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## **ISOLATION OF *ENTEROBACTER* SPECIES FROM HENS' EGGS SOLD IN ASSIUT AND QUENA CITIES, EGYPT WITH REFERENCE TO THEIR ANTIBIOTIC RESISTANCE**

(With 4 Tables)

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**عزل الانتيروباكتري من بيض الدجاج المباع في مدينتي أسيوط وقنا مع الاشارة  
الى مقاومتهم للمضادات الحيوية**

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أجريت هذه الدراسة على 300 بيضة من بيض الدجاج المعد للاستهلاك الأدمى ويشمل بيض دجاج المزارع وبيوت الفلاحين وذلك في مدينتي أسيوط وقنا ( 60 مجموعة). وقد تم فحص العينات لمعرفة مدى تواجد ميكروب الانتيروباكتري على قشر وفي محتوى البيض الداخلى. وأظهرت النتائج تواجد الانتيروباكتري في 33.33 و 53.33 % من قشر بيض المزارع ، أما في قشر بيض بيوت الفلاحين فكانت النسبة 46,66 و 20 % من مدينتي أسيوط وقنا على التوالي. في حين وجدت في 6.6 و 53.33 % في المحتوى الداخلى لبيض المزارع في أسيوط وقنا على التوالي. وفي المحتوى الداخلى لبيض بيوت الفلاحين بنسبة 46.66% في كلا المدينتين. وكانت نسبة عزل الانتيروباكتري كلكا هي الأعلى من بين عترات الانتيروباكتري. وقد تم دراسة مقاومة العترات المعزولة لثمانية من المضادات الحيوية، وقد أظهرت الدراسة مقاومة 25 عترة من الانتيروباكتري لأكثر من نوع من المضادات الحيوية.

### **SUMMARY**

The prevalence of *Enterobacter* species in 300 hens' eggs of poultry farms and farmers' houses in Assiut and Quena cities, Egypt was determined. The 300 eggs representing 15 groups of either poultry farm or farmers' houses eggs from each city. For each group of eggs, *Enterobacter* species was examined on egg shells and in contents. Regarding the shells of farm hens' eggs, the incidence of *Ent. spp.* was 33.33 and 53.33%, while that of farmers' houses hens' eggs was 46.66 and 20% from Assiut and Quena cities, respectively. Whereas, *Ent. spp.*

incidence in the content of farm hens' eggs was 6.6 and 53.33% in Assiut and Quena cities, respectively. While that of farmers' houses hens' eggs was 46.66 of either city. The most prevalent isolated species was *Ent. cloacae*. The resistance of isolated strains to eight antibiotics was determined using the disc diffusion method, 25 isolates exhibited resistance to more than one antibiotic.

**Key words:** *Enterobacter species, hens' eggs, Antibiotic resistance.*

## INTRODUCTION

Eggs are familiar, versatile, nutritious, economical, quick and easy to prepare food, also they provide a unique well balanced source of nutrients for all ages. Moreover, their high quality, low caloric value and ease of digestibility make eggs valuable in many therapeutic diets for adults (Burley and Vadehra, 1989; Bufano, 2000). However, the nutrients that make eggs a high-quality food for human are also a good growth medium for bacteria (Frazier and Westhoff, 1986). Eggs were considered a vehicle for transmission of certain pathogens to man if such eggs are consumed raw or semi-raw. Bacteria on egg shells have been implicated as a source of bacterial contamination of broken out eggs (Solowey *et al.*, 1946; Kraft *et al.*, 1967). Motile bacteria on shells may easily penetrate the shells to the interior (Board, 1968). The rate of penetration is influenced by humidity and storage temperature at which the eggs were produced and stored (Board and Fuller, 1994; Cox *et al.*, 2000).

*Enterobacter* species are found in natural environment (water, sewage, soil, vegetables). Some species are found in human and animal species (Nazarowec-White and Farber, 1997). They are opportunistic pathogens that rarely cause disease in healthy individuals. *Enterobacter* spp. particularly *Ent. cloacae* and *Ent. aerogenes*, are important nosocomial pathogens responsible for various infections, including bacteremia, lower respiratory tract infection, skin and soft-tissue infections, urinary tract infections, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis and ophthalmic infections. This bacterium's virulence similar to other members of the *Enterobacteriaceae* family seems largely to be due to an endotoxin that it produces (Fraser *et al.*, 2008).

