

Original Research Article

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Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus aureus* from the Retail Meat Shops and Customers

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ABSTRACT

This study was completed to know the prevalence of Methicillin-Susceptible (MSSA) and Methicillin-Resistant *S. aureus* (MRSA) in swab samples from retail meat shops (RM) and customers (CU) in five districts of Punjab, India. An aggregate of 182 swabs samples was aseptically collected from RM shops and customers. The collected samples were processed for an isolation of *S. aureus* isolates. The phenotypic resistance of *S. aureus* isolates was most noteworthy to Penicillin (PEN, 97.83%) trailed by Ciprofloxacin (CPH, 56.52%), Tetracycline (TET, 36.96%), Trimethoprim-Sulfamethoxazole (TSH, 34.78%) and Erythromycin (ERY, 17.39%). However, low resistance was observed to Clindamycin, Chloramphenicol, Oxacillin, Ceftriaxone, and that fluctuated from 2%-7%. None of the isolates was phenotypically resistant to vancomycin (MIC 0.5-2 µg/ml). A large portion of *S. aureus* isolates (58.69%, 95% CI 43.63-61.93) were Multi-drug resistant (MDR) and carried resistant genes to penicillin (*blaZ*), oxacillin (*mecA*), gentamicin (*aacA-aphD*), erythromycin (*ermB*, *ermC*) and tetracycline (*tetK*, *tetM*). Two *S. aureus* isolates were borderline oxacillin resistant (BORSA) with MIC 4 µg/ml and one isolate was MRSA (Oxacillin MIC 16 µg/ml) with a genotypic profile, *mecA*⁺*blaZ*⁺*aacA-aphD*⁺*tetK*⁺*ermC*⁺. Among the erythromycin-resistant or intermediate resistant isolates, none expressed inducible macrolide lincosamide and streptogramin (MLS_B) phenotype (ERY+/CLI-, D+) except for one MSSA isolates from CU hand swab sample that demonstrated a constitutive MLS_B phenotype (Erm+/Cli+, D-).

Keywords

Staphylococcus aureus, Multidrug resistance, Epsilometer test, MRSA, BORSA, Swab

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Introduction

Antibiotic resistance and its exchange to different microorganisms are turning into a rising and serious pattern in developing countries like India. Community related sources are one such source that are

imperative in harboring and dissemination of drug resistant microorganisms like *S. aureus*. *S. aureus* is universal in nature and ordinarily present on the skin and mucous membrane of animal and human, in soil and water (Irlinger 2008). It is likewise an imperative food-borne pathogen (Morgan 2008). In spite of the fact

that it could be found in other animal and environment, a human is the noteworthy reservoir for *S. aureus* (Moellering, 2006). *S. aureus* resistance to oxacillin carrying *mecA* gene, presently known as MRSA, was first reported in the year 1961 and from that point forward it has been reported in isolates from hospitals, food, animals, community and environment (Ogata *et al.*, 2012). MRSA isolates have been recognized in people in the community who do not have the conventional risk factors for *S. aureus* infection.

The CA-MRSA infections are of specific general wellbeing concern since they result in serious infections including necrotizing fasciitis and necrotizing pneumonia. The high morbidity, mortality, and cost of care related with organism features the requirement for public health agencies, hospitals, and other research facilities to precisely recognize these microorganisms.

Before, the development of MRSA infection was for all intents and purposes constrained to individuals who had history of ongoing hospitalization. Other than its presence in hospital environment, MRSA has likewise been reported from community settings (Roberts *et al.*, 2011). MRSA has been isolated from understudy homes, university campus and public transportation system in the community (Roberts *et al.*, 2011).

The development of antibiotic resistance in India is a major issue as antibiotic resistance particularly MRSA has been reported from hospitals in India (INSAR 2013, Nadig *et al.*, 2012, D'Souza *et al.*, 2010). However, antibiotic resistance of *S. aureus* isolates from retail meat shops and customers has not been pursued aggressively, particularly in Punjab. Consequently, the point of this study was to determine the prevalence, MRSA and furthermore characterize AR pattern of *S. aureus* phenotypically and genotypically.

Materials and Methods

Collection of samples

A total of 182 swab samples from retail meat shops and customers were collected for the isolation of *S. aureus* from 5 districts of Punjab, India. Swab samples from retail meat shops (RM) included samples from chopping block (34), butcher's hand (37), and chopping knife (38). Region wise collection of different swab samples in the present study is given in Table 1. Swabs were transported back to the laboratory on ice and handled for isolation of *S. aureus* within 6 hours of collection.

