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Characterization of *staphylococcus* species isolated from livestock, poultry and human

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ABSTRACT

The study was performed to characterize *Staphylococcus* spp. isolated from human, livestock and poultry. A total of 100 samples were collected from human (n=30), livestock (n=50) and poultry (n= 20). Samples were enriched in nutrient broth at 37 °C over night. Enriched cultures were streaked onto mannitol salt agar, nutrient agar and blood agar and incubated at 37 °C for 24 hrs for the isolation of bacteria in pure culture. Identification of bacteria was performed by cultural characteristics, staining and biochemical tests. The isolated *Staphylococcus* spp. was tested for antibiotic sensitivity against methicillin and vancomycin. A total of 58 Staphylococci were isolated, among them 33 were coagulase positive *Staphylococcus aureus* (CPSA) and 25 were other coagulase negative *Staphylococcus* spp. (CNS). Among the 33 coagulase positive *S. aureus* isolates, 17 (51.51%) produced golden, 9 (27.27%) produced yellow and 7 (21.21%) produced whitish pigments in mannitol salt agar media. A total of 25 (75.75%) *S. aureus* were β hemolysis producer on the blood agar media. Other *Staphylococcus* spp. were CNS and non-hemolytic. Antibiotic resistant pattern of the CPSA indicated two isolates of broilers and one isolate of cattle were resistant to methicillin but these isolates were sensitive to vancomycin. The results of this study suggested that MRSA (Methicillin Resistant *Staphylococcus aureus*) is present in cattle and poultry which might constitute risk of transmission of MRSA to humans and other animals. More survey data are required to estimate the accurate prevalence of MRSA isolates in human, livestock and poultry.

Introduction

Staphylococcus species are gram positive, nonmotile, nonspore forming, facultative anaerobic cocci occurring in pairs or irregular clusters similar to grapes (Ryan et al., 2004). The genus *Staphylococcus* has more than 20 species, most are harmless and reside normally on the skin and mucous membranes of human and other organisms (Madigan et al., 2005). The most ubiquitous of these species is *Staphylococcus epidermidis* which is found on the skin of human and animals and rarely causes disease. *S. epidermidis* is one of five most common organisms that cause nosocomial infections due to the increase in usage of biomaterials in the clinical environment (Mack et al., 2007). *Staphylococcus aureus* is the most common species of *staphylococcus* to cause Staph infections. The reason *S. aureus* is a successful pathogen is a combination of bacterial immune-evasive strategies. The growth of *S. aureus* in foods is a potential public safety hazard since many of its strains produce enterotoxins that cause food poisoning when ingested. *Staphylococcus intermedius* and *hyicus* have also been shown to produce enterotoxins in food. *Staphylococcus saprophyticus* is known only to cause urinary tract infections. The coagulase test is used to differentiate *Staphylococcus aureus* from the other species since it is the only one to produce the coagulase enzyme (Downes et al., 2001, Holt et al., 2001, Doyle et al., 1994).

Antimicrobial resistance is a public health issue of growing concern. The use of antimicrobials can lead to the development of antimicrobial resistance in bacterial species (Tenover & McGowan, 1996; Acar & Rostel, 2001). Antimicrobial use in food animal production may become a public health issue when resistant organisms or their resistance genes spread from animals to humans by direct contact or through the food chain (Aarestrup, 2005; Wassenaar, 2005). The MRSA is currently causing a pandemic in hospitals around the world and is also emerging in the community (Chambers, 2001). Recently, MRSA has been identified in food production animals and people in contact with these animals (Voss et al., 2005). The finding of this new zoonotic reservoir of MRSA has led to several research initiatives to investigate its implications. These studies strongly suggest that people working with livestock are at a potential risk of becoming MRSA carriers and hence are at an increased risk of infections caused by MRSA. To date, there is no comprehensive data on the situation of MRSA in Bangladesh. The aim of this study was to evaluate the occurrence of MRSA in people in contact with livestock, in farm animals, and in food of animal origin, and to investigate phenotypic resistance data of isolated strains.

