قسم : الصحة ومراقبة الأغذيـــــة .
كليـة : الطب البيطرى ـ جامعة أسيوط.
رئيس القسم : أ.د / علي يوسف لطفـي .

التلوث البكتريولوجي لقشر البيض

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لما كان للبيض من أهمية كبيرة من حيث قيمته الغذائيه ، فهو أيضا يعـــد مصدرا مضرا للبكتريا الممرضه مما يشكل خطورة على صحة الانسان ، لذلك قمنـــا بجمع عدد ١٢٥ بيضة يمثلون ٢٥ مجموعة عشوائيا من أسواق مدينة أســـــيوط ومحلات البقاله المختلفه وقمنا بالتعرف على مدى تلوث قشر البيض من الخـــارج بالبكتريا المختلفـــة .

ولقد تبين أن متوسط العدد الكلي للبكتريا الموجوده على سطح البيـض كان ٣٧×١٠^{٦} بينما متوسط العدد الكلي لـ

Coliforms, Faecal coliform, Enterococci and S. aureus.

كان كما يلي ٣×١٠^{٣} ، ٦×١٥^{٢} ، ١٠٠ لكل قشرة على التوالي .

وكذلك تم عزل وتصنيف عدد ١٧ ميكروب وهي :

S.aureus, Staph. epidermidis, Micrococcus, Enterococci, E. coli, Enterobacter, Citrobacter, Kelbsiella, Arizona, Acinetobacter, Pseudomonas and Serratia.

ولقد ناقش البحث علاقة هذه الميكروبات بتلوث محتويات البيض من الداخل وكذلك منتجاته أثناء التصنيع ومدى خطورته على صحة الانسان . وكذلك الطـرق الواجب اتباعها لمنع تلوث البيض بهذه البكتريا .

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BACTERIAL CONTAMINATION OF EGG SHELLS

(With 2 Tables)

By
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(Received at 30/4/1984)

SUMMARY

125 of commercial hen's eggs were collected at random from Assiut markets and different groceries. The egg samples were examined superficially for bacterial contamination which may be present on shells. The mean value of total bacterial count, Coliform, faecal coliform, Enterococci and S. aureus counts were, $37x10^6$, $3x10^3$, $6x10^2$, $16x10^2$ and / 100/shell, respectively. Different isolates (117) were identified as, S. aureus, Staph. epidermidis, Micrococcus, Strept. faecalis, strept. faecium, intermediate, E.coli, Enterobacter, Citrobacter, Klebsiella ozanae, Arizona hinshawii, Acinetobacter calcoaceticus, Pseudomonas aurogenosa and Serratia.

INTRODUCTION

Microbiology of eggs and egg shells has received inadequate attention so far, from the view points of public health and economic. Bacteria on egg shells have been implicated as a source of contamination of broken out eggs (SOLOWEY et al. 1946 and KRAFT et al. 1967). Moreover, bacteria on shells may also, under certain conditions, penetrate through the shells into the interior and cause spoilage (BOARD, 1968).

Levels of bacterial contamination ranging from 10^2 - 10^8/shell, have been reported by HAINES, 1938; ROSSER, 1942; FORSYTHE et al. 1953 and BOARD et al. 1964. Reports recorded by HAINES, 1938; ZAGAEVSKY and LUTIKOVA, 1944 and BOARD et al. 1964, showed that Coliform, Enterococci, Staphylococci, Micrococcus, Flavobacterium, Pseudomonas and other aerobic gram-negative rods, could be isolated from shells of commercial hen's eggs. On the other hand, BOARD et al. (1964) detected one strain of salmonella from shell of a slightly soiled egg. In a study described by MOATS (1979), the level of bacterial contamination/shell ranged from 2.1×10^3 - 5.11×10^6, while Coliforms, Staphylococci and Enterococci counts were / 50 - 2.05×10^5, 500 - 49.5×10^4 and / 250 - 22×10^3/shell, respectively. Prevalence of salmonella organisms on eggs as reported by BAKER and GOFF (1980) was 0.21% of examined egg shells. MOATS (1980) found that, log 10 of 4.26 - log 10 of 6.46 organisms/shell was the level of bacterial contamination of examined egg shells. Also, he added that, Coliform, Strept. faecalis, Micrococcus and Staphylococci were 10, 8, 5 and 41% of the isolates recovered from egg shell, while 3% of the isolates were found to be S. aureus.

