Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 9 (5): 251-264, 2014
ISSN 1816-4935 / DOI: 10.3923/jm.2014.251.264

Detection of *Staphylococcus aureus* in Frozen Chicken Rinse through Bacteriological and *Nuc* Gene Specific PCR Methods and their Drug Resistance Patterns in Southern Chittagong, Bangladesh

[1]Nazneen Naher Islam, [1]Mahmuda Akter, [1]Zinat Farzana [1]Abdul Jabber Bin Kader, [2]Inkeyas Uddin, [2,3]A.M.A.M. Zonaed Siddiki and [2]K.M. Kamaruddin

[1]Department of Genetic Engineering and Biotechnology, University of Chittagong, Chittagong, 4331, Bangladesh

[2]Poultry Research and Training Centre (PRTC), CVASU, Chittagong, 4225, Bangladesh

[3]Department of Pathology and Parasitology, Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, 4225, Bangladesh

Corresponding Author: Nazneen Naher Islam, Department of Genetic Engineering and Biotechnology, University of Chittagong, Chittagong, 4331, Bangladesh Tel: +88(031)726311-4

ABSTRACT

Staphylococcus aureus are gram positive cocci that can cause sporadic cases and outbreaks of food borne illness. The aim of the present study was to detect and identify this organism in samples of refrigerated chicken rinse obtained from different super stores in Chittagong city. The prevalence of infection and antimicrobial susceptibility of *Staphylococcus aureus* were also studied. The PCR was performed to detect these microorganisms in a chicken rinse microbial consortium and the traditional cultural techniques were performed based on bacteriological analytical manual. To compare PCR and bacterial culture methods for detection of *S. aureus*, 150 chicken rinse samples from different supermarkets in the Chittagong city were collected and tested. Samples were cultured on selective mannitol salt agar media and contamination by *Staphylococcus* was confirmed by gram staining, catalase test and coagulase test. Overall 95.83% of the samples were found to be infected with *S. aureus*. About 68.53% samples were coagulase positive *Staphylococcus* and 31.46% were negative. Bacterial counts of 100000 or more CFU cm^{-2} were found on 16.67% of the frozen chicken samples ($p \leq 0.01$). Simultaneously, total DNA obtained by thermal extraction from samples was subjected to PCR using a set of primers designed for specific regions of *Staphylococcus nuc* gene and PCR products were analyzed by agarose gel electrophoresis. Culture sensitivity test and antibiogram study was done to determine the antibiotic sensitivity pattern of *Staphylococcus* isolates against eight commercially available antibiotic discs (Ampicillin, Amoxycillin, Cephalexin, Ciprofloxacin, Erythromycin, Gentamycin, Doxycycline hydrochloride and Oxytetracycline). All of the samples were resistant to two or more than two antibiotics. The samples showed 100% resistant to Ampicillin, more than 80% were resistant to Oxytetracyclin, Doxycycline hydrochloride and Amoxicillin. Ciprofloxacin showed 77.5%, Cephalexin 38.33% and Gentamycin showed the least resistance 13.33%. The results of this study indicate that the PCR can permit a rapid and reliable means of assessing the bacteriological safety of food and should provide an alternative methodology than conventional viable culture method. The PCR may permit sufficient sensitivity and specificity for the direct detection of *Staphylococcus* in food samples.

Key words: *Staphylococcus aureus*, frozen chicken, PCR, antibiotic suseptibility, Bangladesh

INTRODUCTION

Over the last several decades poultry is a major fast growing source of meat in the world today (Kearney, 2010). The production and consumption of poultry meat is gradually increasing and the consumers expect safe and hygienic products without contamination with pathogenic microorganisms (Mor-Mur and Yuste, 2010). Poultry and poultry products are important vehicle for transmission of food borne pathogen (Tauxe and Blake, 1992). *Staphylococcus aureus* is one of the leading microorganism associated with food poisoning causing outbreaks with an incidence of 11.5% (Altabari and Al-Dughaym, 2002). *Staphylococcus aureus* is gram-positive, facultative anaerobes, non-sporulating bacteria in which most of them are recognized on the skin, mucous membranes of humans and animals and also as environmental contaminants (Feizi *et al*., 2012). In almost all developing countries poor hygienic standards in poultry slaughterhouses coupled with old processing facilities, handling, transportation with significant contamination rates of market chicken products (Boonmar *et al*., 1998). Heavy bacterial loads enter the processing operations with the living birds and these bacteria can be disseminated throughout the plant during processing. Although normal cooking destroys *S. aureus*, recontamination can occur during post cooking handling at the factory (Corner *et al*., 2001). Cellulitis folliculitis, carbuncles, scalded skin syndrome and abscesses to life-threatening diseases such as pneumonia, meningitis, osteomyelitis are associated with *S. aureus*. Treatment of human illness from these organisms has been complicated due to the emergence of drug-resistant strains (Moran *et al*., 2006). To promote the growth of chicken, several antimicrobial agents are used as food supplements and therapy which influence the emergence of drug-resistant bacteria and the transfer of drug-resistant bacteria from animals to humans (Chaslus-Dancla *et al*., 2000). Although in developing countries the culture of chicken rinse samples is considered as a gold standard method for the detection of pathogen but there are several limitations associated with bacterial culture (Meiri-Bendek *et al*., 2002; Phuektes *et al*., 2001). The development of PCR based method provides a promising option for the rapid identification of the pathogen and it can be used as a rapid diagnosis method. The identification of bacterial species by conventional culture method requires the days but PCR has high sensitivity and specificity and can improve the level of detection within few hours (Khan *et al*., 1998; Tamarapu *et al*., 2001). The aim of the present study was: (1) To detect and identify *Staphylococcus aureus* in samples of refrigerated chicken rinse obtained from different superstores. (2) To develop a sensitive, reliable and rapid method of identification of *S. aureus* using PCR. (3) To prove the advantage of molecular methods (PCR) over conventional viable culture method in terms of rapidity, reliability, sensitivity and specificity. (4) To perform CS test to reveal antimicrobial susceptibility of Staphylococcal isolates against antibiotic discs.

