J Food Sci Technol (March 2019) 56(3):1116–1126
https://doi.org/10.1007/s13197-019-03572-5

ORIGINAL ARTICLE

Virulent methicillin resistant *Staphylococcus aureus* (MRSA) in street vended foods

M. Sivakumar[1] · Zunjar B. Dubal[1] · Ashok Kumar[1] · Kiran Bhilegaonkar[1] · Obli Rajendran Vinodh Kumar[2] · Suman Kumar[1] · Anukampa Kadwalia[1] · Bi. Shagufta[1] · M. R. Grace[1] · T. P. Ramees[1] · Anamika Dwivedi[1]

Revised: 20 December 2018 / Accepted: 1 January 2019 / Published online: 12 February 2019

Abstract Street foods are one of the important sources of foodborne infections and *Staphylococcus aureus* is an important infectious agent transmitted through various sources including street foods. The methicillin-resistant *S. aureus* (MRSA) are of public health significance, hence the study was taken to assess the street foods as a source of MRSA, for which 430 street vended foods of animal origin (meat, milk, eggs and their products) and associated environmental samples were processed for isolation and characterization. A total of 52 (12.1%) *S. aureus* were isolated and resistant was observed to oxacillin (36.5%), cefoxitin (25%) and penicillin G (82.7%) by disc diffusion test. On genotypic screening, *mec*A and *blaZ* have detected in 17.3% and 69.2% isolates, respectively. The virulence typing identified *nuc*, *coa*, *clfA*, *spA*, *FnbA* and enterotoxin A (*sea*) genes in 100%, 96.2%, 30.8%, 55.8, 50% and 7.7% isolates, respectively. Genetic diversity among the isolates was observed by enterobacterial repetitive intergenic consensus PCR with a D value of 0.77. The presence of virulent MRSA in street vended foods trigger the public health concern and emphasis to educate the consumers and street food vendors about quality and safety of such foods.

Keywords Street vended foods · MRSA · Food safety · India

✉ Zunjar B. Dubal
drzunjar@yahoo.co.in

[1] Division of Veterinary Public Health, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, UP 243 122, India

[2] Division of Epidemiology, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, UP 243 122, India

Introduction

As per the UNICEF data, the global under-five mortality rate had dropped from 93 deaths per 1000 live births in 1990 to 41 in 2016 and India has also recorded 62% reduction in under-five mortality rate since 1990 (Ahmad et al. 2000), even though the proportional mortality for diarrheal diseases still remains high. According to a new Global Burden of Disease published in The Lancet Infectious Diseases journal in September 2017, India and Nigeria are responsible for 42% of child deaths on account of diarrheal diseases. It is well known that the diarrhoeal diseases are chiefly caused by foodborne pathogens such as bacteria, viruses, parasites or chemical substances which are usually infectious or toxic in nature, enter the body through contaminated food or water. These pathogens can cause severe diarrhea or debilitating infections including meningitis. *Salmonella*, *Campylobacter*, *Listeria monocytogenes* and *Enterohaemorrhagic Escherichia coli* are among the most common foodborne pathogens that affect millions of people annually, sometimes with severe and fatal outcomes (Bhaskar 2017). On the other hand, staphylococcal food poisoning is a gastrointestinal illness caused by eating foods contaminated with toxins of *Staphylococcus aureus*, a commensal bacterium found on the skin and in the nose of about 25–50% of healthy people and animals (Ouidri 2018).

According to FAO, about 2.5 billion people eat street food every day (FAO 2012). These foods are not only cherished for their unique flavors, convenience and the role they play in the cultural and social heritage of societies. They have also become important and essential for maintaining the nutritional status of the populations (FAO 1997). There are several kinds of vegetarian and non-vegetarian foods (animal origin foods consist of Milk,

Kebab, Tikka, Paneer, Kala jamun, Omelet, Lassi, Burfi etc.) available on the roadsides of the Indian cities. On the other hand, the infrastructure facilities of these vendors are relatively limited for potable water, toilets, refrigeration, washing and waste disposal. Hand and dishwashing are usually done in plastic containers due to lack of running water and sometimes without soap (WHO 1996). Due to inadequate infrastructure during preparation and processing, the raw materials (meat, milk, eggs or fishes) are usually contaminated with feces (either of human or animal) and/or with unsafe water (during cleaning and processing), may increase the health risk potential. Thus, a majority of countries reported contamination of these foods with *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* (three agents of greatest concern listed by WHO), *Salmonella* spp., *Listeria monocytogenes*, *Pseudomonas* spp. and *Proteus* spp. Nevertheless, many of the Indian is likely to have street food frequently because of delicacy, poverty, obligations, and idleness, regardless of the safety of health and life. Therefore, the microbial safety of street foods is questionable where foods and surrounding environment thought to be a source of not only virulent pathogens but also antimicrobial resistant pathogens (Iroha et al. 2011; Chrun et al. 2017). The traceability of food origin is also one of the important factors for food safety (El Sheikha and Xu 2017; El Sheikha 2017). Moreover, the consumption of antibiotics in India is expected to double by 2030 which may increase the burden of antimicrobial resistance (AMR) pathogens. However, improved water quality could significantly reduce the burden of childhood diarrheal diseases, which in turn would reduce antibiotic consumption (Nandi et al. 2017).

Resistant bacteria enter the food during various manufacturing and processing operations and are a potential threat to consumers. The crude infectious disease mortality rate in India is 416.75 per 100,000 persons (Laxminarayan et al. 2017) and there was a steep increase in MRSA from 29 to 47% during 2009–2014 (CDDEP 2015). Emerging reports from India documented the superbugs in food-producing animals (Pruthvishree et al. 2017; Nirupama et al. 2018). According to the Indian Network for Surveillance of Antimicrobial Resistance (INSAR), there is a widespread existence of superbugs throughout the country including a startling 41% of MRSA. The mortality due to *S. aureus* bacteremia remains approximately 20–40% despite the availability of effective antimicrobials (Mylotte et al. 1987). Several mechanisms play a key role in the development of resistance, for e.g. methicillin resistance in *S. aureus* is mediated through an altered protein called low-affinity penicillin-binding protein (PBP2a) which is encoded by *mecA* gene, present in chromosomal mobile genetic element called Staphylococcal cassette chromosome mec (SCCmec) (Matsuhashi et al. 1986; Katayama et al. 2000). Since the patient's life is in danger, it is necessary to diagnose, treat and manage the MRSA promptly. Detection of methicillin resistance in India is based on cefoxitin and oxacillin disc diffusion methods with limited reports on MIC determination and detection of *mecA* gene by polymerase chain reaction (PCR) (Bhave et al. 2016; Sharma et al. 2017). In India the street vended foods are not regularly monitored, rather the report on AMR pathogens from street foods are very scanty. Keeping in view, we screened the street vended foods and their environmental samples for virulent MRSA, a pathogen of public health concern.

