

Detection of methicillin-resistant *Staphylococcus aureus* within raw milk and cheese samples

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Abstract

In consequence of previous study of bacterial propagation in milk and yogurt samples, present investigation endeavored to determine the existence of drug resistant *Staphylococcus aureus* especially the methicillin resistant *S. aureus* (MRSA) in raw milk and cheese samples collected from different areas in Dhaka City, Bangladesh. Total 80 samples (40 raw milk and 40 cheese samples) were microbiologically analyzed through cultural and the confirmative biochemical identification. Out of 80 samples studied, 17 (9 raw milk and 8 cheese) were found to harbor *S. aureus*. The drug resistance pattern of the staphylococcal isolates was determined against six antibiotics i.g. novobiocin (30µg), ciprofloxacin (5 µg), nalidixic acid (5 µg), methicillin (5 µg), oxacillin (1 µg) and erythromycin (15 µg) by the agar well diffusion method on Muller-Hinton agar. Three isolates from cheese samples and 8 isolates from raw milk samples were found to be resistant against at least two commonly used antibiotics. The highest resistance was found against methicillin (5µg, 58.82%) and oxacillin (1µg, 100%). The presence of methicillin resistant staphylococcal species as found from this study may pose a great public health threat, especially to the children.

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Keywords

Raw milk

Cheese

Staphylococcus aureus

MRSA

Multi-drug resistance (MDR)

Public health

Introduction

Food is the good territory of proliferation and colonization of disease causing microorganisms (Rahman and Noor, 2012; Acharjee *et al.*, 2013; Ahmed *et al.*, 2013; Noor *et al.*, 2013; Ahmed *et al.*, 2014; Marjan *et al.*, 2014). Considering the nutritive value, milk is widely consumed by the people as an ideal food (Oliver *et al.*, 2005; Javaid *et al.*, 2009; Marjan *et al.*, 2014). Milk and milk products are good source for the proliferation of pathogens, especially *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., *Lactobacillus* spp., *Streptococcus* spp., *Staphylococcus* spp. and *Micrococcus* spp. (Bramley and McKinnon, 1990; McManus and Lanier, 1987; Jayarao *et al.*, 2004; Jayarao *et al.*, 2006; Okpalugo *et al.*, 2008; Marjan *et al.*, 2014). Among the common contaminants, *S. aureus* is very prominent in milk and milk products as a contagious pathogen which triggers the onset of several contagious diseases (Barthel, 1910; Ghosh and Maharjan, 2002; Gubala and Proll, 2006; Jakee *et al.*, 2009; Allgeyer *et al.*, 2010; Zinke *et al.*, 2012). The entrance of such pathogens in milk and milk products may take place from different body parts of cows during milking or the surroundings of the milking area including the air dust, flies, insects and rodents, water supply, hands and clothes of the milker, utensils, bottles,

etc. (Parekh and Subhash, 2008; Torkar and Teger, 2008). However, in Bangladesh, the overall sanitary maintenances of the production, processing of milk and hygiene policies have unfortunately been reported not to be within the required standard (Khan *et al.*, 2008; Addo *et al.*, 2011; Marjan *et al.*, 2014).

In addition to the proliferation of pathogens in milk and milk products, another important concern has risen with the increase in antibiotic resistance which might pose a major clinical obstacle in diseases medication especially in the developing countries like Bangladesh (Tenover, 2006; Bennett, 2008; Canton, 2008; Jilani *et al.*, 2008; Acharjee *et al.*, 2013; Ahmed *et al.*, 2013; Dutta *et al.*, 2013; Noor *et al.*, 2013). In addition to the existence of a range of microorganisms, several studies have indicated that the presence of *Staphylococcus aureus* in the raw milk sample is very common even in pasteurized milk because of their ability to produce heat-stable enterotoxin (Loir *et al.*, 2003; Zinke *et al.*, 2012; Shanebandi *et al.*, 2014). Approximately, 60 years ago the strain *S. aureus* became resistant against the therapeutic agent methicillin and was introduced into the field of clinical microbiology as the methicillin resistant *S. aureus* (MRSA) (Rammelkamp, 1942; Chambers, 2001; Zinke *et al.*, 2012; Shanebandi *et al.*, 2014). Additionally, the presence of MRSA may be considered as indicator of resistance of the isolates