MATERIALS AND METHODS

Chicken rinse test sampling: A total of 150 frozen chicken rinse samples were randomly collected from broiler chickens, representing 5 renowned supermarkets in Chittagong city of Bangladesh. The whole chicken were rinsed in 250 mL of 0.1% (w/v) peptone water for 2 min in sterile plastic bags, put on ice in coolers and sent to the laboratory for testing.

Isolation and identification of *Staphylococcus aureus* by conventional culture method: In the laboratory the chickens rinse were filtrated through sterilized cheesecloth. The filtrated rinses were centrifuged at 15000×g for 10 min at 4°C. The supernatant was discarded and the pellet suspended in 5 mL of nutrient broth. After incubation of 24 h at 37°C, the cultures were

streaked onto a selective mannitol salt agar media. The plates were incubated at 37°C at 24-48 h. Then the suspected yellow or white colonies grown on mannitol salt agar were examined by biochemical tests such as gram staining, catalase and coagulase test.

Enumeration of *S. aureus*: Mannitol salt agar, a selective medium for the counting of *S. aureus* was used for the enumeration of *Staphylococcus aureus*. Samples were serially diluted and an aliquot of 1 mL of each serial dilution was transferred to petridishes. Plates were gently swirled to mix the sample and incubated at 37°C for 24 h. The countable range is 30-300 colonies on all plates. Enumeration data was calculated as colony forming units CFU mL^{-1} and then converted to CFU cm^{-2} following the formula of Thomas and Archer (1989).

DNA extraction: DNA extraction was carried out as described by Salehi and Bonab (2006). Pure bacterial culture was subcultured in nutrient broth medium from nutrient agar slant. The 1 mL broth culture was taken in eppendorf tube and centrifuged at 10000 rpm for 5 min. After the supernatant was discarded, any remaining liquids were removed by soaking and the pellet was collected. To dissolve the pellet, 200 μL autoclaved deionized water was added to the pellet. The cap of the eppendorf tube was pierced by sterile needle. The eppendorf tube was boiled in water bath at 100°C for 10 min. Then kept in ice for 10 min and centrifuged at 10000 rpm for 10 min. At last, 100-150 μL supernatant containing bacterial chromosomal DNA was collected.

Oligonucleotide primers: Primers were selected on the basis of the 966-bp *Nuc* gene derived from the *S. aureus* Foggi strain (Shortle, 1983). A set of primers were selected and applied the PCR for amplification of a sequence of the *Nuc* gene by using the two primers that targeted the gene. The sequences of the two synthetic oligonucleotide primers of 21 and 24 bases were: 5'-GCGATTGATGGTGATACGGTT-3′ (forward primer) and 5;-AGCCAAGCCTTGACGAACTA AAGC-3' (reverse primer), respectively (Brakstad *et al*., 1992).

PCR assay: The reaction mixture consisted of 10 μL PCR master mix (2X) (Cat. No. K0171), 1 μL forward primer, 1 μL reverse primer, 3 μL of bacterial lysate and 5 μL of nuclease free water to make a final volume of 20 μL. The analysis was performed according to Brakstad *et al*. (1992) with minor modifications. A total of 40 PCR cycles were run under the following conditions: Primary denaturation at 94°C for 4 min, denaturation at 94°C for 1 min, primer annealing at 55°C for 0.5 min, extension at 72°C for 1.5 min and final extension at 72°C for 3.5 min. The PCR product was separated by electrophoresis in 1.5% agarose gel at 120V for 30 min, visualized by 500 μg mL^{-1} ethidium bromide staining, illuminated by UV transilluminator (Biometra GmBH, Germany). A 100 bp sharp DNA ladder was used as a size reference. A positive control with known *S. aureus* DNA template and a negative control (water instead of extracted DNA) were used as known standards.

Culture sensitivity test: Each bacterial isolates was cultured on Mueller Hinton Agar media. Discs dipped with considered antibiotic were located in the plates in addition to control disc (soaked with autoclaved distilled water). The plates were incubated at 37°C for 24 h. Then "sensitive", "intermediate" or "resistant" samples were measured with respect to the data available from National Committee for Clinical Laboratory Standards (NCCLS, USA).

Statistical analysis: The bacterial loads isolated from contaminated chicken rinses were represented as $\log_{10}$ CFU cm^{-2} (Dawson *et al*., 2006) and means were calculated. Microbial counts were compared by ANOVA using SPSS software 12.0, with significance defined at the p-value.

RESULTS

Prevalence of *Staphylococcus aureus*: A total of 150 samples were collected from five different supermarkets in Chittagong metropolitan area. The overall prevalence of *S. aureus* was above 95%. Supermarket 3 and 5 showed 100% prevalence. While supermarket 1, 2 and 4 showed the prevalence of 96, 94.28 and 88.88%, respectively. The prevalence of *S. aureus* in different super shops of Chittagong region is shown in Table 1.

Bacteriological analysis: In conventional culture method, 143 out of 150 samples (95.83%) were studied and identified as *Staphylococcus aureus* based on their positive result in mannitol salt agar plate, gram staining and catalase test. After incubation period of 24 h yellow and pink colored colonies were found on MSA plate (Fig. 1). All the colonies (143) were also found catalase positive (Fig. 2a). All gram and catalase positive bacterial isolates were subjected to coagulase test in order to determine their ability of coagulation (Fig. 2b). From this study 68.53% samples were found to be infected with coagulase positive *Staphylococci* and 31.46% with coagulase negative *Staphylococci* (Fig. 3). The overall bacteriological analysis of frozen chicken rinse samples has been presented in Table 2.

