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ISOLATION AND IDENTIFICATION OF BACTERIA FROM THE WORKERS OF LIVE BIRD MARKETS AT MYMENSINGH, BANGLADESH

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Abstract

Context: Identification of bacteria from the workers of live bird markets is important factor for zoonotic aspects and for implementing appropriate control strategies.

Objectives: To determine the occurrence of bacteria especially *Salmonella* sp. and *Escherichia coli* from the workers of live bird markets.

Materials and Methods: A total of 40 samples were collected from hand washes (n=20) and nasal swabs (n=20) of the associated workers in urban and suburban live bird markets. Bacteria were isolated in different media, and identification was performed based on the staining, cultural and some biochemical tests. For *Salmonella* sp., DNA was extracted using a DNA isolation kit and *rfbs* gene was amplified by using commercial PCR kit.

Results: The bacteria such as *Salmonella* sp. and *E. coli* were detected in the samples by several microbial tests. The prevalence of *Salmonella* sp. was 40% and 30%, and *E. coli* was 70% and 40% in the hand washes and nasal swabs respectively of the workers of urban and periurban live bird markets.

Conclusion: The results obtained in this study suggest that the appropriate precautions should be taken during and subsequent to the handling of live birds to minimize the risk of zoonotic diseases.

Key words: *Salmonella*, *Escherichia coli*, live bird markets, isolation and identification

Introduction

In Bangladesh, large number of people in both urban and suburban locations are entirely dependent on live bird marketing. Transmission of bacteria from live bird markets to workers also occurred most probably due to low level of biosecurity practices. Common pathogenic bacteria that may be the cause of food borne diseases in human including strains of *Salmonella* sp. and *Escherichia coli* (Sockett 1991). In many developing countries, lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques were responsible for bacterial infection or contamination in human (Joshi *et al*. 2003). But literature is not available on the transmission of bacteria from the live bird markets to workers. Few reports are available for enteric pathogenic bacteria especially *Salmonella* sp. and *E. coli* of human that can also be isolated from migrating birds. These organisms are important in the context of transmission of potential human pathogens by migrating birds and the handling of birds by workers (Murray 2000, Tizard 2004). Therefore, the present study was undertaken to determine the prevalence of bacteria especially *Salmonella* sp. and *E. coli* in the hand washes and nasal swabs of the workers of live bird markets.

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Materials and Methods

Collection and culture of samples: The total of 40 samples of which twenty (20) hand wash (10 from urban and 10 from suburban) and 20 nasal swabs (10 from urban and 10 from suburban) were collected from the people working in live bird markets.

Test tubes containing samples were incubated for 24 h at 37 ºC. From the cultured samples, subcultures were also made on nutrient agar, BGA agar, EMB agar and blood agar and incubated at 37ºC for overnight. The identification of the organisms was performed by following the tests of Merchant and Packer (1967), Carter (1979), Freeman (1985) and Cheesbrough (2000). On the basis of colony, and staining characters and biochemical tests, the organisms were isolated and identified. The representative bacteria were stained using modified Gram's staining (Lillie 1928). Biochemical tests were performed according to the methods described by Merchant and Packer (1967).

Cultivation of Salmonella isolates and DNA extraction: The purity of *Salmonella* sp. was verified by cultural, staining and biochemical tests (Cheesbrough 2000). One colony from each sample was inoculated into 10 ml of LB broth (Oxoid Ltd. Bahingstoke, Hamshire, England) and incubated at 37ºC for 18 h. One ml from each culture was taken into a sterile eppendorf tube for extraction of genomic DNA.

DNA was extracted from five field samples culturing in LB broth by using Wizard® Genomic DNA Purification Kit (Promegra Corporation. 2800 Woods Hollow Road. Madison, USA). The extracted DNA was quantified using a spectrophotometer's (Spectronic® Genetics™ New York, USA) and expressed in ng/ µl.

Oligonucleotide Primer and Amplification of DNA by PCR: One pair of oligonucleotide primer (SG1: 5´-tca-cga-ctt-aca-tcc-tac -3´ and SG2: 5´-ctg-cta-tat-cag-cac-aac-3´) was used to amplify the rfbS gene of *Salmonella* sp. that is genus specific. Primers were diluted with appropriate amount of TE Buffer (p^H: 8.0) and stored at -20ºC until use. The expected product size of this primer is 720 bp.

PCR amplification was performed in a final volume of 10µl containing 2µl (50 ng/ µl) of DNA template, Taq buffer-A 1µl, dNTPs 1µl, primer-F 0.5µl, primer-R 0.5µl, Taq DNA polymerase 0.2µl and 4.8µl nuclease free water. Three independent reactions with the primers were made for DNA template. Amplification was carried out in Gene amplification PCR system 9600 Thermocycler (eppendorf, Germany), using condition modified from Doran *et al*., (1996). The pre-mix was then mixed well through spinning. Initial denaturation was at 94°C for 1 min, 94°C for 60 sec, annealing at 50°C for 60 seconds and extension at 72°C for 21 seconds, with a final extension at 72°C for 7 minutes for total 33 cycles and held for 4°C. The amplified products were separated by electrophoresis on 1.5% agarose gel containing 5 µl ml^{-1} ethidium bromide with a 100 bp ladder (Promega, Madison, WI, USA) as molecular weight marker (Oliveira *et al*. 2003).