To prevent the occurrence of disease in humans, it is important to investigate the transmission routes from animals to humans and from humans to humans as well. The role of MRSA as a food pathogen needs more research. Microbiologists should investigate the pathogenicity and the capacity for transmission between humans of this particular novel strain to assess the potential threat for public health. At the same time, cooperation between epidemiologists and microbiologists in the human and veterinary field will be required to create a complete overview of all aspects of this problem and to develop cost-effective prevention strategies in both the human and animal populations. Considering the above problems the present research work was undertaken to determine the prevalence of *Staphylococcus* spp. in various samples of human and animals with characterization of *Staphylococcus* spp and their antimicrobial sensitivity profiles against methicillin and vancomycin.

Materials and Methods

Study area

The study was conducted in the laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh during the period from January 2012 to June 2012.

Sources of samples

A total of 100 samples were collected from humans (n=30), livestock (n=50) and poultry (n= 20) with proper aseptic precautions. In case of livestock, a total of 30 nasal swabs viz. 10 slaughter cattle, 10 dairy cattle and 10 breeding goat were collected from slaughter house of Mymensingh, BAU dairy farm and goat farm respectively. Ten abscess samples viz. 5 cattle and 5 goat/sheep from BAU veterinary clinic were collected aseptically for the study. For assessment the MRSA contaminated meat, 10 samples were collected from local market, Mymensingh. The samples was collected with proper aseptic precaution and carried to the laboratory for inoculation into culture media with specific identification marks. In case of Human, a total of 30 nasal swabs viz. 10 slaughter house employees, 10 dairy farm workers and 10 goat breeding farm workers were collected from slaughter house of Mymensingh, BAU dairy farm and goat breeding farm respectively. Immediately after collection the swab sticks were put into sterile test tubes and carried to the laboratory of the Department of Microbiology for inoculation into culture media with specific identification marks.

Experimental design

The entire study was divided into three major steps: The first step included collection of samples from different areas, their transportation to the laboratory and inoculation into nutrient agar, blood agar and mannitol salt agar. In the second step isolation and identification of the *Staphylococcus spp.* was done on their cultural character including pigment production, haemolytic activity, Gram's staining character and catalase activity. In the third step characterization of the organism was done using coagulase test and basic sugar fermentation test. Finally their antibiotic sensitivity test was also performed by using commercially available antibiotic discs (Oxoid, England) following the procedure described by Monica Chessbrough (1985).

Hemolytic activity

Haemolytic activities of *S. aureus* were observed as per the method described by Chatterjee *et al*., (1990). All the strains were tested for the production of alpha (α) and beta (β) haemolysis by growing them on bovine BA plates and were then incubated at 37 °C for 24 hours to determine their hemolytic property. The colony developed on the BA was examined for various types of hemolysis. The hemolytic pattern of the bacteria was categorized according to the types of hemolysis produced on BA plates (Alpha (α) hemolysis: a zone of greenish discoloration around the colony manifested by partial hemolysis. Beta (β) hemolysis: complete clear zone of hemolysis around the colony: Gamma (γ) hemolysis: no detectable hemolysis).

Biochemical tests

Sugar fermentation test, Indole and MR-VP test, catalase test, coagulase test according to the procedure described by Cowan (1985). Test for pigment production was performed according to the procedures described by Chatterjee *et al*., (1990).

Isolation and identification of *Staphylococcus* spp.

For the isolation and identification of bacterial flora, the procedure suggested by Carter, 1979 was followed throughout the experiment. Isolation of *Staphylococcus* spp. from the collected samples was made by inoculating the samples on NA, BA and MSA. The inoculated media were then incubated aerobically at 37 °C for 24 hours for growth. The isolates were identified on Staphylococcus based on their morphology and cultural characteristics and biochemical characters (Ellner 1978). The coagulase test was performed for the identification of the pathogenic *Staphylococcus aureus* from non-pathogenic ones. All the coagulase positive staphylococcal strains were further tested for pigment production, haemolysis on nutrient and blood agar, respectively. Stock culture was prepared and maintained for subsequent studies. To differentiate between *Staphylococcus aureus* and other *Staphylococcus* spp. it was considered the hemolysis, pigment production and coagulase test.