Table 1: Prevalence of *S. aureus* in different super shops of Chittagong

Source	No. of samples	No. of positive samples	No. of negative samples	*S. aureus* prevalence (%)
Supermarket 1	25	24	1	96.00
Supermarket 2	35	33	2	94.28
Supermarket 3	30	30	0	100.00
Supermarket 4	36	32	4	88.88
Supermarket 5	24	24	0	100.00
Total	150	143	7	95.83

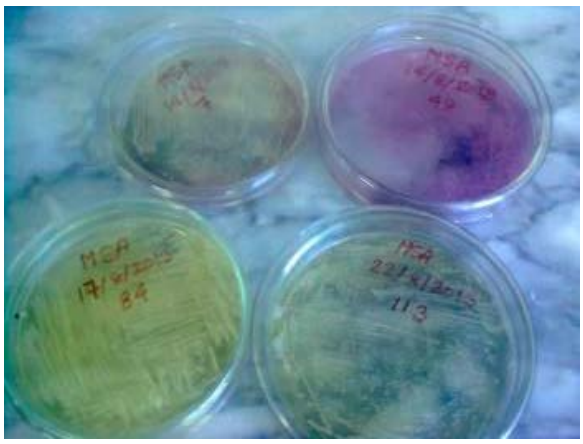

Fig. 1: *Staphylococcus* grown on MSA plate

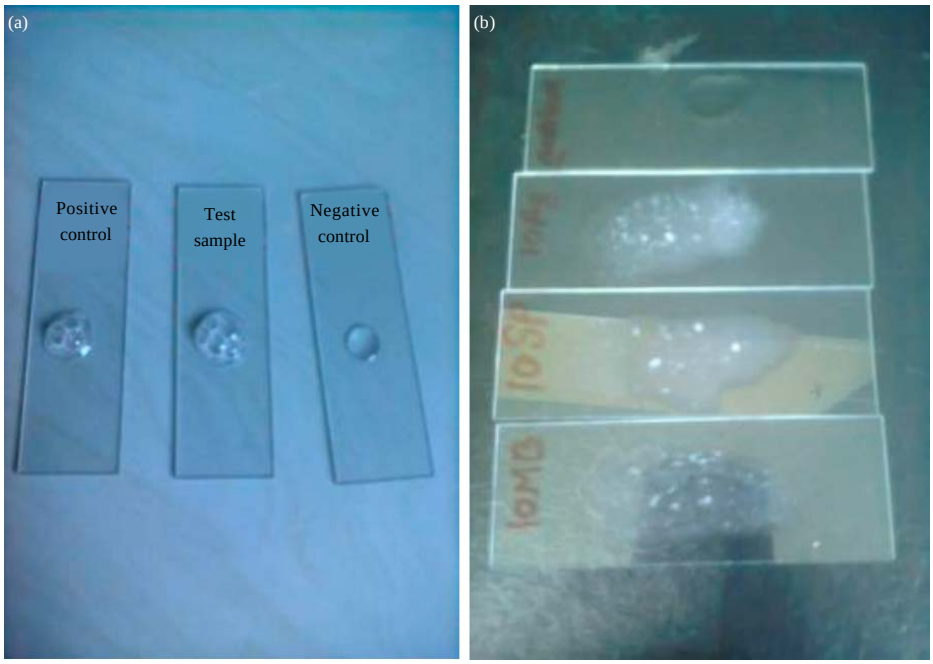

Fig. 2(a-b): (a) Catalase test and (b) Slide coagulase test. Catalase positive shows vigorous bubbling. No bubble and no coagulation forms in both negative control

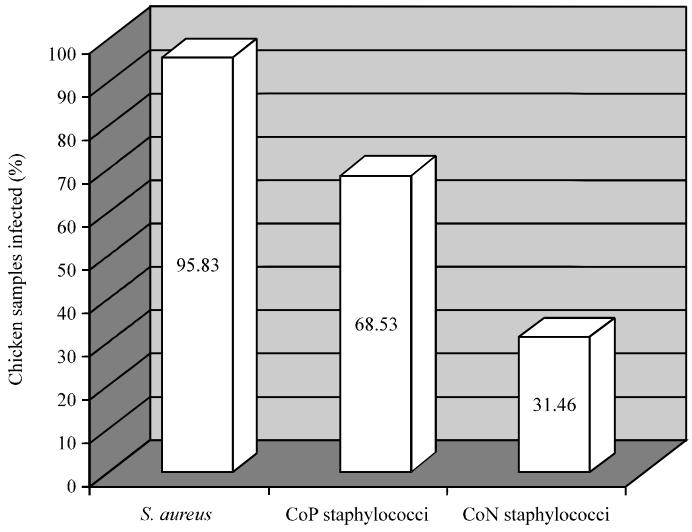

Fig. 3: Percentage of chicken rinse samples infected with *Staphylococcus aureus*. Here, CoP: Coagulase positive and CoN: Coagulase negative

Table 2: Results of different biochemical tests of frozen chicken rinse samples

Total samples	Mannitol salt test (positive)	Gram staining (positive)	Catalase test (positive)	Coagulase test (positive)
150	143	143	143	98

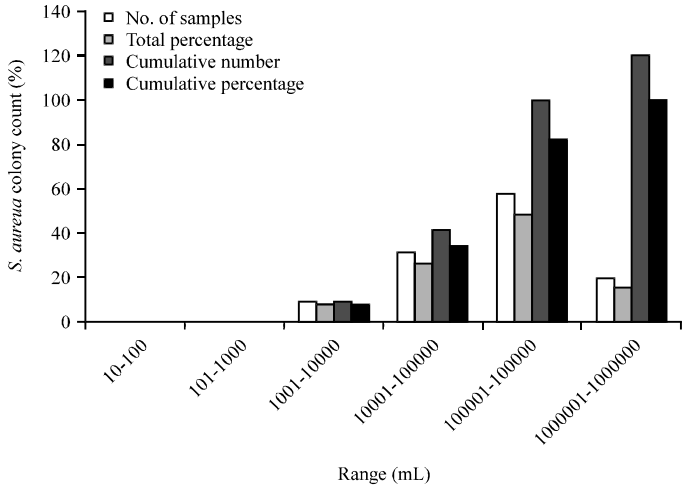

Fig. 4: *Staphylococcus aureus* colony count (per mL) calculated from frozen chicken rinse

