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Spa Typing and Prevalence of Methicillin-Resistant *Staphylococcus aureus* Isolated in Retail Meats from Silchar, Assam, India

Sagolsem Yaiphathoi, Indu Sharma*

Department of Microbiology, Assam University, Silchar-788011, India.

Abstract | **Background**: The study involves the presence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in animal food origin which is a growing concern as their presence makes them highly pathogenic and difficult to treat. **Materials and methods**: A total of 414 samples consisting of pork 50 (12.07%), chicken 86 (20.77%), beef 74 (17.87%), mutton 100 (24.15) and fish 104 (25.12%) were randomly purchased from different retail meat shops located at Silchar, Assam, India. All the isolates were tested against cefoxitin (30µg) and oxacillin (1µg) and classified as MRSA isolates. *Spa* typing of randomly selected 136 MRSA isolates was done followed by antimicrobial susceptibility testing. **Results:** Overall prevalence rate of MRSA was observed as 38/104 (36.53%) in fish, 33/86 (38.37%) in chicken, 26/74 (35.13%) in beef, 26/100 (26%) in mutton and 13/50 (26%) in pork. Of 136 MRSA isolates, only 12 isolates (8.82%) that harboured the *spa* gene amplified at 371bp. The frequently found environmental and human associated *spa* type t021 (clonal complex CC30, 83.33%) and a less frequently found *spa* type t448 (16.66%) were recovered from chicken, mutton, beef and pork. All tested MRSA isolates were found to be 100% resistant to cefoxitin followed by oxacillin and clindamycin but resistance to other antibiotics were variable. **Conclusion:** This is the first pilot study to be conducted in Silchar retail meat markets demonstrating the occurrence of MRSA in chicken, mutton, beef, pork and fish, thus, we strongly recommend regular monitoring and surveillance, implementation of good hygienic practices be incorporated to control the dispersion of MRSA to the community.

Keywords | MRSA, Retail meat, *Spa*-typing, Antibiotic resistance.

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***Correspondence** | Indu Sharma, Department of Microbiology, Assam University, Silchar-788011, India; **Email**: drsharma7652@gmail.com
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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* were found responsible for causing several primary infections both in humans and animals (Reacher et al., 2000). Identification of MRSA strains in animal based food leads to strong concerns world widely regarding the food-borne contamination and their intoxicationssince MRSA has been procured in retail meats (Tauxe, 2002). *S. aureus* has the ability to become methicillin resistant by acquisition of *mecA* gene which encodes the protein 2a where penicillin gets binded with extremely low affinities for β-lactams. Thus several methods, based on phenotypes were developed to detect the methicillin resistance in *S. aureus* isolates, but the PCR based detection for *mecA* gene is applicable due to its high sensitivity and specificity (Chambers, 1997).

Both community-acquired and hospital-acquired MRSA infections are of global concern thereby making their treatment complicated due to resistance for various antimicrobials and strain variations. Emergence of Livestock-associated MRSA has become an important concern with animal contact which is considered as a leading source ofhuman MRSA infections caused by livestock-associated MRSA strains (Wulf et al., 2008). Besides animal-human transmission, livestock acts as an MRSA transporter into the communities which are situated nearby animal farms. Upon entering the food chain, they might serve as convenient vehicles for bacterial transfer, possibly threatening food handlers and consumers (Boost et al., 2013, Crombe et al., 2013; Monaco et al., 2013). ST398 is circulating

Table 1: Primers and PCR conditions used for *Spa*-typing of MRSA.

PCR	Genes	Primer sequence (5′–3′)	Denaturation		Annealing		Elongation		No. of cycles
			Temp (°C)	Time (sec)	Temp (°C)	Time (sec)	Temp (°C)	Time (min)	
Simplex	*spa*-F	TAAAGACGATCCTTCGGTGAGC	94	40	61	30	72	2	30 cycles
	spa-R	CAGCAGTAGTGCCGTTTGCTT							

well in between animals and professionals (veterinarians, meat seller, employees of farm). Nevertheless, a strong suggestion for the existence of ST398 type with the exception of livestock which include retail meats has been seen because it was observed that some human infections cause by MRSA is not related with animal based food (Stinear et al., 2014) and recently reports have also supported the presence of ST398 in retail meats (Pu et al., 2009; Jackson et al., 2013). Since MRSA strains have now been well established in retail meats requires a strict epidemiological surveillance to understand the association between transmission dynamics and the risks factorsfrom animal production to slaughtering methods and its processing. Since, limited information in regard to prevalence and dissemination of MRSA *spa* types in retail meats in Silchar, Assam, India persists; the following study would therefore be highly helpful in assessing the involvement of risk factors to consumers and in monitoring the emergence and dissemination of MRSA virulence factors in retail meats.