Poultry remains a vehicle of important pathogens such as *Enterobacteriaceae*. Among the available methods for the control of

these pathogens, the most widely practiced is the use of various antimicrobials such as antibiotics in the poultry's diet. Nevertheless, it is well known that the extended and continuing use of a range of antimicrobials in animals' rations has been an important factor in promoting the emergence of resistant strains of gram positive and gram negative bacteria that could be passed to humans through the food chain (Threlfall *et al.*, 1993; Weinstein, 1993). The prevalence of resistance to antibiotics among bacteria isolated from eggs has been reported, emphasizing the potential to cause therapeutic problems in consumers (Papadopoulou *et al.*, 1997; Adesiyun *et al.*, 2006).

The present study was therefore conducted to determine the prevalence of *Enterobacter* species on the shells and in the egg contents of commercial hens' eggs as well as the antibiotic resistance of these bacteria.

## **MATERIALS and METHODS**

### **Collection of samples**

A total of 300 commercial hens' eggs (60 groups); were collected from different supermarkets, groceries and farmers' houses in Assiut and Quena cities, Egypt. Each 5 eggs constituted a group, so there are 15 groups representing poultry farms and house hold hens' in each city. Each 5 eggs (one group) were placed in a sterile plastic bag and dispatched to the laboratory without delay where they were prepared and examined for *Enterobacter* species.

### **Preparation of samples**

- **Egg shells:** Egg shells were tested by a surface rinse method as described by Moats (1979).
- **Egg contents:** Eggs were prepared for evacuation of their contents according to Speck (1976).

### **Cultural Techniques**

Isolation of *Enterobacter* species was adopted as recommended by Food and Drug Administration (2002); Enrichment of samples using Enterobacteriaceae enrichment broth, incubated at 37 °C for 24h. From each enrichment culture, a loopful was inoculated into violet red bile agar plates (VRBA) and incubated overnight at 36 °C. Then colonies were streaked onto Trypticase Soy Agar and incubated at 25 °C for 48-72h. Only yellow pigmented colonies were selected and confirmed as

*E. sakasakii*. The other *Enterobacter spp.* were differentiated by biochemical testing according to Farmer and Kelly (1992).

### Antibiotic Sensitivity test

The antibiotic sensitivity test and their interpretation were done using the disc diffusion method following the NCCLS standards (1997) for all isolated strains. The following antibiotics discs (Oxoid) were used to determine the pattern of resistance; Tetracycline 30 µg, streptomycin 10 µg, ampicillin 10 µg, amoxicillin with clavunalic acid 30 µg, gentamicin 10 µg, kanamycin 30 µg, chloramphenicol 30 µg and erythromycin 15 µg.

## RESULTS

**Table 1:** Prevalence of *Enterobacter* species recovered from egg shells of the examined egg groups' samples.

| <i>Enterobacter</i><br>Species | Assiut          |       |                            |       | Quena           |       |                            |       |
|--------------------------------|-----------------|-------|----------------------------|-------|-----------------|-------|----------------------------|-------|
|                                | Farm hens' eggs |       | Farmers' Houses hens' eggs |       | Farm hens' eggs |       | Farmers' Houses hens' eggs |       |
|                                | No./15          | %     | No./15                     | %     | No./15          | %     | No./15                     | %     |
| <i>E. cloacae</i>              | 3               | 20    | 3                          | 20    | 4               | 26.66 | 2                          | 13.33 |
| <i>E. agglomerans</i>          | 1               | 6.66  | 1                          | 6.66  | 2               | 13.33 | 1                          | 6.66  |
| <i>E. sakasakii</i>            | -               | -     | 1                          | 6.66  | 1               | 6.66  | -                          | -     |
| <i>E. gergoviae</i>            | 1               | 6.66  | 2                          | 13.33 | 1               | 6.66  | -                          | -     |
| Total                          | 5               | 33.33 | 7                          | 46.66 | 8               | 53.33 | 3                          | 20    |

