The Bangladesh Veterinarian (2015) 32 (1) : 13 – 18

Bacteria in chicken rolls sold by fast food restaurant and their public health significance

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Abstract

This study determined bacterial quality of chicken rolls sold in a fast food restaurant at Bangladesh Agricultural University (BAU) campus. Fifteen chicken rolls (ten pre-microwaved and five post-microwaved) were collected. Samples were inoculated into selective media, Eosin Methylene Blue (EMB) agar, Salmonella Shigella (SS) agar, Thiosulphate Citrate Bile Salts Sucrose (TCBS) agar and Mannitol Salt (MS) agar. The total viable count (TVC) and total Staphylococcal count (TSC) of pre-microwaved samples were 4.4 log CFU/g and 4.2 log CFU/g, respectively. In post-microwaved samples, the TVC and TSC were 2.7 log CFU/g and 2.6 log CFU/g, respectively. Microwave treatment significantly reduced the TVC and TSC in the chicken rolls ($P<0.05$). Bacteria were recovered only from samples inoculated onto MS agar. Colonies on MS agar were characteristics of *Staphylococcus* spp, confirmed by sugar fermentation, catalase and coagulase tests and polymerase chain reaction (PCR) assay. This study recorded coagulase negative staphylococcus (CNS) resistant to three antibiotics, ampicillin, cephalexin and vancomycin. It is suggested that chicken rolls sold in the fast food restaurant contaminated with resistant CNS might pose a public health hazard. (*Bangl. vet*. 2015. Vol. 32, No. 1, 13 – 18)

Introduction

The term fast food refers to food sold in a restaurant or store with preheated or precooked ingredients, and served in a package for take-away (Harun *et al*., 2013). Chicken roll is one of the most popular fast foods to peoples of all ages for its nutritious value and taste. It is made from chicken, chillies and spices, rolled in flour, egg white and bread crumbs. No study has been done on the microbial quality of chicken rolls sold at fast food restaurant in Bangladesh. The objectives of this work were to determine the prevalence of food-borne bacteria, the bacterial load, and the antibiotic sensitivity profiles of bacteria, in chicken rolls.

Materials and Methods

Collection of samples

Fifteen chicken rolls were collected from Masud Confectionary, at KR market in BAU. Ten were pre-microwaved (chicken rolls without heat treatment), and five were post-microwaved (treated heat prior to sale or offered to consumer).

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Isolation of bacteria

Homogenized samples were enriched in nutrient broth by overnight incubation at 37°C. Enriched cultures were streaked in duplicate onto Mannitol salt (MS) agar, Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar and Thiosulphate Citrate Bile Salts Sucrose (TCBS) agar, and incubated at 37°C for 24 hrs. Colonies on the surface of MSA, EMB and MacConkey agars were sub-cultured on the same media until a pure culture was obtained.

Characterization of bacteria

Bacteria were characterised by recording morphology of colonies (size, margin, elevation and colour), Gram stain, and sugar fermentation, catalase, coagulase, Methyl Red , Voges-Proskauer , indole tests (Cheesbrough, 1985).

Polymerase chain reaction (PCR) for Staphylococcus spp.

A genus-specific PCR was performed to amplify 16S rRNA of *Staphylococcus* spp. using previously published primers (Stuhlmeier and Stuhlmeier, 2003) (Table 1).

Table 1: PCR primers with sequence of *Staphylococcus* spp.

Primers	Sequences	Size (bp)
Staphylococcus 16S (F)	5′-GGAGGAAGGTGGGGATGACG-3′	241
Staphylococcus 16S (R)	5′-ATGGTGTGACGGGCGGTGTG-3′	

(F = Forward, R = Reverse, bp = Base pair)

Antibiotic sensitivity

Antibiotic sensitivity was tested using 0.5 McFarland turbidity standard inoculum and freshly prepared, dried Mueller Hinton agar (Oxoid, UK) against ampicillin, vancomycin, gentamicin, cephalexin, chloramphenicol and ciprofloxacin. Two isolates of *E. coli* and *Staphylococcus* spp. were selected randomly for the test. Disc diffusion or Kirby–Bauer method (Bauer *et al.,* 1966) was used. The results were expressed as resistant, intermediate or sensitive according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2007).

Results and Discussion

Total viable count (TVC) of chicken rolls

The TVC of ten pre-microwaved chicken rolls samples ranged from 3.4 to 5.6 log CFU/g (Table 2). On the other hand, the TVC of five post-microwaved samples ranged from 0 to 3.6 log CFU/g (Table 3). The mean TVC of pre-microwaved samples were 4.4 ± 0.7 log CFU/g and post-microwaved samples 2.7 ± 1.5 log CFU/g.

Total staphylococcal count (TSC) of chicken rolls

The TSC of pre-microwave samples (n = 10) ranged from 3.4 to 5.2 log CFU/g (Table 4). On the other hand, the TSC of post-microwave samples (n = 5) ranged from 0 to 3.3

log CFU/g (Table 5). The mean TSC of pre-microwaved samples were 4.2 ± 0.5 log CFU/g and post-microwaved samples were 2.6 ± 1.4 log CFU/g.

