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ISOLATION AND ANTIBIOGRAM OF STAPHYLOCOCCUS SPECIES FROM EGGS OF JAPANESE QUAIL IN AN ORGANISED FARM

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Abstract: This study was undertaken to analyse and characterize *Staphylococcus* species isolated from the surface and contents of quail eggs on their phenotypic properties, biochemical reactions and antibiotic sensitivity pattern was also studied. The study included 42 strains of the genus *Staphylococcus* isolated from the egg whites, yolks and shells of table quail eggs from 150 samples (26.7%). Among the species isolated, the most frequently occurring strains were of *Staphylococcus hominis* (26.1%), followed by *S. aureus* (14.28%), *S. xylosus* (16.6%), *S. lentus* (14.28%) *S. sciuri* (7.14%), *S. epidermidis* (4.76), *S. caprae* (4.76%), *S. hyicus* (4.76%), *S. cohnii* (2.38%), *S. simulans* (2.38%), *S. auricularis* (2.38%). The greatest number of strains (78.57%) were isolated from shells, while 14.28% of isolates were obtained from yolks and 7.14% from the whites of the eggs. The antibiotic sensitivity testing showed 15.5% of the strains to be resistant to one or more of the therapeutic agents tested. The antibiotic sensitivity testing showed 16.6% of the strains to be resistant to one or more of the therapeutic agents tested. Moreover, some isolates exhibited intermediate sensitivity to the drugs, particularly to gentamicin (23.9%), neomycin (26.2%), streptomycin (50.1%) and Linco-Spectin (45.4%), amoxicillin (11.9%), amoxicillin with clavulanic acid (7.1%), cephalexin (2.3%), doxycycline (4.8%), enrofloxacin (2.4%), linco-spectin (45.4%), oxytetracycline (2.3%), trimethoprim /sulfamethoxazole (9.6%), Norfloxacin (14.4%).

Keywords: *Staphylococcus* spp., quail egg contamination, biochemical properties, drug resistance.

Introduction

Among the bacteria predominantly involved in diseases, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food (Manie et al., 1998). Staphylococcal food poisoning is due to the absorption of Staphylococcal enterotoxins preformed in the food (Loir et al., 2003). Considerable importance is currently given to the role of coagulase-negative staphylococci, which are potentially pathogenic for birds and mammals. Of particular significance in the etiology of infections in humans are the species *Staphylococcus epidermidis, Staphylococcus haemolyticus* and *Staphylococcus hominis*. Coagulase-negative staphylococci that have most frequently been isolated from clinical

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samples and can be potentially pathogenic for humans. Their resistance to antibiotics can be zoonotic as well.

Staphylococci isolated from animals may differ substantially in terms of physiology and resistance to antibiotics and phages; moreover, they do not all adapt easily to the human organism. Resistant microorganisms are also a source of genes responsible for antibiotic resistance in other bacteria that acquire them.

Increasing attention has been given to the role of poultry and poultry products, including eggs, as a potential source of infections in humans induced by antibiotic- resistant *Staphylococcus* strains (Abulreesh and Organji, 2011). Microbial flora on the eggshell and in the egg contents may reduce hatchability (Board and Tranter, 1995) because of trans-shell contamination of hatching eggs. However, table eggs are consumed worldwide in various forms and are considered a very nutritious and inexpensive source of protein. Staphylococci constitute an important component of the flora which can be isolated from the surface and contents of table eggs. They have the potential to cause spoilage and enter the food chain causing infection in consumers.

The shell can be infected when passing through the vent, but many researchers suggest that contamination mainly occurs within a short period after laying due to contact with contaminated surfaces. Bacterial contamination of egg contents could result from the penetration of the shell by bacteria deposited on the surface of the egg after it has been laid (Bahrouz and Al-Jaff, 2007).

In view of the growing popularity of Japanese quail (*Coturnix coturnix japonica*) breeding and of retail sale of quail eggs, the present study was attempted to analyse and characterize *Staphylococcus* isolated from the shell surface and the contents of quail eggs, taking into account their phenotypic properties, biochemical reactions, antibiotic sensitivity patterns.

Sample collection

The study included 150 table quail eggs purchased in an organised quail farm. The eggs were clean, with no cracks or visible defects in the shells and the eggs were fresh.