Our investigation was undertaken with the object of ascertaining the level of microbial contamination as well as, the different microorganisms which may found on shell.

MATERIAL and METHODS

Collection of samples:

125 eggs (25 groups) were collected, at random, from Assiut markets and different groceries. Every 5 eggs (one group) were placed in a sterile plastic bag and dispatched to the laboratory.

Preparation of samples:

Eggs were tested by a surface rinse method, where each egg was immersed in 100 ml. of Tryptic soy broth in a jar and shaken for 15 min. on a mechanical rotatory shaker. The rinse solution obtained from the five eggs of each group were combined and subjected to the following examination:

1- Total bacterial count

Serial dilutions of rinse solution were prepared using 0.1% sterile peptone water. Standard plate count was determined with duplicate plates of standard plate agar as described in Standard Methods (A.P.H.A. 1978).

2- Coliform count

Duplicate plates of Violet red bile agar were used for each dilution as described in Standard Methods (A.P.H.A., 1978).

3- Faecal coliform by sing Violet red bile agar at 44C° (KLEIN and FUNG, 1976).

4- Enterococci count by using E.S.D. agar of EFTHYMIOU and JOSEPH (1974) by the technique of A.P.H.A. (1978).

5- Isolation and enumeration of S. aureus:

Numbers of S. aureus were determined by using Baird-parker agar plates (BAIRD - PARKER, 1962). Duplicate plates were prepared and incubated 48 h. at 37C°. Furthermore, appropriate amount of rinse solution of each sample was inoculted into a tube of Sod. chloride broth, which was then incubated at 37C°. for 24 h. (BAILEY and SCOTT, 1978). A loopful from the incubated tubes was streaked onto a plate of mannitol salt agar (BAILEY and SCOTT, 1978). Confirmation of colonies suspected to be S. aureus was accomplished by the DNase test of LACHICA et al. (1971).

6- Isolation and identification of other Staphylococci and Micrococcus were the same as described by BAILEY and SCOTT, (1978).

7- Isolation and identification of Enterobacteriacae were performed by using API 20E strips (anlytab products, Plainview, N.Y., U.S.A.).

8- Isolation of salmonella was carried out according to the method recommended by SPECK (1976), then the isolates were identified by API 20E strips.

RESULTS

All results obtained from the examined samples are recorded in Tables 1 & 2.

DISCUSSION

The results presented in Table 1, show the max., min., and mean values of total bacterial count, Coliform, faecal coliform, Enterococci and S. aureus count. A lower count of total bacteria was found by HAINES (1938); ROSSER (1942); FORSYTHE et al. (1953) and MOATS (1979), while BOARD et al. (1964) recorded a max. of 10^8 bact./shell. Lower percentage of samples containing

Coliforms was stated by BOARD et al. (1964), while higher counts of Coliform ranged from / 50 - 2.05 x 10^5 /shell have been detected by MOATS (1979). Higher level and incidence of Enterococci were obtained by MOATS (1979) and BOARD et al. (1964). In other instances, a lower incidence of Enterococci was recorded by ZAGAEVSKY and LUTIKOVA (1944) and MOATS (1980). Evidence of contamination with faecal matter was indicated by isolation of faecal coliform. Also, our results reveal a higher incidence of S. aureus (80%) than that reported by MOATS (1980), but a higher level of S. aureus/shell was detected by MOATS (1979).

