Journal of Global Antimicrobial Resistance 16 (2019) 152–158

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Prevalence, multidrug resistance and molecular typing of methicillin-resistant *Staphylococcus aureus* (MRSA) in retail meat from Punjab, India

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ARTICLE INFO

Article history:
Received 6 April 2018
Received in revised form 28 August 2018
Accepted 2 October 2018
Available online 9 October 2018

Keywords:
Staphylococcus aureus
Multidrug resistance
MRSA
SCC*mec* type
ST5
spa t442

ABSTRACT

Objectives: This study reports the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in retail meat from Punjab, India.
Methods: Classical microbiological methods were applied to isolate and identify *S. aureus* isolates. Isolates also underwent Etest. PCR and sequencing were used to identify and characterise antimicrobial resistance genes. MLST, SCC*mec* and *spa* typing were performed.
Results: A total of 408 meat and 101 swab samples were processed for *S. aureus* isolation. Phenotypic resistance was highest to penicillin (90.97%), followed by ciprofloxacin (61.80%), tetracycline (45.14%) and erythromycin (11.11%). Isolates from chicken samples showed significantly higher MICs for tetracycline than chevon and pork samples and significantly higher MICs for trimethoprim/sulfamethoxazole and gentamicin than chevon and swab samples ($P < 0.05$). No isolates were phenotypically resistant to vancomycin (MICs of 0.5–2 μg/mL). Most isolates (52.78%, 95% CI 44.63–60.93%) were multidrug-resistant and carried resistance genes to penicillin (*blaZ*), oxacillin (*mecA*), gentamicin (*aacA-aphD*), erythromycin (*ermB*, *ermC*) and tetracycline (*tetK*, *tetL*, *tetM*). MRSA was only found in chicken samples (2.72%; 4/147). Seven *S. aureus* (5.07%) were borderline oxacillin-resistant (MIC range 4–8 μg/mL). All MRSA were SCC*mec*V-*pvl*$^+$-t442, among which three isolates were ST5. Their genotype was *mecA*$^+$, *blaZ*$^+$, *aacA-aphD*$^+$, *tetK*$^+$, *ermC*$^{+/-}$. Among the erythromycin-resistant isolates, 25% were MRSA, of which 12.5% isolates expressed an inducible macrolide–lincosamide–streptogramin B (iMLS$_B$) phenotype.
Conclusion: These data confirm the presence of ST5-t442-MRSA-SCC*mec*V-*pvl*$^+$ and iMLS$_B$ MRSA in meat samples, indicating a potential role of meat in the dissemination of multidrug-resistant *S. aureus* strains and successful MRSA lineages in Punjab.

1. Introduction

Antibiotics are used extensively in the food animal production system for growth promotion and disease prevention, which has resulted in the rise of antimicrobial-resistant micro-organisms. Antimicrobial −resistant *Staphylococcus aureus* in food animals and food of animal origin is common due to its commensal association. Antimicrobial-resistant *S. aureus*, in particular methicillin-resistant *S. aureus* (MRSA), is a matter of concern as it can cause fatal infections that are difficult to treat [1]. MRSA has been isolated from retail milk, meat and meat products, and meat industry workers, with several reports of MRSA contamination of meat, particularly raw meat, in retail markets [2–6].

The genetic mechanism responsible for the development of resistance to methicillin/oxacillin is via the acquisition and insertion of staphylococcal chromosome cassette *mec* (SCC*mec*) elements, which carry antimicrobial resistance determinants [7]. Several major sequence types (STs) differ in their SCC*mec* types, which may have arisen by independent acquisitions of the *mec* gene. Recently, ST5 MRSA, prevalent among healthcare-acquired MRSA (HA-MRSA), has been identified from pigs, retail meat and farm workers [8,9].

The emergence of antimicrobial resistance in India is an important issue and antimicrobial resistance, especially MRSA, has

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https://doi.org/10.1016/j.jgar.2018.10.005

been reported from hospitals [10–12]. However, detection of antimicrobial resistance of *S. aureus* isolates from meat has not been greatly pursued, especially in Punjab, India. Therefore, the aim of this study was to determine the phenotypic and genotypic patterns of antimicrobial resistance, the prevalence of antimicrobial-resistant *S. aureus* and the types associated with meat samples of Punjab, India.