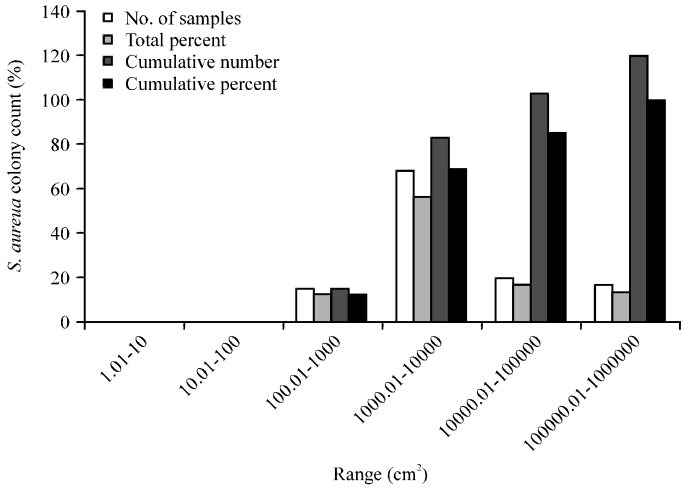

Fig. 5: *Staphylococcus aureus* colony count (per cm^2) calculated from frozen chicken rinse

Results of colony count: Viable bacterial counts were recovered from 100% of the samples. *S. aureus* colony count (per mL) calculated from chicken rinse fluids has been displayed in Fig. 4. The majority 48.33% samples were in the range of 100001-1000000 CFU mL^{-1}. Only 16.67% samples were estimated in the range of ≥10000000 CFU mL^{-1}. *Staphylococcus aureus* colony count (per cm^2) calculated from chicken rinse fluid has been presented in Fig. 5. About 18% samples were estimated in the range of 100-1000 CFU cm^{-2}. The majority 56.67% of the samples were recovered at levels of 1000-10000 CFU cm^{-2}. The 14% of the samples were in the range of ≥1000000 CFU cm^{-2}. The highest detection limit was 2×10^7 CFU mL^{-1} or 1.0×10^6 CFU cm^{-2}. The lowest detection limit was 3×10^4 CFU mL^{-1} or 1.5×10^2 CFU cm^2. The mean Log_{10} CFU cm^{-2} was 4.67. The frequency distribution result in the examined frozen chicken rinse sample is shown in Table 3. The statistical value of t-test was 4.6725 ($p\leq0.01$) and highly significant. According to

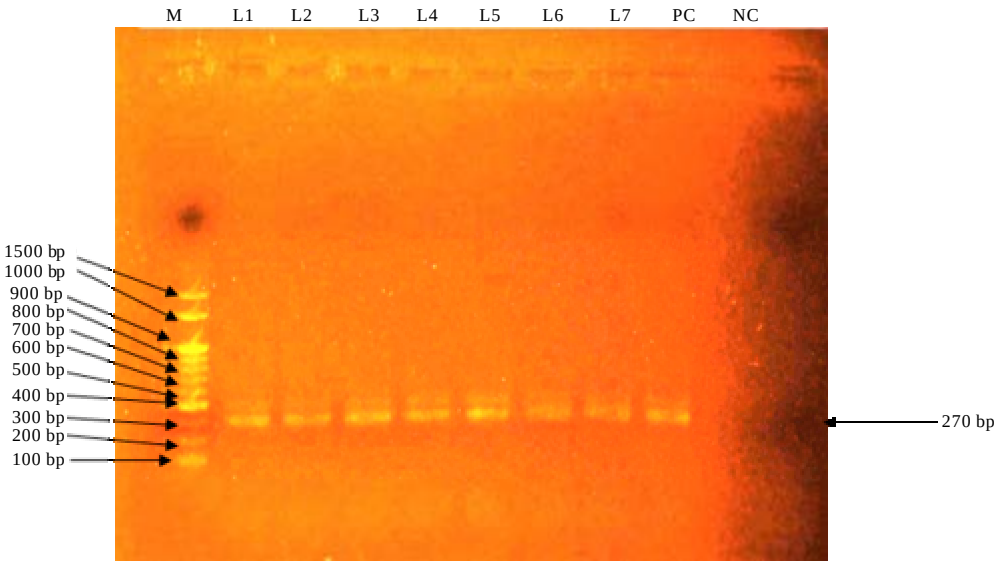

Fig. 6: Molecular detection of *S. aureus*. Here, the product of PCR that had amplified 270 bp gene (*Nuc* gene specific fragment) of S. *aureus* is showing on 1.5% agarose gel. Lane M: Marker DNA, Lanes 1-7: Positive samples, Lane PC: Positive control and Lane NC: Negative control

Table 3: Frequency distribution results in the examined frozen chicken rinse samples

Log (CFU mL^{-1})	No. of samples	Total percentage	Log (CFU cm^{-2})	No. of samples	Total percentage
>3	0	0.00	>2	0	0.00
3-4	0	0.00	2-3	0	0.00
4-5	40	33.33	3-4	30	25.00
5-6	48	40.00	4-5	70	58.33
>6	32	26.67	5-6	20	16.67

Log CFU mL^{-1}: Logarithm of colony forming units per ml and Log CFU cm^{-2}: Logarithm of colony forming units per cm^2

Gong *et al.* (2002) if the number of *S. aureus* in poultry products is $>10^5$ CFU cm^{-2} or 5 $\log_{10}$ CFU cm^{-2} then the products is considered to be at high risk potential or not fit for human consumption. The bacterial load of only 16.67% samples were above the level of $>10^5$ CFU cm^{-2}.

Results of PCR: From the PCR analysis 7.14% (7 out of 98) coagulase positive *Staphylococci* samples were confirmed as *S. aureus*. Overall 5.83% samples were confirmed as *S. aureus*. The *S. aureus* strain was identified on the basis of the 270 bp PCR product corresponding to the sequence of *Nuc* gene on 1.5% agarose gel. The positive band found at the same position of positive control and no band was found in the negative control lane. The results of PCR assay have been shown in Fig. 6.