The resutls summarized in Table 2, show the different types of isolates recovered from egg shells. The incidence of Staphylococci and Micrococcus on egg shells was higher than that obtained by ZAGAVESKY and LUTICOVA (1944) and BOARD et al. (1964). As far can be ascertained, the widewly distribution of Staphylococci in nature and on skin of warm blooded animal (BAIRD - PARKER, 1963) can prove the high incidence of such organism. E.coli and Enterobacter were recovered from egg shells in higher incidence than that recorded by BOARD et al. (1964) and MOATS (1980). Pseudomonas was isolated, but in a lower percentage than that of HAINES (1938). It has been proposed that Pseudomonas, Coliforms and E.coli were among the main types causing spoilage of intact egg (SCOTT et al. 1950-1951 and FLORIAN and TRUSSEL, 1957). Salmonella could not be detected in our examined samples, however PAKER and GOFF (1980) isolated salmonella (0.21%) from the examined egg shells. Two isolates presumed to be Sigella, but could not be assured due to lack of antiserum. Different isolates were recovered from the examined egg shells, including Citrobacter, Arizona hinshawii, Klebsiella ozaenae, Acinetobacter and Serratia.

It is pertinent to note that bad storage of eggs under a very humid condition, could support the multiplication of microorganisms present on egg shells (HAINES, 1983 and FORSYTHE et al. 1953). Furthermore, these bacteria may contaminate the content of egg either by penetration or withdrowal through the shell pores, following cooling of freshly laid contaminated egg (MCLAURY and MORAN, 1959). It has been documented that members of these bacteria have been implicated in cases of enteritis, epidemic or summer diarrhoea in infants, urinary infiction, food poisoning and intestinal disorders (SMITH and CONANT 1960 and FRAZIER, 1967). Also, many of such bacteria have been implicated in spoilage of eggs, leading to economic losses. Moreover, the contamination of egg shells may be responsible for lowering the microbiological quality of egg products by the use of broken-out or cracked eggs.

This study can be used to deduce the most probable sources of contamination to which eggs are exposed, including dust, soil faecal matter, as well as, bad handling. In conclusion, hygienic measures and educational program should be imposed in poultry farms and on egg producers.

REFERENCES

A.P.H.A. (1978): Standard methods for examination of dairy products, 14th Ed. American Public Health Association, Washington, DC.

Bailey, W.R. and Scott, E.G. (1978): Diagnostic microbiology. A text bood for the isolation and identification of pathogenic microorganisms. the C.V. Mosby Company Saint Louis.

Baird-Parker, A.C. (1962): An improved and selective medium for isolating Coagulase positive staphylococci. J. Appl. Bact. 25, 1: 12-19.

Baird-Parker, A.C. (1963): A classification of micrococci and stphilococci based on physiological and biochemical tests. J. Gen. Microbiol. 30: 409-427.

Baker, R.C. and J.P. Goff (1980): Prevalence of salmonella on eggs from poultry farms in New York state. Poultry Sci. 59: 289-292.

Board, R.G.; J.C. Ayers; A.A. Kraft and R.H. Forsythe (1964): Theh microbiological contamination of egg shells and egg paking materials. Poultry Sci. 43: 584-595.

Board, R.G. (1968): Microbiology of egg (a review) in T.C. Carter (ed). Egg quality: a study of the hen's egg. Oliver and Boyd, Edinburgh. (Cited after Moats, 1980).

Efthymiou, C.J. and Joseph, S.W. (1974): Development of a selective enterococcus medium based on manganese ion defficiency, sod. azide and alkakine pH. Appl. Microbiol. 28, 3: 411-416.

Florian, M.L.E. and Trussel, P.C. (1957): Bacterial spoilage of shell egg: IV identification of spoilage organisms Food Technol. 11: 56.

Forsythe, R.H.; J.C. Ayres and J.L. Radlo (1953): Factors affecting microbiological population of shell eggs. Food Technol. 7: 49-56.

Frazier, W.C. (1967): Food Microbiology 2nd Ed. T.A.T.A. McGtw-Hill publishing Company LTD Bombay New Belhi.

Haines, R.B. (1938): Observation on the bacterial flora of the hen's egg with a description of new species of Proteus and Pseudomonas causing rot in egg. J. Hyg. Camb. 38: 338-355. (Cited after Board et al. 1964).