2. Materials and methods

A total of 509 samples, comprising 408 raw meat samples [chicken, $n = 147$; pork, $n = 131$; and chevon (goat meat), $n = 130$] and 101 swab samples (chopping block, $n = 29$; butcher's hand, $n = 34$; and chopping knife, $n = 38$) from retail meat shops in 11 districts of Punjab, India, were collected for isolation of *S. aureus*. Region-wise collection of the various samples is given in Table 1. All of the meat samples were collected in sterile Nasco sampling bags (HiMedia, Mumbai, India) and, after proper labelling, were immediately transported to the Zoonoses Laboratory of the School of Public Health and Zoonoses, Guru Angad Dev Veterinary and Animal Sciences University (Ludhiana, India). Samples were either processed immediately or were kept at −20 °C for further analysis. Likewise, swabs were transported to the same laboratory on ice and were processed for isolation of *S. aureus* within 6 h of collection.

Isolation of *S. aureus* from meat and swab samples was done as per the method recommended by the Bacteriological Analytical Manual 2001 [13]. Colonies with typical morphology on Baird–Parker agar were then subjected to Gram staining and catalase test. Gram- and catalase-positive isolates were biochemically identified as *S. aureus* using the HiStaph™ Identification Kit (HiMedia) and the *S. aureus* isolates were purified and maintained at −20 °C in 20% (v/v) glycerol.

Isolates confirmed as *S. aureus* were further characterised as coagulase–positive and –negative by tube coagulase test using rabbit plasma (HiMedia) according to the manufacturer's instructions. Isolates that showed any degree of clotting within 4 h were considered coagulase-positive *S. aureus* and those showing no clot formation were considered coagulase-negative *S. aureus*.

Antimicrobial susceptibility testing of the *S. aureus* isolates was performed by Etest (Ezy MIC™ Strip; HiMedia) to the following antimicrobials: oxacillin; penicillin; tetracycline; chloramphenicol; trimethoprim/sulfamethoxazole (SXT); ceftriaxone; gentamicin; erythromycin; ciprofloxacin; and vancomycin. Antimicrobial susceptibility testing to amoxicillin/clavulanic acid (AMC) for β-lactamase hyperproduction as well as for inducible clindamycin resistance (D-test) was performed by the disk diffusion method according to Clinical and Laboratory Standards (CLSI) guidelines (M100-S21) [14].

The *S. aureus* isolates were also screened for the presence of the antimicrobial resistance genes *blaZ* (penicillin resistance), *mecA* (oxacillin resistance), *aacA-aphD* (gentamicin resistance), *ermA*, *ermB* and *ermC* (erythromycin resistance), *tetK* and *tetL* efflux genes and *tetM* and *tetO* ribosomal protection proteins (tetracycline resistance) and *vanA* (vancomycin resistance) by amplification of the gene using multiplex PCR. The primers and PCR protocols are presented in the Supplementary material.

Antimicrobial resistance genes for which a positive control was not available were amplified from the phenotypically resistant field strain, and the amplified gene was purified and sequenced (Invitrogen™; Thermo Fisher Scientific, Waltham, MA). The sequenced gene was aligned for similarity in the nucleotide database using the National Centre for Biotechnology Information (NCBI) nucleotide BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/). The aligned sequence was then submitted to NCBI to obtain an accession number. *Staphylococcus aureus* ATCC 33591 and ATCC 33592 were used as MRSA (*mecA*-positive) and methicillin-susceptible *S. aureus* (*mecA*-negative) controls, respectively. *Staphylococcus aureus* field isolates (accession nos. KU872013, KP834338/KP834339, KP658721, KP658723, KP886833, KT454736 and KT454737) from this study were used as positive control for genes *blaZ*, *aacA-aphD*, *tetK*, *tetL*, *tetM*, *ermB* and *ermC*, respectively.

Multiplex PCR for SCC*mec* typing and subtyping of MRSA isolates was performed according to the method described by Zhang et al. [15]. Primers and PCR protocols for SCC*mec* typing and *pvl* gene identification are presented in the Supplementary material. *Staphylococcus aureus* strain ATCC 700699 and *S. aureus* field isolate accession nos. KR610412, KT005393 and KP896298 were used as positive controls for SCC*mec* types II, IV and V and the *pvl* gene.

Multilocus sequence typing (MLST) and staphylococcal protein A (*spa*) typing was performed on the MRSA isolates as described by Enright et al. [16] and Shopsin et al. [17], with slight modifications (Supplementary material). The amplified PCR product of seven housekeeping genes and the *spa* gene were purified and sequenced (SciGenome Labs, Cochin, India).