Antibiotic sensitivity test

Staphylococci were tested for antimicrobial drug susceptibility against 02 commonly used antibiotics belonging to different groups by disc diffusion

method or Kirby-Bauer method (Bauer *et al*., 1966). The antibiogram of isolates (*Staphylococcus* spp.) were determined on freshly prepared, dried up Mueller Hinton agar using by the Kirby-Bauer Disc Diffusion Method (Bauer *et al*., 1966) according to Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS, 2007) procedures. The tested antibiotics included: Methicillin (5 µg/ disc), Vancomycin (30 µg/ disc), and (Oxoid, England). Antimicrobial agents with their disc concentrations and zone diameter interpretive standards for *Staphylococcus* spp were followed as per recommendation by CLSI (2007). The isolates resistant to three or more antibiotics were considered as multi-drug resistant (MDR) strains.

Turbidity standard for inoculums preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension) were used.

Results and Discussion

Isolation of *Staphylococcus*

Out of 100 samples, 58 samples were tested positive for *Staphylococcus* spp. (Table 1). On the basis of cultural characteristics 33 isolates were characterized as *S. aureus* and 25 isolates were other *Staphylococcus* spp. (Table 2).

Cultural and microscopic examination

The growth of staphylococci in NB was characterized by diffused turbidity and in few occasions pellicle was seen. Small, circular and smooth raised gray white or yellowish colonies in *S. aureus* (Fig. 1A) and white colonies in other *Staphylococcus* spp. (Fig. 1C) were observed on nutrient agar media. Staphylococci were gram positive, cocci and arranged in grapes like cluster (Fig. 1E). Staphylococci showed different colonies on the mannitol salt agar (MSA). *S. aureus* fermented MSA with the production of yellowish colonies (Fig. 1B). On the other hand, whitish colonies without fermentation of MSA indicated the growth of other *Staphylococcus* spp. (Fig. 1D). Colonies *of Staphylococcus* spp. on blood agar media were circular, small, smooth raised with gray white or yellowish in color. No hemolysis was noticed in case of other *Staphylococcus* spp. (Fig. 2) but β-hemolysis was seen for *S. aureus* on BA media (Fig. 2).

Table 1. Isolation of *Staphylococcus* spp. from samples of human, livestock and poultry.

Source of samples		Name of samples	No. of positive isolates {n (%)}
Human	Slaughter employees	Nasal swab	6 (60)
	Cattle farm workers	Nasal swab	5 (50)
	Goat farm workers	Nasal swab	3 (30)
Livestock	Cattle (Slaughter house)	Nasal swab	6 (60)
	Cattle (Dairy farm)	Nasal swab	6 (60)
	Goat (Goat farm)	Nasal swab	5 (50)
Poultry		Nasal swab	4 (80)
	Broiler	Cloacal Swab	2 (40)
		Nasal swab	3 (60)
	Layer	Cloacal Swab	1 (20)
Diseased animals	Cattle	Abscess	5 (100)
	Goat	Abscess	5 (100)
Market meat	Cattle	Beef	4 (80)
	Goat	Chevon	3 (60)

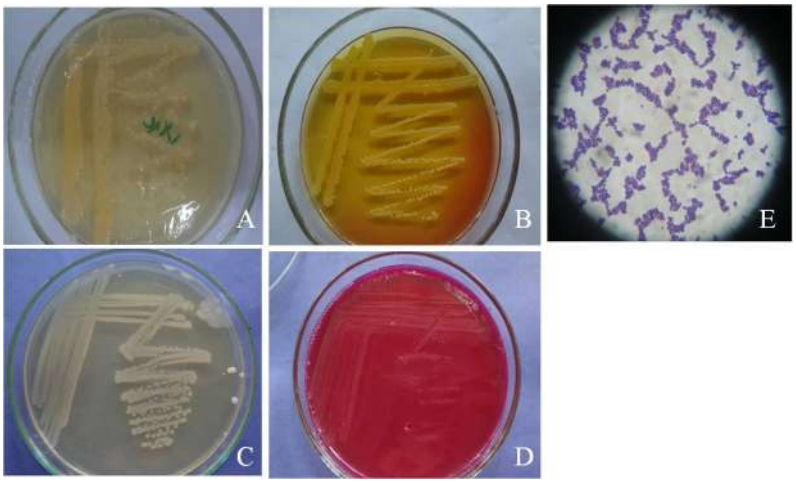

Fig. 1. (A) *Staphylococcus aureus* in nutrient agar , B) *Staphylococcus aureus* in MSA, C) Other *Staphylococcus* spp. in nutrient agar , D) Other *Staphylococcus* spp. in MSA, E) *Staphylococcus* spp. under microscope