Antimicrobial susceptibility pattern: The antimicrobial susceptibility pattern was examined for 120 staphylococcal isolates by antibiotic disc diffusion method (Fig. 7). The result showed resistance to Ampicillin (100%), Oxytetracyclin (99.17%), Doxycycline hydrochloride (88.33%),

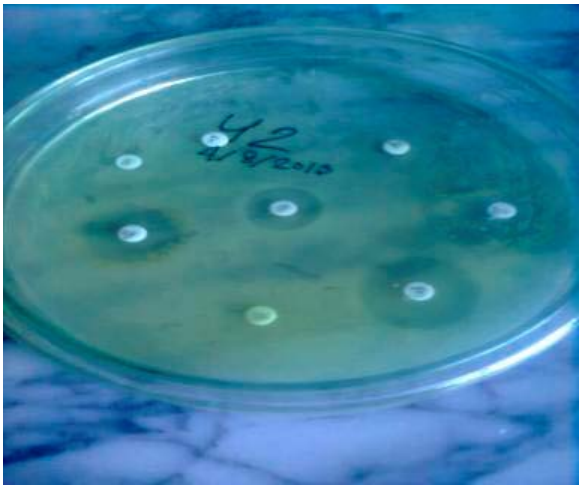

Fig. 7: Culture plates after Culture Sensitivity (CS) test

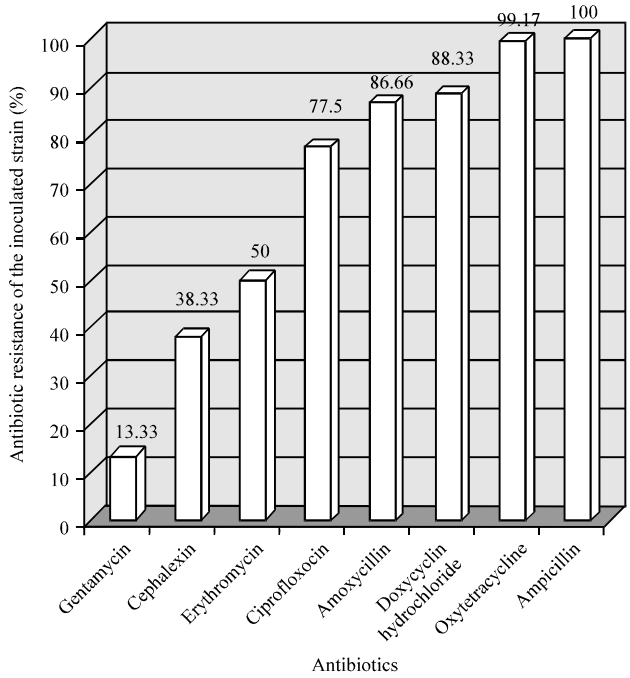

Fig. 8: Antibiotic resistant profiles of *Staphylococcus aureus* from chicken rinse samples

Amoxycillin (86.66%), Ciprofloxacin (77.5%), Cephalexin (38.33%) and Gentamycin showed the least resistance (13.33%). Antibiotic resistance profiles of *S. aureus* from chicken rinse samples have been presented in Fig. 8. The highest susceptibility was found toward Gentamycin (85.83%). In general 20.78% of isolated species were susceptible to selected antibiotics, 10.05% were moderate and 69.71% were resistant. Results of antimicrobial susceptibility pattern and antibiogram study have been presented in the Table 4 and 5, respectively.

Table 4: Antimicrobial susceptibility of *Staphylococcus* isolates

	Susceptible (S)		Intermediate (I)		Resistant (R)	
Antibiotic (μg disc^{-1})	No.	%	No.	%	No.	%
GEN* 10	103	85.83	1	0.83	16	13.33
AMP 25	0	0.00	0	0.00	120	100.00
E 15	17	14.17	43	35.83	60	50.00
OT 30	1	0.83	0	0.00	119	99.17
CIP 5	13	10.43	14	11.66	93	77.50
DO 30	3	2.50	11	9.17	106	88.33
AMX 30	16	13.33	0	0.00	104	86.66
CN 30	47	39.16	27	22.50	46	38.33

*GEN: Gentamycin, AMP: Ampicillin, E: Erythromycin, OT: Oxytetracycline, CIP: Ciprofloxacin, DO: Doxycycline hydrochloride, AMX: Amoxycillin and CN: Cephalexin

Table 5: Antibiogram pattern to multiple antibiotic resistance isolates

No. of antibiotics to which the isolates were resistant	No. of total isolates	Drug resistance patterns
2	1 (105)	AMP, DO
3	1 (106)	AMP, OX, AMX
3	1 (116)	GEN, AMP, OX
3	5 (117, 127, 128, 137, 145)	AMP, OT, DOX
4	3 (5, 72, 111)	AMP, E, OT, AMX
4	2 (17, 90)	AMP, E, OT, AMX
4	8 (41, 74, 91, 93, 112, 124, 132, 136,)	AMP, OT, DO, CL
4	4 (84, 135, 140, 143)	AMP, OT, CIP, DO
4	1 (130)	GEN, AMP, OT, DO
5	2 (2, 6)	AMP, OT, CIP, AMX, CL
5	3 (8, 9, 53)	AMP, E, OT, AMX, CL
5	4 (12, 52, 140, 144)	AMP, E, OT, DO, AMX, CL
5	16 (18, 26, 33, 43, 64,67, 41, 42, 43, 46,116, 119, 126, 139)	AMP, OT, DO, AMX, CL
5	2 (34, 46)	AMP, E, OT, CIP, DO,
5	1 (36)	AMP, OT, CIP, DO, AMX
6	2 (4, 13)	AMP, E, OT, CIP, AMX, CL
6	5 (71, 109, 113, 114, 123)	GEN, AMP, OT, CIP, DO, AMX
6	10 (7, 14, 48, 55, 61, 65, 76, 100, 103, 125)	AMP, OT, CIP, DO, AMX, CL
6	3 (10, 75, 92)	AMP, E, OT, DO, AMX, CL
6	23 (19, 20, 21, 24, 27, 29, 30, 32, 35, 37, 40, 44, 49, 51, 54, 5, 58, 62, 79, 102, 115, 120, 129)	AMP, E, OT, CIP, DO, CL
7	16 (1, 11, 15, 16, 22, 23, 31, 42, 45, 50, 57, 63, 66, 70, 77, 80)	AMP, OT, AMX, CL, DO, E, CIP
7	1 (59)	GEN, AMP, E, OT, CIP, AMX, CL
7	2 (28, 78)	GEN, AMP, E, OT, AMX, DO, CL
8	4 (25, 47, 60, 85)	AMP, OT, AMX, CL, DO, E, CIP, GEN

