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Research Article

CHARACTERIZATION OF DRUG RESISTANT BACTERIA ISOLATED FROM SUBURBAN RAILWAY STATION PREMISES

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ABSTRACT
Pathogens in public areas *e.g.* public transport system can be a critical public health issue due to ease of transfer. Present study deals with isolation and characterization of pathogenic bacteria from touch surfaces of railway station premises. A total of 53 swab samples taken from different touch surfaces were processed by classical microbiological procedures. Isolates were subjected to antibiotic sensitivity test. Further modified disinfectant challenge test was used to compare effectiveness of four commonly used disinfectants. Overall total 73 isolates were obtained. After morphological, cultural and biochemical analysis 7 isolates were identified as Methicillin resistant *Staphylococcus aureus* (MRSA) and 6 isolates as extended spectrum β-lactamase producing (ESBL) *Escherichia coli.* By virtue, these all were multidrug resistant. A disinfectant challenge test revealed Sodium hypochlorite, Dettol, MEDNTEK - R82 were effective while Santavis, a locally available disinfectant cum floor cleaner was not effective at recommended dilutions. Presence of multi drug resistant pathogenic bacteria in premises of railway station is extremely worrisome finding. Appropriate cleaning and disinfection measures required to prevent dissemination of these pathogens.

Keywords: Public health, Methicillin resistant *Staphylococcus aureus,* Extended spectrum β-lactamase, *Escherichia coli,* Disinfection.

1. INTRODUCTION
Personal and environmental hygiene plays crucial role in spread of infectious disease agent. Pathogenic microbes have been isolated from various public places worldwide. Rise in antimicrobial resistance is alarming issue in entire medical fraternity. Drug resistant bacteria like MRSA (Methicillin resistant *Staphylococcus aureus*), ESBL (Extended spectrum β- lactamase) producing bacteria could easily get transmitted through public transportation creating a big public health issue [1]. There are only two studies illustrating presence of drug resistant pathogenic bacteria in public transportation systems in India [2, 3].
Overcrowded metropolitan city like Mumbai and its suburban region is prime hub of trade and business in state of Maharashtra, India. There is daily heavy commuter load on suburban railway system. Unhygienic and humid conditions may help in survival of many pathogenic microbes in station premises [4]. This study aims to isolate bacteria from various touch sites of suburban train station premise and characterize them on the basis of drug and disinfectant sensitivity.

2. MATERIAL AND METHODS
2.1. Sample Collection [2]
Sterile cotton swabs moistened with saline were used to wipe around 10 to 20 cm^2 areas of touch surfaces in station premises. Total 53 (N=53) swab samples obtained from Kalyan junction railway station premises as follows - Taps (n=11), chair armrests (n=17), escalator hand grips (n=10), stair railings (n=13), ticket vending machine (n=1), and lift keypad (n=1). All universal safety precautions were taken while collection, transportation, handling and processing of specimens. All specimens were processed within one hour of collection. The swab samples were transferred to tube containing sterile brain heart infusion broth and kept for incubation at 37°C for 48 hours. Only MRSA & ESBL were included in the present study while all other aerobic, anaerobic pathogens, Mycobacterium species, protozoa & viruses were excluded from the study.

2.2. Isolation and identification of bacteria [3, 12]

After enrichment a loopful sample from tubes showing turbidity was inoculated on sterile mannitol salt agar plate and sterile Macconkey agar plate. Plates were incubated at 37°C for 48 hours and observed for typical colonies of *Staphylococci spp.* and enteric microbes on mannitol salt agar and Macconkey agar respectively. Isolates were maintained on tryptic soy agar slants and identified using specific classical biochemical tests (Catalase test, Tube coagulase test, IMViC tests etc.). MeReSa chrome agar was to confirm MRSA (Himedia Laboratories, India).