Therefore, the aim of the study was to investigate the prevalence of MRSA *spa* types and antimicrobial resistance pattern in retail meats from Silchar, Assam, India.

MATERIALS AND METHODS

Sample Collection and Processing

A total of 414 samples consisting of pork 50 (12.07%), chicken 86 (20.77%), beef 74 (17.87%), mutton 100 (24.15) and fish 104 (25.12%) were randomly purchased from different retail meat shops located at Silchar, Assam, India. The packed samples were kept at 4°C in portable cooling containers during transportation, and microbiological analyses were conducted within 4 hours of purchase.For initial enrichment of the samples, 3g of the meat and fish samples was inoculated in 10 ml peptone water followed by incubation of 24 hours at 37°C. Growth was depicted in the broth by observing the turbidity. A loopful of sample was inoculated onto selective Manitol salt agar (MSA) and Baird-Parker agar (BP) (Hi-Media, India) followed by aerobic incubation of plates at 35°C for 24–48 h. MRSA colonies based on appearance (golden yellow-colour, round colonies in MSA andblack, convex, shiny colonies on Baird-Parker agar) were selected for further analysis. Confirmation of staphylococci by gram's stain; catalase testing and coagulase tests were performed.

Methicillin-resistance was further screened by antimicrobial susceptibility testing, performed by following the Clinical and Laboratory Standard Institute guidelines (CLSI 2013). All the isolates were examined by using cefoxitin (30µg) and oxacillin (1µg) and classified as MRSA when the inhibition zone diameter was ≤ 17 mm for oxacillin and ≤ 22 mm for cefoxitin (Mehdi Goudarzi et al., 2016; Omer et al., 2017).

Spa-typing of MRSA

The *spa*-typing of randomly selected 136 MRSA isolates from positive samples was performed by using methods described earlier by Duarte et al. (2002). DNA templates were extracted from fresh MRSA culture by using the cell lysis by simple boiling method as described by Jose et al. (2016). The isolates were inoculated in Brain Heart Infusion Broth and incubated for 48 hours at 35°C, distributed in 1-ml aliquots into microfuge tubes, and was centrifuged at 14000 rpm for duration of five minutes. After discarding the supernatant, the pellets were subjected to DNA extraction protocols. After that in 200 µl of TE buffer (Tris-HCl [10 mM]: EDTA [1 mM]) suspension of bacterial pellets was done and subjected to 15 minutes of boiling. Immediately after boiling, the microfuge tubes were placed in an ice bath for 15 minutes and then at room temperature, the centrifugation step was done 14,000 rpm for 5 minutes. The supernatant containing 100µl DNA template was place to another sterile tube and kept at –20°C. The conditions of primer sequences and amplification are stated in Table 1. A volume of 20µl was used for the overall PCR reactions which included 2 µl of DNA template (primary PCR product). Oligonucleotide primers were used at final concentrations of 0.3 µM in reactions. The reaction mixture contained PCR Buffer, the 4deoxynucleoside triphosphate for a concentration of 0.2 mM and 0.75 U of Ampli Taq polymerase.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility test was performed by using the disc agar diffusion method on Mueller–Hinton agar (CLSI, 2013).

Overnight suspensions of *S. aureus* cultures were balanced to turbidity of 0.5 McFarland standards. The swabs were

dipped in suspensions and streaked onto Muller Hinton agar (MHA) and further it was kept to dry for some time. Then the antibiotic discs were suspended aseptically on the petri plates and after 24hrs of incubation period the results were interpreted. Further, the isolates were tested for antimicrobial agents representing the major class of antibiotics against: Ampicillin (10 µg), Cefoxitin (30µg), Gentamicin (10µg), Erythromycin (15µg), Clindamycin (2µg), Oxacillin (1µg), Levofloxacin (5µg), Nitrofurantoin (300µg), Ciprofloxacin (5µg) and Tetracycline (30µg).

Data Analysis

The data were entered into an excel spreadsheet for analysis. The variables used in the analysis were prevalence of *spa* type in each areas of sample collection that werecompared using chi-square test (χ^2) by using Microsoft excel (version 2007).

RESULTS

Prevalence of Mrsain Animal Foods

Methicillin-resistant *Staphylococcus aureus* was found in 136 (32.85%) isolates of 414 samples. The overall prevalence rate of MRSA was observed as 38/104 (36.53%) in fish, 33/86 (38.37%) in chicken 26/74 (35.13%) in beef, 26/100 (26%) in mutton and 13/50 (26%) in pork. All MRSA isolates did not carry the *spa* genes. Amongst the 136 MRSA positive strains analysed, only 12 isolates (8.82%)harboured the *spa* gene which amplified at 371bp. The highest MRSA prevalence was observed in chicken samples 33/86 (38.37%) which was followed by fish 38/104 (36.53%), beef 26/74 (35.13%), mutton 26/100 (26%) and pork 13/50 (26%). MRSA prevalence rate was significantly different among all the animal based food samples collected.