**Table 2:** Prevalence of *Enterobacter* species recovered from egg contents of the examined egg groups' samples.

| <i>Enterobacter</i><br>Species | Assiut          |      |                            |       | Quena           |       |                            |       |
|--------------------------------|-----------------|------|----------------------------|-------|-----------------|-------|----------------------------|-------|
|                                | Farm hens' Eggs |      | Farmers' Houses hens' eggs |       | Farm hens' eggs |       | Farmers' Houses hens' eggs |       |
|                                | No./15          | %    | No./15                     | %     | No./15          | %     | No./15                     | %     |
| <i>E. cloacae</i>              | 1               | 6.66 | 3                          | 20    | 5               | 33.33 | 5                          | 33.33 |
| <i>E. agglomerans</i>          | -               | -    | 1                          | 6.66  | 1               | 6.66  | -                          | -     |
| <i>E. sakasakii</i>            | -               | -    | 1                          | 6.66  | 1               | 6.66  | 2                          | 13.33 |
| <i>E. gergoviae</i>            | -               | -    | 2                          | 13.33 | 1               | 6.66  | -                          | -     |
| Total                          | 1               | 6.6  | 7                          | 46.66 | 8               | 53.33 | 7                          | 46.6  |

**Table 3:** Frequency of resistance of *Enterobacter* species isolated from egg shells and egg contents to eight antibiotics.

| <i>Enterobacter</i><br>species | No. of<br>strains<br>tested* | No. (%) of isolates resistant to |          |           |          |         |         |         |         |
|--------------------------------|------------------------------|----------------------------------|----------|-----------|----------|---------|---------|---------|---------|
|                                |                              | TET**                            | S        | AMP       | AMC      | GEN     | K       | CHL     | E       |
| <i>E. cloacae</i>              | 26                           | 10 (38.5)                        | 6 (23.1) | 5 (19.2)  | 4 (15.4) | 3(11.5) | 1(3.8)  | 1 (3.8) | 1 (3.8) |
| <i>E. agglomerans</i>          | 7                            | 4 (57.1)                         | 3 (42.9) | 2 (28.6)  | 1(14.3)  | 1(14.3) | 2(28.6) | -       | -       |
| <i>E. gergoviae</i>            | 7                            | 2 (28.6)                         | 3 (42.9) | 3 (42.9)  | 1 (14.3) | -       | -       | -       | -       |
| <i>E. sakasakii</i>            | 6                            | 2 (33.3)                         | 2 (33.3) | 2 (33.3)  | -        | -       | -       | -       | -       |
| Total                          | 46                           | 18(39.1)                         | 14(30.4) | 12 (26.1) | 6 (13)   | 4(8.7)  | 3(6.5)  | 1 (2.2) | 1(2.2)  |

\* From egg shells and contents, Resistant to one or more antibiotic.

\*\* TET, tetracycline; S, streptomycin; AMP, ampicillin; AMC, amoxicillin and clavulanic acid; GEN, gentamycin; K, Kanamycin; CHL, chloramphenicol and E, erythromycin.

**Table 4:** Frequency of antibiotic resistance of *Enterobacter* species isolated from egg shells and egg contents.

| <i>Enterobacter</i> | No. of | No. (%) | Shells | Contents |
|---------------------|--------|---------|--------|----------|
|---------------------|--------|---------|--------|----------|

| species               | strains tested | of strains resistant* | Farm hens' eggs | Farmers' Houses hens' eggs | Farm hens' eggs | Farmers' Houses Hens' eggs |
|-----------------------|----------------|-----------------------|-----------------|----------------------------|-----------------|----------------------------|
|                       |                |                       | No. (%)*        | No. (%)*                   | No. (%)*        | No. (%)*                   |
| <i>E. cloacae</i>     | 26             | 15 (57.7)             | 7 (26.9)        | 1 (3.8)                    | 6 (23.1)        | 1 (3.8)                    |
| <i>E. agglomerans</i> | 7              | 4 (57.1)              | 3 (42.9)        | -                          | 1 (14.3)        | -                          |
| <i>E. gergoviae</i>   | 7              | 3 (42.9)              | 2 (28.6)        | -                          | 1 (14.3)        | -                          |
| <i>E. sakasakii</i>   | 6              | 3 (50)                | 1(16.7)         | 1 (16.7)                   | 1 (16.7)        | -                          |
| Total                 | 46             | 25 (54.3)             | 13 (28.3)       | 2 (4.3)                    | 9 (19.6)        | 1 (2.2)                    |

\* Resistant to one or more antibiotic.