*GEN: Gentamycin, AMP: Ampicillin, E: Erythromycin, OT: Oxytetracycline, CIP: Ciprofloxacin, DO: Doxycycline hydrochloride, AMX: Amoxycillin and CN: Cephalexin

DISCUSSON

Staphylococcus aureus as a ubiquitous bacterium is the leading cause of superficial infection at the clinical environment for decades and also considered as the third most important cause of reported food-borne illnesses in the world (Normanno *et al*., 2005). Detection of *S. aureus* in poultry carcasses is important for the identification of the source of outbreaks associated with the consumption of improperly cooked poultry meat, assess microbiological safety and storage quality of products (Tompkin, 1983). Conventional culture method generally used for the identification of *S. aureus*. In this study gram staining, catalase and coagulase tests were performed for the identification of *S. aureus* from chicken rinse samples. Kateete *et al*. (2010), El-Hadedy and Abu El-Nour (2012), Thaker *et al*. (2013), Al-Mussawi (2014), Rohinishree and Negi (2011), Nkwelang *et al*. (2009), Nandy *et al*. (2009) also performed above tests for the identification of *S. aureus*.

During this study, all 150 chicken samples were cultured on Mannitol Salt Agar plate (MSA) of which 143 samples were detected as positive on MSA plate. Several biochemical tests are performed for confirmation of bacterial isolates in any selective media. Gram positive, cocci and arranged in irregular, grapelike clusters which are characteristics of staphylococcal species are the result of Gram staining (Holt *et al*., 1994). All the colonies (143) were also catalase positive.

All gram and catalase positive colonies were subjected to coagulase test. Out of 150 samples 98 samples (68.53%) were positive in slide coagulase test and 22 samples (31.46%) were coagulase negative. Citak and Duman (2011) found 92 (47.2%) Coagulase Positive Samples (CPS) out of 195 samples and 103 (52.8%) were coagulase negative. Generally coagulase test is considered as one kind of confirmatory test for identification of *S. aureus*. But coagulase positive samples may be the *S. intermedius* and *S. hyicus* (Devriese *et al*., 2009). Muftah (2011) reported 3-5% non-aureus CPS. Among 487 CPS, 82.1% were *S. aureus*, 17.7% were *S. hyicus* and 0.2% were *S. intermedius*.

The prevalence of *S. aureus* was found in 95.83% samples isolated from chicken rinse which was collected from different supermarkets. The prevalence rate may vary from different investigational report of different countries. Jakee *et al*. (2008) observed the prevalence of *S. aureus* from 223 of 370 meat samples (60.3%). Hanson *et al*. (2011) isolated *S. aureus* from 27 of 165 samples giving an overall prevalence of 17.8%. Shareef *et al*. (2012) investigated the prevalence of *Staphylococcus aureus* among US meat and poultry samples (136 samples) and found the contamination in 47%. Kozacinski *et al*. (2006) observed the prevalence rate of *S. aureus* was 30.30% (66 samples).

The Food and Agricultural Organization (FAO) of the United Nations and the World Health Organization (WHO) stated that illness due to contaminated food is perhaps the most widespread health problem. Certain strains of *S. aureus* produce enterotoxins which causes food poisoning. Toxin levels within this range are typically reached when *S. aureus* populations exceed 100,000/g or $>10^5$ CFU cm^{-2} or 5 $\log_{10}$ CFU cm^{-2} (Ash, 1997; Gong *et al*., 2002). In this study the load of bacteria was not significant or pathogenic for human consumption. But the different scientists observed high level of *S. aureus* pathogenecity in chicken meat. Ahmad *et al*. (2013) found the microbial load as 7.22 $\log_{10}$ CFU cm^2, Kozacinski *et al*. (2006) amounted to 4.72±0.38 $\log_{10}$ CFU g^{-1} and Waters *et al*. (2011) found 41% contamination.

Molecular (i.e., PCR based) diagnostic methods allow a better differentiation among species, serotypes. Such techniques are rapid, sensitive and specific which makes them very useful tools to improve the diagnosis and to understand the mechanisms implicated in pathogenecity, resistance and survival of the raw strains (Tamarapu *et al*., 2001). According to PCR results, 7 out of

98 (7.14%) coagulase positive samples were detected as *S. aureus*. Zhang *et al.* (2012) analyzed a total 15 strains of coagulase positive *S. aureus*. The *Nuc* primer set amplified an expected PCR product, amplicon of 270 bp in all 15 coagulase positive isolates of all *S. aureus*. From total 120 samples 7 (5.83%) were positive in PCR. Musa *et al.* (2009) found 16 samples positive in PCR while 18 samples were positive in biochemical test. In this experiment, the result of different data which were found from conventional and molecular methods revealed a significant difference. The 143 out of 150 samples which were positive by biochemical method but only 7 samples were detected as *S. aureus* by PCR. Musa *et al.* (2009) found similarity in biochemical tests and PCR method.

The antibiotic sensitivity tests are routinely done to select best drug against *Staphylococcus*. During this study 120 samples were subjected to Culture Sensitivity (CS) test. The activities of antibiotics against *S. aureus* showed that varying levels of multiple antibiotic resistances. According to this result, Ampicillin showed resistance (100%), Oxytetracyclin (99.17%), Doxycycline hydrochloride (88.33%), Amoxycillin (86.66%), Ciprofloxacin (77.5%), Cephalexin (38.33%) and Gentamycin showed the least resistance (13.33%). The highest susceptibility (85.83%) of *Staphylococcus* was found toward Gentamycin and Ampicillin showed no susceptibility. Lin *et al.* (2009) observed 50% resistant to tetracycline (64 microgm mL^{-1}) and 23.2% (48 of 207) to Erythromycin. Momtaz *et al.* (2013) showed that Tetracycline had the highest resistant profile (97.56%) followed by Gentamicin (29.26%), Enrofloxacin (28.04%), Ampicillin (26.82%), Chloramphenicol (20.73%) and Cephalothin (17.07%). Akbar and Anal (2013) found resistant to Ciprofloxacin (7.89%), whereas, the resistance against Gentamicin, Streptomycin and Chloramphenicol were recorded 13.15, 18.42 and 21.05%, respectively. The isolates about 28.94% showed resistance to Sulfamethoxazole/Trimethoprim. The highest number of the isolates 44.73% and 55.26% were resistant to Tetracycline and Ampicillin respectively. It has been observed that all isolate showed resistance to at least one antibiotic. Tula *et al.* (2013) showed 71.1, 68.9, 64.4 and 57.8% resistant to Amoxicillin-Clavulanate, Gentamycin, Cloxacillin and Ciprofloxacin, respectively. Though the above scientists observed significant resistance to Gentamycin but this study showed the highest susceptibility to Gentamycin. Increasing antimicrobial resistance of *S. aureus* can caused by various reasons such as widespread and in appropriate use of antimicrobials, along with lacking of efficient antimicrobial administration, large scale use of antimicrobial agents in animal feed as growth enhancers and increased international population movements (Cohen, 1992; Swartz, 1997).