The consensus sequences obtained for each locus for each isolate were assigned allele numbers on the PubMLST database (https://pubmlst.org/saureus/; accessed 20 December 2017) for *S. aureus*. The alleles at each of the seven loci defined the allelic profile, which corresponds to its sequence type (ST).

The consensus sequences of the amplified *spa* gene of the isolates were interpreted using specific sequence analysis software (DNAGear; http://w3.ualg.pt/~hshah/DNAGear/). Analysis of the sequences resulted in identification of short sequence repeat types that were assigned codes, and *spa* types were generated based on the sequence of these repeat codes.

Table 1
Prevalence of *Staphylococcus aureus* in meat and swab samples from different districts of Punjab, India.

District	Meat [*n*/*N* (%)]				Swabs [*n*/*N* (%)]			
	Chicken	Chevon	Pork	Total meat samples	Butcher's hand	Chopping block	Butcher's knife	Total swab samples
Ludhiana	10/25 (40.0)	2/13 (15.4)	3/16 (18.8)	15/54 (27.8)	4/6 (66.7)	1/5 (20.0)	3/6 (50.0)	8/17 (47.1)
Amritsar	4/13 (30.8)	2/12 (16.7)	4/16 (25.0)	10/41 (24.4)	3/5 (60.0)	0/5 (0)	2/6 (33.3)	5/16 (31.3)
Patiala	4/11 (36.4)	3/12 (25.0)	3/16 (18.8)	10/39 (25.6)	–	–	–	–
Pathankot	5/10 (50.0)	2/13 (15.4)	2/14 (14.3)	9/37 (24.3)	–	–	–	–
Hoshiarpur	5/13 (38.5)	2/11 (18.2)	2/11 (18.2)	9/35 (25.7)	4/12 (33.3)	2/11 (18.2)	7/13 (53.8)	13/36 (36.1)
Moga	1/12 (8.3)	2/16 (12.5)	3/18 (16.7)	6/46 (13.0)	3/7 (42.9)	0/6 (0)	3/7 (42.9)	6/20 (30.0)
Ferozepur	2/12 (16.7)	1/8 (12.5)	2/8 (25.0)	5/28 (17.9)	–	–	–	–
Bathinda	2/13 (15.4)	4/14 (28.6)	2/12 (16.7)	8/39 (20.5)	3/4 (75.0)	0/2 (0)	3/6 (50.0)	6/12 (50.0)
Barnala	2/16 (12.5)	2/11 (18.2)	3/10 (30.0)	7/37 (18.9)	–	–	–	–
Gurdaspur	4/14 (28.6)	2/12 (16.7)	2/6 (33.3)	8/32 (25.0)	–	–	–	–
Rupnagar	0/8 (0)	1/8 (12.5)	1/4 (25.0)	2/20 (10.0)	–	–	–	–
Total	39/147 (26.5)	23/130 (17.7)	27/131 (20.6)	89/408 (21.8)	17/34 (50.0)	3/29 (10.3)	18/38 (47.4)	38/101 (37.6)

2.1. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics v.22.0 (IBM Corp., Armonk, NY). The number of sample types positive for *S. aureus*, the number of antibiotics to which isolates were resistant, and the minimum inhibitory concentrations (MICs) were compared using Kruskal–Wallis test with α set as 0.05. Post-hoc analysis was performed using the Wilcoxon–Mann–Whitney test with Bonferroni correction. For analysis of categorical data, isolates showing intermediate resistance were classified as 'resistant'. The logistic regression model using the status of each isolate as a binary outcome, i.e. susceptible or non-susceptible [combining intermediate-resistant (IR) and resistant (R)], with sample type as explanatory variable was used to model resistance profiles modulated by type of meat sample. For each antibiotic, the odds of an isolate being non-susceptible were computed against sample type.

BioNumerics software v.7.6 (Applied Maths, Sint-Martens-Latem, Belgium) was used for analysis of the MIC distribution and construction of a dendrogram based on the antibiogram of the respective sample type. The colour scale is defined based on the colour range. In the MIC data set, the minimum value for all antibiotics was set to 0 and the maximum value to respective higher MIC values (after which the isolate was considered resistant as per CLSI guidelines). All pairwise similarity values were calculated with a similarity coefficient (Pearson correlation). The resulting similarity matrix was then converted into a dendrogram using the unweighted pair-group method with arithmetic mean (UPGMA) clustering algorithm.