Materials and methods

A cross-sectional study was carried out from September 2015 to May 2016 in which a total of 430 samples comprising foods of animal origin and associated environmental swabs were collected randomly from different vendors located in Delhi (n = 114 from New Delhi, Old Delhi, Hazrat Nizamuddin and Anand Vihar) and Bareilly (n = 316 from Delapeer, Air Force station, Sahdana, Rajendra Nagar and Izatnagar). For each location, the sample size calculation was carried out using Epitools software (http://epitools.ausvet.com.au/content.php?page=home) with 95% CI, 80% power with *Staphylococcus aureus* prevalence between 5 and 20% (Chon et al. 2017). In Bareilly region the number of samples were collected marginally higher than the required samples size because of the easy convincing of the street vendors.

For convenience and as per the method of cooking/processing, the samples were categorized as environmental [Hand Swab (HS), Table Swab (TS), Cloth Swab (CS) and Plate Swab (PS)], raw foods (chicken, egg, milk, paneer and fish), ready to eat foods (lassi, rasmalai, burfi, pedha, curd, rasgulla, salad, chutney and masala) and cooked foods (chicken gravy, omelette, cooked fish, boiled egg and boiled milk). Based on availability of the type of samples and willingness of the vendors, 92 environmental swabs, 193 raw, 107 ready to eat and 38 cooked food samples were collected. Swab samples were collected by rubbing on 10 × 10 cm area of the table, plate, cloth and entire surface area of hand (palm) by moistened swabs. The collected swabs were kept in a separate screw-capped tube or test tube containing 10 mL sterile maintenance medium (0.9% NSS + 0.1% peptone) (Vaidya et al. 2007). Approximately 50–100 g each food sample was collected separately in sterile polythene bags. All the samples were transported under cold chain and immediately processed for isolation of *S. aureus* as per FSSAI (2012) followed by identification by Gram staining

and biochemical characterization by catalase and coagulase test (BAM 2001).

Confirmation of *S. aureus* isolates by PCR

Genomic DNA of *Staphylococcus* isolates was extracted by snap chill method (Swetha et al. 2015) and analyzed for the presence of *nuc* gene by species-specific primers (Brakstad et al. 1992). PCR was optimized using different concentrations of reagents and cyclic conditions that affect the sensitivity and specificity. The optimization was carried out with different annealing temperature (as per Tm and TA value of the primer); primer concentration (5–20 pmol); template volume (2–10 µL) and *Taq* DNA polymerase (1–3 U) (Thermo Scientific, USA). After optimization, the PCR was carried out in 0.2 mL tube containing reaction mixture (25 µL) comprised of 2.5 µL of 10 × *Taq* buffer with 25 mM $MgCl_2$, 2.5 µL of 2 mM of each dNTP, 10 pmol of each primer (forward and reverse), 1 U of *Taq* DNA polymerase, 2 µL of DNA template and nuclease-free water to make volume up to 25 µL. The details of primer used, cycling conditions and product size were listed in Table 1. PCR products were analyzed by resolving on 1.5% agarose gel containing ethidium bromide by electrophoresis (Sambrook and Russell 2001) and photographed using a gel doc system (UVP, UK).

Isolation and identification of methicillin-resistant *S. aureus* (MRSA)

The confirmed *S. aureus* isolates were streaked on MeReSa agar (Hi-Media, India) and incubated at 35–37 °C for 18–48 h. Light pink colored colonies suspected of MRSA were selected for further confirmation and characterized for virulence and AMR genes.

Phenotypic detection of MRSA and beta-lactamase resistance by disc diffusion assay

Antibiotic sensitivity of the confirmed *S. aureus* isolates was carried out by the Kirby–Bauer disc diffusion technique using Mueller–Hinton agar (CLSI 2013). Briefly, each culture was inoculated into sterile BHI broth (Hi-Media, India) and incubated at 37 °C for overnight. The turbidity of the inoculum was compared with 0.5McFarland standards. Pure broth culture of each isolate was spread on to the Mueller–Hinton agar (Hi-Media, India) supplemented with 2% NaCl and kept for drying. Antibiotic discs viz. oxacillin (1 µg), cefoxitin (30 pg), and penicillin G (10 units) (BD BBL Sensi-Disc, USA) were aseptically placed over the dried agar surface of plates and incubated at 35 °C for 24 h. Inhibition zone diameter of < 21 mm, < 12 mm and < 28 mm were considered as resistant to cefoxitin, oxacillin, and penicillin G, respectively (CLSI 2013).

Genotypic detection of MRSA, beta-lactamase resistance and virulence genes by PCR

The primers and PCR cycling conditions used for amplification of MRSA and beta-lactamase resistance genes (*mecA* and *blaZ*) and virulence (*coa*, *clfA*, *spA*, *FnbA*, and *sea*) genes were given in Table 1. All the primers were custom synthesized from M/s. Eurofins India Ltd. The procedure for PCR amplification and agar gel electrophoresis was carried out as described earlier.

Enterobacterial repetitive intergenic consensus (ERIC)-PCR

The details of ERIC-PCR primer and cycle conditions were given in Table 1. The amplicons were electrophoresed in 1.5% agarose gel for determining phylogenetic relationship among the isolates by dice coefficient using unweighted pair group method with arithmetic mean (UPGMA) method (UVP, UK). The discriminatory power (D) value was calculated by using an online calculator for discriminatory power (http://insilico.ehu.es/mini_tools/discriminatory_power/).

Statistical analysis

Statistical analysis was done with SPSS version 20.0 on Windows platform. The association between the location, type of samples, number of *S. aureus* and MRSA was tested by Chi square test with Yates correction/Fisher's exact (two-tailed).

