefotaxime*), ciprofloxacin and erythromycin was detected (9).

Japan:
Surface and subway trains (16 train lines) in Tokyo and Niigata in Japan were studied. Surfaces of the straps and handrails of 349 trains rubbed with cotton swabs and processed. Of 349 trains examined, eight (2.3%) were positive for MRSA. The MRSA isolated belonged to sequence types (STs) 5, 8, 88, and 89, and included community infection-associated ST8 MRSA (with novel type IV staphylococcal cassette chromosome *mec*) and the ST5 New York/Japan hospital clone (10).

Jordan:
Different fomites in public facilities in northern Jordan were screened for distribution of MRSA and MSSA. 2600 swab samples were collected from 14 fomite surfaces in a variety of public facilities including transportation sites. 380 *S. aureus* isolates was confirmed. Among them, 158 (41.6%) were MRSA while the rest of the isolates, 222 (58.4%) were MSSA. Percentages of MRSA in public facilities were significantly higher in hospitals and transportation fomites. Multidrug-resistant patterns were higher in MRSA than in MSSA. (11)

Nepal:
28 different vehicles operating in Kathmandu valley were screened for MRSA with 56 wet swab samples and analysed by classical procedure. 35 (25.9%) were found to be *S. aureus* growth positive 11 (31.4%) of them being MRSA (12).

Portugal:
85 public urban buses circulating in Oporto, Portugal, were screened for the occurrence of MRSA. Twenty-two (26%) buses showed MRSA contamination. Genotypic characterization classified the isolates into three clonal types. Majority of the isolates belonged to a single clone (PFGE A, spa types t747, t032, t025 or t020, ST22, SCCmec type IVh) exhibiting the characteristics of the pandemic EMRSA-15 strain which was prevalent in hospital settings in same city. PVL gene was absent in all MRSA strains (13). Similar study performed in another urban area of Portugal– Lisbon, the capital where in hand touched surfaces of 199 public buses in Lisbon were screened for MRSA contamination. Subsequently, the hands of 575 passengers who frequently use these bus lines were also screened for hand and carriage of MRSA. Genotypic characterization revealed that 36.2% buses were contaminated with three clones of MRSA namely clone A (EMRSA, 29%), clone B (The New York/Japan, 21%) and clone C (USA300, 26%). Only 15 passengers shown hand carriage and 4 were nasal carriers of nosocomial clone A and B (14). One more study screened hand rails of buses and trains form Porto city of Portugal. Nasal carriage was also check among local university students. The prevalence of MRSA was 16.1% and 8.9 % in buses and train indicating the difference was not significant. Among students 37% carried S aureus and only one student showed MRSA carriage. EMRSA-15 was prevalent clone as described in earlier studies. One unique single ST30-IVa isolate indicated SCCmec acquisition by an MSSA background in the community (15).

Serbia:
The most cited study explores the occurrence of methicillin-resistant *staphylococci* in a large urban public transport system of Belgrade being densely populated city in Serbia. Total 1400 swabs taken from 55 vehicles (trolleybuses, trams and buses) were examined. 30.1% samples were positive for the presence of methicillin-resistant coagulase-negative *staphylococci* (MRCoNS), but none for methicillin-resistant *Staphylococcus aureus* (MRSA). MRCoNS were isolated from all 55 vehicles. Nearly 50% of MRCoNS isolates displayed resistance not only to beta-lactams, but at least to two or more other classes of antimicrobials as well. This study demonstrated widespread occurrence of MRCoNS on hand rails in public transport vehicles. MRSA was not detected (16).

Turkey:
Handles in public transport trams, metro buses, and buses of Istanbul city were screened using swab samples for microbiological analysis. Total aerobic bacterial and fungal counts in samples collected in the evening were higher than those in samples collected in the morning. *S. aureus*, coagulase-negative *staphylococcus*, and *Enterococcus spp*. were isolated from these samples (17).

United Kingdom:
One study investigated presence of MSSA and MRSA using dip slides. One hundred and eighteen hand-touch surfaces were sampled, comprising public areas of St Thomas' Hospital, underground trains and stations, touch sites of buses and seven miscellaneous sites. Bacterial contamination was significantly high (95%) but only 8% sites showed MSSA. None of the site was contaminated with MRSA (18).