Isolation and identification of *S. aureus*

Isolation of *S. aureus* from the swab samples was endeavored according to method suggested by Bacteriological Analytical Manual 2012 (Bennett and Lancefield, 2001) after making necessary modifications according to Zehra *et al.*, 2017. Colonies with typical morphology were then exposed to Gram staining and catalase test. Gram and catalase positive isolates were biochemically recognized as *S. aureus* utilizing the HiStaph™ Identification Kit (HiMedia Labs, Mumbai). These *S. aureus* isolates were purified and maintained in 20% (v/v) glycerol at -20°C.

Antibiotic susceptibility testing of *S. aureus* isolates

The antibiotic susceptibility testing (AST) of *S. aureus* isolates was performed by the Epsilometer test (E-test, Figure 1). All the *S. aureus* isolates were tested for their affectability to different antibiotics *viz.* Oxacillin, Penicillin, Tetracycline, Chloramphenicol, Co-trimazole, Ceftriaxone, Gentamicin, Erythromycin, Ciprofloxacin, and Vancomycin using Ezy MIC™ strip (HiMedia Lab, Mumbai). AST for

amoxicillin/clavulanic acid (β -lactamases hyperproduction) and D-test (inducible clindamycin resistance) was performed by disc diffusion method according to CLSI guidelines (M100-S21).

Identification of antibiotic-resistant genes

All the *S. aureus* isolates were likewise screened for the presence of following antibiotic resistance genes: *blaZ*, *mecA*, *aacA-aphD*, *erm* (*ermA*, *ermB*, *ermC*), *tet* (*efflux genes tetK and tetL*, *tetM and tetO of the ribosomal protection (RP) family*) and *vanA* encoding for Penicillin, Oxacillin, Gentamicin, Erythromycin, Tetracycline and Vancomycin resistance, respectively, by amplification of the existing gene utilizing multiplex Polymerase Chain Reaction (PCR) (Table 1).

A *S. aureus* strain ATCC 33591 and ATCC 33592 was used as MRSA (*mecA* +ve) and MSSA (*mecA* -ve) positive control, respectively. KU872013, KP834338/KP834339, KP658721, KP658723, KP886833, KT454736, KT454737, *S. aureus* isolates were used as positive control for genes *blaZ*, *aacA-aphD*, *tetK*, *tetL*, *tetM*, *ermB*, *ermC*, respectively.

The isolation of genomic DNA from *S. aureus* strains was done using HiPurA™ bacterial genomic DNA purification kit (HiMedia Lab, Mumbai). Each isolate was subjected to a separate multiplex PCR assays for a detection of each group (gp) of genes: gp1 (*16S rDNA*, *nuc*, *mecA*); gp2 (*tetK*, *tetL*, *tetM* and *tetO*); gp3 (*ermA*, *ermB*, *ermC* and *aacA-aphD*); gp4 (*coa* and *blaZ*) as detailed in Zehra *et al.*, (2017). Separate PCR was run for *vanA* gene. The cycling conditions of multiplex PCR for gp1, gp2 and gp3 and of single PCR for *vanA* gene were as per methodology of Strommenger *et al.*, 2003 (with little modifications as per Zehra *et al.*,

2017) and Saha *et al.*, 2008, respectively. However, cycling condition for *coa* and *blaZ* was as per the methodology of Zehra *et al.*, (2017).

Statistical analysis

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

Results and Discussion

Prevalence of *S. aureus*

A total 46 swab samples out of 182 were positive for *S. aureus* culturally and affirmed through genus and species-specific PCR, resulting in an overall prevalence of 25.27% (95% CI 15.97-34.57; Table 2, Figure 2).

Among the swab samples from RM shops, *S. aureus* was frequently isolated from butcher's Knife (47.37%) trailed by butcher's hand (45.94%), chopping block (11.76%) and CU hand (9.6%) (Table 2). The recurrence of *S. aureus* isolation from butcher hands and knife were significantly different ($P < 0.05$) than from Cu hands. The odds of finding *S. aureus*-positive swab samples from RM workers were at least 2 times higher than the hand of customers.

These outcomes of the present study feature the requirement for good hygiene amid transportation, dealing with at retail outlets and customers to diminish the risk of transmission of *S. aureus*.