Results and discussion

Indians have the delicacy and practice of consuming street vended foods, where, poor quality raw foods, unhygienic and inadequate sanitary practices are rampant which may disseminate harmful bacteria. The National Policy for Urban Street Vendors/Hawkers in India stated that street vendors constitute approximately 2% of the population of a metropolis (Bhowmik 2005). It is well known that the foods of animal origin such as chicken meat and raw milk are prone to many bacterial contaminations including MRSA (Rodriguez-Lazaro et al. 2017). Therefore, the present study was aimed to determine the AMR and virulence genes in *Staphylococcus aureus*, for which a total of 430 street vended foods of animal origin and associated environmental samples were processed for isolation and identification of *S. aureus*. Of the 430 samples, 52 (52/430,

Table 1 Primer sequence used for amplifying corresponding genes in PCR assay

Sl. no.	Gene	Primer	Annealing temperature (°C)	Product size (bp)	References
1.	*nuc*	F- CGATTGATGGTGATACGGTT R-ACGCAAGCCTTGACGAACTAAAGC	94 °C × 5 m/94 °C × 30 s − 57 °C × 1 m − 72 °C × 1 m (30 Cycles)/72 °C × 7 m	279	Brakstad et al. (1992)
2.	*coa*	F-ATAGAGATGCTGGTACAGG R-GCTTCCGATTGTTCGATGC	94 °C × 5 m/94 °C × 1 m − 58 °C × 1 m − 72 °C × 1 m (35 Cycles)/72 °C × 7 m	Variable	Hookey et al. (1998)
3.	*spA*	F-CACCTGCTGCAAATGCTGCG R-GGCTTGTTGTTGTCTTCCTC	94 °C × 5 m/94 °C × 1 m − 58 °C × 1 m − 72 °C × 1 m (35 Cycles)/72 °C × 7 m	Variable	Sekr et al. (2013)
4.	*clfA*	F-GGCTTCAGTGCTTGTAGG R-TTTTCAGGGTCAATA TAAGC	94 °C × 5 m/94 °C × 1 m − 58 °C × 1 m − 72 °C × 1 m (35 Cycles)/72 °C × 7 m	975	Stephan et al. (2001)
5.	*fnbA*	F-GCGGAGATCAAAGACAA R-CCATCTATAGCTGTGTGG	95 °C × 5 m/95 °C × 1 m − 49 °C × 1 m − 72 °C × 1 m (35 Cycles)/72 °C × 7 m	1280	Booth et al. (2001)
6.	*entA* (*sea*)	F-AAAGTCCCGATCAATTTATGGCTA R-GTAATTAACCGAAGGTTCTGTAGA	94 °C × 5 m/94 °C × 30 s − 58 °C × 1 m − 72 °C × 1 m (35 Cycles)/72 °C × 7 m	216	Kalorey et al. (2007)
7.	*mec*A	F-AAGCAATAGAATCATCAGAT R-AGTTCTGCAGTACCGGATTTGC	95 °C × 5 m/94 °C × 30 s − 57 °C × 1 m − 72 °C × 1 m (35 Cycles)/72 °C × 7 m	451	Kamal et al. (2013)
8.	*blaZ*	F-AAGAGATTTGCCTATGCTTC R-GCTTGACCACTTTTATCAGC	95 °C × 5 m/94 °C × 30 s − 56 °C × 1 m − 72 °C × 1 m (35 Cycles)/72 °C × 7 m	517	Haveri et al. (2005)
9	ERIC	F-ATGTAAGCTCCTGGGGATTCAC R-AAGTAAGTG ACTGGGGTGAGCG	94 °C × 5 m/94 °C × 30 s − 36 °C × 1.5 m − 72 °C × 2 m (40 Cycles)/72 °C × 7 m	120–2500	Ye et al. (2012)

Table 2 Details of *S. aureus* isolates from different street vended food samples collected from Delhi and Bareilly

Description of samples	Type of samples	Delhi			Bareilly		
		No. of samples analysed	No. of *S. aureus* isolates	No. of MRSA isolates^{+}	No. of samples analysed	No. of *S. aureus* isolates	No. of MRSA isolates^{+}
Environmental	HS	5	0	0	17	3	0
	TS	1	0	0	16	0	0
	CS	0	0	0	16	1	0
	PS	3	0	0	18	0	0
	Water	0	0	0	16	0	0
	Sub-total	9	0^{Aa}	0^{Ba}	83	4.81% (4)^{Ca}	0^{Da}
Raw foods	Raw chicken	14	3	0	25	7	2
	Raw egg	17	1	0	16	0	0
	Raw milk	10	5	2	39	17	5
	Paneer	21	3	0	26	5	0
	Channa	0	0	0	5	2	0
	Raw fish	0	0	0	8	0	0
	Raw kabab	0	0	0	12	0	0
	Sub-total	62	19.35% (12)^{Ab}	3.22% (2)^{Bb}	131	23.66% (31)^{Db}	5.34% (7)^{Db}
Ready to eat foods	Lassi	2	0	0	2	0	0
	Rasmalai	0	0	0	5	2	0
	Burfi	0	0	0	5	1	0
	Pedha	2	0	0	3	0	0
	Curd	4	0	0	3	1	0
	Rasgulla	2	0	0	2	0	0
	Salad	14	0	0	28	0	0
	Chutney	7	0	0	20	0	0
	Masala	0	0	0	8	1	0
	Sub-total	31	0^{Ac}	0^{Bc}	76	6.57% (5)^{Cc}	0^{Dc}
Cooked foods	Chicken gravy	1	0	0	1	0	0
	Omelette	3	0	0	7	0	0
	Cooked fish	1	0	0	0	0	0
	Boiled egg	4	0	0	6	0	0
	Boiled milk	3	0	0	4	0	0
	Cooked kabab	0	0	0	8	0	0
	Sub-total	12	0^{Ad}	0^{Bd}	26	0^{Cd}	0^{Dd}
Total		114	10.52% (12)	1.75% (2)	316	12.65% (40)	2.21% (7)

Values with different uppercase superscripts in a column differ significantly ($p < 0.05$)

Values with different lowercase superscripts in a row differ significantly ($p < 0.05$)

HS hand swab, *TS* table swab, *CS* cloth swab, *PS* plate swab, *NS* non-significant

$^{+}$No. of MRSA isolates carrying *mecA* gene

Table 3 Antimicrobial resistance and virulence profile of *S. aureus* isolates from street vended foods of animal origin and associated environment