3. Results and discussion

A total of 89 of 408 meat samples and 38 of 101 swab samples were positive for *S. aureus* on culture and were confirmed by targeting genus- and species-specific genes by PCR, resulting in an overall prevalence of 21.8% [95% confidence interval (CI) 17.8–25.8%] and 37.6% (95% CI 28.2–47.0%), respectively (Table 1; Fig. 1). Approximately 432 *S. aureus* isolates were recovered from the total 127 positive field samples, of which 144 isolates were unique in their biochemical characteristics. Among these 144 isolates, 129 isolates were coagulase-positive and 15 were coagulase-negative.

Staphylococcus aureus contamination was higher in chicken (26.5%), followed by pork (20.6%) and chevon (17.7%) meat samples; however, this difference was not statistically significant ($P > 0.05$) (Table 1). Among the swab samples from retail meat shops, 50.5% of *S. aureus* isolates were from butchers' hands, 47.4% from butchers' knives and 10.3% from chopping blocks. The difference among swab samples was also non-significant ($P > 0.05$) (Table 1); however, the frequency of *S. aureus* isolation from meat (chevon/pork) and swab samples was significantly different ($P < 0.05$). The odds of finding *S. aureus*-positive swab samples were at least two times higher than pork/chevon samples.

Variable prevalence levels have been reported in different studies, which may be due to differences in handling practices of raw meat sample types and their geographical location. However, in a meta-analysis by Ou et al., no significant difference in the pooled prevalence rates of *S. aureus* was identified in various raw meat products, with an overall pooled prevalence rate of 29.2% (95% CI 22.8–35.9%) [18]. Moreover, in their subgroup analyses, the prevalence of *S. aureus* contamination in chicken products was highest in Asian studies, whilst in European studies the prevalence rates of *S. aureus* contamination in chicken and pork products were lower. The pooled prevalence rates of *S. aureus* contamination in chicken and pork products were significantly higher in samples collected from retail sources than in samples collected from slaughterhouses and processing plants. These results, as well as the present study, highlight the need for good hygiene during transportation and handling at retail outlets

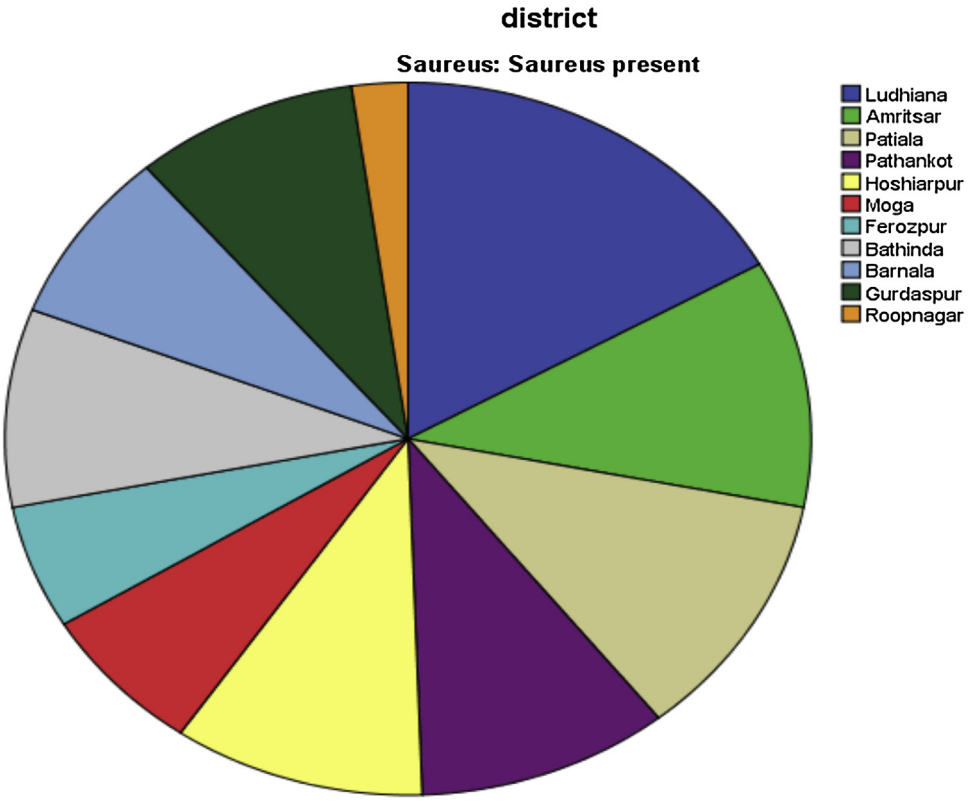

Fig. 1. District-wise distribution of *Staphylococcus aureus*.

to reduce the risk of transmission of *S. aureus* from meat products to humans.