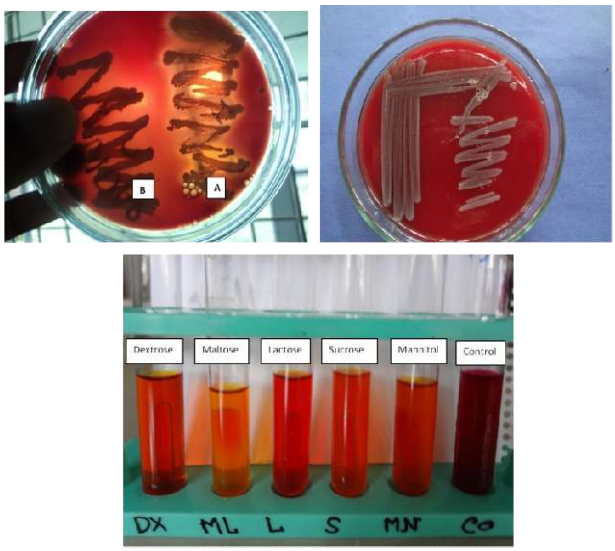

Fig. 2. Coagulase positive *Staphylococcus aureus* on blood agar. (Upper left) A showing the positive reaction (hemolysis) and B indicating the negative reaction (Non-hemolysis). (Upper right) Other *Staphylococcus* spp. on blood agar. (Below) sugar fermentation test- only acid was produced, no gas was observed in Durham´s tube.

Table 2. Summary of cultural characteristics of *Staphylococcus* spp. isolated from human, livestock and poultry

Colony characteristics on			Interpretation
Nutrient agar	Mannitol salt agar	Blood agar	
Small, circular, smooth raised gray white colonies	Small, circular, whitish colonies without fermentation	Small, circular, smooth raised gray white colonies without hemolysis	Coagulase negative *Staphylococcus* spp.
Small, circular, smooth raised yellowish colonies	Small, circular yellowish colonies with fermentation	Small, circular, smooth raised yellowish colonies with β hemolysis	*S. aureus*

Biochemical test

Fermentation test

All the isolates fermented dextrose, maltose, lactose, sucrose and mannitol and produced only acid. Acid production was indicated by the color change from reddish to yellow (Fig. 2).

Catalase test

Catalase test was performed to differentiate Staphylococci (catalase producer) from Streptococci (non-catalase producer). Hydrogen peroxide was breakdown into water and oxygen. Production of oxygen was indicated by the bubble formation (Fig. 3). All *Staphylococcus* isolates were catalase positive.

Coagulase test

A total of 33 *Staphylococci* isolates gave positive reaction in coagulase test indicated that they were *S. aureus* (Fig. 3). On the other hand 25 isolates were found to be coagulase negative which was other *Staphylococcus* spp. (Fig. 3). The summary of coagulase positive and negative *Staphylococcus* spp. isolated from humans, livestock and poultry samples are listed in Table 3.

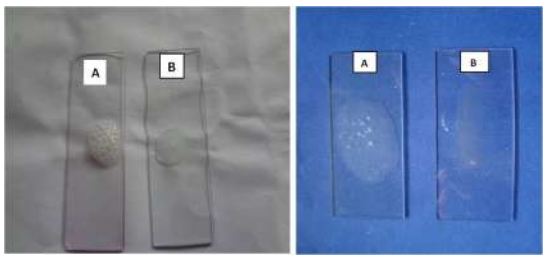

Fig. 3. Catalase test (Slide test). (Left) showing the positive reaction i.e. catalase producing bacteria (*Staphylococcus* spp.) and B indicating the negative reaction. (Right) A showing the positive reaction, card like clot formation. (*Staphylococcus* spp.) and B indicating the negative reaction.

Table 3. Summary of the results of coagulase test of *Staphylococcus* spp. isolated from human, livestock and poultry.

