

Detection of Methicillin Resistant *Staphylococcus aureus* in Dairy Products and Anterior Nares of Dairy Workers

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ABSTRACT

Objectives: To isolate methicillin resistant *Staphylococcus aureus* (MRSA) from anterior nares of dairy workers and dairy products and assess the antibiotic susceptibility pattern of the isolates.

Methods: Swab samples collected from anterior nares of dairy workers and dairy product (butter) were inoculated into mannitol salt agar and incubated at 37°C for 24 hours. Identification was done based on colony characteristics, Gram's staining, catalase, oxidase and coagulase test. Antibiotic susceptibility testing was done by modified Kirby Bauer disc diffusion method. MRSA was confirmed by using cefoxitin disc.

Results: A total of 109 *S. aureus* (98 from dairy workers and 11 from butter samples) were isolated. Out of them 32 MRSA were isolated from dairy workers and 4 from butter samples. The association between age group and MRSA was found insignificant ($p = 0.115$). The association of MRSA between male and female workers was found significant (>0.05). About 86% of the MRSA isolates were susceptible to Gentamicin (86.11%) followed by Ciprofloxacin (77.78%).

Conclusion: Detection of MRSA among dairy workers and dairy products warrants proper handling and adequate control measures to prevent transmission of MRSA from dairy industry.

Key words: MRSA, dairy products, dairy workers, carriage

INTRODUCTION

Staphylococcus aureus is considered to be one of the leading causes of food borne illness. Milk, dairy products and meats are often contaminated with this bacterium. Foodstuff contamination may occur directly from infected food producing animals or may result from poor hygiene during production processes or the retail and storage of foods, since humans may carry the microorganisms (Hennekinne et al. 2010). *S. aureus* colonizes skin and mucosa of humans and animals, with nasal carriage rates between 30% and 50% among the adult human population (Sakr et al. 2018). While colonization of the anterior nares is usually asymptomatic, it serves as a reservoir for the spread of the organism. Carriers are at increased risk to develop bacteremia which in 80% of cases is caused by the strain colonizing their nares (Hassoun et al. 2017). The rapid emergence of antibiotic resistance among *S. aureus* is also known to play a crucial role in the epidemiology of

staphylococcal infections (Wertheim et al. 2005).

Methicillin resistant *S. aureus* (MRSA) has become an increasingly important and serious public health concern due to the morbidity, mortality and cost that is associated with these infections annually. Once exposed to MRSA, animals may become colonized, and serve as reservoirs to transmit the infection to other animals or humans. Data has shown that with human to animal transmission, there is a possibility that until the animal is free of infection, re-transmission from the animal and subsequent human to human transmission can occur (American Veterinary Medical Association 2009). Therefore, this study aims to isolate MRSA from anterior nares of dairy workers and dairy products and assess the antibiotic susceptibility pattern of the isolates.

MATERIALS AND METHODS

This descriptive cross-sectional study was carried out

Date of Submission: October 25, 2019

Published Online: December, 2019

Date of Acceptance: December 1, 2019

DOI: <https://doi.org/10.3126/tujm.v6i0.26585>

in the quality control laboratory of Dairy Development Corporation, Balaju, Kathmandu, Nepal. A total of 109 samples from dairy workers and dairy products were investigated.

Sample collection and processing

Samples were collected aseptically from nasal swabs of both noses from dairy workers of different working units. The samples were also collected from butter samples by means of sterile cotton swab in separate sterile screw-capped test-tube. All the samples were labeled properly and processed in the laboratory as soon as possible. In case of delay, samples were stored at refrigerator (2-8°C).

Each sample was inoculated into mannitol salt agar plates and incubated at 37°C for 24 hours. Identification of *S.aureus* was done based on colony characteristics, Gram’s staining, catalase, oxidase and coagulase test (Chakraborty 2011). All identified *S. aureus* isolates were processed for antibiotic susceptibility testing by modified Kirby Bauer disc diffusion method as recommended by Clinical Laboratory Standards Institution (CLSI, 2013). Antibiotic discs (Hi Media

Laboratories, Pvt. Limited, India) such as ciprofloxacin (5 µg), clindamycin (2 µg), gentamicin (10 µg), penicillin G (10 U), teicoplanin (10 µg) were used for antibiotic susceptibility tests.

