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Methicillin resistant *Staphylococcus aureus* (MRSA) from community associated settings

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ABSTRACT

Antibiotic resistance is a cause of concern worldwide. Community settings are important reservoir of drug resistant microorganisms like *Staphylococcus aureus*. The present study was to determine the prevalence, phenotypic and genotypic antibiotic resistance pattern of *S. aureus* isolated from different community settings of university campus. A total of 300 swab samples were collected for isolation of *S. aureus* from different community settings at university campus of Guru Angad Dev Veterinary and Animal Sciences University and Punjab Agriculture University, Ludhiana, India. Confirmed *S. aureus* isolates were further subjected to antibiotic sensitivity by Epsilometer test (E-test) and detection of antibiotic resistance genes. The prevalence of *S. aureus* in the community samples was 12% (36/300). Methicillin Resistant *S. aureus* (MRSA) contamination among community was 3.33% (10/300). Among *S. aureus* isolates from community samples 63.8% (23/36) and all the MRSA isolates were multidrug resistant (MDR). Five out of 10 MRSA carried SCC*mec* type IVa, and 4 were *pvl positive gene*, therefore, designated as community associated MRSA (CA-MRSA). Phenotypic resistance to antibiotics ciprofloxacin, chloramphenicol, ceftriaxone clindamycin and trimethoprim-sulfamethoxazole was 69.4% (MIC ≥32 µg/ml), 63.9% (MIC 32 µg/ml), 16.7% (MIC 16–64 µg/ml), 16.7% (MIC 256 µg/ml) and 8.3% (MIC 12–64 µg/ml), respectively. Resistance genes *bla*Z, *mec*A, *tet*K, *tet*M, *erm*B and *aac*A-*aph*D were present. Presence of MRSA and MDR variant in community settings is a public health concern, as cell phone, offices telephone, computer keyboard and tap faucet are commonly shared or touched by people. Therefore, have potential to disseminate widely, not only in the community settings but also in hospitals environment, complicating treatment.

Keywords: Antibiotic resistance (AR), Antibiotic resistance genes, Community, Epsilometer test, MIC values, Multidrug resistance (MDR), *Staphylococcus aureus*

Antibiotic resistance is a public health issue worldwide. Community settings are important source of drug resistant microorganisms like *S. aureus*. It is commonly present on skin, mucous membrane of animal and human, in soil and water (Irlinger 2008). It is also an important food-borne pathogen (Morgan 2008). Though it could be found in other animals and environment, human is the major reservoir for *S. aureus* (Moellering 2006). Control and treatment options for this organism are complicated by its remarkable ability to adapt to its environment especially through the acquisition of antibiotic resistance determinants. The MRSA especially, Community Associated-MRSA (CA-MRSA) infections are of particular public health concern because they result in serious diseases including necrotizing fasciitis and necrotizing pneumonia. The high morbidity, mortality and cost of care associated with the organism highlights the need for public health agencies, hospitals, and other laboratories to accurately identify these microorganisms.

In the past, MRSA infection was limited to people who had history of recent hospitalization, (Moellering 2006). However, it has also been reported from community settings such as student homes, university campus and public transportation system in the community (Roberts *et al*. 2011).

It is important to identify antibiotic resistant (AR) patterns of *S. aureus* strains from different sources, especially from community settings due to their changing patterns of resistance and its potential to serve as a reservoir of AR genes. Emergence of antibiotic resistance in India is also a concern. People frequently come in contact with community settings unaware of health risk posed by antibiotic resistant microorganisms, especially, *S. aureus*. Therefore, the aim of this study was to determine the prevalence, and antibiotic resistance pattern of *S. aureus* isolated from different community settings.