Source of samples		Name of samples (n)	No. of coagulase positive isolates {n (%)}	No. of coagulase negative isolates {n (%)}
Human	Slaughter employees	Nasal swab (10)	3 (50)	3 (50)
	Cattle farm workers	Nasal swab (10)	2 (40)	3 (60)
	Goat farm workers	Nasal swab (10)	1 (33.33)	2 (66.67)
Livestock	Cattle (Slaughter house)	Nasal swab (10)	4 (66.67)	2 (33.33)
	Cattle (Dairy Farm)	Nasal swab (10)	3 (50)	3 (50)
	Goat (Goat farm)	Nasal swab (10)	2 (40)	3 (60)
Poultry	Broiler	Nasal swab (5)	3 (75)	1 (25)
		Cloacal swab (5)	1 (50)	1 (50)
	Layer	Nasal swab (5)	2 (66.67)	1 (33.33)
		Cloacal swab (5)	1 (100)	0 (0)
Diseased animals	Cattle	Abscess lesion (5)	4 (80)	1 (20)
	Goat	Abscess lesion (5)	4 (80)	1 (20)
Market meat	Cattl e	Beef (5)	2 (50)	2 (50)
	Goat	Chevon (5)	1 (33.33)	2 (66.67)

Table 4. Pigment production and β hemolysis of coagulase positive *Staphylococcus* spp. isolates of humans, livestock and poultry

Source of samples		Number of coagulase positive isolates	Pigment production			β-Haemolysis production {n (%)}
			Golden yellow {n (%)}	Yellow {n (%)}	Whitish {n (%)}	
Human	Slaughter employees	3	2 (66.67)	1 (33.33)	0 (0)	2 (66.67)
	Cattle farm workers	2	1 (50)	0 (0)	1 (50)	1 (50)
	Goat farm workers	1	1(100)	0 (0)	0 (0)	1(100)
Livestock	Cattle (Slaughter house)	4	2 (50)	1 (20)	1 (20)	3 (75)
	Cattle (Dairy Farm)	3	2 (66.67)	1 (20)	0 (0)	3 (100)
	Goat (Goat farm)	2	1 (50)	0 (0)	1 (50)	1 (50)
		3	2 (66.67)	1 (33.33)	0 (0)	2 (66.67)
Poultry	Broiler	1	0 (0)	0 (0)	1 (100)	0 (0)
	Layer	2	0 (0)	1 (50)	1 (50)	1(50)
		1	0 (0)	0 (0)	1 (100)	0 (0)
Diseased animals	Cattle	4	3 (75)	1 (25)	0 (0)	4 (100)
	Goat	4	2 (50)	1 (25)	1 (25)	4 (100)
Market meat	Cattle	2	1 (50)	1 (50)	0 (0)	2 (100)
	Goat	1	0 (0)	1 (100)	0 (0)	1 (100)

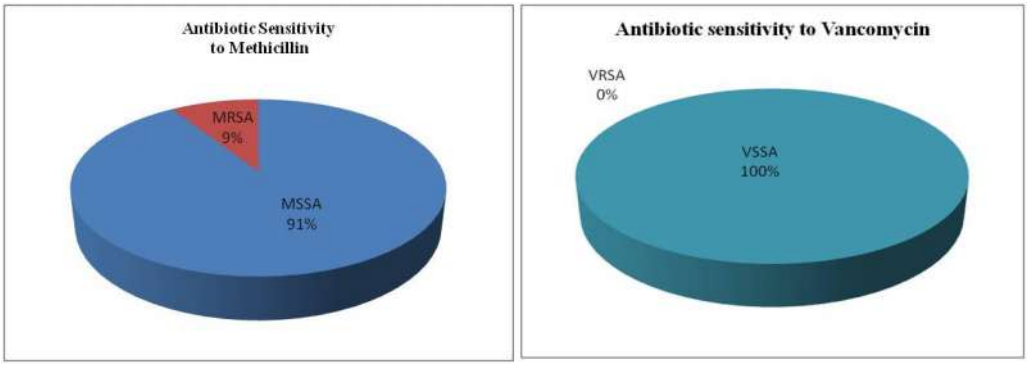

Fig. 4. Antimicrobial profiles of CPSA isolated from human, livestock and poultry against methicillin and vancomycin. MRSA = Methicillin Resistant *Staphylococcus aureus;* MSSA = Methicillin Susceptible *Staphylococcus aureus;* VRSA = Vancomycin Resistant *Staphylococcus aureus;asxyx*VSSA = Vancomycin Susceptible *Staphylococcus aureus*

Pigment production and haemolytic activity

Production of different types pigment by coagulase positive *Staphylococcus aureus* on mannitol salt agar and production of β hemolysis on blood agar media is presented in Table 4.