The isolates showing zone of inhibition of ≤ 21 mm around cefoxitin disc (30 µg) were considered as MRSA (CDC 2019). Data were analyzed by IBM SPSS statistics 21 software. Frequency and percentage for descriptive and Chi Square test with cross tab for inferential statistics were used.

RESULTS

Altogether 109 *S. aureus* were isolated of which 98 (89.90%) were from dairy workers and 11 (10.10%) from dairy products. Out of 98 isolates recovered, 63.26% (n= 62) were from male and 36.34% (n= 36) were from female workers. Similarly, 7 (63.63%) of *S. aureus* were isolated from plain butter (exported) and 4 strains (36.36%) were from table butter.

The maximum numbers of MRSA were isolated from dairy workers of age group 40-49 years followed by 30-39 years. However, the number of MRSA and age was not statistically significant (Table 1).

Table 1: Distribution of MRSA among different age groups of dairy workers

Age in years	No. of <i>S. aureus</i>	MRSA N (%)	p- value*
20-29	8	2 (25.00)	0.115
30-39	28	11 (39.28)	
40-49	47	15 (31.91)	
50-59	15	4 (26.67)	
Total	98	32	

*p-value calculated using chi-square test

MRSA was isolated more from male workers as compared to female workers (Table 2).

Table 2: Distribution of MRSA among different gender of dairy workers

Gender of workers	No. of <i>S. aureus</i>	MRSA N (%)	p- value*
Male	56	20 (35.71)	0.001
Female	42	12 (28.57)	
Total	98	32	

*p-value calculated using chi-square test

Antibiotic susceptibility pattern showed that most of the MRSA isolates were susceptible to Gentamicin followed by Ciprofloxacin (Table 3).

Table 3: Antibiotic susceptibility pattern of MRSA isolates

Antibiotics used	Susceptible N (%)	Resistant N (%)
Ciprofloxacin	28 (77.78)	8 (22.22)
Clindamycin	26 (72.22)	10 (27.78)
Gentamicin	31 (86.11)	5 (13.89)
Penicillin G	24 (66.67)	12 (33.33)
Teicoplanin	27 (75.00)	9 (25.00)

DISCUSSION

Isolation of 109 strains of *S. aureus* from dairy workers and dairy sample i.e. table butter and plain butter (exported) in our study is similar to that conducted by Crago et al. (2014). Andre et al. (2008) found that 75% (3/4) of the investigated persons were colonized with *S. aureus*. Huber et al. (2010) found that a nasal carriage rate was approximately 27% in healthy adult populations. Two studies with pre-clinical medical students showed that 35.2% and 29% were *S. aureus* nasal carriers (Bischoff et al. 2005). Our result is consistent with these findings.

Similarly, 11 strains of *S. aureus* were detected from butter sample which was 10.10% of total isolates. Among them 7 strains (63.63%) were isolated from plain (exported) butter whereas 4 strains (36.36%) were from table butter. Due to predominantly manual handling during butter packaging, contamination with *S. aureus* might occur. Highest number of MRSA in our study were found at the age of range of 40-49 years. However, the association between age groups and MRSA was found statistically insignificant which signifies that presence of MRSA does not depend on the age group. The detection of MRSA in workers and butter suggests that healthy human could play a role in the spread of MRSA among animals, workers, and the food environment (Crago et al. 2014).

Livestock derived food product that could lead to MRSA food intoxication or serve as vehicle for MRSA transmission is raw milk, when contaminated raw milk is used for the production of cheese. This was reported in Italy, where two MRSA strains of unknown origin were found in dairy cheese products (Normanno et al. 2007). As these strains were found to harbor genes for expression of common staphylococcal enterotoxins, they had the potential to cause food poisoning (Normanno et al. 2007).

The major limitation of our study is that this we have not correlated the strains isolated from dairy workers and dairy product. Further studies should be done to correlate the strains upto sequence level.

CONCLUSION

Detection of MRSA among dairy workers and dairy products warrants proper handling and adequate control measures to prevent transmission of MRSA from dairy industry.

ACKNOWLEDGEMENTS

The authors acknowledge the concerned persons at Dairy Development Corporation (DDC) Balaju unit, Kathmandu, Nepal and Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal for their contributions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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