Isolate code	Type of sample	Type of vendor	Antimicrobial resistance				Virulence profile
			Phenotypic resistant profile			Genotypic resistant profile	
			Cefoxitin	Oxacillin	Penicillin G		
DS2	Paneer	Milk vendor	S	S	S	–	*nuc*, *spA*, *coa*, *fnbA*
DS3	Paneer	Milk vendor	R	R	R	*blaZ*	*nuc*, *spA*, *coa*, *fnbA*
DS7	Channa	Milk vendor	S	S	R	–	*nuc*, *clfA*, *spA*, *coa*, *fnbA*
DS9	Burfi	Milk vendor	S	S	R	*blaZ*	*nuc*, *spA*, *coa*, *fnbA*
DS13	Curd	Milk vendor	S	S	S	–	*nuc*, *coa*, *fnbA*
DS14	Raw milk	Milk vendor	R	R	R	*mecA*	*nuc*, *spA*, *coa*, *fnbA*
DS18	Channa	Milk vendor	S	S	S	–	*nuc*, *spA*, *coa*, *fnbA*
DS20	Hand swab	Milk vendor	S	S	R	*blaZ*	*nuc*, *clfA*, *spA*, *coa*, *fnbA*
DS22	Raw milk	Milk vendor	R	R	S	*mecA*, *blaZ*	*nuc*, *clfA*, *spA*, *coa*, *fnbA*
DS29	Paneer	Milk vendor	S	S	R	*blaZ*	*nuc*, *clfA*, *spA*, *coa*
DS31	Paneer	Milk vendor	S	S	R	*blaZ*	*nuc*, *clfA*, *spA*, *coa*
DS32	Raw chicken	Meat vendor	R	R	R	*mecA*, *blaZ*	*nuc*, *clfA*, *spA*, *coa*, *fnbA*
DS33	Raw chicken	Meat vendor	S	R	R	*blaZ*	*nuc*, *spA*, *coa*
DS36	Raw chicken	Meat vendor	R	S	R	*mecA*, *blaZ*	*nuc*, *spA*, *coa*
DS42	Raw chicken	Meat vendor	S	R	S	*blaZ*	*nuc*, *spA*, *coa*
DS43	Raw chicken	Meat vendor	S	S	S	*blaZ*	*nuc*, *clfA*, *spA*, *coa*
DS45	Paneer	Milk vendor	S	S	R	*blaZ*	*nuc*, *coa*
DS49	Raw milk	Milk vendor	R	R	R	*blaZ*	*nuc*, *clfA*, *spA*, *coa*, *fnbA*
DS50	Paneer	Milk vendor	S	R	R	*blaZ*	*nuc*, *spA*, *coa*
DS52	Raw milk	Milk vendor	S	S	R	–	*nuc*, *clfA*, *spA*, *coa*, *fnbA*
DS53	Raw milk	Milk vendor	S	R	R	–	*nuc*, *clfA*, *spA*, *coa*, *fnbA*
DS54	Raw milk	Milk vendor	S	R	R	–	*nuc*, *clfA*, *spA*, *fnbA*
DS55	Cloth swab	Milk vendor	S	R	S	–	*nuc*, *spA*, *coa*
DS56	Raw milk	Milk vendor	S	R	R	*blaZ*	*nuc*, *clfA*, *spA*, *coa*, *fnbA*
DS61	Raw chicken	Meat vendor	S	S	R	*blaZ*	*nuc*, *coa*, *entA*
DS63	Raw chicken	Meat vendor	S	S	R	*blaZ*	*nuc*, *spA*, *coa*, *fnbA*
DS70	Raw chicken	Meat vendor	S	S	R	–	*nuc*, *spA*, *coa*, *entA*
DS71	Raw chicken	Meat vendor	S	S	R	–	*nuc*, *coa*, *fnbA*
DS73	Paneer	Milk vendor	S	S	R	*blaZ*	*nuc*, *coa*
DS74	Raw milk	Milk vendor	R	R	R	*mecA*, *blaZ*	*nuc*, *coa*, *entA*
DS76	Rasamalai	Milk vendor	S	S	R	*blaZ*	*nuc*, *coa*
DS77	Raw milk	Milk vendor	R	R	R	*mecA*, *blaZ*	*nuc*, *coa*, *entA*
DS78	Hand swab	Milk vendor	S	R	R	*blaZ*	*nuc*, *coa*
DS79	Raw egg	Egg vendor	S	S	R	–	*nuc*, *coa*
DS80	Hand swab	Milk vendor	S	S	R	*blaZ*	*nuc*, *coa*
DS81	Raw milk	Milk vendor	S	S	S	–	*nuc*, *coa*
DS82	Masala	Egg vendor	R	S	R	*blaZ*	*nuc*, *spA*, *coa*, *fnbA*
DS83	Rasamalai	Milk vendor	S	S	R	*blaZ*	*nuc*, *coa*, *fnbA*
DS84	Raw milk	Milk vendor	S	S	S	–	*nuc*, *spA*, *coa*
DS85	Raw chicken	Meat vendor	R	S	R	–	*nuc*, *spA*, *coa*, *fnbA*
DS86	Raw milk	Milk vendor	S	S	R	*blaZ*	*nuc*, *clfA*, *coa*, *fnbA*
DS87	Raw milk	Milk vendor	S	S	R	*blaZ*	*nuc*, *coa*, *fnbA*
DS88	Raw milk	Milk vendor	S	S	R	*blaZ*	*nuc*, *coa*, *fnbA*
DS89	Raw milk	Milk vendor	S	R	R	*blaZ*	*nuc*, *coa*, *fnbA*

Table 3 continued

Isolate code	Type of sample	Type of vendor	Antimicrobial resistance				Virulence profile
			Phenotypic resistant profile			Genotypic resistant profile	
			Cefoxitin	Oxacillin	Penicillin G		
DS90	Paneer	Milk vendor	S	S	R	*blaZ*	*nuc*, *clfA*, *coa*,
DS91	Raw milk	Milk vendor	S	S	R	*blaZ*	*nuc*, *coa*
DS92	Raw milk	Milk vendor	S	S	R	*blaZ*	*nuc*, *coa*
DS93	Raw milk	Milk vendor	R	R	R	*mecA*, *blaZ*	*nuc*, *coa*
DS94	Raw milk	Milk vendor	R	R	R	*mecA*	*nuc*, *clfA*, *coa*, *fnbA*
DS95	Raw milk	Milk vendor	R	R	R	*mecA*, *blaZ*	*nuc*, *clfA*, *coa*, *fnbA*
DS96	Raw milk	Milk vendor	S	S	R	*blaZ*	*nuc*, *spA*, *coa*
DS97	Raw milk	Milk vendor	S	S	R	*blaZ*	*nuc*, *spA*
Total						*mecA*-9, *blaZ*-36	*nuc*-52, *clfA*-16, *spA-29*, *coa-50*, *fnbA*-26, *entA*-4

