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Original

Isolation and Identification of Pathogenic Gram-Positive Bacteria from Egg Shell of Hen and to See Their Antimicrobial Susceptibility Pattern

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Abstract

***Background**: Food-borne disease is a major public health problem affecting developed as well as developing countries. Inaccurately treated eggs can be one of its causes. So we designed this study to observe the possibility of transmission of pathogenic Gram-positive bacteria from market eggs to the community. **Objectives**: To identify different Gram-positive bacteria in eggs and to observe their antimicrobial susceptibility. **Materials and Methods**: This observational study was conducted in the department of Microbiology, Dhaka Medical College, Dhaka. Shells of 150 eggs collected from different markets of Dhaka city were tested. Bacteria were isolated and identified by culture and relevant biochemical tests. **Results**: Out of 150 egg shells, 120 (80%) yielded growth of different bacteria. Of them, Staphylococcus spp. were 80 (66.67%), Streptococcus spp. 8 (6.67%), Bacillus subtilis 20 (16.67%) and Bacillus cereus 12 (10%). Out of 80 Staphylococcus spp., 30 (25%) were Staphylococcus aureus and 50 (41.67%) were Staphylococcus saprophyticus. Most of the Gram-positive bacteria were sensitive to ciprofloxacin, ceftriaxone and imipenem. No MRSA and VRSA were found. **Conclusion**: It can be concluded from this study that Gram-positive bacteria from market eggs may be an important source of infection to the community.*

***Key words**: Gram-positive bacteria; Culture; Eggs; Antibiotic susceptibility*

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Introduction

Food-borne illness is a major public health problem and an important cause of diarrheal diseases affecting all developed and developing countries.[1] Inaccurately treated eggs can cause food-borne illness.[2] Absence of standard structures and drainage system in the market and relatively high humidity could have contributed to the high microbial growth.[3] Most retailers do not store eggs in refrigerators, thus the eggs are exposed to weather conditions, resulting in contamination.[4] In spite of the antibacterial factors, it can be infected with different bacteria such as *Salmonella spp., Esch coli, Campylobacter jejuni, Proteus spp., Klebsiella spp.* and different Gram-positive bacteria like *Stapylococcus spp.*, *Streptococcus spp.*, *Listeria monocytogens*, and *Bacillus spp.*[5] *Staphylococcus spp*. is a major disease-producing organism for poultry and approximately 50% of typical and atypical *S. aureus* strains produce enterotoxins that can cause food poisoning in human being.[6] Streptococcosis in avian species is worldwide in distribution, occurring as both acute septicemic and chronic infections with mortality ranging from 0.5–50%.[6] Although *Bacillus spp.* is a saprophytic organism mainly but it also causes food poisoning.[5]

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The present study is carried out to isolate the pathogenic Gram-positive bacteria from egg shells of hen by culture and to see their antimicrobial susceptibility pattern.

Materials and Methods

This observational study was carried out on egg shells of 150 eggs collected from different markets of Dhaka city. The study was done in the department of Microbiology, Dhaka Medical College (DMC), Dhaka from July 2012 to June 2013. Undamaged and clean eggs without fecal contamination and cracks were included in this study.

Sample processing with enrichment

For each egg, one sterile swab stick was made wet by trypticase soy broth (TSB) and shell swab was taken from the entire surface of the egg and was immediately inoculated in a test tube containing 9 mL TSB for enrichment.[6]

Isolation of organisms

After processing, TSB media were incubated at 37ºC for 24 hours. Then one or two loopful inoculum was streaked on blood agar medium from TSB and again incubated at 37ºC for 24 hours and examined after 24 hours for visible colony of bacteria.[6,7] For confirmation of *Staphylocoocus spp*. subculture was done on mannitol salt agar medium from blood agar medium and incubated at 37ºC for 24 hours and examined after 24 hours.[8]

Identification of organisms

All the isolated organisms were identified by their colony morphology, staining characters and further confirmed by relevant biochemical tests including catalase test, coagulase test and oxidase test. *Staphylococcus aureus* from *S. saprophyticus* were differentiated by novobiocin sensitivity test. *Bacillus subtilis* were differentiated from *B. cereus* by mannitol fermentation test.[9]