CONCLUSION

This study is the first document for the identification of *S. aureus* in chicken rinse collected from different supermarkets of Bangladesh. Though bacteriological methods are used for the detection of pathogen but PCR is more accurate and specific for the confirmation of pathogen. From the comparison of above mentioned result it has been revealed that the prevalence of *Staphylococcus aureus* infections in supermarket is alarming for future because of its epidemiological importance. Proper management should be taken in the supermarkets to minimize the zoonotic disease transmissible from frozen chicken meat to humans.

ACKNOWLEDGEMENT

The authors are grateful to all the supermarkets for providing the chicken rinse samples for this study. The study was funded by UGC (University Grant Commission) of Bangladesh.

REFERENCES

Ahmad, M.U.D.A., M.I. Sarwar, M.I. Najeeb, A.A. Nawaz, M.A. Anjum, N.M. Ali and N. Mansur, 2013. Assessment of microbial load of raw meat at abattoirs and retail outlets. J. Anim. Plant Sci., 23: 745-748.

Akbar, A. and A.K. Anal, 2013. Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat. Asian Pacific J. Trop. Biomed., 3: 163-168.

Al-Mussawi, A.A., 2014. Detection of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) from human clinical specimens using conventional biochemical tests and chromogenic media. Indian J. Applied Res., 4: 7-9.

Altabari, G. and A.M. Al-Dughaym, 2002. The role of sanitary inspection of meat in relation of food poisoning. Proceedings of the 2nd Annual Scientific Meeting for Environment Hygiene, (Meat Hygiene), (ASMEH'02), Riyadh, Saudi Arabia, pp: 180-203.

Ash, M., 1997. *Staphylococcus aureus* and Staphylococcal Enterotoxins. In: Foodborne Microorganisms of Public Health Importance, Hocking, A.D., G. Arnold, I. Jenson, K. Newton and P. Sutherland (Eds.). 5th Edn., AIFST (NSW Branch), Sydney, Australia, pp: 313-332.

Boonmar, S., A. Bangtrakulnonth, S. Pornrunangwong, N. Marnrim, K. Kaneko, M. Ogawa, 1998. Salmonella in broiler chickens in Thailand with special reference to contamination of retail meat with *Salmonella enteritidis*. J. Vet. Med. Sci., 60: 1233-1236.

Brakstad, O.G., K. Aasbakk and J.A. Maeland, 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. J. Clin. Microbiol., 30: 1654-1660.

Chaslus-Dancla, E., J.P. Lafont and J.L. Martel, 2000. Spread of resistance from food animals to man: The French experience. Acta Veterinaria Scandinavica Supplementum, 93: 53-60.

Citak, S. and T. Duman, 2011. *Staphylococcus aureus* and coagulase-negative *Staphylococcus* from raw chicken samples in Turkey: Prevalence and antimicrobial resistance. J. Food Agri. Environ., 9: 156-158.

Cohen, M.L., 1992. Epidemiology of drug resistance: Implications for a post-antimicrobial era. Science, 257: 1050-1055.

Corner, D.E., M.A. Davis and L. Zang, 2001. Poultry-Borne Pathogens: Plant Considerations. In: Poultry Meat Processing, Sams, A.R. (Ed.). Chapter 9, CRC Press LCC., Boca Raton, FL., pp: 137-158.

Dawson, P., I. Hon, M. Cox, C. Black and L. Simmons, 2006. Residence time and food contact time effects on transfer of *Salmonella typhimurium* from tile, wood and carpet: Testing the five-second rule. J. Applied Microbiol., 102: 945-953.

Devriese, L.A., K. Hermans, M. Baele and F. Haesebrouck, 2009. *Staphylococcus pseudintermedius* versus *Staphylococcus intermedius*. Vet. Microbiol., 133: 206-207.

El-Hadedy, D. and S. Abu El-Nour, 2012. Identification of *Staphylococcus aureus* and *Escherichia coli* isolated from Egyptian food by conventional and molecular methods. J. Genet. Eng. Biotechnol., 10: 129-135.

Feizi, A., M. Nazeri and A. Pilevar, 2012. Isolation of *Staphylococcus* spp. genera from broiler breeder flocks in East Azerbaijan Province of Iran: Prevalence and antimicrobial susceptibility. Afr. J. Microbiol. Res., 6: 5819-5823.

Gong, J., R.J. Forster, H. Yu, J.R. Chambers, P.M. Sabour, R. Wheatcroft and S. Chen, 2002. Diversity and phylogenetic analysis of bacteria in the mucosa of chicken ceca and comparison with bacteria in the cecal lumen. FEMS Microbiol. Lett., 208: 1-7.

Hanson, B.M., A.E. Dressler, A.L. Harper, R.P. Scheibel and S.E. Wardyn *et al.*, 2011. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. J. Infect. Public Health, 4: 169-174.

Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., Lippincott Williams and Wilkins, Baltimore, USA., ISBN-13: 978-0683006032, Pages: 787.

Jakee, J., A.S. Nagwa, M. Bakry, S.A. Zouelfakar, E. Elgabry and W.A. Gad El-Said, 2008. Characteristics of *Staphylococcus aureus* strains isolated from human and animal sources. Am-Euras. J. Agric. Environ. Sci., 4: 221-229.