2.3. Antibiotic susceptibility testing [5]

Antibiotic susceptibility was checked by Kirby Bauer disk diffusion method following CLSI guidelines (2018). Antibiotic discs used were as follows - Penicillin G (10 units), Ampicillin (10mcg), Cefoxitin (30mcg), Cefuroxime (30 mcg), Ceftazidime (30 mcg), Ceftazidime/ Clavulanic acid (25/5 mcg), Aztreonam (30 mcg), Rifampicin (5mcg), Tetracycline (30 mcg), Gentamicin (10 mcg), Erythromycin (15 mcg), Clindamycin (2 mcg), Nitrofurantoin (300 mcg), Linezolid (30 mcg), Enoxacin (10 mcg), Lomefloxacin (30 mcg), Ofloxacin (5 mcg), Levofloxacin (5 mcg), Moxifloxacin (5 mcg), Norfloxacin (10 mcg), Tobramycin (10 mcg), Prinstinamycin (30 mcg), Chloramphenicol (30 mcg), Trimethoprim (5 mcg), Co-trimoxazole (25 mcg). All the antibiotics were procured from Himedia Laboratories, India. Methicillin resistance was detected by virtue of cefoxitin resistance. Extended spectrum β-lactamase production was checked by phenotypic confirmatory disc diffusion test. Inducible clindamycin resistance was determined through D test. MRSA ATCC 43300 and *K. pneumoniae* ATCC 700603 were the standard reference strains used.

2.4. Disinfection challenge test [6]

A simple qualitative disinfectant challenge test was devised by modification of quantitative test to check comparative efficacy of four disinfectants-1) Sodium hypochlorite [0.05%, Loba chemie Pvt Ltd], 2) Dettol [1:40 dilution, Reckitt Benckiser Ltd], 3) MEDNTEK - R82 [1: 256 dilution, IMAEC MEDNTEK Ltd], 4) Santavis [1:400 dilution, SH Home Care Ltd.]. All disinfectants were diluted as per recommendation with distilled water. Each 1 ml culture suspension of bacteria (10^8 cfu/ml) was transferred to 4 ml of disinfectant solution. A loopful of this mixture was drawn at different time intervals (0 sec, 30 sec, 60 sec, 120 sec, 150 sec, 180 sec & 300 sec) and plated on sterile tryptic soy agar plates. Plates were incubated at 37°C for 48 hours to check appearance of growth. MRSA ATCC 43300 and *K. pneumoniae* ATCC 700603 were the standard reference strains used. Assay was performed in duplicate and data presented in tabular form as + (growth) and - (no growth).

3. RESULTS AND DISCUSSION

3.1. Isolation and identification of bacteria

Heavy growth was observed in broth after enrichment of all swab samples. When plated on media, total 68 isolates were obtained from 53 swab samples. The number and characterization of isolates from samples of different touch surfaces depicted in table 1 and 2. Out of 68 isolates, 54 (79.41%) were *Staphylococci spp*. (categorized from mannitol salt agar plates) and 14 (20.58%) were Gram negative isolates (categorized from MacConkey's agar plates). Out of 54 *Staphylococcus* species isolated, 7 (12.96%) were *S. aureus* and all turned out to be MRSA, which were confirmed through growth on MeReSa chrome agar. Prevalence of MRSA was 12.96 %, remaining isolates identified as coagulase negative *staphylococci* and gram positive rods were excluded from study. Out of 14 isolates considered from Macconkey agar plates, 6 (42.85%) were identified as *E. coli*. Interestingly all were ESBL producers through phenotypic confirmatory disc diffusion test prevalence of ESBL positive *E. coli* was 42.85 %. Not to surprise, all sites were contaminated with bacteria. Presence of *Staphylococci spp*. and *E. coli* could be attributed to frequent dermal contact, absence of routine cleaning, poor sanitation practice and lack of awareness among commuters. MRSA was found on taps, chair armrests and stair railings while ESBL producing *E. coli* was isolated from taps, chair armrests and stair railings and escalator hand grip. This is troublesome finding and proper measures for the sanitation of the said sites through appropriate procedures are recommended.