12.09%) samples were positive for *S. aureus* by cultural, morphological, biochemical and PCR assay. Relatively higher number of *S. aureus* was isolated in raw food samples (22.27%), followed by ready to eat foods (4.67%) and environmental samples (4.34%). Methicillin-resistant *S. aureus* has emerged as a pathogen of global importance due to public health significance and rising antibiotic resistance pattern (Ferreira et al. 2012). It can be detected by a number of methods like bacteriological isolation in differential chromogenic media, disc diffusion agar test with oxacillin or cefoxitin. In the present study, while analyzing 52 *S. aureus* isolates for methicillin-resistant pattern by disc diffusion assay, it was observed that 13 (25%), 19 (36.5%) and 43 (82.7%) isolates were resistant to cefoxitin, oxacillin, and penicillin G, respectively (Table 3). As per the CLSI (2013) guidelines, if any isolate is resistant to any one of the two antimicrobials namely cefoxitin and oxacillin, it may be considered as methicillin-resistant. Therefore, 42.30% (22/52) isolates were considered as methicillin-resistant. Thus, overall presence of methicillin resistance was observed as 5.11% (22/430) in street vended foods and its associated environment, of which majority were from milk and milk products (16/430, 3.72%). Of the 22 resistant *S. aureus* isolates, only nine isolates showed characteristic pink color colonies on MeReSa agar. However, to rule out the false positivity in the phenotypic test, it is equally important to confirm these isolates by a molecular method like PCR targeting *mec*A gene. On genotyping screening, the 9 isolates which showed characteristic growth on MeReSa agar harbored *mec*A gene. Thirteen out of 22 MRSA (detected by cefoxitin disc diffusion method) isolates did not carry *mecA* gene, indicated existence of some other mechanism of resistance, either by *mecC* gene or unknown. Adhikari et al. (2017) also noted that the *mecA* gene was absent in 7 of the MRSA isolates detected by the cefoxitin disc diffusion method. Further, the *mecA* gene was absent in methicillin- susceptible S. aureus (MSSA) isolates. Presence of Methicillin-sensitive and Methicillin-resistant *S. aureus* in foods of animal origin sold at the street was also reported by Lozano et al. (2016). Even in India, *S. aureus* was reported from street vended foods sold at Gangtok, Nainital and Tumkur cities (Kharel et al. 2016; Sudeep Kumar et al. 2017). However, the prevalence of MRSA in these foods has not been studied in detail. Recent reports suggest that human clinical samples have a higher prevalence of MRSA i.e. 53.74% and 76.75% from Moradabad and Jaipur, India (Kumar and Bhadauria 2016; Gupta and Sinha 2017).

Resistance to penicillin G can be considered as beta-lactamase resistance in *S. aureus* (CLSI 2013). The detection of the *blaZ* gene by PCR is equally important to know the presence of beta-lactamase producing gene. Of the 82.69% (43/52) phenotypic beta-lactam resistant isolates, 69.23% (36/52) isolates harbored *bla*Z gene. Among 9 MRSA isolates, phenotypic and genotypic beta-lactam resistance was detected in 8 and 7 isolates, respectively. Beta-lactamase production was also observed by Adhikari et al. (2017) in 71.82% of the *S. aureus* isolates.

The *nuc* and *coa* genes are considered as important virulent determinants of *S. aureus* (Chesneau et al. 1993; Da Silva and Da Silva 2005). In this study, all the 52 *S. aureus* isolates harbored *nuc* gene (100%) and 50 isolates carried *coa* gene (96.15%). Similar findings have been

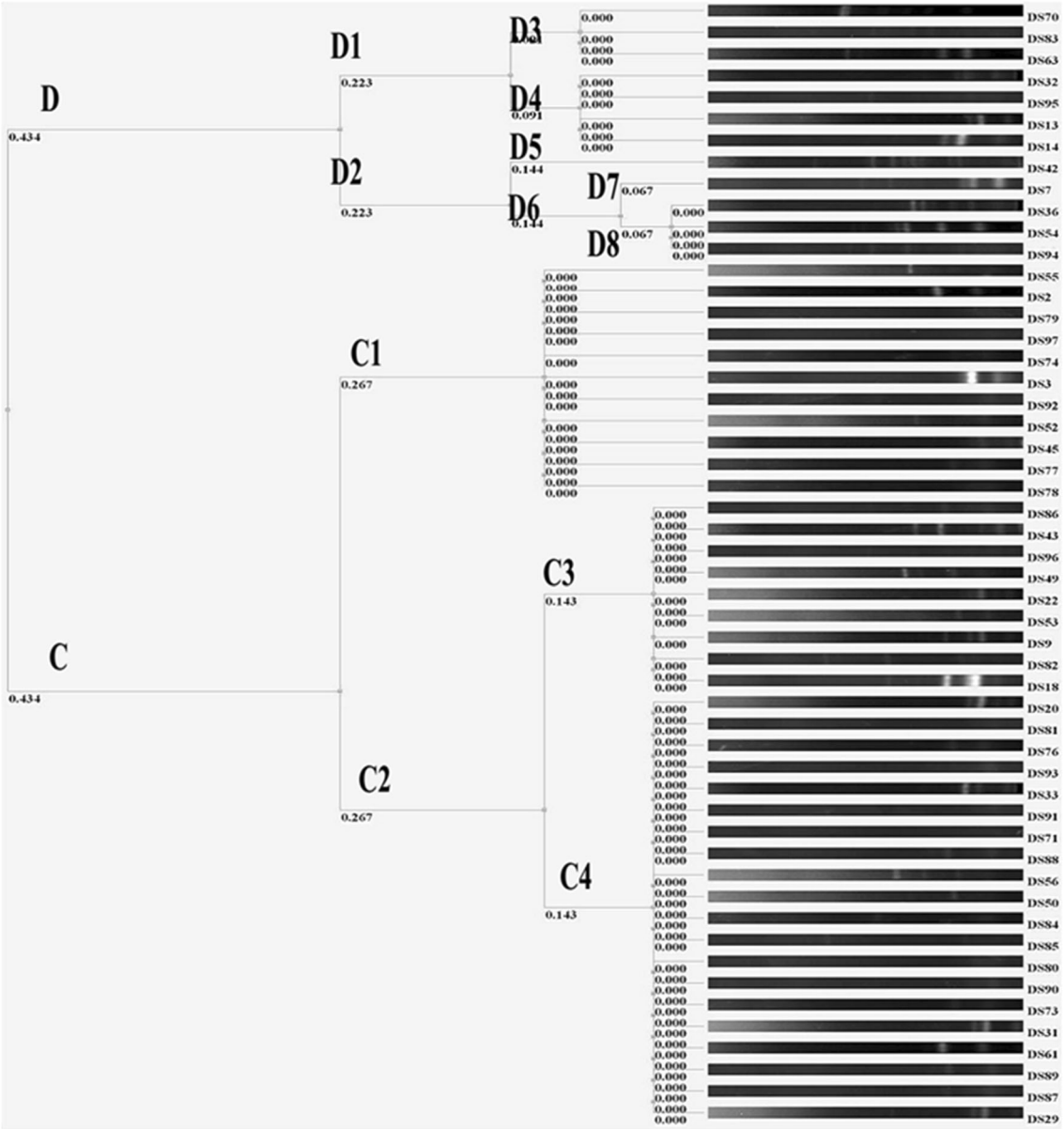

Fig. 1 Phylogenetic analysis of *S. aureus* isolates isolated from street vended foods of animal origin and associated environment