United States of America:
12 Metro stations in the underground train in Washington, D.C. were studied for presence of aerobic bacteria. 5 alpha haemolytic *streptococci*, 5 coagulase negative *staphylococci*, and 2 *S. aureus* were isolated. These bacteria were present in significantly smaller numbers indicating lower risk to commuters (19). Another study attempt isolated a subset of 14 suspected *Staphylococcus spp.* colonies based on phenotype and 16s RNA sequencing after processing 70 samples from bus and trains from mid-sized US city namely Portland, Oregon. 16S similarities were found with the following taxa: *S. xylosus, S. saprophyticus, S. cohnii, S. haemolyticus, S. epidermidis, and S. warneri.* Of the 14 isolates sequenced, 11 were *staphylococci*, and of these, five were resistant to penicillin and ampicillin, while only two displayed intermediate resistance to bacitracin. All 11 isolates were sensitive to trimethoprim-sulfamethoxazole, vancomycin, and tetracycline. Resistance in isolates was low and interestingly *S. aureus* was absent (20). In another study 16S amplicon and shotgun metagenomic sequencing was used to profile microbial communities on multiple transit surfaces across train lines and stations in the Boston metropolitan transit system. All surfaces were dominated by human skin and oral commensals such as *Propionibacterium*, *Corynebacterium*, *Staphylococcus*, and *Streptococcus*. Microbial communities on transit surfaces maintained from a metapopulation of human skin commensals and environmental generalists. It was observed that transit environment was not found to be a reservoir of antimicrobial resistance and virulence genes. This is the only report which used latest technology to categorize microbes from touch surfaces of transport system (21). The first study which reports presence of MRSA on public transportation vehicles in the United State screens 237 surface samples from 40 buses using electrostatic wipes. *Staphylococcal* isolates were subjected to various phenotypic and genotypic analyses. It was found that 68% (27/40) were contaminated with S aureus, and 63% (25/40) were contaminated with MRSA. Seats and seat rails were the surfaces most frequently contaminated, followed by the back door and stanchions. Most (62.9%) of the MRSA isolates were classified as community-associated MRSA clones (SCCmec type IV), and 22.9% were health care associated MRSA clones (SCCmec type II). Of the MRSA strains, 65% (5/20) were multidrug resistant (22).

Table. 1 Studies done so far worldwide to isolate drug resistant *Staphylococci* from public transportation systems

City and Country	Type of transportation system studied	Sampling site	Method of sampling	Phenotypic characterization (Overall)	Genotypic characterization of MRSA	Prevalence of MRSA	Resistance pattern of MRSA	Reference
Cittagong, Bangladesh	Bus	Grab rail, armrest and vinyl seat	Swab	12 MRSA	ND	26.66%	Resistance to ceftazidime only	Chowdhury*et. al.* 2016
Goiânia, Brazil	Bus, platforms and terminals	Fixed bars of doors, turnstiles	Swab	*S. aureus* (18.4%), MDR (39.5%)	PVL (5.7%) iMLSB (40.8%) cMLSB (0.6%)	2.5%	NA	Neves*e t. al.* (2012-2013)– abstract
Gaungzhou, China	Metro system	Hand rails, seats, stanchions, Ticket Vending Machine	Swabs moistened with saline	*Staphylococci* (75.6%) MDR (79.8%) MSSA (8.75%) MRCoNS (8.56%) MSCoNS	SSCmec type – I (12.5%) II (37.5%) III (25%) NT (25%) Sequence type –	2.5%	Cefoxitin (75%), Clindiamycin (87.5%), Rifampicin (62.5%), Moxifloxacin (50%), Tobramycin	Peng*et. al.* 2015

		s (TVMs), and escalators		(57.81%) *Staphylococci*-negative (24.38%)	ST398 (37.5%) ST125 (25%) ST5 (12.5%) ST15 (12.5%) ST30 (12.5%)		(62.5%), Trimethoprim (62.5%), Penicillin (100%), Linezolid (25%), Teicoplanin (12.5%), Erythromycin (87.5%), Gentamicin (75%)	
	Metro stations	Waiting rooms	Aerial sample (settle plate)	Different *Staphylococcus spp.*	mecA(28%)	ND	ND	Zhou *et. al.* 2013
Mekelle, Ethiopia	Bus	Handle surface	Swab	*S. aureus* *E. coli* *Enterobacter spp.*	ND	5.7%	ND	Kahsay *et. al.* 2019
Lyon, France	Metro	Hand rails, ticket machines	Liquid Stuarts Double Swab	Presumptive MRSA (21.81%)	ND	8% (VITEK-MS), 0% (PBP2a Culture Colony Test and PCR)	Intermediate level resistanc to cefoxitin and penicillin	Gaymard*et. al.* 2016
India	Bus	Hand rail, seat rail, hand grip and seat	Swab	*S. aureus* (20%) MDR (17.5%) CONS (26%)	ND	9%	Penicillin G (100%), Carbenicillin (100%), Chloramphenicol (100%), Gentamicin (100%), Clindamycin (22.7%), Linezolid (22.7%), Amikacin (44.4%), Netilmicin (61.1%), Vancomycin (0%)	Vinodkumar*et. al.*2017
	Suburban Railway	Handles	Swab	*Staphylococcus spp.* (85%) S. aureus (40%) MDR (35%)	ND	35%	Penicillin G (100%) Cefoxitin (100%) Cefotaxime (100%) Ciprofloxacin (100%), Erythromycin (100%)	Aruna K. *et al.* 2022
Tokyo, Niigata,	Surface and	Straps and	Swab	ND	Sequence types-	2.3%	Clindamycin (37.5%),	Yama moto