Antibiotic resistance of *S. aureus*

Among 46 *S. aureus* isolates from various swab samples, a large portion of the isolates

showed resistance to Penicillin (PEN, 97.83%) trailed by Ciprofloxacin (CPH, 56.52%), Tetracycline (TET, 36.96%), Trimethoprim-Sulfamethoxazole (TSH, 34.78%) and Erythromycin (ERY, 17.39%). Nonetheless, low resistance was seen to Clindamycin, Chloramphenicol, Oxacillin, Ceftriaxone, and that varied from 2%-7% (Table 3). None of the isolates was discovered resistant to Vancomycin.

Most of the *S. aureus* isolates (58.69%, 95% CI 43.63-61.93) in the present study were resistant to atleast three antibiotics and they were designated as multidrug-resistant *S. aureus* (MDR). *S. aureus* isolates were resistant to antibiotics somewhere in the range 0 and 5, with a median of 2 on account of CU hand swab samples and with a median of 3 on account of RM swab samples. However, the quantity of antibiotics to which *S. aureus* isolates were resistant did not vary significantly between swab samples ($p>0.05$).

Antibiotic resistance gene

S. aureus isolate (1/46=2.17%) was assigned as MRSA dependent on the presence of methicillin resistance gene (*mecA*, Figure 1). This MRSA isolates was found only in CU hand swab sample from Gurdaspur district (0.55%, 1/182) which accounted for 1.37% (1/73) MRSA prevalence in CU hand swab samples.

MRSA in the food of animal origin and community settings represents a significant risk to human wellbeing. In this study, the CU hand swab sample was the only sample contaminated with MRSA. Regardless of sample size variations, these studies suggested that MRSA contamination of various source can change by location due to the difference in management practices, hygienic measures and molecular distinction may exist among MRSA isolates of different origin.

Isolates that was *mecA* positive, indicated resistance to oxacillin (MIC 16 $\mu\text{g/ml}$). Nonetheless, two *S. aureus* (2/46, 4.35%) isolates that were phenotypically resistant to oxacillin yet genotypically without *mecA* had MIC 4 $\mu\text{g/ml}$ and assigned as Borderline Oxacillin Resistant *S. aureus* (BORSA). Martineau *et al.*, (2000) likewise reported the presence of isolates that were phenotypically oxacillin resistant however negative for the *mecA* gene. Similarly, Pereira *et al.*, (2009) found 38% of *S. aureus* isolates resistant to oxacillin yet just 0.68% of the isolates demonstrated the presence of the *mecA* gene. Borderline oxacillin-resistant *S. aureus* (BORSA) has been frequently observed phenotype amongst *S. aureus* isolates. These isolates are cefoxitin/ceftriaxone susceptible and do not carry the *mecA* or *mecC* genes, however, are indicated oxacillin resistance MIC between 1-8 $\mu\text{g/ml}$ (Shore and Coleman 2013). Such pattern may be a direct result of hyperproduction of β -lactamases, a creation of typical PBP with modified binding capacity or variant of *mecA* gene (Martineau *et al.*, 2000, Laurent *et al.*, 2012). To bar the likelihood of hyperproduction of β -lactamase, amoxyclave antibiotic disk diffusion test was performed (Martineau *et al.*, 2000). The results of this study showed that isolates from RM (oxacillin resistant, *mecA* negative) to be ceftriaxone susceptible and β -lactamases hyperproducers (*blaz*⁺ and amoxicillin/clavulanic acid disc diffusion size > 20 mm).

Gentamicin resistant isolates were observed just in RM swab samples (3/39, 7.69%) with MIC 16-32 $\mu\text{g/ml}$. These isolates were positive for the *aacA-aphD* gene (Figure 3). On the other hand, 8.69% (4/46) *S. aureus* isolates possessed *aacA-aphD* gene yet were phenotypically sensitive to gentamicin. This might be because of the absence of expression or partial expression of *aac(6')/aph(2'')* gene (Martineau *et al.*, 2000, Choi *et al.*, 2003). In

this study, the *aacA-aphD* gene was found in MRSA isolates. This Gentamicin-resistant MRSA is ordinarily experienced in community isolates and is less as often as possible found among clinical isolates (Ida *et al.*, 2001).