Results

The prevalence of *Salmonella* sp. in hand wash of urban and suburban workers of live bird markets were 40% and 30% respectively. The prevalence of *E. coli* in nasal swabs of urban and suburban workers were 70% and 40% respectively (Table 1). The findings revealed that the prevalence of isolated bacteria was higher in urban than suburban live bird markets.

Table 1. Prevalence of bacteria in hand washes and nasal swabs

Isolated bacteria	Urban LBMs workers			Periurban LBMs workers		
	Sample tested	Occurrence of isolates	Prevalence among tested cases (%)	Sample tested	Occurrence of isolates	Prevalence among tested cases (%)
Salmonella sp.	10	4	40.00	10	3	30.00
Escherichia coli	10	7	70.00	10	4	40.00

Table 2. Biochemical activities of different isolated bacteria

Isolated bacteria	Carbohydrate fermentation test					Indole	MR	VP test
	D	Ma	S	L	Mn			
Salmonella spp.	AG	AG	-	-	AG	-	+	-
Escherichia coli	AG	AG	-	AG	AG	+	+	-

Plate 1. *Salmonella* sp. as rod shaped, gram-negative with short to long chain forming (Modified Gram's stain × 830).

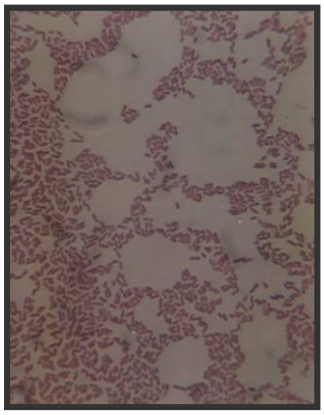

Plate 2. *Escherichia coli* showing a short rod, gram-negative varying from coccoid to bipolar shape (Modified Gram's stain, X 830).

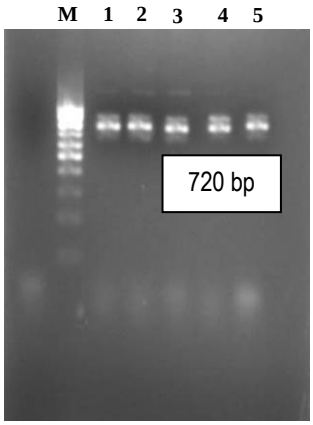

Plate 3. Agarose gel electrophoresis of amplified PCR products. Lane1-3: *Salmonella* isolates from workers of urban LBMs; Lane 4-5: *Salmonella* isolates from workers of periurban LBMs; Lane-M: Molecular marker.

From the smear of pure cultures, *Salmonella* sp. revealed that the morphology of the isolated bacteria was rod shape, gram-negative and short to long chain forming (Plate 1). *E. coli* in smears from pure cultures, revealed that the morphology of the isolated bacteria was short rod, gram-negative varying from coccoid to bipolar shape (Plate 2). All of the *Salmonella* isolates fermented dextrose, maltose and mannitol but did not ferment lactose and sucrose, and all of the isolates were indole negative, methyl red positive and VP negative (Table 2). Among seven isolates, five isolates of which three isolates were from urban workers and two isolates were from suburban workers that were identified by standard culture and biochemical methods. Among five isolates, all *Salmonella* isolates were successfully amplified and gave an amplified product of 720 bp (Plate 3)

Discussion

The colony characters of *Salmonella,* showed pink white opaque colored colonies on brilliant green agar was corresponded with the findings of others (Old 1990, Yuno *et al*. 1995, Sharma and Katok 1996, Perez *et al*. 2004). *Escherichia coli* grew well on EMB agar and produce greenish colony with metalic sheen after 24 hours at 37°C which was similar with the findings of others (Jones *et al*. 1997, Mishra *et al*. 2002).

Salmonella isolates fermented dextrose, maltose and mannitol and all of the isolates were indole negative, methyl red positive and VP negative which are special biochemical characters for *Salmonella* sp. was previously suggested by Christensen *et al*. (1993) and Robinson *et al*. (2000). *E. coli* on the otherhand failed to ferment sucrose whereas dextrose, lactose, maltose and mannitol were fermented with production of acid and gas, and all of the isolates were indole positive, methyl red positive and VP negative which are special biochemical characters for this bacterium was similar to the reports of others (Jones *et al*. 1997, Mishra *et al*. 2002). Among five isolates, all *Salmonella* isolates were successfully amplified and gave an amplified product of 720 bp and this result was coincides with the result of Park *et al.* (2001).

Conclusion

The presence of *salmonella* sp. and *E. coli* in hand washes and nasal swabs of workers in live bird markets indicates the biosecurity index. In near future, the attempts should be taken to identify the specific causative agents. The findings of this investigation will help the veterinarian or physician to take necessary steps to prevent zoonotic diseases.

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