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Prevalence and phylogenetic analysis of *E. coli* from retail chicken meat shops in Bengaluru, Karnataka, India

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Abstract
The present study was undertaken to study the prevalence, distribution of *E. coli* in 100 chicken retail outlets in Bengaluru and to phylogenetically group these isolates. A total of 400 samples (100 each of chicken meat, cloacal swabs, water sample and cutting board swabs) were tested based on isolation and the overall prevalence of *E. coli* in the present study was 57.25 percent (229/400). The distribution found was 62.00 percent in Chicken meat, 88.00 percent in cloacal swab, 29.00 percent in water samples and 50.00 percent in cutting board swabs. A highly significant ($P \leq 0.01$) difference in the prevalence was observed among the different samples obtained from the retail outlets. Phylogenetic analysis of 229 *E. coli* isolates from the retail outlets revealed that 32 belonged to group A (13.97%), 31 belonged to group B1 (13.53%), 136 belonged to group B2 (59.39%), and 30 belonged to group D (13.10%). The results clearly indicated that *E. coli* isolated in the present study were virulent and extra-intestinal strains, whereas only 13.97 percent could be categorized as commensal.

Keywords: *E. coli,* chicken meat, retail outlets, phylogenetic analysis

Introduction

Poultry meat is relatively cheap compared to other meat types, it is easily accessible, has dietary characteristics demanded by consumers (low fat content) and lacks religious obstacles to consumption, all of which contribute to its global acceptance [1]. In the recent times poultry industry is growing rapidly in India due to high rate of urbanization with large scale operations making it hygienic and economic. However in semi-urban and rural areas of India, even today the poultry supply chain is operated at small scale retail outlets or wet markets with increased risk of microbial hazards. A typical retail chicken meat outlet operations comprises of maintenance of live birds, slaughter, dressing and cutting all in a single room by the same person (s) without any separation between these sections. However, unorganized and unauthorized slaughtering, dressing and retailing leads to supply of unhygienic and poor quality fresh meat and chicken which definitely hold public health risks [2].

Microbial food safety and food-borne infections are important public health concern worldwide [3, 4]. *E. coli* has been suggested as an indicator microorganism to monitor quality of water and foods because it reliably reflects faecal contamination and indicates a possible contamination of enteric pathogens [5]. However, *E. coli* are a commensal organism in the intestinal tract of the birds and hence isolation does not warrant the organism to be pathogenic. In order to establish the pathogenecity of the isolates phylogenetic analysis has shown that *E. coli* strains fall into four main groups (A, B1, B2, and D). It has been found that pathogenic *E. coli* strains causing extraintestinal infections mainly belong to group B2 and a lesser extent to group D whereas commensal strains belong to group A and B1 [6]. Hence the present study was designed to isolate *E. coli* from various samples of retail chicken shops and to characterize the pathogenecity of the isolates based on phylogenetic analysis.

Materials and Methods
Study area and period
Samples were drawn from 100 retail chicken outlets located using proportionate random sampling method from 8 zones of Bengaluru (Karnataka state) during December 2017 to April 2018. Sampling was done using proportionate random sampling based on number of licensed

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chicken shops distributed in each zones according to data provided by BBMP (Bruhath Bengaluru Mahanagara Palike).

Isolation and Identification of *E. coli*
A total of 400 samples were collected from 100 selected chicken retail outlets and analyzed in the laboratory. From each retail outlet four (4) different samples *viz.,* Meat sample, Water used for processing, Swab from Knife/ cutting board used and Cloacal swab from live bird before slaughter in case of outlets which slaughtered live birds. Isolation of *E. coli* was done as per the method described in BAM [7]. After pre-enrichment in BHI Broth, loop-full each of enriched cultures were transferred by streaking separately onto Eosin Methylene Blue (EMB) Agar media (Himedia Laboratories, Mumbai). The plates were then incubated at 37±1 °C for 24 ± 3 hours. Typical characteristics colony of *E. coli* on EMB agar as greenish metallic sheen was isolated. Identification of *E. coli* was done by biochemical test viz., IMViC, catalase, oxidase, nitrate reduction, sugar fermentation and motility tests [8].