3.1. Antimicrobial resistance profile of Staphylococcus aureus isolates

Among the 144 *S. aureus* isolates from meat and swab samples, most isolates showed resistance to penicillin (90.97%), followed by ciprofloxacin (61.80%), erythromycin (49.31%, IR + R) and tetracycline (45.14%). However, low resistance rates, ranging from 2–9%, were observed for clindamycin, chloramphenicol, oxacillin and ceftriaxone (Table 2). None of the isolates were resistant to vancomycin.

Approximately 38, 37, 31 and 29 unique phenotypic resistance profiles were found among the chicken, swab, pork and chevon isolates, respectively (Supplementary material). From the dendrogram analysis, it was observed that the resistance profiles were distributed randomly. There was no relationship among the antimicrobial resistance profile of isolates with the districts or sample types. Similarly, no correlation was found between the resistance profile of isolates from swab (hand/knife/chopping block) and the corresponding meat sample. None of the *S. aureus* isolates from swab samples were resistant to oxacillin, and no significant variations in oxacillin MICs were observed between the samples. However, significant variation in the MICs for tetracycline, clindamycin, ceftriaxone, SXT, chloramphenicol and gentamicin were observed between sample types (Table 3). *Staphylococcus aureus* isolates from chicken meat samples showed significantly higher MICs for tetracycline than chevon and pork samples and significantly higher MICs for SXT and gentamicin than chevon and swab samples ($P < 0.05$) (Table 3), thereby indicating that the chances of finding an *S. aureus* isolate with a higher MIC (eventually making them resistant) to tetracycline, gentamicin and SXT are greater in chicken meat compared with other sample types. This may be attributed to the common use of these antibiotics in poultry farming in India [19].

Most of the *S. aureus* isolates (52.78%, 95% CI 44.63–60.93%) were resistant to three or more antibiotics and were designated as multidrug-resistant (Table 4). *Staphylococcus aureus* isolates were resistant to a varied number of antibiotics (between 0 and 7), with a median of 2 in the case of chicken and chevon samples and 3 in the case of pork and swab samples. However, the number of antibiotics to which *S. aureus* isolates were resistant did not differ significantly between chicken, chevon, pork and swab samples ($P > 0.05$).

Furthermore, all of the *S. aureus* isolates were screened for antimicrobial resistance genes. There was a statistically significant positive correlation ($P < 0.05$) between phenotypic and genotypic resistance in *S. aureus* isolates to the antibiotics penicillin, tetracycline, gentamicin and erythromycin. Isolates that were *mecA*-positive showed resistance to oxacillin (MICs of 4–32 μg/mL). However, seven *S. aureus* isolates (7/138; 5.07%) that were phenotypically resistant to oxacillin but genotypically devoid of the *mecA* gene had MICs ranging from 4–8 μg/mL and were designated as borderline oxacillin-resistant *S. aureus* (BORSA). BORSA is a frequently observed phenotype amongst *S. aureus* isolates [20,21]. These isolates are susceptible to cefoxitin/ceftriaxone and do not carry the *mecA* or *mecC* genes but show oxacillin resistance with MICs between 1–8 μg/mL [22]. Such a pattern may be because of hyperproduction of β-lactamases, production of normal penicillin-binding protein (PBP) with altered binding capacity, or a variant of the *mecA* gene [20,23,24]. To exclude the possibility of hyperproduction of β-lactamases, the AMC antibiotic disk diffusion test was performed [20]. The result showed that isolates from chevon (oxacillin-resistant, *mecA*-negative) were susceptible to ceftriaxone and hyperproduced β-lactamases, but variable results were found in the case of isolates from pork samples

Table 2
Antimicrobial resistance[a] of *Staphylococcus aureus* isolates from meat and swab samples in Punjab, India.