MATERIALS AND METHODS

A total of 300 random samples of community origin from August 2013 to March 2014 were collected from different places of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) and Punjab Agricultural University (PAU) campus, Ludhiana, Punjab, India

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Table 1. Swab samples collected from different community places at GADVASU and PAU campus

Source	No. of swab samples collected
Cell Phone	29
Office telephone	18
Door handle	31
Tap faucet	25
Computer keyboard	46
Computer Mouse	30
Hand swab	73
Nasal swab	18
Kitchen counter top	24
Chair arm	6
Total	300

(Table 1). The sterile normal saline (0.85%) moistened swabs was rubbed firmly over the entire surface of the specified object and immediately put back in the sterile swab tube. If the surface area was large, additional swabs were used and all were counted as one sample. For nasal swabbing, two dry swabs, one each for each side of the nose were used. After swabbing, swabs were put in a test tube containing sterile saline. The collected swabs were immediately transported to laboratory on ice and processed for isolation of *S. aureus* within two hours or stored in refrigerator and processed next day.

Isolation and identification of S. aureus: *Isolation* of *S. aureus* from swab samples was performed as per the Bacteriological Analytical Manual (Food and Drug Administration). Trypton soy broth (TSB) plus 7% sodium chloride (NaCl) and 1% sodium pyruvate was used for enrichment and Baird-Parker agar (BPA) supplemented with egg-yolk tellurite emulsion was used as a selective agar. Biochemical identification was done as mentioned by Zehra *et al.* (2017). Biochemically positive isolates were further confirmed my molecular detection targeting 16SrDNA (genus specific detection) and *femA* gene (for species detection) using primers described by Strommenger *et al.* (2003) and Duran *et al.* (2010), respectively, in a multiplex PCR mentioned ahead. The confirmed *S. aureus* isolates were maintained in 20% (v/v) glycerol at –80°C till further processing.

Antibiotic susceptibility testing of S. aureus *strains:* The antibiotic susceptibility testing (AST) of *S. aureus* isolates was performed by the Epsilometer test (E-test). All the selected *S. aureus* isolates were tested for their sensitivity to various antibiotics, viz. oxacillin, penicillin, tetracycline, chloramphenicol, co-trimazole, ceftriaxone, gentamicin, erythromycin, ciprofloxacin, and vancomycin using Ezy MIC^{TM} strip (HiMedia Lab, Mumbai).

Identification of methicillin resistant (*mecA*) *gene: S. aureus* strains were also tested for the presence of methicillin resistance genes *(mec*A) in a multiplex PCR also targeting 16SrDNA and *fem A genes*. Isolation of genomic DNA from biochemically positive *S. aureus* strains was done using HiPurATM bacterial genomic DNA purification kit (HiMedia Lab, Mumbai).

S. aureus strains ATCC 33591 and ATCC 33592 were used as MRSA (*mec*A +ve) and MSSA (*mecA* -ve) control, respectively.

The cycling conditions of multiplex PCR were as per methodology of Strommenger *et al.* (2003) and Zehra *et al.* (2017).

SCCmec typing, subtyping and pvl *gene:* The multiplex PCR for the typing and subtyping of *SCCmec* was performed using primers and method described by Zhang *et al*. (2005)

ATCC 700699 was used as *SCCmec* type II positive/*pvl* negative control, KP896298, KR610412 and KT005393 were used as *pvl, SCCmec type* IV and V positive control.

The PCR targeting *pvl* gene was also performed using primers and method of Wang *et al.* (2012). All the PCR amplicons were visualized using a UV light box after electrophoresis on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide.

Statistical analysis: Microsoft excel was used for statistical analysis. The categorical variables were compared using a Pearson Chi-square or Fisher's exact test, as appropriate. Differences were considered significant when the P-value was < 0.05.