3.2. Antibiotic susceptibility testing

The susceptibility of MRSA and ESBL positive *E. coli* isolates to different antibiotics is described in Table 3 and 4 respectively. Graph 1 and 2 represent per cent susceptibility of bacteria under test. Among MRSA isolates maximum resistance was towards penicillin-G, cefoxitin, moxifloxacin, enoxacin, levofloxacin, lomefloxacin & linezolid. One of the MRSA isolates was

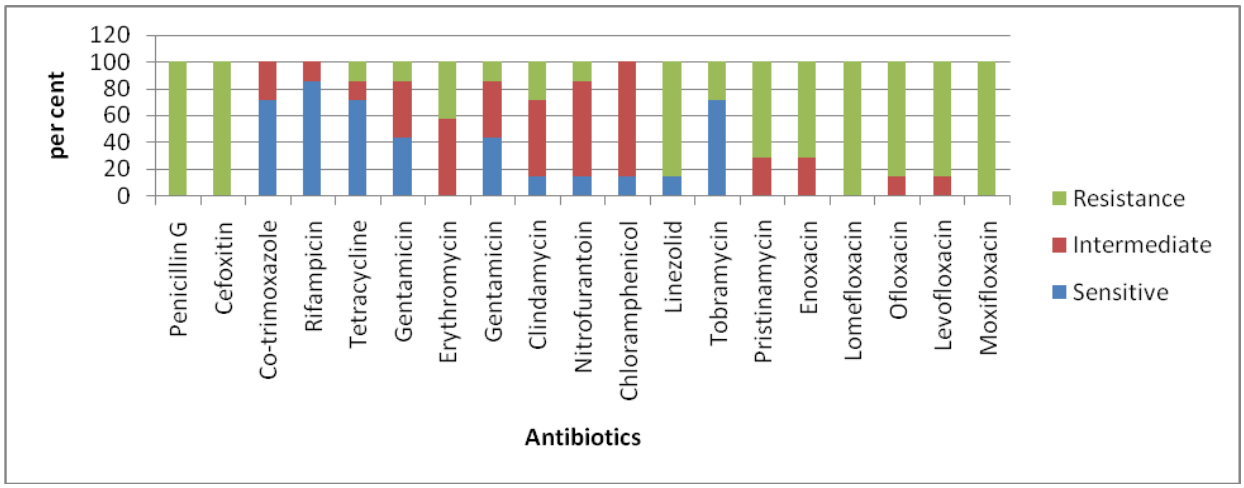
positive for inducible clindamycin resistance (D test positive). Among *E. coli* isolates maximum resistance was towards all β-lactam group antibiotics tested namely ampicillin, ceftazidime, ceftazidime/clavulanate, cefazolin, cefuroxime and aztreonam. Intermediate resistance to chloramphenicol higher in MRSA as well as *E. coli* isolates. For MRSA sensitivity was higher for co-trimoxazole, tetracycline, tobramycin & rifampicin while for *E. coli* it was for trimethoprim, ofloxacin and enoxacin. Among MRSA isolated from public transportation, resistance profile revealed to be similar with other studies [7-10] with highest resistance to penicillin-G, cefoxitin, erythromycin, ciprofloxacin. In case of *E. coli* isolated from public transport, very few studies revealing resistance pattern are available [11, 12]. When we compared those with present study, similarity was observed for resistance to ampicillin, co-trimoxazole, and chloramphenicol.