Klien, H. and Fung, D.Y. (1976): Identification and quantification of faecal coliforms using violet red bile agar at elevated temp. J. Milk Food Technol. 39: 768-770.

Kraft, A.A.; G.S. Torry; T.C. Ayers and R.H. Forsythe (1967): Factors influencing bacterial cntamina tion of commercially produced liquid eggs. Poultry Sci. 46: 1204-1210.

Lachica, R.V.; Genigeorgis, C. and Hoeprich, P.D. (1971): Metachromatic agar diffusion methods for detecting staphylococcal nuclease activity. Appl. Microbiology, 21: 585-588.

Mclaury, D.W. and Moran, A.E. (1959): Ky. Agr. Exper. Sta. Bull. 665. (Cited after Ahmed, A.A. 1969. Studies on salmonella infection in eggs and poultry excretion in thesis presented for M.D., Faculty of Vet. Med., Cairo Univ.).

Moats, W.A. (1979): The effect of washing eggs under commercial conditions on bacterial loads on egg shells. Poultry Sci. 58: 1228-1233.

Moats, W.A. (1980): Classification of bacteria from commercial egg washer and washed and unwash-ed eggs. Appl. and Enviromental Microbiol. 40, 4: 710-714.

Rosser, F.T. (1942): Preservation of eggs. 11. Surface contamination on egg shell in relation to spoilage. Canad. J. Res. 20 D: 291-296. (Cited after Board et al. 1964).

Scott, W.J. et al. (1950-1951): Studies in the preservation of shell eggs. Aust. J. Appl. Sci. 1: 208, 2: 205. (Cited after Frazier, 1967).

Smith, T.D. and Conant, N.T. (1960): Zinsser Microbiology 12th Ed. Appleton- Century Croft, Inc. New York.

Solowy, M.; E.H. Spaulding and H.E. Goreslin (1946): An investigation ofa source and mode of entry of salmonella organisms in spray-dried whole egg powder. Food Res. 11: 380-390.

Speck, M.L. (ed) 1976. Compendium of methods for the microbiological examination of foods. Ameri-can Public Health Association, Washington, DC.

Zagaevsky, J.S. and P.O. Luticova (1944): Sanitary measures in the egg-breaking plant. U.S. Egg Poultry Mag. 50: 17-20, 43-46. (Cited after Moats, 1980).

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Table (1): Count of some microorganisms recovered from egg shells

Types	Positive samples No./25	%	Min./shell	Max./shell	Mean/shell
Total bact. count	25	100	6×10^5	81×10^7	37×10^6
Coliform count	16	64	*/ 100	24×10^3	3×10^3
Faecal coliform count	6	24	*/ 100	8×10^3	6×10^2
Enterococci count	16	64	*/ 100	19×10^3	16×10^2
S. aureus	20	80	*/ 100	*/ 100	*/ 100

* No colonies could be detected on the plates.

Table (2): Incidence and frequency distribution of different isolates recovered from egg shells

Isolates	No. of positive samples No./25	%	Isolates No.	%
Staph. aureus	20	80	20	17.09
Staph. epidermidis	10	40	10	8.55
Micrococcus	14	56	14	11.97
Strept. faecalis	15	60	15	12.82
Strept. faecium	5	20	5	4.30
Intermediate	3	12	3	2.56
E.coli	12	48	12	10.26
Enterobacter : aerogenes	1	4	1	0.85
- agglomerans	3	12	3	2.56
- cloacae	3	12	3	2.56
- hafinae	8	32	8	6.84
Citrobacter:				
- freundii	4	16	4	3.42
- diversus	1	4	1	0.85
Klebsiella ozaenae	8	32	8	6.84
Arizona hinshawii	1	4	1	0.85
Serratia :				
- liquefaciens	3	12	3	2.56
- rubidaea	2	8	1	1.71
Acinetobacter colcoaceticus	1	4	1	0.85
Shigella species	2	8	2	1.71
Pseudomonas aurogenosa	1	4	1	0.85
			117	100