reported by Salem-Bekhit et al. (2010) and Momtaz et al. (2013). The *Staphylococcal* protein A (*spA*), fibronectin binding proteins A and B (*Fnb*A and *Fnb*B), collagen-binding protein, and clumping factor (*clf*) A and B proteins are necessary for attachment of *S. aureus* to the host cell surface to initiate the colonization (Foster and Hook 1998). In the present study, 30.76% (16/52) isolates harbored *clf*A; 55.76% (29/52) *spA*; 50% (26/52) *Fnb*A and 7.69% (4/52) *ent*A (*sea*) genes. Similarly, Momtaz et al. (2013) reported 76.92% isolates with clumping factor A and 26.82% isolates with *spA* gene. In chicken and bovine mastitis samples, 4.5–25.5% isolates carried *sea* gene (Hwang et al. 2010; Mashouf et al. 2015). In contrast, Kumar et al. (2011) reported *Fnb*A gene in all the isolates from mastitis milk samples while, Kalorey et al. (2007) could not detect any of the isolate carrying *sea* gene among 37 *Staphylococcal* isolates screened in Nagpur, India. Surprisingly, all the MRSA isolates recovered in the present study was from raw food samples and the majority of them were from milk and milk products (16/22, 72.72%) (Tables 2, 3). It was also observed that egg, meat, and their products were less contaminated by *S. aureus* as compared to milk and its products.

The raw food items that showed high isolation rates from Delhi were raw milk (50%); raw chicken (21.42%); paneer (14.28%) and raw egg (5.88%) with the overall

isolation rate of 19.35%. In Bareilly, items that revealed high isolation rates were raw milk (43.58%), channa (40.0%), rasmalai (40.0%), curd (33.33%), raw chicken (28.0%), burfi (20.0%), paneer (19.23%), hand swab (17.64%), masala (12.5%) and cloth swab (6.25%) with an overall of 12.65% (40/316). The food item wise analysis for presence of *S. aureus* and MRSA isolates in Delhi and Bareilly region showed that raw foods of Bareilly region carried significantly ($p < 0.05$) higher number of *S. aureus* isolates than environment, ready to eat and cooked foods. Between the two places there was no significant difference ($p > 0.05$) in the presence of *S. aureus* and MRSA in different food samples (Table 2). The isolation rate from milk vendors, meat vendors, and egg vendors were 17.7%, 13.6%, and 2.12%, respectively. The present study revealed the highest isolation rate of *S. aureus* in milk samples, and similar findings were reported by Kamal et al. (2013) and Kalorey et al. (2007). This might be due to well adaptation of *S. aureus* with the udder tissue and cause of mastitis. Further, this pathogen is sturdy in the environment and could contaminate the surfaces and hands of street vendors, especially the milk vendors. There are only a few studies on *S. aureus* isolation from street vended foods from India and the studies carried out in Silchar city, Assam by Sharma and Mazumdar (2014) revealed that 14.2% street vended food samples were positive for *S. aureus* while in Gangtok and Nainital, it was detected in 19.5% and 33% street vended food samples (Kharel et al. 2016). Various workers reported that between 2.42% and 69.74% restaurant/street food samples harbored MRSA (Rhee and Woo 2010; Rizek et al. 2011; Ranjbar et al. 2017), thus responsible for health implications to the consumer. In another study, enterotoxigenic *S. aureus* was detected in 91 (60%) samples of coriander sauce, 87 (58%) samples of coconut slices and 129 (86%) samples of ready-to-eat salads in New Delhi and Patiala City (Ghosh et al. 2007). In contrast to our study, Sharma and Mazumdar (2014) and Ghosh et al. (2007) reported a high isolation rate of *S. aureus*.

All the 52 isolates were characterized by ERIC PCR to determine the genetic diversity and phylogenetic relationship among the isolates. All the isolates were typeable by ERIC PCR. The Dendrogram analysis of 52 *S. aureus* isolates revealed 8 distinct types/clades with discriminatory power (D value) of 0.77. These isolates formed two main clusters (C and D) with the heterogeneity of 43.4%. The two main clusters further divided into sub-clusters and formed 8 clades (D3, D4, D5, D7, D8, C1, C3, and C4) (Fig. 1). A low D value was observed in this study compared to Ye et al. (2012) who reported that ERIC-PCR classified 35 *S. aureus* isolates into 28 ERIC types with a D value of 0.98. Only 12 isolates were grouped in D clade while remaining all in clade C in which 11 isolates (from one cloth swab, three paneer, one hand swab, one raw egg and five raw milk) formed a subgroup C1 and five on them were MRSA. It is surprising to note that, of the 12 isolates grouped in clade D, all the isolates were either sensitive or resistant to both cefoxitin and oxacillin except the 3 isolates (two from raw chicken and one from raw milk) which were either resistant to one. Remaining 10 phenotypically confirmed MRSA isolates were grouped in C2 and C3 clade. The similarities between the ERIC profiles among isolates from diverse sources as identified in clades C1, C2 and C3 indicate that ERIC PCR fingerprints were effective in differentiating the isolates from various sources. Further, 100% type ability of ERIC PCR reaffirms the fact that this technique is very reliable in genotyping of isolates and hence is a useful tool in food microbiology. The ERIC-PCR has been reported as an effective tool in typing *S. aureus* isolates from various sources including animals (Arslan and Mtulu 2016).

Conclusion

The present study revealed that raw foods, environmental samples and ready to eat food samples were a major source of *S. aureus* and MRSA which is a public health concern. Though all the environmental swabs and many ready-to-eat foods were contaminated, all cooked food samples were free from *S. aureus* and MRSA. The ERIC-PCR analysis showed relatedness between the isolates from different sources indicated that a common source of contamination and warrants proper hygiene measures. There is also an urgent need for continuous surveillance of street vended foods to tackle the threat of antibiotic resistance.

Acknowledgements The authors are thankful to the Director and Joint Director (Research), Indian Veterinary Research Institute, Izatnagar, India for providing necessary facilities for the study. The work supported by grants from Indian Council of Agricultural Research, New Delhi, India is duly acknowledged.