Spa-typing of MRSA

Amongst the 136 MRSA isolates, two different spa types were detected (Table 2). The frequently found environmental and human associated spa type t021 (clonal complex CC30, 83.33%) was the very frequent spa type found in the three different animal based food that is, pork mutton, beef and a less frequently found Spa type t448 (16.66%), was recovered from chicken. In pork samples, *spa* type t021 was most prevalent (30.76% isolates) followed by beef s (15.38% isolates) and mutton samples (7.69% isolates). The spa type found in chicken sample was diverse from the other three samples which may be due to the presence of less common spa type t448 (6.06 % isolates).

Antimicrobial Susceptibility Testing

The prevalence of antimicrobial resistant in MRSA isolates within each different meat sampleswas notably different. All tested MRSA isolates were 100% resistant to cefoxitin followed by oxacillin and clindamycin but resistance to other antibiotics was variable. All MRSA isolates (100%) isolated from five different meat samples (fish, chicken, pork, beef and mutton) were sensitive towards ciprofloxacin (100%). Isolates from fish, chicken and mutton were found to be sensitive towards gentamicin (71.32%) whereas isolates from pork and beef expressed their resistance (28.67%). Isolates from chicken, mutton and pork were resistant to erythromycin (52.94%), whereas, isolates from fish and beef were sensitive (47.05%). All isolates were found to be sensitive towards nitrofurantoin (90.44%), except isolates from pork expressed their resistance (9.55%). It was noted that ampicillin (56.61%) was resistant to MRSA isolates isolated from fish, pork and beef but sensitive to chicken and mutton (43.38%) isolates. Chicken, mutton and pork isolates showed resistance towards tetracycline (52.94%) whereas isolates from fish and beef were observed to be sensitive (47.05%) for the same. Further, levofloxacin was sensitive towardsfish, chicken and mutton isolates (71.32%) whereas isolates from pork and beef (28.67%) resulted into intermediate sensitivity. Overall results revealed that isolates from pork showed the highest resistance and isolates from fish showed the least resistance.

Table 2: Identified *Spa*-types from various foods of animal origin

Sample Identification number	Sample source	Identified *Spa*-types
SP1	Pork	t021
SP2	Mutton	t021
SP3	Chicken	t448
SP4	Beef	t021
SP5	Pork	t021
SP6	Mutton	t021
SP7	Beef	t021
SP8	Beef	t021
SP9	Chicken	t448
SP10	Pork	t021
SP11	Pork	t021
SP12	Beef	t021

Total isolates=136
Pork=4 *spa* types (30.76%)
Mutton=2 *spa* types (7.69%)
Chicken=2 *spa* types (6.06%)
Beef=4 *spa* types (15.38%)

Data Analysis

Significant differences in the prevalence of *spa* types from different areas of sample collections were observed where the P-value ≤0.05 was considered as statistically significant at 95% confidence interval.

DISCUSSION

Contaminated foods of animal origin have been repre-

sented as a source of MRSA infection forhumans (Lee, 2003) which are now a major global health issues. The cause of the severity of their illnesses (Livermore, 2000) relies on poising risk of incidence of Community associated MRSA (CA-MRSA) and Livestock associated MRSA (LA-MRSA) infections due to increased developing countries (Doufour et al., 2002). The first MRSA infection was described in 1961 (Jevons, 1961) and since then, human infections caused by multi-drug-resistant MRSA have being on raise (Waness, 2010) including MRSA infections from contaminated retail meats (de Boer et al., 2009; Pu et al., 2009; Buyukcangaz et al., 2013). Besides these, MRSA have also raised serious concerns anddrawn attentions though beinga critically important human pathogen, in disseminating its potential capability of infection and colonization in human by the animal based foods as a source.