RESULTS AND DISCUSSION

The prevalence of *S. aureus* from community associated samples revealed their presence in total of 36 samples (12% (36/300): 95% CI, 11.96 to 12.04%) Table 2. The maximum contamination of *S. aureus* was reported from chair arms (50%), and minimum from kitchen counter top (4.2%). computer keyboards (10.9%) and the door handles (6.5%) which are frequently used in offices were also contaminated. These results are in concordance with the other studies. Oie *et al.* (2002) reported 27% door handles of 196 rooms contaminated with *S. aureus*. Tambekar *et al.* (2007) found 41 samples (13.2%) positive for *S. aureus* out of 310 samples consisting of doctor's mobile phone, wound/pus and hand swabs. Ugwu *et al.* (2013) isolated 53 *S. aureus* isolates from nostril of 100 healthy people. Al-Abdalall *et al.* (2010) identified 56.58% *S. aureus* contamination of mobile phones in the city of Dammam, Saudi Arabia. Based on these results it can be concluded that contamination of community associated resources with *S. aureus is* common as these organisms are part of normal flora of human and animals. They are able to survive on dry surfaces relatively better than other bacterial agents. Since S. *aureus* is also known to cause minor skin infections to sever necrotizing pneumonia. Therefore, presence of these organisms on surfaces and places commonly shared in the community is a cause of concern.

All the *S. aureus* isolates irrespective of their sources were resistant to penicillin (Table 3). Resistance to antibiotic erythromycin was also high (97.2%, 35/36). Antibiotics ciprofloxacin and chloramphenicol was also ineffective in high number with resistance percentage of 69.4%, MIC ≥32 µg/ml and 63.9%, MIC 32 µg/ml, respectively (Table 3). Complete resistance (100%) to ciprofloxacin was reported

Table 2. Prevalence of *S. aureus* in different community samples

Samples source	No. of samples	Samples Positive for *S. aureus*	Prevalence (%)
Cell phone (CP)	29	5	17.3%
Telephone (T)	18	5	27.78%
Door handle (DH)	31	2	6.5%
Tap faucet (TF)	25	4	16%
Computer keyboard (CK)	46	5	10.9%
Computer Mouse (CM)	30	2	6.67%
Hand swab (HS)	73	7	9.6%
Nasal swab (NS)	18	2	11.1%
Kitchen counter top (KCT)	24	1	4.2%
Chair arm (CA)	6	3	50%
Total	300	36	12%

in isolates from door handle, tap faucet, chair arm and nasal swabs. Though low but intermediate resistance to ceftriaxone is of concern because ceftriaxone (third generation of cephalosporin) is used to treat animals and humans especially children (Frye and Fedorka-Cray, 2007).

The resistant isolates were commonly reported from office telephone, tap faucet, computer keyboard, hand swab and cell phones. Two isolates from computer keyboard, one isolate from nasal swab and one from chair arm were resistant to tetracycline and co-trimoxazole, respectively. All the *S. aureus* isolates obtained during the study were sensitive to gentamicin except one from computer keyboard. Majority of isolates were in susceptible range to vancomycin which is the drug of choice in people with MRSA infection, though 5 isolates were close to intermediate resistance, which included one each from office telephone, tap faucet, chair arm and two from hand swab, this need further confirmation with broth dilution method. A number of *S. aureus* isolates, 17 and 18 showed phenotypic resistant to antibiotics oxacillin and methicillin, respectively (Table 3).

Resistance of *S. aureus* to various antibiotics has also been observed in different studies. Similar to the observation of this study, high resistance to antibiotic penicillin has been documented by others (Tambekar *et al.* 2007, Aso *et al.* 2011 and Dan *et al.* 2013). Tambekar *et al.* (2007) found 100% of their *S. aureus* isolates from hand swab and cell phone resistant to penicillin. Resistance to other antibiotics such as co-trimoxazole, erythromycin, ceftriaxone and vancomycin was also reported in *S. aureus* isolates from hands and cell phone but with varying resistance pattern in their study. In another study as per Aso *et al.* (2011), which was basically done on samples in hospital with sampling from door knob, cell phone, pillow, bed sheet and stethoscope reported high resistance to penicillin (98.37%). Resistance to antibiotics chloramphenicol, ciprofloxacin and erythromycin ranged from 4.8% to 29.2% which is lower as compared to resistance pattern of isolates in present study. MIC values of isolates to vancomycin in present study, close to intermediate resistance range is a cause of

Table 3. Antibiotic resistance spectrum of *S. aureus* isolates from community associated samples