References

Adhikari R, Pant ND, Neupane S, Neupane M, Bhattarai R, Bhatta S, Chaudhary R, Lekhak B (2017) Detection of methicillin-resistant *Staphylococcus aureus* and determination of minimum inhibitory concentration of vancomycin for *Staphylococcus aureus* isolated from pus/wound swab samples of the patients attending a tertiary care hospital in Kathmandu, Nepal. Can J Infect Dis Med Microbiol. https://doi.org/10.1155/2017/2191532

Ahmad OB, Lopez AD, Inoue M (2000) The decline in child mortality: a reappraisal. Bull World Health Organ 78:1175–1191

Arslan E, Mtulu EG (2016) Genotyping of *Staphylococcus aureus* strains isolated from bovine mastitis in Turkey by using ERIC-PCR method. Pak J Zool 48(6):1747–1752

BAM (US Food and Drug Administration) (2001) Bacteriological analytical manual, chapter 12, *Staphylococcus aureus*. http://

www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071429.htm
Bhaskar SV (2017) Food borne diseases: disease burden. In: Food safety in the 21st century, 1st edn. Elsevier. pp 3–12. https://doi.org/10.1016/B978-0-12-801773-900001-7
Bhave PP, Ramteerthakar MN, Kartikeyan S, Patil NR (2016) Hospital-based study of methicillin-resistant *Staphylococcus aureus* in surgical site infections with special reference to determination of environmental and human sources. Int J Res Med Sci 4(9):4131–4135. https://doi.org/10.18203/2320-6012.ijrms20162948
Bhowmik SK (2005) Street vendors in Asia: a review. Econ Political Wkly 4:2256–2264
Booth MC, Pence LM, Mahasreshti P, Callegan MC, Gilmore MS (2001) Clonal associations among *Staphylococcus aureus* isolates from various sites of infection. Infect Immun 69:345–352
Brakstad OG, Aasbakk K, Maeland JA (1992) Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. J Clin Microbiol 30(7):1654–1660
CDDEP (Center for Disease Dynamics, Economics and Policy) (2015) State of the world's antibiotics, 2015. Washington, D.C. https://cddep.org/sites/default/files/swa_2015_final.pdf
Chesneau O, Allignet J, El Solh N (1993) Thermonuclease gene as a target nucleotide sequence for specific recognition of *Staphylococcus aureus*. Mol Cell Probes 7:301–310
Chon J, Sung K, Khan S (2017) Methicillin-resistant *Staphylococcus aureus* (MRSA) in food-producing and companion animals and food products. In: Frontiers in *Staphylococcus aureus*. In Tech, London, pp 48–101. https://doi.org/10.5772/66645
Chrun R, Hosotani Y, Kawasaki S, Inutsu Y (2017) Microbiological hazard contamination in fermented vegetables sold in local markets in Cambodia. Biocontrol Sci 22(3):181–185. https://doi.org/10.4265/bio.22.181
CLSI (2013) Performance standards for antimicrobial susceptibility testing, CLSI approved standard M100-S23. Clinical and Laboratory Standard Institute. CLSI document, Wayne, pp 108–111, 132–134
Da Silva ER, Da Silva N (2005) Coagulase gene typing of *Staphylococcus aureus* isolated from cows with mastitis in southeastern Brazil. Can J Vet Res 69:260–264
El Sheikha AF (2017) Traceability and inspection: for safer food supply. Asia Pac J Food Saf Secur 3:1–2
El Sheikha AF, Xu J (2017) Traceability as a key of seafood safety: reassessment and possible applications. Rev Fish Sci Aquac 25(2):158–170
FAO (1997) Street foods. Report of an FAO technical meeting on street foods, Calcutta, India. FAO food and nutrition paper no. 63. Rome FAO Food Nutr Pap 63:1–76
FAO (2012) Selling street and snack foods. http://www.fao.org/docrep/015/i2474e/i2474e00.pdf. Accessed 6 Aug 2018
Ferreira JP, Correa MT, Lyman R, Anderson KL (2012) A review of methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy cattle. Bov Pract 46:1–9
Foster TJ, Hook M (1998) Surface protein adhesins of *Staphylococcus aureus*. Trends Microbiol 6:484–488. https://doi.org/10.1016/S0966-842X(98)01400-0
FSSAI (2012) Manual of methods of analysis of foods. Microbiological testing. Food Safety Standard Authority of India, New Delhi, p 102
Ghosh M, Wahi S, Kumar M, Ganguli A (2007) Prevalence of enterotoxigenic *Staphylococcus aureus* and *Shigella* spp. in some raw street vended Indian foods. Int J Environ Health Res 17:151–156. https://doi.org/10.1080/09603120701219204
Gupta BP, Sinha S (2017) Prevalence of methicillin-resistant *Staphylococcus aureus* in clinical samples of Teerthankar Mahaveer Medical College Hospital and Research Centre (TMMCH & RC), Moradabad (UP), India. Int J Med Res Health Sci 6(6):17–20
Haveri M, Suominen S, Rantala L, Honkanen-Buzalski T, Pyorala S (2005) Comparison of phenotypic and genotypic detection of penicillin G resistance of *Staphylococcus aureus* isolated from bovine intramammary infection. Vet Microbiol 106:97–102. https://doi.org/10.1016/j.vetmic.2004.12.015
Hookey JV, Richardson JF, Cookson BD (1998) Molecular typing of *Staphylococcus aureus* based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. J Clin Microbiol 36:1083–1089
Hwang SY, Park YK, Koo HC, Park YH (2010) spa typing and enterotoxin gene profile of *Staphylococcus aureus* isolated from bovine raw milk in Korea. J Vet Sci 11:125–131. https://doi.org/10.4142/jvs.2010.11.2.125
Iroha IR, Ugbo EC, Ilang DC, Oji AE, Ayogu TE (2011) Bacteria contamination of raw meat sold in Abakaliki, Ebonyi State Nigeria. J Pub Health Epidemiol 3(2):49–53
Kalorey DR, Shanmugam Y, Kurkure NV, Chousalkar KK, Barbuddhe SB (2007) PCR based detection of genes encoding virulence determinants in *Staphylococcus aureus* from bovine subclinical mastitis cases. J Vet Sci 8:151–154. https://doi.org/10.4142/jvs.2007.8.2.151
Kamal RM, Bayoumi MA, El Aal SFA (2013) MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt: a mini-survey. Food Control 33:49–53. https://doi.org/10.1016/j.foodcont.2013.02.017
Katayama Y, Ito T, Hiramatsu K (2000) A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 44:1549–1555. https://doi.org/10.1128/AAC.44.6.1549-1555.2000
Kharel N, Palni U, Tamang JP (2016) Microbiological assessment of ethnic street foods of the Himalayas. J Ethn Foods 3(3):235–241. https://doi.org/10.1016/j.jef.2016.01.001
Kumar S, Bhadauria S (2016) Increasing trend of methicillin-resistant *Staphylococcus aureus* in Jaipur, Rajasthan, India. Afr J Med Res 10(34):1417–1421. https://doi.org/10.5897/AJMR2016.7995
Kumar R, Yadav BR, Singh RS (2011) Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. J Biosci 36:175–188. https://doi.org/10.1007/s12038-011-9004-6
Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N (2017) Antibiotic resistance the need for global solutions. Lancet Infect Dis 13(12):1057–1098. https://doi.org/10.1016/s1473-3099(13)70318-9
Lozano C, Gharsa H, Slama KB, Zarazaga M, Torres C (2016) *Staphylococcus aureus* in animals and food: methicillin resistance, prevalence and population structure. A review in the African continent. Microorganisms 4:12. https://doi.org/10.3390/microorganisms4010012
Mashouf RY, Hosseini SM, Mousavi SM, Arabestani MR (2015) Prevalence of enterotoxin genes and antibacterial susceptibility pattern of *Staphylococcus aureus* strains isolated from animal originated foods in West of Iran. Oman Med J 30:283. https://doi.org/10.5001/omj.2015.56
Matsuhashi M, Song MD, Ishino F, Wachi M, Doi M, Inoue M, Ubukata K, Yamashita N, Konno M (1986) Molecular cloning of the gene of a penicillin-binding protein supposed to cause high resistance to beta-lactam antibiotics in *Staphylococcus aureus*. J Bacteriol 167:975–980. https://doi.org/10.1128/jb.167.3.975-980.1986
Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A, Momeni M (2013) Virulence genes and antimicrobial resistance profiles of *Staphylococcus aureus* isolated from chicken meat in Isfahan province,