Phylogenetic Analysis
A rapid technique for determining the phylogenetic groups of *E. coli* strains based on PCR detection of the *chuA* and *yjaA* genes and DNA fragment TSPE4. C2 as described by Clermont *et al.* [9]. As a first step, PCR was performed with a standard protocol. Each reaction was carried out by using a 25-µl mixture containing 12.5 µl of 2× Master mix, 20 pmol of each primer, $2\,\mu$L of DNA (approximately 100 ng) and Nuclease free water was added to make a final volume of 25µl. The PCR was performed with a thermal cycler with under the following conditions: denaturation for 5 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at 5 5°C, and 30 s at 72 °C; and a final extension step of 7 min at 72 °C. Primers used in the present study are presented in Table 1.

Results and Discussion
The overall prevalence of *E. coli* in the present study was 57.25 percent (229/400). The distribution found was 62.00 percent in Chicken meat, 88.00 percent in cloacal swab, 29.00 percent in water samples and 50.00 percent in cutting board swabs (Table 2). Chi-square test revealed a highly significant (P 0.01) difference of prevalence among the different samples obtained from the retail outlets. Among the various samples, the highest prevalence was recorded in case of the cloacal samples (88%) followed by meat (62%), cutting board (60%) and water (29%). The results of the present study were in accordance with Sharma and Singh [10] and Bhoomika *et al.* [11] who reported an *E. coli* prevalence of 61.76 and 66.32 percent in retail poultry meat in Himachal Pradesh and tribal districts of Chhattisgarh, India, respectively. Similar prevalence of *E. coli* in poultry meat have been documented by Chavhan *et al.* [12] in Nagpur (71.1%), Noori and Alwan [13] in Baghdad (52.5%) and Cohen *et al.* [14] in Morocco (48.4%). In the present study it was evident that the prevalence of *E. coli* in the cloacal sample set was higher followed by meat, cutting board and the lowest value was observed in water samples from the retail outlets. *E. coli* being a natural inhabitant of the intestinal tract of birds as well as the environment, the high presence of *E. coli* clearly indicated lack of cleanliness and faecal contamination of meat during processing and handling [15]. The higher prevalence in the cutting board sample set in the present study could be attributed to the fact that majority of the retail outlets in Bengaluru utilize wooden boards for cutting of chicken meat and these wooden blocks absorb moisture from meat and serve as a good media for the growth of microflora. In addition, cleaning of these wooden logs was generally accomplished by scrapping the cutting surface, which could remove only the visible meat pieces and the invisible microbes might be still present resulting in cross-contamination of meat.

The results of phylogenetic analysis of *E. coli* isolated from different samples obtained from retail outlets in Bengaluru are depicted in Table 3. All the 229 *E. coli* isolates in the present study were analyzed and their phylogenetic groups were as follows: 32 belonged to group A (13.97%), 31 belonged to group B1 (13.53%), 136 belonged to group B2 (59.39%), and 30 belonged to group D (13.10%). Determination of *E. coli* phylogenetic type is being of great epidemiological importance because there is a relation between the genetic background and the type of the extended spectrum ß-lactamase [16]. Phylogenetic analyses have shown that *E. coli* strains fall into four main phylogenetic groups (A, B1, B2, and D) and that virulent extra-intestinal strains belong mainly to group B2 and, to a lesser extent, to group D whereas most commensal strains belong to group A [9]. The results clearly indicated that the majority *E. coli* isolated were virulent and extra-intestinal strains, whereas only 13.97 percent could be categorized as commensal.

Table 1: Primer sequences used for phylogenetic analysis

Target gene	Sequence	PCR product (bp)
chuA	F: ATG GTA CCG GAC GAA CCA AC R: TGC CGC CAG TAC CAA AGA CA	288
yjaA	F: CAA ACG TGA AGT GTC AGG AG R: AAT GCG TTC CTC AAC CTG TG	211
TspE4C2	F: CAC TAT TCG TAA GGT CAT CC R: AGT TTA TCG CTG CGG GTC GC	152

Table 2: Prevalence of *E. coli* in different samples from retail outlets in Bengaluru

Sample	Tested	No. of samples positive	Prevalence of *E. coli* (%)
Chicken Meat	100	62	**62.0**
Cloacal swab	100	88	**88.0**
Water	100	29	**29.0**
Cutting board	100	50	**50.0**