In the present study, *tetK* and *tetM* genes were present in *S. aureus* isolates (Figure 4). Of the total *S. aureus* isolates, 36.96% (17/46) were positive for *tetK* and 4.35% (2/46) were positive for both *tetK* and *tetM* genes. None of the isolates were positive for *tetL* and *tetO*

gene. An MRSA isolate was positive for the *tetK/tetM* gene. The wide circulation of *tetK* and *tetM* among *S. aureus* and MRSA isolates have been connected to the way that these genes are situated on mobile genetic elements (Chopra and Roberts 2001). Among the Erythromycin resistant genes, just *ermB* and *ermC* genes were present in *S. aureus* isolates (Figure 3). The majority of the *S. aureus* isolates that were carrying any of the *erm* genes demonstrated complete or intermediate resistance to Erythromycin.

Table.1 Primers used for detection of antibiotic resistant genes in *S. aureus*

Gene	Oligonucleotide sequence (5`-3`)	Amplicon size	Reference
<i>aacA-aphD</i>	TAA TCC AAG AGC AAT AAG GGC GCC ACA CTA TCA TAA CCA CTA	227	Strommenger <i>et al.</i> , (2003)
<i>ermA</i>	AAG CGG TAA ACC CCT CTG A TTC GCA AAT CCC TTC TCA AC	190	Strommenger <i>et al.</i> , (2003)
<i>ermB</i>	CTATCTGATTGTTGAAGAAGGATT GTTTACTCTTGGTTTAGGATGAAA	142	Martineau <i>et al.</i> , (2000)
<i>ermC</i>	AAT CGT CAA TTC CTG CAT GT TAA TCG TGG AAT ACG GGT TTG	299	Strommenger <i>et al.</i> , (2003)
<i>tetK</i>	GTA GCG ACA ATA GGT AAT AGT GTA GTG ACA ATA AAC CTC CTA	360	Strommenger <i>et al.</i> , (2003)
<i>tetM</i>	AGT GGA GCG ATT ACA GAA CAT ATG TCC TGG CGT GTC TA	158	Strommenger <i>et al.</i> , (2003)
<i>tetL</i>	GTCGTTGCGCGCTATATTCC GTGAACGGTAGCCACCTAA	696	Huys <i>et al.</i> , (2005)
<i>tetO</i>	AATGAAGATTCCGACAATTT CTCATGCGTTGTAGTATTCCA	781	Huys <i>et al.</i> , (2005)
<i>mecA</i>	AAA ATC GAT GGT AAA GGT TGG C AGT TCT GCA GTA CCG GAT TTG C	532	Strommenger <i>et al.</i> , (2003)
<i>blaZ</i>	ACT TCA ACA CCT GCT GCT TTC TGA CCA CTT TTA TCA GCA ACC	173	Martineau <i>et al.</i> , (2000)
<i>vanA</i>	ATGAATAGAATAAAAAGTTGC TCACCCCTTTAACGCTAATA	1032	Saha <i>et al.</i> , (2008)
<i>16SrDNA</i> (<i>Staphylococcus</i> genus specific)	CAG CTC GTG TCG TGA GAT GT AAT CAT TTG TCC CAC CTT CG	420	Strommenger <i>et al.</i> , (2003)
<i>Nuc</i>	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	279	Brakstad <i>et al.</i> , (1992)

Table.2 Prevalence of *S. aureus* in RM shops and CU hand samples from different districts of Punjab

Districts	Swabs				Hand (Customers) (%)
	Butcher Hand (%)	Chopping block (%)	Butcher Knife (%)	Total Swab samples (%)	
Ludhiana	4/6 (66.7)	1/5 (20)	2/7 (28.57)	7/18 (38.89)	1/15 (6.67)
Amritsar	2/5 (40.0)	0/6 (0.0)	3/6 (50.0)	5/17 (29.41)	2/16 (12.5)
Gurdaspur	5/13 (38.46)	3/14 (21.42)	7/13 (53.85)	15/40 (37.5)	3/12 (25.0)
Patiala	3/6 (50.0)	0/6 (0)	3/7 (42.86)	6/19 (31.58)	0/13 (0)
Barnala	3/7 (42.85)	0/3 (0.0)	2/6 (33.33)	5/16 (31.25)	1/17 (5.89)
Total	17/37 (45.94)	4/34 (11.76)	18/38 (47.37)	39/109 (35.78)	7/73 (9.59)

Table.3 Antibiotic resistance of *S. aureus* isolates isolated from RM shops and CU hand swab samples

