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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 الانتيروباكتر من بيض الدجاج المباع فى مدينتى أسيوط وقنا مع الاشارة الى مقاومتهم للمضادات الحيوية

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أجريت هذه الدراسة على ٣٠٠ بيضة من بيض الدجاج المعد للاستهلاك الآدمي ويشمل بيض دجاج المزارع وبيوت الفلاحين وذلك فى مدينتى أسيوط وقنا (٦٠ مجموعة). وقد تم فحص العينات لمعرفة مدى تواجد ميكروب الانتيروباكتر على قشر وفى محتوى البيض الداخلى. وأظهرت النتائج تواجد الانتيروباكتر في ٣٣,٣٣ و ٥٣,٣٣ % من قشر بيض المزارع ، أما فى قشر بيض بيوت الفلاحين فكانت النسبة ٤٦،٦٦ و ٢٠ % من مدينتى أسيوط وقنا على التوالي. فى حين وجدت فى ٦,٦ و ٥٣,٣٣ % فى المحتوى الداخلى لبيض المزارع في أسيوط وقنا على التوالي. وفى المحتوى الداخلى لبيض بيوت الفلاحين بنسبة ٤٦,٦٦% فى كلا المدينتين. وكانت نسبة عزل الانتيروباكتر كلواكا هى الأعلى من بين عترات الانتيروباكتر. وقد تم دراسة مقاومة العترات المعزولة لثمانية من المضادات الحيوية، وقد أظهرت الدراسة مقاومة ٢٥ عترة من الانتيروباكتر 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.

Enterobacter 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	%
E. cloacae	3	20	3	20	4	26.66	2	13.33
E. agglomerans	1	6.66	1	6.66	2	13.33	1	6.66
E. sakasakii	-	-	1	6.66	1	6.66	-	-
E. gergoviae	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.

Enterobacter 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	%
E. cloacae	1	6.66	3	20	5	33.33	5	33.33
E. agglomerans	-	-	1	6.66	1	6.66	-	-
E. sakasakii	-	-	1	6.66	1	6.66	2	13.33
E. gergoviae	-	-	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.

Enterobacter species	No. of strains tested*	No. (%) of isolates resistant to							
		TET**	S	AMP	AMC	GEN	K	CHL	E
E. cloacae	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)
E. agglomerans	7	4 (57.1)	3 (42.9)	2 (28.6)	1(14.3)	1(14.3)	2(28.6)	-	-
E. gergoviae	7	2 (28.6)	3 (42.9)	3 (42.9)	1 (14.3)	-	-	-	-
E. sakasakii	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 clavunalic 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.

Enterobacter species	No. of strains tested	No. (%) of strains resistant*	Shells		Contents	
			Farm hens' eggs	Farmers' Houses hens' eggs	Farm hens' eggs	Farmers' Houses Hens' eggs
			No. (%)*	No. (%)*	No. (%)*	No. (%)*
E. cloacae	26	15 (57.7)	7 (26.9)	1 (3.8)	6 (23.1)	1 (3.8)
E. agglomerans	7	4 (57.1)	3 (42.9)	-	1 (14.3)	-
E. gergoviae	7	3 (42.9)	2 (28.6)	-	1 (14.3)	-
E. sakasakii	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.

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