Iran. J Appl Poult Res 22:913–921. https://doi.org/10.3382/japr.2012-00673

Mylotte JM, McDermott C, Spooner JA (1987) Prospective study of 114 consecutive episodes of *Staphylococcus aureus* bacteremia. Rev Infect Dis 9:891–907

Nandi A, Megiddo I, Ashok A, Verma A, Laxminarayan R (2017) Reduced burden of childhood diarrheal diseases through increased access to water and sanitation in India: a modeling analysis. Soc Sci Med 180:181–192. https://doi.org/10.1016/j.socscimed.2016.08.049

Nirupama KR, Vinodh Kumar OR, Pruthvishree BS, Sinha DK, Murugan MS, Krishnaswamy N, Singh BR (2018) Molecular characterisation of *bla*OXA48 carbapenemase, extended spectrum beta-lactamase (ESBL) and Shiga toxin producing *Escherichia coli* isolated from farm piglets of India. J Global Antimicrob Resist 13:201–205.

Ouidri MA (2018) Screening of nasal carriage of methicillin-resistant *Staphylococcus aureus* during admission of patients to Frantz Fanon Hospital, Blida, Algeria. New Microbe New Infect 23:52–60. https://doi.org/10.1016/j.nmni.2018.02.006

Pruthvishree BS, Vinodh Kumar OR, Sinha DK, Malik YPS, Dubal ZB, Desingu PA, Shivakumar M, Krishnaswamy SB (2017) Spatial molecular epidemiology of carbapenem-resistant and New Delhi metallo beta-lactamase (blaNDM)-producing Escherichia coli in the piglets of organized farms in India. J Appl Microbiol 122(6):1537–1546. https://doi.org/10.1111/jam.13455

Ranjbar R, Shahreza MHS, Rahimi E, Jonaidi-Jafar N (2017) Methicillin-resistant *Staphylococcus aureus* isolates from Iranian restaurant food samples: Panton-Valentine Leukocidin, SCCmec phenotypes and antimicrobial resistance. Trop J Phar Res 16(8):1939–1949. https://doi.org/10.4314/tjpr.v16i8.26

Rhee CH, Woo GJ (2010) Emergence and characterization of foodborne methicillin-resistant *Staphylococcus aureus* in Korea. J Food Prot 73(12):2285–2290

Rizek CF, Matté MH, Dropa M, Mamizuka EM, de Almeida LM, Lincopan N, Matte GR, Germano PML (2011) Identification of *Staphylococcus aureus* carrying the *mec*A gene in ready-to-eat food products sold in Brazil. Foodborne Pathog Dis 8(4):561. https://doi.org/10.1089/fpd.2010.0706

Rodríguez-Lázaro D, Oniciuc EA, García PG, Gallego D, Fernández-Natal I, Dominguez-Gil M, Eiros-Bouza JM, Wagner M, Nicolau AI, Hernández M (2017) Detection and characterization of *Staphylococcus aureus* and methicillin-resistant *S. aureus* in foods confiscated in EU borders. Front Microbiol 8:1344. https://doi.org/10.3389/fmicb.2017.01344

Salem-Bekhit MM, Muharram MM, Ibrahim M, Alhosiny M, Hasim ESY (2010) Molecular detection of genes encoding virulence determinants in *Staphylococcus aureus* strains isolated from bovine mastitis. J Appl Sci Res 6(2):121–128

Sambrook J, Russell D (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory, Harbor

Sekr K, Sakurada J, Seong HK, Murai M, Tachi H, Ishii H, Masuda S (2013) Occurrence of coagulase serotype among *Staphylococcus aureus* strains isolated from healthy individuals. Microbiol Immunol 42:407–409. https://doi.org/10.1111/j.1348-0421.1998.tb02302.x

Sharma I, Mazumdar JA (2014) Assessment of bacteriological quality of ready to eat food vended in streets of Silchar city, Assam, India. Indian J Med Microbiol 32(2):169. https://doi.org/10.4103/0255-0857.129809

Sharma S, Srivastava P, Kulshrestha A, Abbas A (2017) Evaluation of different phenotypic methods for the detection of methicillin resistant *Staphylococcus aureus* and antimicrobial susceptibility pattern of MRSA. Int J Community Med Public Health 4(9):3297–3301. https://doi.org/10.18203/2394-6040.ijcmph20173832

Stephan R, Annemuller C, Hassan AA, Lammler C (2001) Characterization of enterotoxigenic *Staphylococcus aureus* strains isolated from bovine mastitis in north-east Switzerland. Vet Microbiol 78(4):373–382. https://doi.org/10.1016/S0378-1135(00)00341-2

Sudeep Kumar M, Veena K, Nagaraj ER (2017) Microbial profile of street food from different locations at Tumkur, India. Pathol UpdateTrop J Pathol Microbiol 3(2):84–89

Swetha SV, Babu AJ, Rao TM, Kumar E (2015) Evaluation of various selective and non selective broths for detection of *Listeria monocytogenes* in pork and for PCR compatibility. Int J Adv Res 3(3):316–327

Vaidya VM, Paturkar AM, Waskar VS, Zende RJ, Dubal ZB (2007) Analysis of sources of contamination in organized poultry slaughterhouse. Indian J Comp Microbiol Immunol Infect Dis 28(1&2):48–52

WHO (1996) Essential safety requirements for street-vended foods. Food Safety Unit Division of Food and Nutrition World Health Organization, Geneva, p 3

Ye Y, Jiang Q, Wu Q, Zhang J, Lu J, Lin L (2012) The characterization and comparison of *Staphylococcus aureus* by antibiotic susceptibility testing, enterobacterial repetitive intergenic consensus-polymerase chain reaction, and random amplified polymorphic DNA-polymerase chain reaction. Foodborne Pathog Dis 9(2):168–171. https://doi.org/10.1089/fpd.2011.0927