Antibiotic sensitivity profiles

Out of 33 caogulase positive *Staphylococcus aureus* (CPSA) 30 were found to be sensitive to methicillin (Fig. 4 & 5). Three CPSA isolates were found to be methicillin resistant of which 2 were isolated from broiler and 1 from the cattle. On the other hand, all of the CPSA isolates were sensitive to vancomycin. The detail results are shown in Table 5.

Fig. 5. Left -Antibiotic sensitivity test in poultry. A (MET) remarks the methicillin resistant and B (VA) remarks the sensitive (17 mm) to vancomycin. Right- Antibiotic sensitivity test in human. A (MET) and B (VA) indicated the methicillin (20 mm) and vancomycin (16 mm) sensitive respectively.

Table 5. Antimicrobial profiles of coagulase positive *Staphylococcus aureus* isolated from human, livestock and poultry against methicillin and vancomycin.

Source of samples		No. of coagulase positive isolates	No. of organisms found resistance					
			Methicillin			Vancomycin		
			R	I	S	R	I	S
Human	Slaughter house employees	3	-	-	3	-	-	3
	Cattle farm workers	2	-	-	2	-	-	2
	Goat farm workers	1	-	-	1	-	-	1
Livestock	Cattle (Slaughter house)	4	-	-	5	-	-	5
	Cattle (Dairy Farm)	3	-	-	4	-	-	4
	Goat (Goat farm)	2	-	-	2	-	-	2
Poultry	Broiler	3	2	-	1	-	-	3
		1	-	-	-	-	-	-
	Layer	2	-	-	2	-	-	-
		1	-	-	1			
Diseased animals	Cattle	4	1	-	3	-	-	4
	Goat	4	-	-	4	-	-	4
Market meat	Cattle	2	-	-	2	-	-	2
	Goat	1	-	-	1	-	-	1
Total		33	3	-	30	-	-	33

Table 6. Antimicrobial profiles of coagulase negative *Staphylococcus* isolated from humans, livestock and poultry against methicillin and vancomycin.

Source of samples		No. of coagulase negative isolates	Isolated strains sensitive to various antibiotic, n					
			Methicillin			Vancomycin		
			R	I	S	R	I	S
Human	Slaughter house employees	3	-	-	3	-	-	3
	Cattle farm workers	3	-	-	3	-	-	3
	Goat farm workers	2	-	-	2	-	-	2
Livestock	Cattle (Slaughter house)	1	-	-	1	-	-	1
	Cattle (Dairy Farm)	2	-	-	2	-	-	2
	Goat	3	-	-	3	-	-	3
Poultry	Broiler	1	-	-	1	-	-	1
		1	-	-	1	-	-	1
	Layer	1	-	-	1	-	-	1
		-	-	-	-	-	-	-
Diseased animals	Cattle	1	-	-	1	-	-	1
	Goat	1	-	-	1	-	-	1
Market meat	Cattle	2	-	-	2	-	-	2
	Goat	2	-	-	2	-	-	2
Total		25	-	-	25	-	-	25

The antibiotic sensitivity profiles were also studied for coagulase negative Staphylococci isolates of human, livestock and poultry. All of the isolates (n = 25) were sensitive to both methicillin and vancomycin (Table 6). Staphylococci are the commensal organism that normally present in the body. According to Das and kanna (1995) the host range of the organism is wide and many strains are potential pathogen which supported the collection of samples from human, animal and birds.

The study revealed 56.89% Coagulase positive *Staphylococcus aureus* (CPSA) and 43.1% Coagulase negative Staphylococci (CNS) which was in agreement with Das et al., (1990) who reported 56.11% prevalence of CPSA. Out of 30 human specimens 14 *Staphylococcus* isolates were recovered. Of 14 isolates 3 (50%), 2 (40%) and 1 (33.33%) were CPSA which were found in slaughter house employees, cattle farm workers and goat farm workers respectively. The isolation rates recorded in this study were higher than Vyletelova *et al*., (2011) and El-Jakee *et al*., (2008). The difference of rate in prevalence might be due to the small size of samples screened in this experiment. Higher prevalence of CPSA was recorded in slaughter house employees as compared to others. Data of this study warrant the need for implementation of adequate hygienic and sanitary measures in the slaughter houses.