This research was focused on the MRSA prevalence in animal food origin which included chicken, beef, mutton, pork and fish and it describes the various impacts on the MRSA prevalence in retail animal foods. The present study observed the overall prevalence rate of MRSA as 38/104 (36.53%) in fish, 33/86 (38.37%) in chicken, 26/74 (35.13%) in beef, 26/100 (26%) in mutton and 13/50 (26%) in pork. The findings of this study revealed that prevalence of MRSA in chicken (38.37%) was higher in comparison to other animal foods (Kwoji et al., 2017). In North East India, generally birds are kept on deep-litter system (Kwoji et al., 2017) where control measures for diseases and pathogen dissemination are usually not practiced by most of the private poultry raisers and further add to this there is indiscriminate misuse or overuse of antibiotics for promoting growth and prophylactics in poultry holders and other food animals without in consultation and prescription with the veterinarians (Broens et al., 2012). This has been commonly practised over time thus constituting reservoirs as a threat to other related infections and diseases (Rodrı´guez Noriega et al., 2010). Poultry farmers can be colonized by MRSA CC398 (Herve et al., 2017). Elevated rates of MRSA carriage were disclosed too in the workers of poultry slaughter house in the Netherlands, with much higher carriage rates among workers who contacted live birds that those who worked only with dead fowl (Herve et al., 2017). Besides this, other geographical differences and farm husbandry practices could have also influenced the MRSA prevalence rate observed in current study.In a previous study, it has been observed that the prevalence rate of *S. aureus* on traded pork products were ranging from 12% to 59.7% (Hanson et al., 2011, Waters et al., 2011, Hanning et al., 2012, Pu et al., 2009, Bhargava et al., 2011, Kelman et al., 2011,O'Brien etal., 2012) and the results from the present study show similar findings to that of the previous published reportssince *S. aureus* was found on 26% of pork products.The prevalence rate of *S. aureus* in traded beef products from other studies has shown a range from 20% to 37% while the*S. aureus* prevalence rate of retail beef from this study was observed at 35.13%. The reason could be the usage of different beef products in this study while comparing to other studies (Jackson et al., 2013).

Presently, it has been reported that contamination in aquaculture systems could be the possible reason for such high prevalence rates of MRSA in fish along with possible cross-contamination of the meat handlers to some extend while processing. As stated in earlier findings about the antibiotic usage in animal tissue and their meat products, similar such uses of antibiotics have also been incorporated indiscriminately to promote aquaculture or the fishery industry (Lipsitch et al., 2002) here at North East India.

Further the prevalence rate in mutton was observed in 26% of the meat samples from the present study, but recent studies have shown that among 717 sheep meat samples, a minimum of 6 that is 0.8% were detected as positive for MRSA (Quddoumi et al., 2006) in Jordan while in Nigeria another study was conducted, in clinical and non-clinical swab samples and reported about the isolation frequency of *S. aureus* from healthy sheep nasal samples was 56·2% (Bamaiyi and Aniesona, 2013) and no MRSA were isolated. Such differences in this study could be possibly due to higher contamination rates in retail than in slaughter samples and the increased number of operations the meat of sheep has been subjected to along with the hygienic-sanitary profile of food handlers which is often unacceptable in terms of health status, personal hygiene practices, and habits, thus, raising the risk of cross contamination in the handled food (Campos et al.,2009; Hammad et al., 2012; Kamal et al., 2013; Ferreira et al., 2014).

We identified 12 *spa* types from 136 isolates isolated from chicken, pork, mutton and beef. The predominant *spa* types among the MRSA isolates (t012) represented more than 7.35% of all the MRSA isolates. The presence of t021 *spa* type in three different animal based foods that is pork, beef and mutton were environmental and human associated *spa* types which belong to clonal complex CC30. This *spa* type can cause infection bycolonizing in humans especially in areas where high livestock farming is practised, thus making the environment vulnerable towards dissemination of infection between humans and the environment (Papadopoulos et al., 2018). Another *spa* type t448 detected in the current study from chicken was in accordance to a study conducted at Chicago where, *spa* typing disclosed that *S. aureus* ST88 *spa* genotypes including t448 were colonizing the animals which was also the lead cause of infection in male mice known as PGA(preputial gland adenitis) (Sun et al., 2018).

All tested MRSA isolates in our study were found to be resistant for a minimum of one antibiotic (Bravo et al.,

2015). The differences in antibiotic resistance patterns could be due to regular continuous check on usage of antibiotics in foods of animal origin. Various countries have adopted some norms of regular surveillance on antibiotic usage and their doses but in Silchar (Assam) no such regulations are present to keep a strict vigilance or check on retail meat markets for handling antibiotics. Differences in the origin of meat samples and different geographic settings are added factors that contribute to such differences in the results. Sometimes possibilities of MRSA strains to obtain resistance gene through genetic mobile elements rather than antibiotics from other bacteria in the environment (Jamrozy et al., 2017) is also observed. Thus such type of research needs more attention as post antibiotic era is soon to approach. Hygiene and food safety practices in regard to food production from 'farm to fork' should be strictly followed in order to keep away MRSA from contaminating food.

CONCLUSION

The existence of MRSA in retail meats is of great public health concern as high transmission from raw meat to meat handlers may strengthen the health associated risk factors involving both animals and humans concerned. Thus, MRSA control by *using* whole genome sequence technology will help in differentiating among human and animal isolates and decrease colonization rate and risk factors to both animal and human health. Besides this, a regular monitoring and vigilance practices will be highly appreciated to keep a check on antibiotic resistance too.

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CONFLICT OF INTEREST

None to declare.

AUTHORS CONTRIBUTION

Sagolsem Yaiphathoi: Performed all the experiments.
Dr. Indu Sharma: Manuscript writing and data compilation.

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