Antibiotic susceptibility test

Using Kirby-Bauer modified disc-diffusion technique, antibiotic susceptibility test was performed as described by Clinical and Laboratory Standards Institute (CLSI).[10,11]Antibiotic dishes of oxacillin (1µg/disc), vancomycin (30µg/disc), amoxiclav (amoxicillin and clavulanic acid), (20/100µg/disc), azithromycin (15µg/disc), cefixime (30µg/disc), ceftriaxone (30µg/disc), doxycycline (30µg/disc), erythromycin (15µg/disc), gentamicin (10µg/disc), imipenem (10µg/disc) (Oxoid Ltd. UK) were used. Pure colonies of isolated organisms were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. Selected antibiotic discs were placed on inoculated Mueller-Hinton agar medium. These plates were incubated at 37ºC for 24 hours. Resistant and sensitive bacteria were defined according to CLSI guidelines.[11]

Detection of methicillin resistant *Staphylococcus aureus* (MRSA)

Isolated *Staphylococcus aureus* were screened for methicillin resistance by phenotypic disc diffusion method. Disc diffusion test was performed following Clinical and Laboratory Standard Institute (CLSI, 2010) using oxacillin (1µg) disc. A 0.5 McFarland standard suspension of the isolate was made and inoculated into Mueller-Hinton agar plate by using a sterile swab. Oxacillin disc was applied on plate using sterile forceps. The agar plates were incubated at 37ºC for 24 hours and diameters of zone of inhibition <10 mm indicated oxacillin resistance.[12]

Detection of vancomycin resistant *Staphylococcus aureus* (VRSA)

Isolated *Staphylococcus aureus* were screened for vancomycin resistance by phenotypic disc diffusion method. Disc diffusion test was performed following Clinical and Laboratory Standard Institute (CLSI, 2010) using vancomycin (30µg) disc. A 0.5 McFarland standard suspension of the isolate was made and inoculated into Mueller-Hinton agar plate by using a sterile swab. Vancomycin disc was applied on plate using sterile forceps. The agar plates were incubated at 37ºC for 24 hours and diameters of zone of inhibition less than 15 mm indicated vancomycin resistance.[12]

Data analysis

After compiling data were analyzed using `Microsoft Office Excel 2007` program.

Results

Out of total 150 egg shells 120 (80.00%) yielded growth of different Gram-positive bacteria. Among the isolated bacteria *Staphylococcus spp*. were the most common (66.67%) organisms and 6.67% were *Streptococcus spp*. (Table I).

According to antimicrobial resistance pattern of Gram-positive bacteria isolated from egg shells to different antibiotics, none of the isolates were resistant to ciprofloxacin and ceftriaxone. No MRSA and VRSA were isolated. All (100%) the *Streptococcus spp*. were resistant to amoxiclav and doxycycline. All (100%) the *Bacillus spp*. were sensitive to imipenem (Table II).

Table I: Frequencies of microbial isolates from egg shells (n=120)

Name of the bacteria	Number	Percentage
Staphylococcus spp.		
Staphylococcus aureus	30	25.00
Staphylococcus saprophyticus	50	41.67
Streptococcus spp.	8	6.67
*Bacillus subtilis**	20	16.67
Bacillus cereus	12	10.00
Total	120	100.00

* In this study, *B. subtilis* were identified in 20 egg shells. Although *B. subtilis* is non-pathogenic but it can cause allergic manifestations in prolonged exposure and can also cause food poisoning as like *B. cereus* in immuno-compromised patients. For this *B. subtilis* were included in the list of pathogenic bacteria.