## DISCUSSION

*Enterobacter* spp. are the sixth most common cause of nosocomial infection and antibiotic resistant strains are observed with increasing frequency (Peters *et al.*, 2000). *Enterobacter* species are not primary human pathogens, however *E. cloacae* have been implicated in a broad range of clinical syndromes (Kaminska *et al.*, 2002; Liu *et al.*, 2004).

The recorded results in Table 1 show that *Ent. spp.* were isolated as 33.33 and 53.33% from the shells of farm hens' eggs and from 46.66 and 20% of the shells of farmers' houses hens' eggs in Assiut and Quena cities, respectively. The findings illustrated in Table 2, revealed that *Ent. spp.* were isolated from the contents of eggs as 6.6 and 53.33% of farm hens' eggs, 46.66 and 46.66% of farmers' houses hens' eggs of Assiut and Quena, respectively. Adesiyun *et al.* (2006) isolated *Enterobacter spp* from 8.2% and 3.3% out of the shells and contents of eggs, respectively. This variation in recovery rate may be attributed to differences in environmental temperature, variation of bird husbandry practices or even variation in methods of isolation (WHO, 1988; ICMSF, 1996). The results agree with many published reports where *Enterobacter spp.* have been recovered from eggs (Papadopoulou *et al.*, 1997; Musgrove *et al.*, 2004; Brito *et al.*, 2006; Edema and Atayese, 2006; Musgrove and Jones, 2007).

The fact that *Enterobacter* species were recovered from eggs was not unexpected since it is known that freshly laid eggs become readily contaminated in their environments (Jones *et al.*, 1995; Indar *et al.*,

1998). Sources of this contamination are numerous as the fecal matter, the lining of the nest, wash water if the eggs are washed, handling and perhaps by the material in which eggs are packed (Board and Fuller, 1994; Cox *et al.*, 2000).

It has been documented that storage of eggs at the sale outlets, depending on storage conditions, particularly the temperature and duration, may affect the microbial load of both egg shells and contents but not the prevalence of bacteria (Jones *et al.*, 2004).

The most prevalent isolated species was *Ent. cloacae* which was isolated from 20% out of the shells of both farm hens' eggs and farmers' houses hens' eggs in Assiut City. While, in Quena samples; it was isolated in an incidence of 26.66, 13.33% from the shells of farm hens' eggs and farmers' houses hens' eggs, respectively. As regarding its prevalence in the contents of farm hens' eggs and farmers' houses hens' eggs, was 6.66, 20% in Assiut, while it was 33.33% of each in Quena city.

Freshly laid eggs are generally semi-sterile, however they may constitute, if contaminated, a public health hazard, and cause economic losses through their spoilage (Perales and Audicana, 1989; ACMSF, 1993).

*Enterobacter sakazakii* with other species were also isolated from eggs samples (Table 1). Musgrove *et al.* (2008) isolated *Enterobacter sakazakii* from eggshells collected from processing plants. However, this organism was never isolated from fully processed eggs. It is considered to be a foodborne pathogen, which used to be known as a "yellow pigmented *Enterobacter cloacae*" until 1980, when it was introduced as a new species (*Ent. sakazakii*). Recently, a taxonomic reclassification of this pathogen to consist of 5 species within a new genus "Cronobacter" was proposed (Baumgartner *et al.*, 2009).

Antibiotic Susceptibility testing results illustrated in Table 4 showed that *Enterobacter spp.* isolated from farm hens' eggs either from the shells or the contents were more resistant to the tested antibiotics than those isolated from farmers' houses hens' eggs, this could be due to the fact that antibiotics are widely used both as growth promoters and control of infections in poultry farms. In contrary to, the farmers' houses hens which are bred sporadically by people seldom receive antibiotics.

