Prevalence of Multiple Drug-Resistant Salmonella and E. Coli in Table Eggs in North India

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Abstract
Salmonellosis and colibacillosis in poultry are two major zoonotic bacterial diseases often transmitted through eggs to the offspring. To detect prevalence of Salmonella and Escherichia coli bacteria in table purpose poultry eggs 85 eggs were sampled from Bareilly and Dehradun retail markets and an organized farm in Izatnagar. Salmonella could be isolated from four eggs and E. coli from 24 eggs. Salmonella isolates belonged to three serovars (S. Virchow, S. Winneba and S. Champaign) and were isolated from egg yolk only. Escherichia coli could be isolated from egg shells (10), egg yolk (14) and egg albumen (2). From an egg sampled from Bareilly market, both Salmonella (S. Winneba) and E. coli were isolated from egg yolk. Each of the two eggs sampled from Bareilly were positive for E. coli on shells and in albumen, the strains from shell had different drug sensitivity patterns than those from albumen of the same egg. Many of the E. coli (30.8%) had multiple-drug-resistance (MDR). Most of the E. coli and Salmonella isolates were resistant to ampicillin and tetracycline but none to enrofloxacin. The study concludes that MDR Salmonella and E. coli may be transferred to egg consumers. Besides frequent isolation of Salmonella and E. coli from eggs (Adesiyun et al., 2005; Poulter, 2006; Suresh et al., 2006), several egg-borne outbreaks of salmonellosis have been reported in egg consumers (Doorduyn et al., 2008; O’Flanagan et al., 2008). However, only few studies reported isolation of Salmonella and E. coli from eggs in India, this study was undertaken to know the occurrence of multi-drug-resistant (MDR) Salmonella and E. coli in fresh table eggs from different commercial outlets in Bareilly, Izatnagar and Dehradun in Northern India.

1. Introduction
In poultry many zoonotic and avian pathogens can be transmitted vertically through eggs but Escherichia coli and Salmonella enterica ssp. enterica serovars are the commonest ones (Wissman, 1996; Chen et al., 2009). Both of the pathogens are often associated with death-in-shell of chick-embryos and early age mortality of chicks. Escherichia coli is the primary cause of vertically transmitted mushy chick diseases or omphalitis or yolk sac infection and mortality may vary from 5-100% in different batches of hatches (Lister and Barrow, 2008). Although Salmonella and E. coli often enter eggs vertically from layers, horizontal transmission is not uncommon and many Salmonella serovars having zoonotic potential have been shown to penetrate intact poultry eggs during incubation and storage, even at cold storage temperature (Saeed and Koons, 1993). Multiplication of E. coli and Salmonella in infected eggs is reported at wide range of storage temperature and the pathogens can survive for several months in eggs (Hu et al., 2001). In light of ability of Salmonella and E. coli to penetrate intact eggs and vertical transmission from carrier hens, it is probable that Salmonella and E. coli may be transferred to egg consumers. Besides frequent isolation of Salmonella and E. coli from eggs (Adesiyun et al., 2005; Poulter, 2006; Suresh et al., 2006), several egg-borne outbreaks of salmonellosis have been reported in egg consumers (Doorduyn et al., 2008; O’Flanagan et al., 2008). However, only few studies reported isolation of Salmonella and E. coli from eggs in India, this study was undertaken to know the occurrence of multi-drug-resistant (MDR) Salmonella and E. coli in fresh table eggs from different commercial outlets in Bareilly, Izatnagar and Dehradun in Northern India.

2. Materials and Methods
Two eggs each were purchased from each of the 22 and 10 retail egg vendor from Bareilly and Dehradun while 3 eggs each were collected from retail outlet of an organized poultry farm at Izatnagar at seven occasions on monthly interval. Eggs were directly taken from vendors into sterile poly bags and brought to laboratory under sterile conditions. In laboratory, taking all aseptic precautions, egg shell, egg yolk and egg albumen from each egg were separated and processed for isolation of E. coli and Salmonella enterica ssp. en-
terica serovars as per standard protocol (ICMSF, 1978; 2002) using enrichment in tetrathionate broth (Hi-Media Mumbai) and EC broth (Hi-Media) for E. coli and brilliant green agar (BGA, Hi-Media) for Salmonella. Three to five suspected colonies were picked on to nutrient agar slants and characterized through growth and biochemical characteristics, and Salmonella isolates were serotyped using group and factor specific O and H antisera (Edwards and Ewing, 1972; Popoff and Leminor, 2002). Antibiotic sensitivity of all the isolates of E. coli and Salmonella was determined against ampicillin (10 g), chloramphenicol (30 g), co-trimoxazole (25 g), enerofoxacin (5 g), gentamycin (10 g), nalidixic acid (30 g), streptomycin (10 g) and tetracycline (30 g) discs (Hi-Media) through disc diffusion method (CLSI, 2006). Isolates resistant to three or more antimicrobials were classified as multi-drug-resistant (MDR).