Table II: Antimicrobial resistance pattern of isolated bacteria from egg shells to different antibiotics

Antimicrobials	*Staphylococcus spp*. (n=80)	*Streptococcus spp*. (n=8)	*Bacillus spp*. (n=32)
Oxacilin	0 (0.00)	0 (0.00)	_
Vancomycin	0 (0.00)	0 (0.00)	_
Erythromycin	30 (37.5)	4 (50.00)	11 (34.35)
Doxycycline	70 (87.5)	8 (100.00)	_
Ciprofloxacin	0 (0.00)	0 (0.00)	0 (0.00)
Ceftriaxone	0 (0.00)	0 (0.00)	0 (0.00)
Azithromycin	0 (0.00)	7 (87.5)	_
Cefixime	40 (50.00)	4 (50.00)	_
Amoxiclav	70 (87.5)	8 (100.00)	_
Tetracycline	_	_	8 (25.00)
Imipenem	_	_	0 (0.00)
Gentamicin	_	_	9 (28.13)

Discussion

Microbial contamination of egg has an important effect on poultry industry and illness from contaminated egg is a serious public health problem around the world.[3] Though Gram-negative bacteria can contaminate eggs but Gram-positive bacteria are the main culprits to contaminate poultry products.[13] Gram-positive bacteria can tolerate dry and harsh conditions and are present in dust, soil and feces, which is the major reason of its presence on egg shells.[14] Food poisoning, and gastroenteritis are the major infections transmitted by feco-oral route in Bangladesh. Eggs are considered as a major source of protein and vitamins for everybody. Eggs are bought from the market and brought to every kitchen irrespective of assessing whether it carries harmful bacteria or not.

Out of 150 eggs collected from different markets, 120 (80%) egg shells yielded growth of pathogenic bacteria. Another study reported that 95% egg shells yielded growth of different bacteria from eggs collected from market.[7] These bacterial contaminations might be from clothes and hands of poultry and market workers, market retailers, use of same tray and environment of the market.[15] In the developing countries, especially in Bangladesh, inadequate refrigeration even no refrigeration during the market storing can increase the percentage of different bacterial contamination on egg shell.

Out of the 150 shells of eggs collected from market, 30 (20%) yielded no growth. Abdullah[6] reported that 5% of the isolated organisms were fungi and 0.1% was anerobic bacteria in his study. In the present study, negative results of egg shells might be due to the fact that these samples were contaminated by fungus or anerobic bacteria or any bacteria which were not isolated in routine culture media. If these fungi and anerobic bacteria could be cultured the diagnostic yield would definitely be better. Alternatively, DNA of these organisms which cannot be cultured in routine laboratory media can be detected by PCR. Warm and moist litters, poor condition in the farm houses are the causes of fungi growth and sporulation.[16]

Among the isolated aerobic bacteria, 80 (66.67%) were *Staphylococcus spp*. In Iran, relatively higher percentage of *Staphylococcus spp*. (75%) was observed. In another study, 28.45% *Staphylococcus spp*. was identified.[8] In this study, 30 (20%) *Streptococcus spp*. and 10 (6.67%) *Bacillus spp*. were identified. Abdullah[6] reported that 4.9% *Streptococcus spp*. was found in his study, which was lower than what we found in the present study. Contamination is more likely linked with cracked egg, dirty shells and storage in contaminated surroundings. It

can be contaminated during formation and laying process.[14] Although Streptococcus and Staphylococcus are present in fecal content but these are the major health concerns causing fever, food poisoning, ocular infection etc.[17]

Drug resistance is a major problem in treating the infectious diseases. In the present study, most of the isolated bacteria showed highest sensitivity to ciprofloxacin and ceftriaxone (100%). In case of *Staphylococcus spp.*, all (100%) serotypes were sensitive to oxacillin and vancomycin. Another study reported that 14.25% *Staphylococcus spp*. were resistant to oxacillin.[18]

From this study, it can be concluded that eggs may be a source of transmission of different Gram-positive bacteria from market to the community. Early detection and proper hygienic practice should be maintained in handling and marketing eggs by the farm handlers and retailers to prevent spread of infection of different Gram-positive bacteria to the community. Ceftriaxone and ciprofloxacin are the most effective drugs against Gram-positive bacteria isolated from market eggs.

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