Isolation of bacteria

The material (whites, yolks and shells) was pre-enriched in buffered peptone water (Buffered Peptone Water, HiMedia Pvt. Ltd.) at 37^{o}C for 18-24 hours (Singh and Prakash, 2008). The material was then transferred onto blood agar (Blood Agar Base, HiMedia Pvt. Ltd), MSA (Mannitol Salt agar, HiMedia Pvt. Ltd.) and the selective agar, Baird Parker Agar (BPA) (HiMedia Pvt. Ltd.). The agar plates are incubated in aerobic conditions at 37^{o}C for 24-48

hours, depending on the rate of growth of the bacteria. Single colonies were then transferred onto blood agar to isolate pure bacterial cultures, and an initial bacteriological characterization was performed by evaluating the morphology of the colonies and the presence and type of hemolysis.

Characteristic appearance of jet black colonies surrounded by a white halo were considered to be presumptive *S. aureus*. The pure cultures were streaked on Nutrient agar (HiMedia Pvt. Ltd.) and incubated for 24 hours at 37°C and were further characterized by biochemical tests.

Morphological characteristics

The smear was prepared from the isolated culture on clean grease free microscopic glass slide and stained with Gram's method of staining. The stained smear was observed under microscope. Smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes.

Biochemical Identification

Biochemical tests were performed to confirm S. aureus using Catalase test, Coagulase test, DNase test, Acetoin production, Oxidase test and D-mannitol fermentation, free coagulase test, tests for bound coagulase (clumping factor), catalase test using the commercially available media and reagents procured from HiMedia Pvt. Ltd.

Antibiogram pattern

The susceptibility of isolates to different anti-microbial agents was done by disk diffusion method using commercial disks (Bauer et al., 1966) procured from HiMedia Pvt. Ltd. The antimicrobial agents tested were the following; Amoxicillin (AMX), amoxicillin with clavulanic acid (AMC), cephalexin (CL), doxycycline (D), enrofloxacin (EX), norfloxacin (NX), gentamicin (GEN), Linco-Spectin (lincomycin/ spectinomycin) (LS), neomycin (N), oxytetracycline (OT), streptomycin (S) and trimethoprim/sulfamethoxazole (SXT).

Sensitivity of the isolated bacterial strains to selected antibiotics and sulfonamides was tested using the Kirby-Bauer disk diffusion method on Mueller-Hinton medium in accordance with accepted international norms (CLSI, 2011). The results were read and interpreted based on the diameter of the zone of inhibition, with the strains designated as resistant (R), of intermediate sensitivity (I) or sensitive (S).

Results

Isolation and identification of bacterial strains

A total of 42 *Satphylococcus* strains were isolated from the material, including 11 strains of *Staphylococcus hominis* (26.1%), 6 of *S. aureus* (14.28%), 7 of *S. xylosus* (16.6%), 6 of *S.*

lentus (14.28%), 3 of *S. sciuri* (7.14%), 2 of *S. epidermidis* (4.76%), 2 of *S. caprae* (4.76%), 2 of *S. hyicus* (4.76%), 1 of *S. cohnii* (2.38%), 1 of *S. simulans* (2.38%), and 1 of *S. auricularis* (2.38%). The greatest number of strains (78.57%) were isolated from shells, while 14.28% of isolates were obtained from yolks and 7.14% from the whites of the eggs. The types of bacteria isolated from different parts of the egg are presented in table 1.

Table 1. The types of bacteria isolated from different parts of the egg

Speccies	Egg shell	Egg white	Yolk	Total (%)
S. hominis	10	-	1	26.1
S. aureus	5	1	-	14.28
S. xylosus	6	-	1	16.6
S. lentus	2	1	3	14.28
S. sciuri	3	-	-	7.14
S. epidermidis	1	1	-	4.76
S. caprae	2	-	-	4.76
S. hyicus	2	-	-	4.76
S. cohnii	1	-	-	2.38
S. simulans	1	-	-	2.38
S. auricularis	-	-	1	2.38
Total (%)	78.57	7.14	14.28	100.0

Biochemical properties of the strains

The *Staphylococcus* strains tested had highly varied biochemical and enzymatic properties. As many as 7 biotypes were distinguished among the 7 isolates of *S.aureus*, 6 biotypes within the species *S. xylosus* (6 strains tested), 5 biotypes among the 6 strains of *S.lentus*, but only 4 among the 11 strains of *S. hominis.* Detailed data are presented in Table 2.