Antibiotic	No. of isolates resistant*(%) n=46	No. of isolates resistant from RM swab samples ¹ (%) n=109	No. of isolates resistant from CU swab samples (%) n=73
OXA	2.17	0.00	1.37
PEN	97.83	34.86	100.00
TET	36.96	15.59	0.00
CLI	2.17	0.00	1.37
CTR	0.00	0.00	0.00
GEN	6.52	2.75	0.00
ERY	17.39	1.83	8.22
CPH	56.52	21.10	4.11
TSH	34.78	14.68	0.00
CHL	4.35	0.00	2.74
VAN	0.00	0.00	0.00

OXA-oxacillin, PEN-penicillin, TET-tetracycline, CLI-clindamycin, CTR-ceftriaxone, GEN-gentamicin, ERY-erythromycin, CPH-ciprofloxacin, TSH-trimethoprim-sulfamethoxazole, CHL-chloramphenicol, VAN-vancomycin. 1-swab samples from hand/knife/chopping block.

*Resistant strains include only those that showed complete resistance

n- no. of isolates under study

Note: For erythromycin resistance estimation only isolates showing complete resistance were consider although 14 other isolates were intermediate resistant.

Fig.1 The Epsilometer test showing the interaction of the inhibition zone with the strip of a *S. aureus* for Ceftriaxone (CTR)

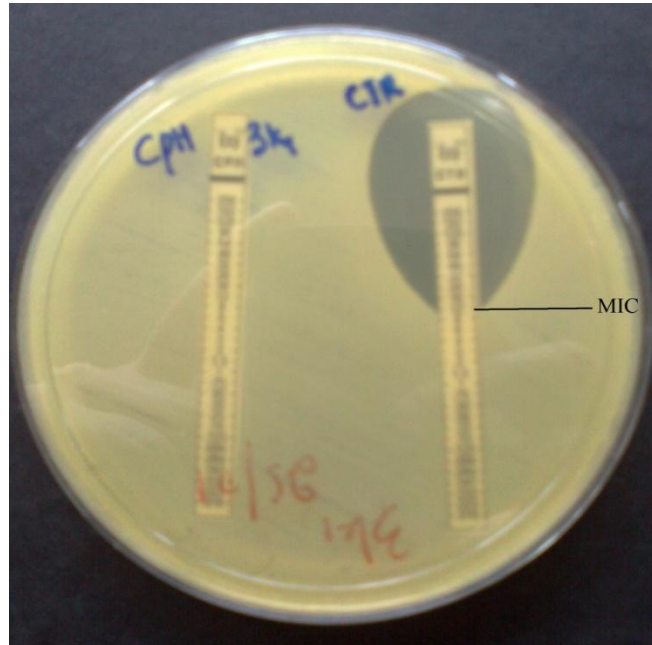


Fig.2 Agarose gel electrophoresis of PCR-amplified products using genus (16S rDNA-420bp)/species-specific (nuc-279bp) and mecA (532bp) primer sets. lane M, 100-bp Plus DNA size marker; lane S, *S. aureus* ATCC 33591 reference strain; lane NTC, no template control



Fig.3 Agarose gel electrophoresis of PCR-amplified products using tetracycline resistance genes primer sets. Lanes 1-5, examined *Staphylococcus aureus* isolates; lane M, 100-bp Plus DNA size marker; lane S, Standard; lane NTC, no template control.



Fig.4 Agarose gel electrophoresis of PCR-amplified products using tetracycline resistance genes primer sets. Lanes 7-11, examined *Staphylococcus aureus* isolates; lane M, 100-bp Plus DNA size marker; lane S, Standard; lane NTC, no template control. Note: Isolate in the lane 9 was isolates from the meat sample not from swab samples.