Antimicrobial agent	% of isolates resistant ($n = 144$)	% of isolates resistant from meat samples ($n = 106$)	% of isolates resistant from chicken samples ($n = 46$)	% of isolates resistant from chevon samples ($n = 29$)	% of isolates resistant from pork samples ($n = 31$)	% of isolates resistant from swab samples[b] ($n = 38$)
OXA	9.03	12.26	13.04	10.34	12.90	0
PEN	90.97	88.68	89.13	86.21	90.32	97.37
TET	45.14	45.28	54.35	37.93	38.71	44.74
CLI	2.08	2.83	6.52	0	0	0
CRO	2.78	3.77	2.17	0	9.68	0
GEN	18.75	22.64	28.26	10.34	25.81	7.89
ERY	11.11	13.21	19.56	6.90	9.68	5.26
CIP	61.80	63.21	56.52	72.41	64.52	57.89
SXT	22.22	16.04	13.04	37.93	0	39.47
CHL	4.17	5.66	0	10.34	9.68	0
VAN	0	0	0	0	0	0

OXA, oxacillin; PEN, penicillin; TET, tetracycline; CLI, clindamycin; CRO, ceftriaxone; GEN, gentamicin; ERY, erythromycin; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; CHL, chloramphenicol; VAN, vancomycin.
[a] Resistant isolates include only those that showed complete resistance.
[b] Swab samples from butchers' hand/knife/chopping block.

Table 3
Post-hoc tests (Mann–Whitney *U*-test) of *Staphylococcus aureus* overall, by sample type (chicken, chevon, pork and swab samples)[a].

Antimicrobial agent	Chicken vs. chevon	Chicken vs. pork	Chicken vs. swab	Chevon vs. pork	Chevon vs. swab	Pork vs. swab
Penicillin	–	–	–	–	–	343.5, 0.003
Tetracycline	402.5, 0.0003	434.5, 0.003	–	–	396.5, 0.046	422.0, 0.041
Ceftriaxone	437.5, 0.011	–	–	–	314.5, 0.000	–
SXT	457.0, 0.022	–	400.5, 0.000	282.00, 0.012	–	194.5, 0.000
Chloramphenicol	384.5, 0.001	–	–	–	400, 0.47	–
Gentamicin	407.5, 0.004	–	547.0, 0.003	312.0, 0.038	–	408.5, 0.027
Clindamycin	–	–	515.0, 0.001	–	222, 0.000	347.0, 0.002

SXT, trimethoprim/sulfamethoxazole.
[a] Mann–Whitney *U*-test value and *P*-value (only statistically significant values are shown).

Table 4
Prevalence of multidrug-resistant (MDR) *Staphylococcus aureus* from meat and swab samples in Punjab, India.

Sample	MDR (*n*/*N*)[a]	Prevalence (95% CI)
Chicken	21/46	45.65 (31.25–60.05)
Pork	17/31	54.84 (37.34–72.34)
Chevon	15/29	51.72 (33.53–69.97)
Swab[b]	23/38	60.53 (45.03–76.03)
Total	76/144	52.78 (44.63–60.93)

CI, confidence interval.
[a] *n*, number of positive isolates; *N*, total number of isolates.
[b] Swab from butchers' hand, knife and chopping block.

(Table 5). These isolates were intermediate or completely resistant to ceftriaxone and one isolate was not a β-lactamase-hyperproducer. Isolates with such characteristics have been described as modified *S. aureus* (MODSA), with mutations in *pbp* genes and/or PBP4 overexpression [24]. It was also observed that two of the four erythromycin-resistant MRSA expressed an inducible macrolide–lincosamide–streptogramin B (iMLS$_B$) phenotype (ERY$^+$/CLI$^-$, D$^+$).

Fig. 2 shows the odds ratio (OR) of an antimicrobial resistance gene against each antimicrobial agent in different samples compared with the reference category, adjusted for the type of sample (chicken, chevon, pork and swab). It is evident that the antimicrobial resistance determinants vary with the type of sample. Tetracycline resistance in *S. aureus* isolates from chevon is more likely to be governed by *tetK* (OR = 2–3) compared with *S. aureus* isolates from swab and chicken samples that are governed by *tetM* (individually or in combination with *tetK* and *tetL*). This may be because of unhygienic handling of chicken that makes the prevalence of *tetK*/*tetM* greater in chicken and swab samples. Likewise, the *ermB* gene is prevalent in chevon, followed by chicken. The combination of *tetK*- and *ermB*-positive isolates was at least two to three times higher in chevon samples than in chicken, pork and swab samples.

3.2. Prevalence of methicillin-resistant *Staphylococcus aureus*

Staphylococcus aureus isolates were designated as MRSA based on the presence of the methicillin resistance gene *mecA*. These MRSA isolates were found only in chicken samples (2.72%; 4/147),

Table 5
Characteristics of isolates resistant to oxacillin but negative for the *mecA* gene by PCR.