Among the livestock population 4 (66.67%), 3 (50%) and 2 (40%) CPSA were found in slaughtered cattle, dairy cattle and goats respectively. Similar rates of isolation were also reported by Schillinga *et al*., (2012) and Megra et al., (2006). This study recorded higher rate of isolation of CPSA in slaughter cattle. On the other hand 25 of 58 *Staphylococcus* isolates were coagulase negative which is higher than the rate reported by Alzohairy (2011). However, the number of CPSA isolates is higher in broiler as compared to layer. This might be due to overcrowding and/or lack of effective sanitary and hygienic measures of the live bird market. The higher isolation rate of CPSA in broiler was also reported by Persoons *et al.,* (2009).

S. aureus is one of the important etiological agents responsible for pyogenic infection in man and animals (Rich M., 2005). In case of abscess**,** the rate of isolation of CPSA is higher both in cattle and goats. These results are in agreement with the findings of Menes et al., (1984). In market meat CPSA isolates were found both in cattle and goat. Isolation rate of CPSA reported in this study is similar with the isolation rate reported by Podpecan *et al.,* (2007). The presence of CPSA in meat sample indicates low hygiene status of market meat and could be a source of transmission of CPSA in humans through food chain.

This chromogenic character recorded in this study were found to be similar to the findings of Chatterjee et al., (1990) who recorded golden, yellow and white color colonies of CPSA. In this experiment out of 33 CPSA 25 isolates (75.75%) produced β-hemolysis on BA. Chatterjee et al., (1990) found 64.63% β-haemolysis production and Das et al., (1990) found 100% β-hemolysis production by the CPSA on 10% sheep blood agar. The variation of rate of hemolysis might be linked to the difference of origin of CPSA isolates.

All CPSA isolates in this study fermented glucose, maltose, lactose, sucrose and mannitol fermentation with only acid production. These findings are in close agreement with that of Chatterjee et al. (1990) who reported 81 (98.78%) strains as mannitol fomenters. Hazarika *et al*., (1995) and Das and Khanna (1994) observed 100% glucose and mannitol fermentation by *S. aureus* strains. A much lower rate of glucose (75.38%) and mannitol (70.76%) fermentation were cuased by *S. aureus* isolated from meat, fish and food handlers (Das and Khanna, 1994).

The antibiotic susceptibility results revealed that all CNS isolates were susceptible to both methicillin and vancomyciin. No resistance was observed against these antibiotics. The findings were in close agreement with Alzohairy (2011). On the other hand 3 of 33 CPSA isolates were found to be resistant to methicillin and rests of the isolates were sensitive. However, All 3 MRSA isolates of this study were sensitive to vancomycin. The antibiotic vancomycin is used to treat MRSA. MiceK (2007) stated that vancomycin remains the reference standard for the treatment of systemic infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA). The findings of this study are in agreement with Kelman *et al*., (2011). The susceptibility to vancomycin to MRSA was also observed by Citak *et al*., (2011) and Rhee et al., (2010). This study recorded the presence of MRSA in broiler and cattle with abscess. The presence of MRSA in broiler might be resulted from indiscriminate or over use of antibiotics. In 2007, the World Health Organization advised to stop intensive routine use of antimicrobials in food animals (Collignon *et al.,* 2009). Data of antibiotic sensitivity profile suggest that MRSA are present on farms, which might be transmitted to animals and humans and would likely to cause serious public and animal health hazard.

The result of the present study indicated that vancomycin is the drug of choice for treatment of methicillin-resistant *S. aureus* (MRSA). The use of antimicrobials in production animals has become a worldwide concern in the face of rising resistance levels potentially threatening treatment options in both veterinary and human medicine. MRSA has entered into farming operations in Bangladesh but still occurring at lower number. This low prevalence suggests that at the moment there is only a limited risk of MRSA transmission from livestock to humans and to food of animal origin. To maintain this situation, further efforts within the field of veterinary public health are of major importance and it is necessary to establish a monitoring system for further trend analysis. Continuous surveillance on resistance patterns of *Staphylococcus* spp. in understanding new and emerging trends is of utmost importance.

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