Antibiotic	Number of resistant isolates									
	No. of isolates resistant (%) n=36	From cell phone (%) n=5	From telephone (%) n=6	From Door handle (%) n=2	From tap faucet (%) n=5	From computer keyboard % n=5	From hand swab (%) n=7	From nasal swab (%) n=2	From kitchen counter top (%) n=1	From chair arm (%) n=3
Oxacillin	17 (47.2%)	2 (40%)	4 (66.7%)	1 (50%)	2 (40%)	2 (40%)	1 (14.2%)	2 (100%)	1 (100%)	2 (66.7%)
Penicillin	36 (100%)	5 (100%)	6 (100%)	2 (100%)	5 (100%)	5 (100%)	7 (100%)	2 (100%)	1 (100%)	3 (100%)
Tetracycline	3 (8.3%)	–	–	–	–	2 (40%)	–	1 (50%)	–	–
Clindamycin	6 (16.7%)	1 (20%)	1 (16.7%)	–	2 (40%)	1 (20%)	1 (14.2%)	–	–	–
Ceftriaxone	6 (16.7%)	1 (20%)	1 (16.7%)	1 (50%)	1 (20%)	1 (20%)	1 (14.2%)	–	–	–
Gentamicin	1 (2.8%)	–	–	–	–	1	–	–	–	–
Erythromycin	35 (97.2%)	5 (100%)	6 (100%)	2 (100%)	5 (100%)	5 (100%)	6 (85.7%)	2 (100%)	1 (100%)	3 (100%)
Ciprofloxacin	25 (69.4%)	3 (60%)	5 (83.3%)	2 (100%)	5 (100%)	3 (60%)	3 (42.8%)	2 (100%)	–	2 (66.6%)
Chloramphenicol	23 (63.9%)	4 (80%)	1 (16.7%)	1 (50%)	3 (60%)	4 (80%)	6 (85.7%)	1 (50%)	–	3 (100%)
Vancomycin	5 (13.8%)	–	1 (16.7%)	–	1 (20%)	–	2 (28.5%)	–	–	1 (33.3%)
Co-trimaxazole	3 (8.3%)	–	–	–	–	2 (40%)	–	–	–	1 (33.3%)
Methicillin	18 (50%)	3 (60%)	3 (50%)	1 (50%)	2 (40%)	1 (20%)	3 (42.8%)	2 (100%)	1 (100%)	2 (66.6%)

Table 4. Prevalence of multi-drug resistance (MDR) *S. aureus* in different community setting, SCC*mec* typing and *pvl* gene

Source	No. of isolates	MDR (%) *S. aureus*	No. of MRSA	MDR (%) MRSA	Isolate	SCC*mec* type/*Pvl*
Cell phone	5	4 (80%)	1 (20%)	1 (100%)	CP-2	IV a/+
Telephone	5	3 (60%)	3 (60%)	3 (100%)	TP-1	NT/+
					TP-2	NT/-
					TP-10	IV a/+
					TP-11	NT/-
Door handle	2	1 (50%)	0	0	-	-
Tap faucet	4	2 (50%)	1 (25%)	1 (100%)	TF-6	IV a
Computer mouse	2	1 (50%)	0	0	-	
Computer keyboard	5	3 (60%)	1 (20%)	1 (100%)	KB-10	NT/+
Hand swab	7	4 (57.1%)	1 (14.2%)	1 (100%)	HS-24	NT
Nasal swab	2	1 (50%)	0	0		
Kitchen counter top	1	1 (100%)	1 (100%)	1 (100%)	KT-17	IV a/+
Chair arm	3	3 (100%)	1 (33.3%)	1 (100%)	CA-4	IV a/+
Total	36	23 (63.8%)	10 (27.7%)	10 (100%)	10	

concern as this antibiotic is considered as an alternative to the treatment of MRSA infections. However, further confirmation by broth dilution method is needed to re-check their susceptibility status to vancomycin before drawing any conclusion.

Prevalence of Methicillin Resistant S. aureus (MRSA): Out of 36 *S. aureus* isolates, from various community sources, 10 (27.8%) were found to have *mec*A gene. None of the isolates from computer mouse, nasal swab and door handle were MRSA (Table 4).

