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Frequency and antimicrobial resistance of enteric bacteria with spoilage potential isolated from table eggs

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Abstract

The prevalence of potential spoilage microorganisms on the shells and in the egg contents of table eggs sold in Trinidad was determined. Table eggs samples were obtained from 23 poultry layer farms, 14 shopping malls and 102 supermarkets across the country. Each farm was visited twice approximately one month apart and 25 pooled eggs constituted a composite sample. Shopping malls were each visited twice usually one month apart while supermarkets were each visited once over a 4-month period. For both mall and supermarkets, six pooled eggs constituted a composite sample. Swabs of egg shells and pooled yolk and albumen (egg content) were tested for selected bacteria using standard methods. The resistance of bacteria to seven antimicrobial agents was detected using the disc diffusion method. Of a total of 184 composite eggs (shells, yolk/albumen or both) sampled, 71 (38.6%) samples were positive for enteric microbes, other than *E. coli*, *Salmonella*, *Campylobacter* spp. and *Listeria* spp. *Enterobacter* spp. and *Klebsiella* spp. were isolated from 15 (8.2%) and 14 (7.6%), respectively, of pooled egg shells alone and from 6 (3.3%) and 3 (1.6%), respectively, of egg content samples alone. Prevalence of enteric bacteria in egg contents was generally higher than found on egg shells with faeces/blood or cracks compared with those without, but the differences were not significant ($P > 0.05$; X^2). The microbial load of egg content was not significantly affected by type of housing of laying birds, source of feeds, use of medicated feeds and temperatures at which eggs were kept at sale outlets. Of a total of 131 bacterial isolates tested, 125 (95.4%) exhibited resistance to one or more antimicrobial agents and resistance was high to streptomycin (90.1%), tetracycline (51.9%) and kanamycin (30.5%). Failure to properly handle or heat table eggs sold in Trinidad poses a potential health hazard to consumers because of their poor microbial quality and high frequency of resistance to antimicrobial agent.

Keywords: Selected enteric bacteria; Antimicrobial resistance; Table eggs; Trinidad

1. Introduction

Table eggs are consumed worldwide in various dishes and are considered very nutritious and a cheap source of protein (Blumenthal, 1990; MAFF, 2000; Papadopoulou et al., 1997). The well known enteric pathogens particularly *Salmonella*, *Escherichia coli*, *Campylobacter* spp. and *Listeria* spp. have been isolated from table eggs and their contents (Adesiyun et al., 2005b; Farber, Daley, & Coates, 1992; Hope, Baker, & Edel, 2002; Leasor & Foegeding, 1989; Shane, Gifford, & Yogasundram, 1986) and have been responsible for egg-borne epidemics globally (CDC, 1990; Danielsson, Mollby, & Brag, 1979; Mazurek, Holbert, & Parrish, 2005; Rocourt, BenEmbarek, & Toyofuku, 2003; Todd, 1996). Other members of the family Enterobacteriaceae such as

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Pseudomonas spp., *Citrobacter* spp., *Alcaligenes* spp. and *Klebsiella* spp. have all been isolated from whole or cracked eggs with a potential to cause spoilage and enter the food chain through table eggs causing infection in consumers (Ibeh & Izuagbe, 1986; Jones, Musgrove, & Northcutt, 2004a; Musgrove, Jones, & Northcutt, 2004; Papadopoulou et al., 1997; Wadstrom & Ljungh, 1991). The prevalence of resistance to antimicrobial agents amongst numerous bacteria isolated from eggs or their washings has been reported by others, emphasizing the potential to cause therapeutic problems in consumers (Papadopoulou et al., 1997).