Table 2: Total viable count in pre-microwaved oven chicken rolls

Sample No.	TVC (log CFU/g)	Mean TVC (mean log CFU±SD/g)
1	4.6	4.4 ± 0.7
2	3.8	
3	3.4	
4	4.8	
5	4.9	
6	4.0	
7	4.7	
8	5.6	
9	3.7	
10	4.7	

TVC = Total viable count, CFU = Colony forming unit

Table 3: Total viable count found in post-microwaved oven chicken rolls

Sample No.	TVC (log CFU/g)	Mean TVC (mean log CFU ± SD/g)
1	3.6	2.7 ± 1.5
2	3.4	
3	3.3	
4	3.3	
5	0	

TVC = Total viable count, CFU = Colony forming unit

Table 4: Total staphylococcal count in pre-microwaved chicken rolls

Sample No.	TSC (log CFU/g)	Mean TSC (mean log CFU ± SD/g)
1	4.3	4.2 ± 0.5
2	3.6	
3	4.3	
4	4.5	
5	4.6	
6	3.7	
7	4.2	
8	5.2	
9	3.4	
10	4.4	

TSC = Total staphylococcal count, CFU = Colony forming unit

Table 5: Total staphylococcal count in post-microwaved chicken rolls

Sample No.	TSC (log CFU/g)	Mean TSC (mean log CFU ± SD/g)
1	3.2	
2	3.3	
3	3.2	2.6 ± 1.4
4	3.2	
5	0	

TSC = Total staphylococcal count, CFU = Colony forming unit

Isolation of bacteria

Bacteria were recovered only from samples inoculated onto MS agar.

Cultural, morphological and staining characteristics

The cultural characteristics of *Staphylococcus* spp. were similar to the findings of other authors (Sharada *et al.,* 1999; Thomas *et al.,* 2005; Konuku *et al.,* 2012). Small whitish colonies appeared on MS agar, which were characteristic of *Staphylococcus* spp. Gram positive cocci, were arranged in grape-like clusters, characteristic of *Staphylococcus* spp.

Biochemical characteristics

Staphylococcus spp. fermented all five basic sugars with acid production (Table 6). Catalase, Methyl Red and Voges-proskauer tests were positive but indole and coagulase tests were negative. These results are similar to those of Thomas (1998); Konuku *et al.* (2012).

Table 6: Summary of sugar fermentation and biochemical tests for identification of *Staphylococcus* spp.

Sugar fermentation reaction profiles					MR test	VP test	Indole production test	Interpretation
DX	ML	L	S	MN				
A	A	A	A	A	+	+	-	*Staphylococcus* spp.

DX = Dextrose, ML = Maltose, L = Lactose, S = Sucrose, MN = Mannitol, A = Acid, MR = Methyl red, VP = Voges-proskauer, + = Positive, - = Negative

Molecular detection of Staphylococcus spp. by PCR

DNA extracted from *Staphylococcus* spp. were used in PCR assay. PCR primers targeting 16S rRNA of *Staphylococcus* spp. amplified 241 bp fragments of DNA confirmed the identity of *Staphylococcus* spp. (Fig. 1).

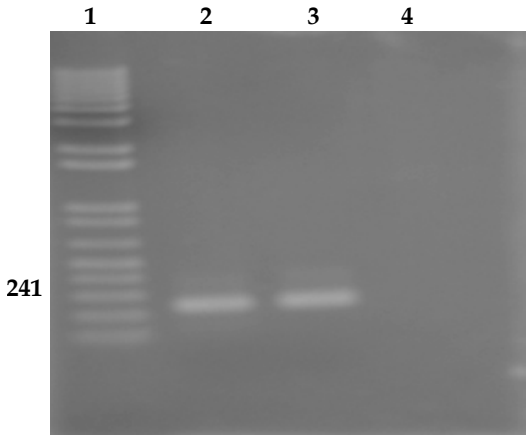

Fig. 1. Identification of *Staphylococcus* spp. by amplification of 16S rRNA gene by PCR. Lane 1: 100 bp size DNA marker (Trackit, Invitrogen, USA); Lane 2: positive control DNA of *Staphylococcus;* Lane 3: DNA of bacteria isolated from chicken roll; Lane 4: negative control without DNA.

Antibiotic sensitivity

Staphylococcus spp. was resistant to ampicillin, vancomycin and cephalexin, and sensitive to ciprofloxacin, chloramphenicol and gentamicin (Table 7). The results are identical to those by Thong and Modarressi (2011); Singh *et al.* (2011); Tagoe *et al.* (2011).

Table 7: Antimicrobial profile of *Staphylococcus* spp.

Antibiotic disc	Diameter of zone of inhibition (mm)	Interpretation
Ampicillin	9	R
Chloramphenicol	17	I
Ciprofloxacin	23	S
Gentamicin	17	S
Cephalexin	10	R
Vancomycin	10	R

Legend: R = Resistant, S = Sensitive, I = Intermediate

Conclusions

Staphylococcus spp. resistant to two or three antibiotics was identified. They may be transmitted to humans through the consumption of contaminated chicken rolls.

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