Table 3: Phylogenetic grouping of *E. coli* isolated from different samples from Chicken retail outlets in Bengaluru

Phylogenetic Group	Meat	Water	Board	Cloaca	Total	% Occurrence
A	7	5	6	14	32	13.97
B 1	9	6	6	10	31	13.53
B 2	42	11	28	55	136	59.39
D	4	7	10	9	30	13.10
Total	62	29	50	88	229	

Conclusion

The higher prevalence of *E. coli* in chicken meat, water, cutting board and cloacal swab of birds clearly indicated low level of hygiene employed in these outlets, resulting in serious public health risk to the consumers. In addition, majority *E. coli* isolated in the present study were virulent and extra-intestinal strains and hence could cause serious public health risk to the consumers.

Acknowledgement

References

1. Magdelaine P, Spiess MP, Valceschini E. Poultry meat consumption trends in Europe. World's Poultry Science Journal. 2008; 64:53-64.
2. Allen V, Tinker D, Wathes C, Hinton M. Dispersal of micro-organisms in commercial defeathering systems. British Poultry Science. 2003; 44:53-59
3. Zhao C, Geb DE, Villena J, Sudler R, Yeh E, Zhao S *et al*. Prevalence of *Campylobacter* spp., *Escherichia coli*, and Salmonella serovars in retail chicken, turkey, pork, and beef from the greater Washington, D.C., area. Appl. Environ. Microbiol. 2001; 67:5431-5436
4. Kiranmayi B, Krishnaiah N, Subhashini N, Amaravathi P, Mani M, Ramya P. PCR analysis of Mutton and chicken samples for The Presence of Shiga Toxigenic *E. coli.* Ind. Med. Pub. J. 2011; 2(4):52-68.
5. Purohit HJ, Kapley A. PCR as an emerging option in the microbial quality control of drinking water. Trends Biotechnol. 2002; 20:325-329.
6. Picard B, Garcia JS, Gouriou S, Duriez P, Brahimi N, Bingen E, *et al*. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect Immun. 1999; 67:546-53.
7. BAM, Bacteriological Analytical Manual, 8 edition publication by FDA, U.S, 1998.
8. Cowan ST, Steele KJ. Characters of Gram positive bacteria. In Cowan and Steel's manual for Identification of Medical bacteria 3 edn. Cambridge Univ. Press. 1993.
9. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic group. Appl. Environ Microbiol. 2000; 66:4555-4558.
10. Sharma M, Singh DP. Recovery of Bacterial Contaminants from Various Foods in the State of Himachal Pradesh, India FAVA -OIE Joint Symposium on Emerging Diseases, 2008.
11. Bhoomika, Shakya S, Patyal A, Gade NE. Occurrence and characteristics of extended-spectrum β-lactamases producing Escherichia coli in foods of animal origin and human clinical samples in Chhattisgarh, India. Veterinary World. 2016; 9(9):996-1000.
12. Chavhan SK, Kalorey DR, NAGDIVE AA. Pathogenic attribute of *Escherichia coli* isolated from commercial broilers. Ind. vet. J. 2012; 89(1):39-40.
13. Noori TE, Alwan MJ. Isolation and Identification of Zoonotic Bacteria from Poultry Meat. Int. J Adv. Res. Biol. Sci. 2016; 3(8):57-66.
14. Cohen N, Ennaji H, Bouchrif B, Hassar M, Karib H. Comparative Study of Microbiology Quality of Raw Poultry Meat at Various Seasons and For Different Slaughtering Process in Casablanca (Morroco). J Appl Poult Res. 2007; 16:502-508.
15. Fratamico PM, Smith JL. Escherichia coli infections. In: Riemann, H.P., Cliver, D.O. (3rd): Food-borne infections and intoxications. Florida: Elsevier Inc. Academic Press. 2006, 205-208.
16. Branger C, Zamfir O, Geoffroy S, Laurans G, Arlet G, Thien HV *et al*. Genetic background of *Escherichia coli* and extended -spectrum-ß-lactamase type. Emerg. Infect. Dis. 2005; 11:54-61.