Multidrug resistance increased over time, especially in infections caused by *E cloacae* (Lockhart *et al.*, 2007). The number of the resistant strains for each tested antibiotic is shown in Table 3. Taking into consideration that antibiotics such as tetracycline, gentamycin and

streptomycin were used for the control of infections by the large breeders, and comparing the resistance of the isolated bacterial strains to these specific antibiotics, it is quite possible that resistant strains could be passed to human through the food chain. So, the results indicate that antibiotic-resistant strains might be transmitted to human by the consumption of eggs containing such multiresistant bacteria, and that the use of antibiotics common both in human and animal care should be avoided.

## REFERENCES

- ACMSF (*Advisory Committee on the Microbiological Specification of Food*) (1993): Report on Salmonella in eggs. Report by the Advisory Committee on the Microbiological Specification of Food. London. HMSO.
- Adesiyun, A.; Offiah, N.; Seepersadsingh, N.; Rodrigo, S.; Lashley, V. and Musai, L. (2006): Frequency and antimicrobial resistance of enteric bacteria with spoilage potential isolated from table eggs. *Food Research International*, 39: 212-219.
- Baumgartner, A.; Grand, M.; Liniger, M. and Iversen, C. (2009): Detection and frequency of Cronobacter spp. (*Enterobacter sakazakii*) in different categories of ready-to-eat foods other than infant formula. *Int. J. Food Microbiol.*, 136 (2): 189-192.
- Board, R.G. (1968): Microbiology of the egg (a review). In: T. C. Carter (Ed.), *Egg quality: a study of the hen's egg*. Oliver and Boyd, Edinburgh.
- Board, R.G. and Fuller, R. (1994): *Microbiology of the Avian Eggs*. 1<sup>st</sup> Ed. Chapman and Hall, pp. 94- 112, 128.
- Brito, J.R.; Gilbreth, S.E.; Musgrove, M.T.; Call, J.E. and Luchansky, J.E. (2006): Characterization of *Enterobacter spp.* Isolated from shell eggs using pulsed-field gel electrophoresis. (Abstract). International Association of Food Protection's Annual Meeting. P-238, pp. 126.
- Bufano, N.S. (2000): Keeping eggs safe from farm to table. *Food Technol.*, 54 (8): 192.
- Burley, R.W. and Vadehra, D.V. (1989): *The Avian Egg Chemistry and Biology*. 1<sup>st</sup> Ed., John Wiley, Sons, New York, Toronto.
- Cox, N.A.; Berrang, M.E. and Cason, J.A. (2000): Salmonella penetration of egg shells and proliferation in broiler hatching eggs. *A Review Poultry Sci.*, 79: 1571-1574.



- Edema, M.O. and Atayese, A.O. (2006): Bacteriological quality of cracked eggs sold for consumption in Abeokuta, Nigeria. *International J. Poultry sci.*, 5 (8): 772-775.
- Farmer, J.J. and Kelly, M.T. (1992): Enterobacteriaceae. P. 360-383. In: Barlows (Ed.) *Manual of Clinical Microbiology*. ASM, Washington, D.C.
- Food and Drug Administration (2002): Isolation and enumeration of *Enterobacter sakasaki* from dehydrated powdered infant formula. Center for Food Safety and Applied Nutrition. Available at <http://www.cfsan.eda.gov/~comm/mmesakaz.html>.
- Fraser, S.L.; Arnett, M. and Sinave, C.P. (2008): *Enterobacter Infections*. e-Medicine specialities- Infectious diseases.
- Frazier, W.C. and Westhoff, D.C. (1986): *Food Microbiology*. TMH Edt, N.Y., pp. 540.
- ICMSF (*International Committee on Microbiological Specifications of Food Pathogens*). (1996): *Microorganisms in Food. Microbiological Specifications of Food Pathogens*. Co.Publ. By James & James Ltd, London.
- Indar, L.; Baccus-Taylor, G. and Commissiong, E. (1998): Salmonellosis in Trinidad: evidence for transovarian transmission of *Salmonella* in farm eggs. *West Indian Medical J.*, 47: 50- 53.
- Jones, D.R.; Curtis, P.R. and Anderson, K.E. (2004): Microbial Contamination in inoculated shell eggs. II. Effects of layer strain and egg storage. *Poultry Science*, 83: 95- 100.
- Jones, F.T.; Rives, D.V. and Carey, J.B. (1995): *Salmonella* contamination in commercial eggs and an egg production facility. *Poultry Science*, 74: 753- 757.
- Kaminska, W.; Patzer, J. and Dzierzanowska, D. (2002): Urinary tract infections caused by endemic multiresistant *Enterobacter cloacae* in a dialysis and transplantation unit. *J. Hospital Infection*, 51: 215- 220.
- Kraft, A.A.; Torrey, G.S.; Ayres, J.C. and Forsythe, R.H. (1967): Factors influencing bacterial contamination of commercially produced liquid eggs. *Poultry Sci.*, 46: 1204-1210.
- Liu, C.P.; Wang, N.Y.; Lee, C.M.; Weng, L.C.; Tseng, H.K.; Liu, C.W.; Chiang, C.S. and Huang, F.Y. (2004): Nosocomial and community-acquired *Enterobacter cloacae* bloodstream infection: risk factors for and prevalence of SHV-12 in