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against the other β -lactam antibiotics (Enright *et al.*, 2002; Naimi, 2003; Zinke *et al.*, 2012; Shanebandi *et al.*, 2014). Along these lines, present study attempted to detect the milk and cheese spoiling MRSA isolates.

Materials and Methods

Sample collection, processing and microbiological analysis

Forty raw milk and 40 cheese samples were aseptically collected in sterile test tubes and polyethylene bags, respectively from different area and super shops in Dhaka city within the time frame of December 2011 to December 2012. After collection, samples were transported immediately to the laboratory in an icebox for the microbiological analysis (APHA, 1998). The samples were aseptically processed and enriched in peptone water (PW) (HiMedia Pvt. Ltd.) for the identification and enumeration of *S. aureus*. An aliquot of 10 ml of each samples were homogenized with 90 ml of peptone water (pH 7.2 ± 0.2) and diluted up to 10^{-4} according to the standard guideline (Cappuccino and Sherman, 1996). In order to determine the presence of *S. aureus*, 0.1ml of samples from each dilution was introduced onto the Baird Parker agar (BPA) (Oxoid Pvt. Ltd). The plates were incubated at 37 °C for 24 hours. All the isolates were subjected to morphological and biochemical confirmation according to the prescribed method (Cappuccino and Sherman, 1996).

Study of antibiogram

The *in vitro* susceptibility of the isolated *S. aureus* against different antibiotics was measured by the Kirby-Bauer method (Bauer *et al.*, 1966, Acharjee *et al.*, 2013, Noor *et al.*, 2013, Marjan *et al.*, 2014). In this study six commonly available antibiotics such as novobiocin (NV, 30 μ g), ciprofloxacin (CIP, 5 μ g), nalidixic acid (NAL, 5 μ g), methicillin (MEC, 5 μ g), oxacillin (OXA, 1 μ g) and erythromycin (ERY, 15 μ g) were used. Suspensions of the test organisms were prepared using Muller-Hinton broth by adjusting the turbidity of the broth with normal saline to match the equivalent turbidity standard of McFarland (0.5 standards) and was incubated for 2 hours. Sterile cotton swabs were dipped into the suspensions and the swabs were then evenly spread over the entire surface of a Muller-Hinton agar plate to obtain uniform inoculums. Antibiotic discs of appropriate concentrations were applied aseptically over the surface of the inoculated plates at appropriate spatial arrangement by means of sterile needle within a distance of 5 mm. Plates were then inverted and incubated at 37 °C. After 24 hours,

plates were examined and the diameters of the zones of complete inhibition were measured and interpreted as susceptible, or resistant (Ferraro *et al.*, 2001).

Results and Discussion

Presence of *Staphylococcus spp.* among raw milk samples and cheese sample

The experimental result of raw milk (40) and cheese (40) samples revealed that all the samples were contaminated with *staphylococcus spp.* Among the 80 samples, 17 samples (raw milk 9 and cheese 8) were found to be highly contaminated with *Staphylococcus aureus*. Several studies have indicated the assortment of pathogenic bacteria especially *staphylococcus spp.* may rise in raw milk and cheese samples may responsible for the nausea, vomiting, abdominal cramps and diarrhea like diseases (Balaban and Rasooly 2000; Omori *et al.*, 2001; Aly and Galal, 2002; Robinson, 2002; Soomro *et al.*, 2002; Lues *et al.*, 2003; Soomro *et al.*, 2003; Chye *et al.*, 2004; Marjan *et al.*, 2014). As we found the staphylococcal contamination in cheese samples, which might occur during the manufacturing process or due to the lack of personal hygienic practice (Aly and Galal, 2002; Soomro *et al.*, 2003; Lues *et al.*, 2003; Chye *et al.*, 2004).