Japan	subway trains	handrails			ST5 (25%) ST8 (50%) ST88 (12.5%) ST89 (12.5)		Chloramphenicol (12.5%) Erythromycin (50%), Fosfomycin (25%), Gentamicin (37.5%), Kanamycin (75%), Levofloxacin (25%), Minocyclin (12.5% intermediate), Tetracyclin (12.5%)	T.*et. al.* 2012
Ramtha and Irbid, Jordan	Buses and taxies	Door handles and chairs	Swabs moistened in sterile 0.1% peptone buffer solution	MSSA 3.2%	NA	5.6%	NA	Jaradat *et. al.* 2021
Nepal	Tempo, bus and microbus	Handles and seats	Swab	*S. aureus* (62.5%) CONS (32.14%)	ND	19.64%	Penicillin G (100%), Erythromycin (72.7%), Gentamicin (0%), Chloramphenicol (0%)	Angnu hang*et. al.* 2018
Oporto, Portugal	Buses	Handrails	Cotton gauzes moistened with brain heart infusion broth with 0.1%tween 80	NA	Three clones 1. EMRSA [PFGE A, spa types t747,t032, t025 or t020, ST22, SCCmec type IVh (91%)] 2. PFGE B, ST5, spa type t002, SCCmecIva(5.4%) 3. PFGE C, spa type t008, ST8, SCCmecIva(3.63%) PVL (0%)	26%	NA	Simo˜es R.R *et. al.* 2011
Lisbon	Buses	Handrails, seat rails, handgrips, stop buttons, validatio	Cotton gauze moistened with Tryptic soy agar	NA	Three clones 1. EMRSA[PFGE A, spa typest2357/t747/t025/t379/t910,	36.2%	ND	Conceic¸a˜o*et. al.* 2013

		n tickets machines			ST22, and SCCmecIVh (29%)] 2. New York/Japan clone [PFGE B-t002/t10682-ST5-II (21%)] 3. Community acquired USA300 [PFGE C-t008-ST8-IVa/IVc/IVg/IVnt/VI (26%)]			
Porto	Buses, Trains	Hand rails, stop buttons	Cotton gauzes moistened with brain heart infusion broth with 0.1%tween 80	MSSA bus 71.4% & trains 64.6%	Three clones 1. EMRSA[PFGE A, spa types t032/t747 /t13285/ t13286, ST22, SCCmec type IVh (82.7%)] 2. PFGE B-t002-ST5-IVa/VI (13.79%) 3. PFGE D-t012-ST30-Iva (3.44%)	Bus 16.1% Train 8.9%	ND	Mendes A.,*et. al*. 2015
Belgrade, Serbia	Trolleybuses, Trams and Buses	Handrails	Swabs moistened in phosphate-buffered saline (pH 7.2)	MRCoNS (30.1%)	ND	0%	NA	Stepanović S.,*et. al.* 2008
Istanbul, Turkey	Trams, Metro buses and Buses	Handles	Swab	*S. aureus* (21.66%) CONS (38.33%)	ND	ND	ND	Tan *et. al.* 2017
United Kingdom	Train, station, ticket machines etc.	Chair arms and grab rails	Dip slides	MSSA (8%)	ND	0%	NA	Otter *et. al.* 2009
Washington, USA	Stations	NA	NA	*S. aueus* CONS	NA	NA	NA	Brook *et. al.* 2008
Boston, USA	Trains, Stations	seat, seat back, horizontal pole, vertical	DNA free cotton swab	*Propionibacterium*, *Corynebacterium*, *Staphylococc*	ND	ND	ND	Hsu *et. al.* 2016

		pole, hanging grip, wall, or touchscreen		*us*, and *Streptococcus*				
Portland, Oregon, USA	Buses and trains	cloth seats on buses, vinyl seats on trains, handholds and handrails, windows, floors, under seats, metal armrests	Swab	*S. xylosus*, *S. saprophyticus*, *S. cohnii*, *S. haemolyticus*, *S. epidermidis*, *and S.warneri*	ND	0%	NA	Yeh*et. al.* 2011
Columbus, USA	Buses	stanchions, seats, seat rails, and vehicle operator's area	Electrostatic wipes	*S. aureus* (68%) MDR (65%)	USA300 or USA 400 [SCCmec type IV (62.9%)], USA100[SCCmec type II (22.9%)]	63%	Erythromycin (80%), Ciprofloxacin (50%), Enrofloxacin (30%), Clindamycin (45%)	Lutz *et. al.* 2014

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