- multiresistant isolates in a medical centre. *J. Hospital Infection*, 58: 63- 77.
- Lockhart, S.R.; Abramson, M.A. and Beekmann, S.E. (2007): Antimicrobial resistance among Gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. *J. Clinical Microbiol.*, 45(10): 3352-3359.
- Moats, W.A. (1979): The effect of washing eggs under commercial conditions on bacterial loads on egg shells. *Poultry Sci.*, 58: 1228-1233.
- Musgrove, M.T. and Jones, D.R. (2007): Enterobacteriaceae and related organisms recovered from retail shell eggs. *International Association for Food Protection Proceedings*.
- Musgrove, M.T.; Jones, D.R.; Northcutt, J.K.; Cox, N.A. and Harrison, M. (2004): Identification of Enterobacteriaceae from washed and unwashed commercial shell eggs. *J. Food Prot.*, 67: 2613-2616.
- Musgrove, M.T.; Northcutt, J.K.; Jones, D.R.; Cox, N.A. and Harrison, M.A. (2008): Enterobacteriaceae and related organisms isolated from shell eggs collected during commercial processing. *J. Poultry Sci.*, 87: 1211-1218.
- NCCLS (*National Committee for Clinical Laboratory Standards*) (1997): Approved standard M2-A6. Performance standards for antimicrobial disc susceptibility tests. 6<sup>th</sup> ed. NCCLS, Wayne, PA.
- Nazarowec-White, M. and Farber, J.M. (1997): *Enterobacter sakasakii*: a review. *International J. Food Microbiol.*, 34, (2): 103-113.
- Papadopoulou, C.; Dimitriou, D.; Levidiotou, S.; Gessouli, H.; Panagiou, A.; Golegou, S. and Antoniadou, G. (1997): Bacterial strains isolated from eggs and their resistance to currently used antibiotics: is there a health hazard for consumers?. *Comparative Immunology Microbiol. and Inf. Dis.*, 20: 35-40.
- Perales, I. and Audicana, A. (1989): The role of hens eggs in outbreaks of salmonellosis in North Spain. *Int. J. Food Microbiol.*, 8: 175-180.
- Peters, S.M.; Bryan, J. and Cole, M.F. (2000): Enterobacterial repetitive intergenic consensus polymerase chain reaction typing of isolates of *Enterobacter cloacae* from an outbreak of infection

in a neonatal intensive care unit. American J. infection control, 28: 123-129.

*Solowey, M.; Spaulding, E.H. and Goresline, H.E. (1946):* An investigation of a source and mode of entry of Salmonella organisms in spray-dried whole-egg powder. Food Research, 11: 380-390.

*Speck, M.L. (Ed.) (1976):* Compendium of Methods for Microbiological Examination of Food. American Public Health Association, Washington, D.C.

*Threlfall, E.; Rowe, B. and Ward, L. (1993):* A comparison of multiple drug resistance in Salmonellas from humans and food animals in England and Wales, 1981-1990. Epidemiology and Infection, 111: 189- 197.

*Weinstein, P. (1993):* Multiple resistant *Staphylococcus aureus* (MRSA) infections in South Australia, 1983- 1990. Comm.Disease Intel. 17, 25: 590- 593.

*WHO (World Health Organisation) (1988):* Report of the WHO Informal Working Group on Foodborne Listeriosis, Geneva 15-19 February (WHO/EHE/FOS/88.5).