Kateete, D.P., C.N. Kimani, F.A. Katabazi, A. Okeng and M.S. Okee *et al.*, 2010. Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Ann. Clin. Microbiol. Antimicrob., Vol. 9. 10.1186/1476-0711-9-23

Kearney, J., 2010. Food consumption trends and drivers. Philos. Trans. R. Soc. B: Biol. Sci., 365: 2793-2807.

Khan, M.A., C.H. Kim, I. Kakoma, E. Morin and R.D. Hansen *et al.*, 1998. Detection of *Staphylococcus aureus* in milk by use of polymerase chain reaction analysis. Anim. J. Vet. Res., 59: 807-813.

Kozacinski, L., M. Hadziosmanovic and N. Zdolec, 2006. Microbiological quality of poultry meat on the Croatian market. Vet. Arhiv., 76: 305-313.

Lin, J., K.S. Yeh, H.T. Liu and J.H. Lin, 2009. *Staphylococcus aureus* isolated from pork and chicken carcasses in Taiwan: Prevalence and antimicrobial susceptibility. J. Food Prot., 72: 608-611.

Meiri-Bendek, I., E. Lipkin, A. Friedmann, G. Leitner, A. Saran, S. Friedman and Y. Kashi, 2002. A PCR-based method for the detectionof *Streptococcus agalactiae* in milk. J. Dairy Sci., 85: 1717-1723.

Momtaz, H., F.S. Dehkordi, E. Rahim, A. Asgarifar and M. Momeni, 2013. Virulence genes and antimicrobial resistance profiles of *Staphylococcus aureus* isolated from chicken meat in Isfahan province, Iran. J. Applied Poult. Res., 22: 913-921.

Mor-Mur, M. and J. Yuste, 2010. Emerging bacterial pathogens in meat and poultry: An overview. Food Bioprocess Technol., 3: 24-35.

Moran, G.J., A. Rishnadasan, R.J. Gorwitz, G.A. Fosheim, L.K. McDougal and R.B. Carey, 2006. Methicillin-resistant *S. aureus* infections among patients in the emergency department. New Engl. J. Med., 355: 666-674.

Muftah, H.H.B., 2011. Identification and antimicrobial resistance of *Staphylococcus aureus* from bovine milk. Master Thesis, Ohio State University, Veterinary Preventive Medicine.

Musa, N.O., K. Eltom, F. Gessler, H. Bohnel, A. Babiker, M.O. Elhaj and S.M. El Sanousi, 2009. Characterization of some *Staphylococcus aureus* subspecies anaerobius isolates by spa and coa genes. J. Anim. Vet. Adv., 8: 2272-2275.

Nandy, P., S. Roy, A.R. Thakur and S.R. Chaudhuri, 2009. Comparative study on characterization of three *Staphylococcal* isolates from varied origin. J. Cult. Collection, 6: 52-60.

Nkwelang, G., J.F.T.K. Akoachere, L.H. Kamga, E.D. Nfoncham and R.N. Ndip, 2009. *Staphylococcus aureus* isolates from clinical and environmental samples in a semi-rural area of Cameroon: Phenotypic characterization of isolates. Afr. J. Microbiol. Res., 3: 731-736.

Normanno, G., A. Firinu, S. Virgilio, G. Mula and A. Dambrosio *et al.*, 2005. Coagulase-positive *Staphylococci* and *Staphylococcus aureus* in food products marketed in Italy. Int. J. Food Microbiol., 98: 73-79.

Phuektes, P., P.D. Mansell and G.F. Browning, 2001. Multiplex polymerase chain reaction assay for simultaneous detection of *Staphylococcus aureus* and *Streptococcal causes* of bovine mastitis. J. Dairy Sci., 84: 1140-1148.

Rohinishree, Y.S. and P.S. Negi, 2011. Detection, identification and characterization of staphylococci in street vend foods: Characterization of *Staphylococcus* isolates. Food Nutr. Sci., 2: 304-313.

Salehi, T.Z. and S.F. Bonab, 2006. Antibiotics susceptibility pattern of *Escheichia coli* strains isolated from chickens with colisepticemia in Tabriz Province, Iran. Int. J. Poult. Sci., 5: 677-684.

Shareef, A.M., R.A. Farag and E.K. Al-Ruthwani, 2012. Evaluation of bacterial load of frozen chicken thighs in Mosul markets. Iraq J. Vet. Sci., 26: 63-69.

Shortle, D., 1983. A genetic system for analysis of staphylococcal nuclease. Gene, 22: 181-189.

Swartz, M.N., 1997. Use of antimicrobial agents and drug resistance. N. Engl. J. Med., 337: 491-492.

Tamarapu, S., J.L. McKillip and M. Drake, 2001. Development of a multiplex polymerase chain reaction assay for detection and differentiation of *Staphylococcus aureus* in dairy products. J. Food Prot., 64: 664-668.

Tauxe, R.V. and P.A. Blake, 1992. Epidemic cholera in Latin America. J. Am. Med. Assoc., 267: 1388-1390.

Thaker, H.C., M.N. Brahmbhatt and J.B. Nayak, 2013. Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. Vet. World, 6: 10-13.

Thomas, W.D. and G.L. Archer, 1989. Mobility of gentamicin resistance genes from staphylococci isolated in the United States: Identification of Tn4031, a gentamicin resistance transposon from *Staphylococcus epidermidis*. Antimicrob. Agents Chemother., 33: 1335-1341.

Tompkin, R.B., 1983. Indicator organisms in meat and poultry products. Food Technol., 37: 107-110.

Tula, M.Y., A.V. Azih and R.O. Okojie, 2013. Antimicrobial susceptibility pattern and plasmid-mediated antibacterial resistance in *Staphylococcus aureus* and Coagulase-Negative Staphylococci (CoNS). Am. J. Res. Commun., 11: 149-166.

Waters, A.E., T. Contente-Cuomo, J. Buchhagen, C.M. Liu and L. Watson *et al*., 2011. Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. Clin. Infect. Dis., 52: 1227-1230.

Zhang, T., C. Wang, X. Wei, X. Zhao and X. Zhong, 2012. Loop-mediated isothermal amplification for detection of *Staphylococcus aureus* in dairy cow suffering from mastitis. J. Biomed. Biotech. 10.1155/2012/435982