Table 1: Distribution of MRSA on different touch surfaces under study

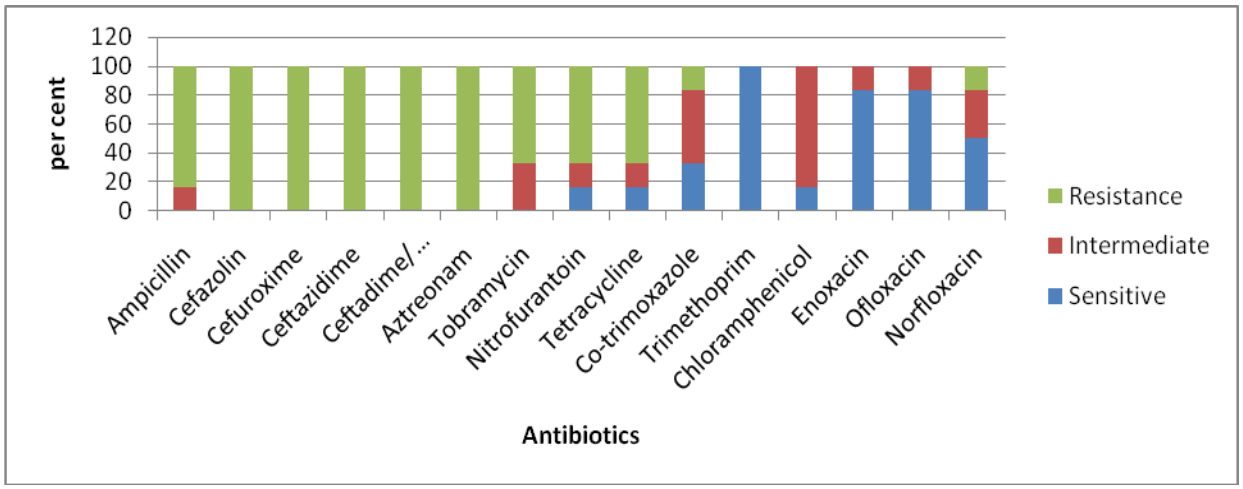
Touch surfaces	Samples (N = 53)	Isolates (Mannitol salt agar)	MRSA
Taps	11	13	4
Chair armrests	17	19	2
Escalator hand grip	10	7	0
Stair railings	13	12	1
Ticket vending machine	01	2	0
Lift keypad	01	1	0
		54	**7 (12.96%)**

Table 2: Distribution of ESBL producing *E. coli* on different touch surfaces under study

Touch surfaces	Samples (N=53)	Isolates (Macconkey agar)	ESBL producing *E. coli*
Taps	11	9	2
Chair armrests	17	3	2
Escalator hand grip	10	1	1
Stair railings	13	1	1
Ticket vending machine	1	0	0
Lift keypad	1	0	0
		14	**6 (42.85%)**



Graph 1: Percent susceptibility pattern of MRSA



Graph 2: Percent susceptibility pattern of ESBL positive *E. coli*

Table 3: Antibiotic sensitivity pattern of MRSA

Isolate ID	Sensitive	Intermediate	Resistant
SA D2	RIF, TE, TB	COT, GEN, NIT, C	PG, CX, E, CD, LZ, EN, LO, RP, OF, LE, MO
SA E5	COT	RIF, E, CD, NIT, C	PG, CX, TE, GEN, LZ, EN LO, TB, RP, OF, LE, MO
SA H2	RIF, TE, GEN, TB	COT, E, CD, NIT, C, EN, OF	PG, CX, LZ, LO, RP, LE, MO
SA I3	COT, RIF, TE	GEN, C, EN	PG, CX, E, CD, NIT, LZ, LO, TB, RP, OF, LE, MO
SA I5	COT, RIF, GEN,TB	TE, CD, NIT, C	PG, CX, E, LZ, EN, LO, RP, OF, LE, MO
SA I6	COT, RIF, TE, TB	GEN, E, CD, NIT, C, RP,	PG, CX, LZ, EN, LO, OF, LE, MO
SA L3	COT, FIR, TE, GEN, CD, NT, LZ, C, TB	E, RP, LE,	PG, CX, EN, LO, OF, MO