Detection of drug-resistance of the pathogenic isolate

Interestingly, the pathogenic isolates found in the samples exhibited the multi-drug resistance (MDR) trait against six commonly used antibiotics; i.e., novobiocin (NV, 30 μ g), ciprofloxacin (CIP, 5 μ g), nalidixic acid (NAL, 5 μ g), methicillin (MEC, 5 μ g), oxacillin (OXA, 1 μ g) and erythromycin (ERY, 15 μ g), coagulase positive *Staphylococcus aureus* was found to be highly resistant against oxacillin (100%) and methicillin (58.82%) (Table 1). The measurement of zone diameter was ≤ 14 mm in case of methicillin and ≤ 18 mm for oxacillin indicated the resistance of the isolate.

In Bangladesh, very few number of study have reported the frequency of hospital acquired MRSA in a range of 32-63%, which is high enough compared to the United States and European countries, estimated as 25-30% and nearly 50%, respectively (Majumder *et al.*, 2001; Butt *et al.*, 2004; Jilani *et al.*, 2008; Dutta *et al.*, 2013). Occurrence of Methicillin resistant *S. aureus* in food samples has been a major concern worldwide (Shanebandi *et al.*, 2014). Considering the consumer health safety, present study focused on the presence of methicillin resistant *S. aureus* (MRSA) in raw milk and cheese samples as the extension of the previous study done by our another research group

Table 1. Antibiotic susceptibility pattern of *Staphylococcus aureus*

Sample no.	Sample name	Antibiotic susceptibility					
		N	CIP	NA	ME	OX	E
S5	Cheese	S	S	S	S	R	S
S7	Cheese	I	S	S	R	R	S
S8	Cheese	I	S	S	R	R	S
S9	Cheese	S	I	S	S	R	R
S10	Cheese	S	R	R	R	R	S
S15	Cheese	S	S	S	S	R	S
S19	Cheese	R	S	R	R	R	R
S3	raw milk	S	R	R	R	R	S
S5	raw milk	S	I	S	S	R	S
S14	raw milk	S	R	S	R	R	S
S15	raw milk	S	I	R	S	S	R
S16	raw milk	S	R	S	R	R	S
S17	raw milk	S	S	R	S	S	R
S22	raw milk	S	S	R	R	R	S
S24	raw milk	S	S	R	S	R	S
S31	raw milk	S	S	S	S	S	R
S38	raw milk	S	S	S	R	R	S

All the experiments have been done three times and the results were reproducible. One representative data have been shown. NOV= novobiocin (30 µg), CIP= ciprofloxacin (5 µg), NAL= nalidixic acid (5 µg), ME= methicillin (5 µg), OX= oxacillin (1 µg), ERY= erythromycin (15 µg), R= resistant, S= sensitive.

Marjan *et al.* (2014). In developing countries like Bangladesh more than 70% of infecting bacteria have been accounted as multi drug resistant strain (MDR) (Prescott, 2000; Jilani *et al.*, 2008; Dutta *et al.*, 2013). However, one of our research groups reported multi drug resistant bacteria isolated from the milk and milk products (Marjan *et al.*, 2014). Furthermore, present investigation unveiled huge existence of MRSA and oxacillin resistant *Staphylococcus aureus* in raw milk and cheese samples. This sort of study attracted more attention from the consumers and the regulatory body of the government in countries where the antibiotic misuse is very common and as such investigation is still infancy in Bangladesh (El-Astal, 2004; Bean *et al.*, 2008; Olukemi and Adeola, 2012).

Conclusion

The presence of methicillin-resistant *S. aureus* in milk and cheese samples is indicative of treatment failure scenario during food borne disease outbreaks. Furthermore, the current findings may provide the proper guidelines of hygienic handling and processing of raw milk and cheeses followed by proper pasteurization and fermentation.

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