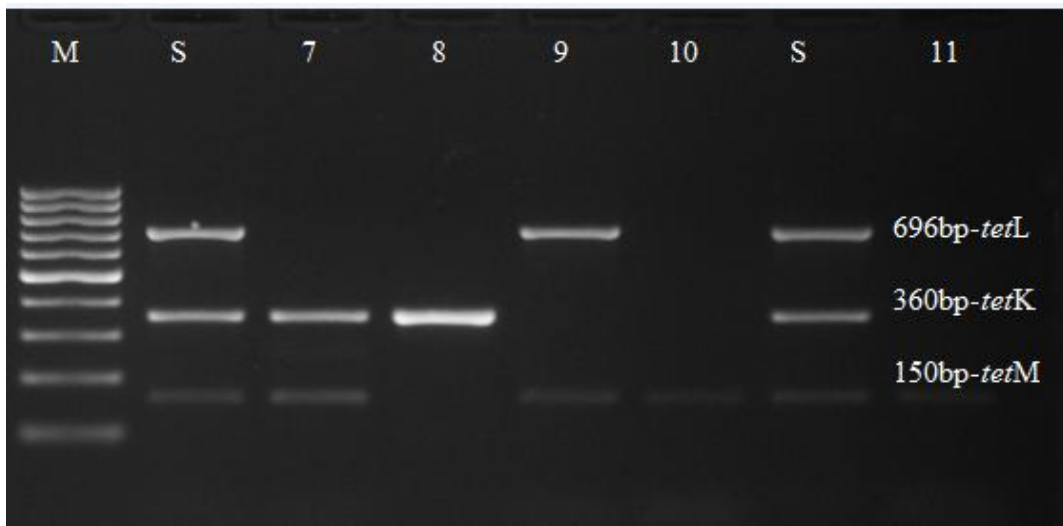
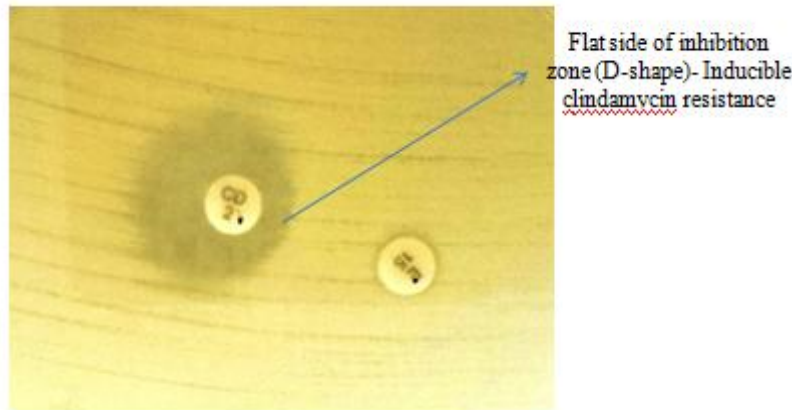


Fig.5 D-shaped zone of inhibition around the Clindamycin disk is designated as the D phenotype which is labeled as D+.



In this study, 19.57% (9/46) of the *S. aureus* isolates were genotypically negative yet demonstrated an intermediate resistance with MIC 1-2µg/ml phenotypically. This intermediate resistance to Erythromycin could be because of different genes like *msrA* or novel gene *ermTR* (Seppälä *et al.*, 1998, Martineau *et al.*, 2000). Similarly, Martineau *et al.*, (2000) additionally reported two *S. aureus* isolates resistant to erythromycin phenotypically however not carrying any of the *erm* resistance genes. It was additionally seen that the isolates from the CU hands were complete resistant to erythromycin unlike samples from RM.

These isolates that were resistant (R) or intermediate resistant (IR) to erythromycin *in vitro* tested for inducible clindamycin resistance (D-test, Figure 5). Among the 22 erythromycin-resistant (R+IR) *S. aureus* isolates, none demonstrated inducible MLS_B phenotype (ERY+/CLI-, D+) aside from one MSSA (methicillin susceptible *S. aureus*) isolates from CU hand swab sample that showed a constitutive MLS_B phenotype (Erm+/Cli+, D-).

In conclusion, this is the short study of the prevalence of *S. aureus* and MRSA in RM and CU hand swab samples from five districts of Punjab. This study detailed a relatively

high prevalence of *S. aureus* and high rates of antimicrobial resistance amongst the isolates. Just one isolate was MRSA and was isolated from the hand of the customer. The information of this study affirm the uneven conceivable sources of MRSA contamination in the food chain that can have a potential role in the dispersal of multidrug-resistant *S. aureus* isolates. This study clearly shows the prerequisite of further investigation at the ranch, retail and customer levels including large sample size over time so as to all the more likely evaluate the presence and cause of MRSA in domesticated animals and the risk to livestock handlers and customers in Punjab.

Practical measures ought to be taken to guarantee the wellbeing of our food products. Likewise, the present study uncovered the prevalence of BORSA that is of public health concern in light of the fact that the occurrences of BORSA have been accounted for in emergency clinics.

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Conflict of interest

The authors declare that they have no competing interests.

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