Key: Rifampicin (RIF, 5 mcg), Tetracycline (TE, 30 mcg), Tobramycin (TB, 10 mcg), Co-trimoxazole (COT, 25 mcg), Gentamicin (GEN, 10 mcg), Nitrofurantoin (NIT, 300 mcg), Chloramphenicol (C, 30 mcg), Penicillin-G (PG, 10 units), Cefoxitin (CX, 30 mcg), Erythromycin (E, 15 mcg), Clindamycin (CD, 2mcg), Linezolid (LZ, 30 mcg), Prinstinamycin (RP, 15 mcg), Enoxacin (EN, 10 mcg), Lomefloxacin (LO, 30 mcg), Ofloxacin (O, 5 mcg), Levofloxacin (LE, 5 mcg), Moxifloxacin (MO, 5 mcg)

Table 4: Antibiotic sensitivity pattern of ESBL positive *E. coli*

Isolate ID	Sensitive	Intermediate	Resistant
EC A1	TR, EN, OF	TB, TE, NX, COT, C,	AMP, CAZ, CAZ/CL, AO, NIT, CZ, CU
EC A2	NIT, NX, TR, EN, OF	COT, C	AMP, CAZ, CAZ/CL, AO, TB, TE, CZ, CU
EC A4	TE, COT, C, TR	TB, NIT, EX, OF	AMP, CAZ, CAZ/CL, AO, NX, CZ, CU
EC A5	NX, COT, TR, EN, OF	C	AMP, CAZ, CAZ/CL, AO, TB, NIT, TE, CZ, CU
EC A6	NX, TR, EN, OF	C	AMP, CAZ, CAZ/CL, AO, TB, NIT, TE, COT, CZ, CU
EC A7	TR, EN, OF	AMP, NX, COT, C	CAZ, CAZ/CL, AO, TB, NIT, TE, CZ, CU

Key: Trimethoprim (TR, 5 mcg), Enoxacin (EN, 10 mcg), Tobramycin (TB, 10 mcg), Ofloxacin (O, 5 mcg), Norfloxacin (NX, 10 mcg), Tetracycline (TE, 30 mcg), Co-trimoxazole (COT, 25 mcg), Chloramphenicol (C, 30 mcg), Ampicillin (AMP, 10 mcg), Ceftazidime (CAZ, 30 mcg), Ceftazidime/ clavulanic acid (CAZ/CL, 25 mcg), Aztreonam (AO, 30 mcg), Nitrofurantoin (NIT, 300 mcg), Cefazolin (CZ, 30 mcg), Cefuroxime (CU, 30 mcg)

3.3. Disinfection challenge test

Table 5 illustrates effect of disinfectants on isolates of MRSA & ESBL positive *E. coli*. Most commonly used disinfectants were quite effective against bacteria under test irrespective of drug resistance level. Hypochlorite, Dettol & R-82 were highly effective in inactivating bacteria under test up to 30 seconds of exposure at specified dilution. However local pine oil based disinfectant cum floor cleaner, Santavis failed to destroy bacteria even after 5 minutes of exposure at

recommended dilution. The disinfectant challenge test performed indicated that hypochlorite, Dettol (chloroxylenol, pine oil, isopropanol, castor oil, soap) and quaternary ammonium compound based MEDNTEK - R82 had best effect on MRSA and ESBL producing *E.coli*. There is paucity of data regarding susceptibility of drug resistant pathogenic bacteria isolated from public transport systems & public places to different disinfectants but number of studies available that explain higher sensitivity to disinfectants with mentioned active ingredients [13-15]. Hypochlorite could be a cheap and best alternative for sanitization even recommended by WHO and CDC during COVID-19 pandemic for environmental surface disinfection [16]. Activity of locally available disinfectant cum floor cleaner was not up to the mark when compared with other disinfectants using mentioned test. Disinfection policies should take into account the reasons and purposes for which disinfectants are used.