3. Results
From 85 eggs sampled from different retail outlets (Table 1) Salmonella could be isolated from four and E. coli from 24 eggs from different parts of eggs. All four isolates of Salmonella enterica ssp. enterica serovars belonging to three serovars (S. Virchow, S. Winneba and S. Champaign) were isolated from egg yolks only. Escherichia coli could be isolated from egg shells (10), egg yolk (14) and egg albumen (2) of several eggs. From one egg from Bareilly both Salmonella (S. Winneba) and E. coli were isolated from egg yolk. From two eggs samples from Bareilly, E. coli isolates with different drug sensitive pattern were detected from egg albumen and egg shells both.

Most of the E. coli strains were resistant to one or more antimicrobials and 30.8% were classified as multiple-drug-resistant (MDR) type showing resistance to three or more drugs (Table 2). Of the 26 E. coli isolates, 16 were resistant to only one drug while two each were resistant to 2, 3, 4, 6 and 7 drugs. Most of the E. coli isolates were resistant to ampicillin (69.2%) but none to enerofoxacin, for other antimicrobials 15-4 to 46.2% isolates were resistant (Table 2). All the four Salmonella isolates were resistant to ampicillin and tetracycline but none was resistant to chloramphenicol and enerofoxacin, two isolates were resistant to three or more antimicrobials to be classified as MDR type (Table 2).

4. Discussion
In the study, Salmonella could be isolated from about 5% eggs, which is much lower than in eggs from Southern India (7.7%), reported earlier (Suresh et al., 2006) but simultaneously much more than isolated from eggs in developed countries (Poulter, 2006). Lower rate of isolation from North Indian eggs might be due to less humid and hot climate than in Southern India, or might be difference in hygienic standards, yet to be explored and reasoned. Isolation of zoonotic pathogens from table eggs and transfer of diseases through eggs either contaminated vertically or horizontally, is not uncommon and several outbreaks of salmonellosis through eggs with varying morbidity and mortality are on record (Palumbo et al., 1996; Doorduyn et al., 2008; O’Flanagan et al., 2008; Wissman, 2006). Although S. Enteritidis and S. Typhimurium are the major Salmonella enterica ssp. enterica serovars isolated and transferred through eggs all over the world including southern India (Adesiyun et al., 2005; Poulter, 2006; Suresh et al., 2006), in the present study none of the four isolates of Salmonella belonged to those common serovars of Salmonella. It might be due to low prevalence
of S. Enteritidis and S. Typhimurium in poultry birds in Northern India (Singh, 2005). However, S. Winneba (1) and S. Champaign (2), isolated for the first time from poultry birds or poultry products in India indicated that Salmonella uncommon serovars in eggs might also be important. Multiple drug resistance in two of the four isolates (one each of S. Champaign and S. Virchow serovars) is in concurrence to similar antimicrobial drug resistance patterns observed in most of the Salmonella serovars in different parts of World including India from environment, birds as well as from other animals and human sources (EFSA, 2006; Suresh et al., 2006; Singh et al., 2006a, b, 2007; Singh et al., 2009:). Resistance of Salmonella isolates particularly against ampicillin and tetracycline observed in the study is also in concurrence of earlier reports (EFSA, 2006; Suresh et al., 2006). It might be due to the fact that ampicillin and tetracycline are the two most commonly used antibacterial drugs prescribed for animals and poultry birds in India due to their low cost and easy availability (Singh et al., 2008). Strains of E. coli were isolated from 24 of the 85 eggs examined (28% eggs); maximum from egg yolk (16%) followed by egg shells (12%) and egg albumen (2%). There are only few reports on prevalence of E. coli in eggs in India and abroad to compare the observations but it is known since long that E. coli is the most common vertically transmitted infection from layers to chicks (Lister and Barrow, 2008). Multiple drug resistance in about 31% E. coli isolates from eggs, towards 3-8 drugs, is alarming as these bacteria can survive in environment for long and can transfer their MDR trait to other E. coli and other potential human and animal pathogens (WHO, 1997; Fridkin et al., 1999). Observation of maximum prevalence of Salmonella and E. coli in eggs of an organized farm at Izatnagar and least prevalence in retail eggs from Dehradun might be associated with difference in environmental and managerial factors reported earlier (Becirevic et al., 1988) to be associated with invasion of Salmonella and E. coli in eggs, however, on the basis of this pilot study on 85 egg samples exact reasons can not be concluded.

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References