Presence of MRSA has been reported from various community sources such as computer keyboard, cell phone, door handles, student home, nasal swabs and university campus (Oie *et al.* 2002, Kaseem *et al.* 2007 and Shaha by *et al.* 2012). In one such study Kaseem *et al.* (2007) isolated *S. aureus* from computer keyboards (20.83%), out of which two were MRSA. In another study Shahaby *et al.* (2012) found mobile phone to be highly contaminated with several pathogenic bacteria including *S. aureus* (27.9%). However, none of their *S. aureus* isolates were MRSA. MRSA contaminating door handles has also been reported, however these isolates were from door handles in wards of university hospital (Oie *et al.* 2002). In that study out of 196 room examined, 53 were contaminated with *S. aureus*, out of which 17 were MRSA, indicating the importance of these isolates in hospital environment and potential to spread from hospital to community. None of the isolates from door handle in current study were MRSA. Presence of *S. aureus* in nasal swab has also been documented. Shopsin *et al.* (2000) in their study reported 35 and 28% of nasal swabs from children and guardian, respectively, contaminated with *S. aureus* and only one swab sample had MRSA, indicating that people can carry MRSA in their nasal cavity which can act as source of contamination to other source through poor hygiene.

Multidrug resistance of S. aureus *isolates:* Most of the *S. aureus* strains (23/36, 63.8%) were resistant to three or more than three antibiotics and were multidrug-resistant *S. aureus* (Table 4). All the MRSA isolates (n=10) were also MDR (Table 4). Majority of the isolates from chair arm, cell phone, kitchen counter top and hand swab were MDR. MDR *S. aureus* has been reported from community sources by Tambekar *et al.* (2007) and Torimiro and Torimiro (2012). Torimiro and Torimiro (2012) in their study found 85% of their *S. aureus* isolates from community associated sources to be MDR and 4 were MRSA isolates. Similarly, Tambekar *et al.* (2007) in their study found 67% of CA-MRSA were MDR.

There was no significant difference between the percentage of antibiotic resistance among the hand/nasal and community *S. aureus* isolates (Fisher's exact test; P = 0.478) but the prevalence of MDR *S. aureus* was higher on inanimate objects (50 to 100%) as compared to hand/nasal samples (50 to 57.1%), although not significant. The data of this study demonstrates that community settings are frequently contaminated with multidrug resistant *S. aureus* which is the public health concern, because direct or indirect contact with individuals colonized with resistant bacteria is the most documented route of transmission in the community and hospitals. Improper self-medication by people, easy availability of drug across the counter are the important factors contributing to MDR among community.

Presence of MDR isolates on community associated sources which are frequently shared necessitates awareness of community on importance of personal hygiene to arrest spread of antibiotic resistance, rational use of antibiotics coupled with regular monitoring of community thriving microorganisms for the development of antibiotic resistance.

SCC mec *typing and subtyping:* On SCC*mec* typing and sub typing of MRSA, five (50%, 5/10) MRSA isolates were SCC*mec* type IVa and 4 of them were carrying the *pvl* gene (Table 4). SCC*mec* type IV and V are frequently associated with CA-MRSA, particularly type IV along with the carriage of the *pvl* gene. Dan *et al*. (2013) reported 22 MRSA isolates from 2,103 nasal swab of healthy students belonging to SCC*mec* type IV (15/22), type V (6/22) and one was non-typeable (NT). They found 10 isolates out of 22 were carrying the *pvl* gene. Roberts *et al*. (2013) isolated 55 MRSA isolates from student home, university campus, local community and water samples and 36 (65.4%) isolates

were SCC*mec* type IV, 5 (9%) were type I, 1 (1.8%) was type II while 13 (23.6%) were non-typeable. Based on the typing results of SCC*mec* element, MRSA in the present study are community associated. These MRSA isolates which are also positive for *pvl* genes can easily disseminate in hospital settings through community sources, establish there, resulting in serious nosocomial infections.

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