Table 2. Biochemical properties of the isolated *Staphylococcus* strains

Property	*S. aureus* n=6	*S.hominis* n=11	*S. xylosus* n=7	*S. lentus* n=6	*S. sciuri* n=3	*S. epidermidis* n=2	*S. caprae* n=2	*S. hyicus* n=2	*S. cohnii* n=1	*S.simulans* n=1	*S.auricularis* n=1
Pigment production	2	10	1	3	1	0	1	0	0	0	0
Coagulase	2	0	0	0	0	0	0	1	0	0	0
β hemolysis	2	0	2	1	1	0	0	0	0	0	0
α hemolysis	4	0	2	0	0	0	0	0	0	0	0
DNase	4	0	0	2	1	0	1	2	0	1	0
Catalase	6	11	7	6	3	2	2	2	1	1	0
Acid production from: – glucose	6	11	7	6	3	2	2	2	1	1	1
– fructose	6	11	7	6	3	2	2	2	2	1	1
– mannose	6	3	7	6	3	2	0	2	1	0	0
– maltose	6	11	7	6	3	2	1	0	0	0	1
– lactose	6	0	7	6	3	2	0	0	1	1	0
– trehalose	6	11	7	6	1	0	2	2	0	1	1
– mannitol	4	0	7	6	3	0	0	0	1	0	0
– raffinose	2	0	3	6	0	0	0	0	0	0	0

-saccharose	6	11	6	6	3	2	2	2	1	1	1
– NAG*	6	0	7	6	1	0	0	0	0	1	0
– MDG**	0	0	7	1	1	0	0	0	0	0	0
– melibiose	0	0	3	5	0	0	0	0	0	0	0
– xylitol	0	0	0	1	0	0	0	0	0	0	0
– xylose	1	0	7	6	1	0	0	0	0	0	0
Production of: – urease	6	5	3	0	0	2	0	2	0	1	0
– ADH***	6	4	0	0	0	2	0	2	0	1	0
– β-galact-osidase	4	4	0	5	1	1	1	0	0	0	1
– alkaline phosphatase	6	11	7	6	3	2	2	2	1	1	1
– nitrate reduction	6	11	5	5	3	2	2	2	0	1	1
– acetoin****	5	10	3	3	1	2	0	0	0	0	0

* breakdown of N-acetylglucosamine, ** breakdown of methyl-α-D-glucopyranoside, ***production of arginine dihydrolase, **** Voges-Proskauer test.

Drug sensitivity of the bacterial strains

Tests of the sensitivity of the 42 *Staphylococcus* strains to antibiotics and chemotherapeutic agents found 7 strains (16.6%) that were resistant to some of the drugs applied. These were *S. hominis* - 1 strain resistant to gentamicin (GEN) and 2 strains resistant to streptomycin (S), *S. epidermidis* - 1 strain resistant to oxytetracycline (OT), *S. hyicus* - 1 strain resistant to gentamicin and streptomycin, *S. cohnii* - 1 strain resistant to Linco-Spectin (LS) and *S. simulans* - 1 strain resistant to doxycycline, gentamicin, norfloxacin, streptomycin and trimethoprim/sulfamethoxazole. None of the strains was found to be resistant in *in vitro* conditions to cephalexin (CL), enrofloxacin (EX), amoxicillin with clavulanic acid (AMC), amoxicillin (AML) or neomycin (N). However, a certain percentage of isolates was observed to have intermediate sensitivity to these agents - 2.4% of strains exhibited intermediate sensitivity to cephalexin and enrofloxacin, 7.1% to amoxicillin with clavulanic acid, 11.9% to amoxicillin, and 26.2% to neomycin.

The antibiotic sensitivity testing showed 16.6% of the strains to be resistant to one or more of the therapeutic agents tested. Moreover, some isolates exhibited intermediate sensitivity to the drugs, particularly to gentamicin (23.9%), neomycin (26.2%), streptomycin (50.1%) and Linco-Spectin (45.4%), amoxicillin (11.9%), amoxicillin with clavulanic acid (7.1%), cephalexin (2.3%), doxycycline (4.8%), enrofloxacin (2.4%), linco-spectin (45.4%), oxytetracycline (2.3%), trimethoprim /sulfamethoxazole (9.6%), Norfloxacin (14.4%).