In Trinidad and Tobago, table eggs are used in several dishes and drinks but in 1992, an epidemic in mental patients which resulted in 10 mortalities was confirmed to be egg nog-borne salmonellosis caused concern in the population (Hyatali et al., 1992). Indar et al. (1998) demonstrated trans-ovarial transmission of *Salmonella* in table eggs sampled from poultry farms in Trinidad. Adesiyun et al. (2005a) reported the prevalence of antimicrobial residues in table eggs sold in the country, emphasizing the health risk they pose to consumers and in another report assessed the potential of table eggs in Trinidad serving as sources of *Salmonella*, *E. coli*, *Campylobacter* spp. and *Listeria* spp. for consumers (Adesiyun et al., 2005b). To date, information is unavailable on the presence of potential spoilage or infectious microorganisms on or in table eggs nor is the effect of storage conditions on the microbial load of eggs known. The frequency of resistance of these bacteria to commonly used antimicrobial agents in the population has also not been reported.

The study was therefore conducted to determine the prevalence of selected enteric microbes on the shells and in the egg contents of table eggs sold at outlets across Trinidad as well as the antimicrobial resistance of these bacteria. The study also investigated the possible effects of management practices at the poultry layer farm level, as well as storage conditions at sale outlets, on the prevalence of these enteric pathogens in the eggs.

2. Materials and methods

Samples of table eggs were obtained from layer farms and sale outlets (shopping mall and supermarkets). Farms rear layers which supply eggs to a majority of sale outlets in Trinidad. Shopping malls are medium to large shopping complexes with several shops including a supermarket. Table eggs are sold refrigerated in these mall supermarkets. Supermarkets, which are in the large, medium and small categories, are outlets across the country selling essentially food items. They represent sale outlets which include street vendors, kiosks, open trucks/vehicles along highways and stores in neighborhoods selling table eggs mostly at ambient or room temperature with a few at refrigeration temperature. A questionnaire was administered to obtain information on location, farm size, number of layers and type of housing for laying birds, problem of rodents and free-flying birds, type (medicated or non-medicated) and source of feeds used and sale outlets for eggs. All farms, all shopping malls and selected supermarkets representative of various regions of the island were sampled. At each farm, a total of 25 eggs were randomly collected on two occasions, approximately one month apart. At the shopping malls and supermarkets, six eggs were randomly sampled from different producers available for sale during the visits. Shopping malls were sampled twice, approximately one month apart, and the supermarkets once. For farms, sterile crates were provided for the collection of 25 eggs while at the shopping malls and supermarkets, the eggs were purchased in the crates used for normal sales at each outlet. The temperatures (refrigeration, ambient and air conditioned room temperature) at which eggs were kept at sale outlets were noted during each visit. Samples were transported to the laboratory within 2 h of collection. All eggs were processed in the laboratory within 24 h of collection and when impracticable, they were stored overnight at the temperatures at which they were sold.

Overall, from 23 farms, a total of 46 composite eggs samples were processed while from 14 shopping malls, a total of 31 composite eggs were processed. From the 102 supermarket outlets studied, a total of 107 composite eggs were processed. The eggs were processed as earlier described (Adesiyun et al., 2005b). Briefly, wearing sterile gloves to handle egg samples from each source, for egg shells of either a pool of 6 (malls and supermarkets) or 25 (farms), one sterile swab moistened in saline was applied to the surface of each egg. The 6 or 25 swab samples were submerged in 6 and 20 ml of saline, respectively, in universal bottles, the contents mixed with VWR Mini- Vortexers (Henry Treemer, USA) and used to inoculate the appropriate enrichment broths or media. For yolk and albumen samples, a pool of 6 or 25 eggs were submerged in 75% ethanol for 5 min after which the pointed end of each egg was disinfected by flaming for 5–10 s with a Bunsen burner. A small hole was then made on the shell with a sterile scalpel blade and the egg content (yolk and albumen) aseptically emptied into a Stomacher bag. The content was blended for 30 s at normal speed in a Stomacher 400 (Seward, London, United Kingdom). The resulting mixture was used to inoculate appropriate enrichment broths or media.

Swabs of egg shells (6 eggs from malls and supermarkets and 25 egg from farms) and swabs of pooled egg contents were used to inoculate MacConkey agar (Difco Ltd., Michigan, USA). The plates were incubated aerobically overnight at 37 °C. Both lactose-fermenting and