Source	Isolate	Oxacillin MIC (μg/mL)	Ceftriaxone MIC (μg/mL)	AMC inhibition zone (mm)[a]	*blaZ* (PCR detection)	β-Lactamase-hyperproducer
Pork	P12a	6	16	50	+	+
Pork	P72	6	16	46	+	+
Pork	P44(CNSA)	4	64	18	+	–
Pork	P59	4	64	30	+	+
Chevon	Che1	4	8	28	+	+
Chevon	Che2	4	8	30	+	+
Chevon	Che72(CNSA)	6	8	45	+	+

MIC, minimum inhibitory concentration; AMC, amoxicillin/clavulanic acid.
[a] Isolates were considered β-lactamases hyperproducers if the diameter of the inhibition zone with amoxicillin/clavulanic acid (20/10 μg) was >20 mm.

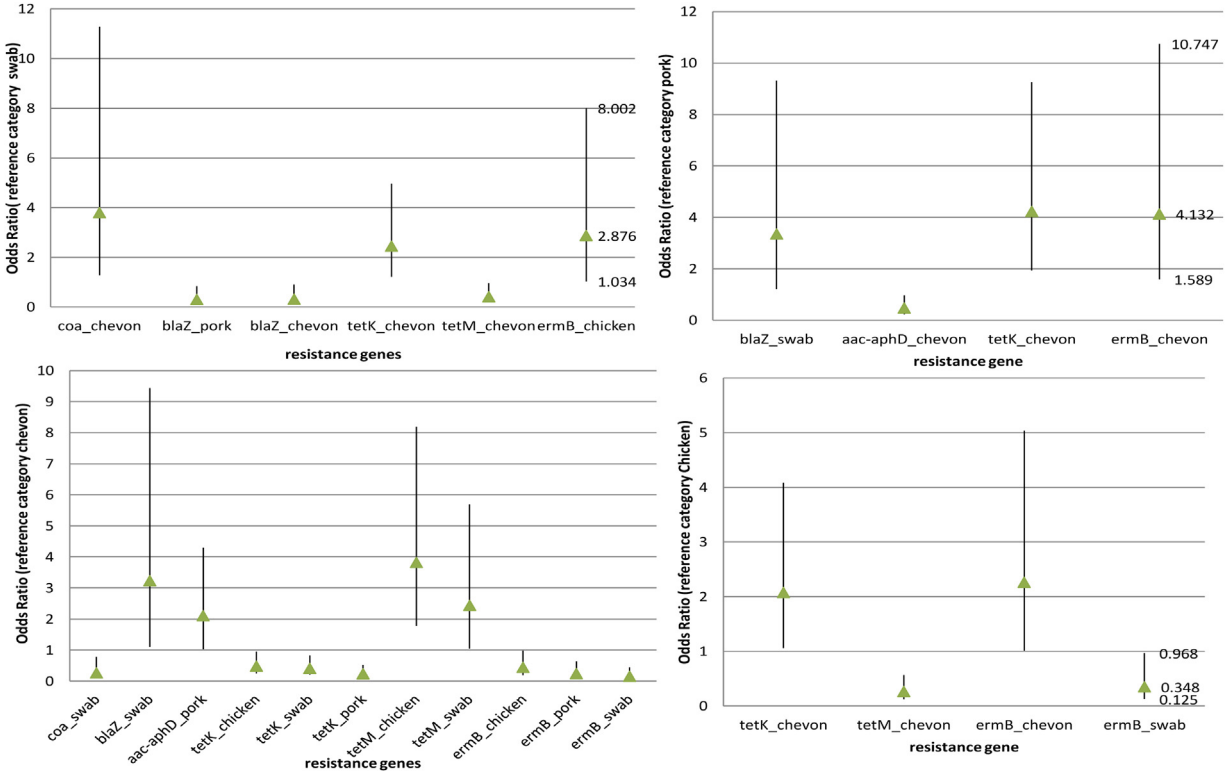

Fig. 2. Results from the logistic regression modelling the risk of antimicrobial resistance gene prevalence against sample type. The *y*-axis represents the odds ratios on an exponential scale.

which accounted for a 0.98% (4/408; 95% CI 0.02–1.94%) MRSA prevalence in meat samples. MRSA-positive meat samples were reported from the Hoshiarpur [3/35 (8.57%) in meat and 3/13 (23.08%) in chicken] and Ferozepur [1/28 (3.57%) in meat and 1/12 (8.33%) in chicken] districts of Punjab, whereas meat samples from the remaining regions were negative for MRSA.