Table 3. Antibiotic sensitivity of the isolated *Staphylococcus* strains

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Amoxicillin (AML)	88.1	11.9	0
Amoxicillin with clavulanic acid (AMC)	88.1	7.1	0

Cephalexin (CL)	95.2	2.3	0
Doxycycline (D)	92.8	4.8	2.3
Enrofloxacin (EX)	97.6	2.4	0
Norfloxacin (NX)	83.3	14.4	2.3
Gentamicin (GEN)	73.8	23.9	2.3
Linco-Spectin (LS)	52.3	45.4	2.3
Neomycin (N)	73.8	26.2	0
Oxytetracycline (OT)	95.4	2.3	2.3
Streptomycin (S)	47.6	50.1	2.3
Trimethoprim /sulfamethoxazole (SXT)	88.1	9.6	2.3

Discussion

The results obtained indicate that a fairly high percentage of the retail quail eggs tested were contaminated with *Staphylococcus* bacteria. While *Staphylococcus aureus* is the most frequent cause of infections in poultry, other *Staphylococcus* species, though far less often described by other authors, cannot be treated exclusively as commensals in birds. They are often a direct infectious agent for both people and animals (Gill et al., 1983). Reports can be found in the literature of the frequent occurrence of coagulase-negative staphylococci, not only in the contents and on the shells of eggs but also in the tissues of birds (Wieliczko et al., 2002).

While most of the staphylococci were found on the shells of the eggs, a certain percentage of strains of the species *S. lentus, S. xylosus*, and *S. hominis* were also noted in the yolk and white. The *S. epidermidis* strains, which were isolated exclusively from the white and yolk, were an exception. The species most frequently isolated from the environment of the egg white were *S. warneri* and *S. epidermidis*, while the species isolated most frequently from the yolk was *S.aureus*, regardless of the source of the eggs (Stępień-Pyśniak et al., 2009).

In the present study, the most frequently isolated species was the coagulase-negative *Staphylococcus hominis*. Analysis of the biochemical properties of the 11 strains of this species, using the tests, found differences in the breakdown of only 4 substrates. The biochemical patterns of 5 of the 11 strains were 100% similar. In the case of the *Staphylococcus aureus* strains, a certain percentage of isolates had an atypical biochemical profile. For this reason additional tests were conducted, in which clumping factor production, and strong DNase activity were found in 100% and 71%, respectively, of the *S. aureus* strains. Only two *S. aureus* strains (33.3%) and one *S. hyicus* strain (50%) produced coagulase in the tube coagulase test. All of the other isolates tested were coagulase-negative. Coagulase production usually occurs only in *Staphylococcus aureus* subsp. *aureus* and in

some strains of *S. intermedius* (Młynarczyk et al., 1997). Some *Staphylococcus* species exhibit highly varied sensitivity to the effects of lysozyme contained in egg white. It is observed that staphylococci that produce an orange pigment, ferment mannitol and produce coagulase are more resistant to lysozyme than strains lacking these biochemical characteristics (Thompson and Khorazo, 1935). This is partially confirmed by the results of the present study, as the only *S. aureus* strain isolated from egg white exhibited all of these traits.

The antibiotic sensitivity tests showed that 16.6% of the strains were resistant to one or more of the therapeutic agents applied. The strains exhibiting resistance to more than one of the antibiotics were *Staphylococcus simulans* and *Staphylococcus hyicus*, which were resistant to five and two of the antibiotics, respectively. A small percentage (2.2%) of the staphylococci tested were resistant to synthetic chemotherapeutic agents of the fluoroquinolone group, which are widely used to treat bacterial infections in poultry. Resistance to this group of antibiotics is observed with increasing frequency among *Staphylococcus* bacteria (Aarestrup et al., 2000). In a study of the antibiotic sensitivity of *Staphylococcus* strains isolated from poultry showed that in *in vitro* conditions, the strains were most sensitive to amoxicillin and amoxicillin with clavulanic acid (Lyon et al., 1987). The results of the present study showed that none of the bacteria tested were resistant to amoxicillin and amoxicillin with clavulanic acid in *in vitro* conditions, but a certain percentage of strains (11.9% and 7.1%) showed intermediate sensitivity to these antibiotics.

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