Table 5: Effect of different disinfectants on survival of MRSA & ESBL positive *E. coli*

		Growth response at different exposure time period in seconds							
Isolate ID	**Name of Disinfectant**	**0**	**30**	**60**	**90**	**120**	**150**	**180**	**300**
MRSA ATCC 43300	Hypochlorite	++	--	--	--	--	--	--	--
	Dettol	++	--	--	--	--	--	--	--
	R-82	++	--	--	--	--	--	--	--
	Santavis	++	++	++	++	++	++	++	++
MRSA (N = 7)	Hypochlorite	++	--	--	--	--	--	--	--
	Dettol	++	+-	--	--	--	--	--	--
	R-82	++	+-	--	--	--	--	--	--
	Santavis	++	++	++	++	++	++	++	++
***K. pneumoniae* ATCC 700603**	Hypochlorite	++	--	--	--	--	--	--	--
	Dettol	++	--	--	--	--	--	--	--
	R-82	++	--	--	--	--	--	--	--
	Santavis	++	++	++	++	++	++	++	++
ESBL (N = 6)	Hypochlorite	++	--	--	--	--	--	--	--
	Dettol	++	--	--	--	--	--	--	--
	R-82	++	+-	--	--	--	--	--	--
	Santavis	++	++	++	++	++	++	++	++

Key: + = growth, - = no growth

4. CONCLUSION

An existence of MDR bacteria such as MRSA and ESBL positive *E. coli*on the touch surfaces of the railway station premises is troublesome finding indicating potential threats of transmission of infections among travellers. This is of a great public health concern as the mass population of different health condition is at risk of direct exposure. Hence it is advisable to consider proper measures for sanitation routinely.

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Conflicts of interests

There are no conflicts of interest.

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6. REFERENCES

1. Cave R, Cole J, Mkrtchyan HV, et al. *Enviro Intern,* 2021; **157(106836):**1-13.
2. Vinod Kumar CS, Prasad S, Patil S, Reddy A, Jha A, Raghu Kumar KG, et al. *Int J Res Med Sci,* 2017; **6(1):**172-176.
3. Rodrigues J, Thommana L, Ravi S, Putta R, Jain S, Lele O, et al. *Int J Enhan Res Sci Tech &Engi,* 2022; **11(3):**54-59.
4. Chowdhury T, Mahmud A, Barua A, Khalil I, Chowdhury R, Ahamed F, et al. *J Enviro Sci Toxicol and Food Technol,* 2016; **10(4):**48-55.
5. CLSI, Performance Standards for Antimicrobial Susceptibility Testing; 28th ed. CLSI Supplement: M100.Wayne, P.A.: Clinical Laboratory Standards Institute; 2018.
6. Kamal M, Khalaf MA, Ahmed ZA, Jakee J, et al. *Veterinary World,* 2019; **12(12):** 2025-2035.

7. Peng Y, Ou Q, Lin D, Xu P, Li Y, Ye X, et al. *Scientific reports,* 2015; **5(16087):**1-5.
8. Iwao Y, Yabe S, Takano T, Higuchi W, Nishiyama A, Yamamoto T, et al. *Microbiol and Immunol,* 2012; **56:**76-82.
9. Angbuhang KB, Neupane M, Adhikari A, Binita KC, Jha S, et al. *Trib Univ J Microbiol.,* 2018; **5(1):**51-56.
10. Lutz JK, Balen J, Crawford JM, Wilkins JR, Lee J, Nava-Hoet RC, et al. *Ame J Infect Con,* 2014; **42:**1285-90.
11. Shen C, Feng S, Chen H, et al. *Clin Infect Dis,* 2018; **67(S2):**17–24.
12. Kahsay AG, Asgedom SW, Weldetinsaa HL, et al. *BMC research notes,* 2019; **12 (337):**1-5.
13. Ghosh S, Bandyopadhyay S, Paul S, Paul A, Adhya S, et al. *J Den Med Sci,* 2016; **15(4):**29-32.
14. Banerjee T, Filgona J, Anupurba S, et al. *Int J Infect Con,* 2013; **9**(11):1-5.
15. Pereira S, Milon de Oliveira H, Turrini R, Lacerda R, et al. *Rev Esc Enferm USP,* 2015; **49**(4): 675-681.
16. Interim gusidance, 15 May 2020, Cleaning and disinfection of environmental surfaces in the context of COVID-19: Interim guidance. World Health Organization, 1-8.