MRSA in food of animal origin poses a considerable threat to human health [25]. In the current study, chicken was the only meat contaminated with MRSA. In a previous study, MRSA contamination was reported in chicken from Korea [5]. Ogata et al. also reported MRSA in poultry meat (1.5%; 3/197) [25], in contrast to studies that have reported MRSA from meat other than chicken, e.g. pork had the highest contamination of MRSA in the USA and Canada [6,26], beef in Korea [5] and poultry in Egypt and the Netherlands [2,27]. However, in a meta-analysis by Ou et al., there was no significant difference in the pooled prevalence of MRSA contamination identified in various raw meat products, with an overall pooled prevalence rate of 3.2% (95% CI 1.8–4.9%) [18]. Despite sample size variations, these studies suggest that MRSA contamination of different meat sources can vary by location owing to differences in management practices.

MLST and *spa* typing of the *mecA*-positive MRSA isolates revealed the presence of ST5 ($n = 3$) and *spa* type t442 ($n = 4$) associated with chicken meat isolates. An MRSA isolates from Firozpur district was untypeable by MLST as two of the alleles (*yqiL* and *pta*) could not be sequenced. All MRSA isolates carried SCC*mec* type V and the *pvl* gene, which are molecular markers for community-associated *S. aureus* (CA-MRSA), indicating the presence of community isolates in food of animal origin (meat). Although ST5 is a pandemic *S. aureus* type and is generally reported as hospital-associated and community-associated *S. aureus* around the globe as well as in India, the majority of cases carry SCC*mec* type II, a molecular marker of hospital-associated *S. aureus* [28,29]. Thus, the present study is the only study reporting this sequence type, *spa* type and SCC*mec* type from chicken samples in Punjab.

Basanisi et al. reported MRSA-SCC*mec*V-*pvl*$^+$ as the most common MRSA isolate in milk samples [30], whereas Kim et al. reported it from meat samples [31]. Feltrin et al. reported that most of the MRSA isolates from pig and cattle holdings carried SCC*mec* type V but were negative for the *pvl* gene [32], and Heikinheimo et al. reported livestock-associated MRSA (LA-MRSA) isolates from fattening pigs that displayed *mecA*, *blaZ/I/R*, *tetK* and SCC*mec* type V [33]. ST5 has been reported from retail pork or pork farms in the USA [34] and from pigs and pig farmers in Canada [8]. ST5 has also been reported from fish samples and the aquatic environment in Kerala, India [35].

spa type t442 has been reported from hospitalised patients suffering from urinary tract, respiratory, bone and joint and skin and soft-tissue infections as well as sepsis, where some of the isolates were categorised as community-associated and some were hospital-associated as per the definition of the US Centers for Disease Control and Prevention (CDC). *spa* type t442 has been also reported from Ivorian milk samples [36] and from intensive care units in Korean hospitals [37]. This indicates the potential role of food in the dissemination of MRSA lineages.

4. Conclusion

To our knowledge, this is the first thorough study of the prevalence of *S. aureus* and MRSA in retail meat samples from different districts of Punjab and is the only study describing the presence of ST5, *spa* type t442-MRSA-SCC*mec*V- *pvl*$^+$ in meat samples in Punjab, India. This study reported a relatively high prevalence of *S. aureus* and high rates of antimicrobial resistance amongst the isolates from meat samples, thus indicating the potential role of meat in the dissemination of multidrug-resistant *S. aureus* strains and MRSA lineages in Punjab and highlighting the health risks for consumers. This study clearly shows the requirement for further study at farm and retail levels, involving a large sample size over time in different locations, in order to better assess the presence and origin of MRSA in livestock and the risk to livestock handlers and consumers in Punjab. Practical measures should be taken to ensure the safety of food products. The present study also revealed the prevalence of BORSA in meat, which is of public-health concern as the occurrence of BORSA has been reported in hospitals. It was also observed that many isolates carried antimicrobial resistance genes but lacked phenotypic expression, thus demonstrating the future potential of these strains to become resistant to the evaluated antimicrobial agents.

Funding

This work was financially supported at the School of Public Health and Zoonoses, GADVASU (Ludhiana, India) under Rashtriya Krishi Vikas Yojana (RKVY).

Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2018.10.005.

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