Serotyping, pathogenicity and antibiogram of Escherichia coli *isolated from raw poultry meat in West Bengal, India*

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Summary
This study has been undertaken to isolate and characterise *Escherichia coli* strains from raw poultry meat in West Bengal, determine their pathogenicity and identify the prevalent serotypes and their antibiogram. A total of 83 raw poultry meat samples were collected from February to July 2004. Thirty-three samples (39.76%) were positive for *E. coli*. The majority of highly pathogenic *E. coli* belonged to O3, O6, O25, O73, O120 whereas the highly enteropathogenic *E. coli* belonged to O6, O25, and O158. Most isolates (84% - 100%) were sensitive to chloramphenicol, amikacin and gentamicin, they were (92% - 100%) also resistant to novobiocin, cefixime, sulphafurazole, vancomycin. Considering the frequency of *E. coli* serogroups O6, O25, O158 which are important zoonotic pathogens, special attention needs to be paid in order to maintain strict hygienic measures in the retail meat shops, so to avoid serious health risks for the retailers and for the consumers.

Caratterizzazione di Escherichia coli *isolata in campioni di carne cruda di pollo nel Bengala occidentale, India*

Parole chiave
Antibiogramma,
Carne di pollo,
Escherichia coli,
India,
Sierogruppo.

Riassunto
Lo studio presentato in questo articolo è stato condotto al fine di isolare e individuare le caratteristiche distintive di ceppi di *Escherichia coli* rilevati in campioni di carne cruda di pollo prelevati nello stato del Bengala occidentale. Lo studio ha avuto, inoltre, anche lo scopo di determinare la patogenicità dei microrganismi, i sierotipi prevalenti e il loro antibiogramma. Nel periodo febbraio-luglio 2004, sono stati prelevati 83 campioni di carne cruda di pollo di cui 33 campioni (39,7%) sono risultati positivi per *E. coli*. La maggior parte dei sierotipi altamente patogeni è risultata appartenere ai gruppi O3, O6, O25, O73, O120, quelli altamente eteropatogeni ai gruppi O6, O25 e O158. La maggior parte degli isolati (84% - 100%) ha mostrato sensibilità nei confronti di cloramfenicolo, amikacina e gentamicina, mentre si è rilevata resistenza (92% - 100%) a novobiocina, cefixime, sulfafurazolo e vancomicina. L'articolo evidenzia la necessità di dedicare particolare attenzione al rispetto delle condizioni igenico-sanitarie negli esercizi che trattano carne cruda di pollo al fine di evitare seri rischi per la salute dei consumatori e degli addetti alla vendita. Tale necessità si fa ancor più pressante se si considera la frequenza di *E. coli* O6, O25, O158 e la loro importanza come agenti di zoonosi.

Introduction

Microbes in meat, especially those causing food borne diseases, have recently become a matter of great public health concern (11). India could earn a considerable profit though meat export, however its poor quality and high level of microbial load deprive the country of such a possibility. As a concequence of the growing demand for animal proteins, slaughterhouses have to face an increasing load of meat to process. Such an increased load came at the cost of the hygienic aspects of meat production, to which is paid less attention that it is requited. Meat, being it a nutrient-rich substrate, can support the growth of a wide range of micro-organisms, which also include *Escherichia coli*. The latter has received much attention as a potential public health threat due to the morbidity and mortality rates associated with outbreaks and sporadic cases of human illness (10). This study has been undertaken to isolate and characterise *E. coli* from raw poultry meat in West Bengal; to determine the pathogenicity; and to identify the prevalent serotypes and their antibiogram.

Materials and methods

Collection of samples

A total of 83 raw poultry meat samples was collected between February and July 2004 from various retail shops situated in different local markets of Nadia and Calcutta districts of West Bengal: namely, Mohanpur (N=30), Jaguli (N=20), Kalibazar (N=17) and Belgachia (N=16) (Figure 1). About 10 to 15 grams of meat samples were randomly collected including neck, breast, back, thigh and abdomen of poultry carcasses.

Isolation and identification

Samples were plated on MacConkey agar (HIMEDIA Laboratories, Mumbai, India) and incubated at 37°C for 24 hrs. The lactose fermenting colonies were reinoculated to Eosin Methylene Blue (HIMEDIA) agar and colonies producing metallic sheen were transferred to Nutrient agar slants and incubated at 37°C for 24 hrs and stored at 4°C for further identification. Identification of isolates was done according to the Cruickshank *et al.* (3) based on staining and biochemical tests.

Serotyping

The isolates were sent to the National *Salmonella* and *Escherichia* Centre, Kasauli, Himachal Pradesh, India for further confirmation and serotyping.

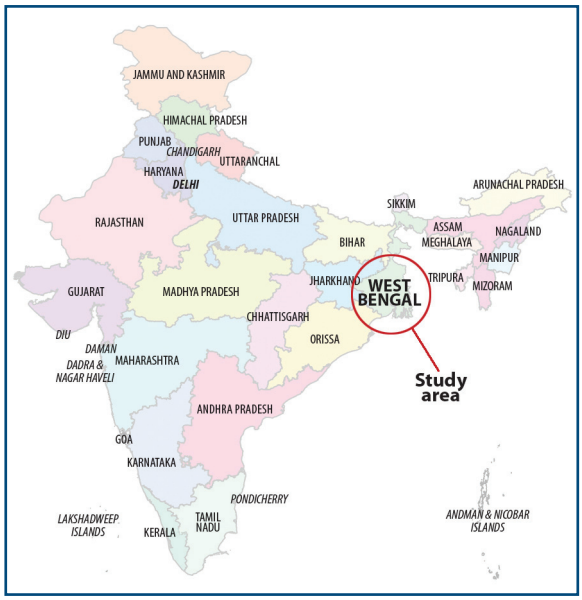

Figure 1. *Map of India showing the study area.*

Pathogenicity testing

In mice

Escherichia coli isolates were tested for pathogenicity in white Swiss albino mice as described by Mukherjee *et al.* (8). Inoculated mice were observed at 6 hrs intervals during the first 24 hrs post inoculation and, subsequently, at 12 hrs intervals for 4 days. Mortality was recorded at each interval observation. Those mice who died due to probable infection were immediately necropsied under strict aseptic condition and the gross pathological changes in the internal organs were recorded and re-isolation of *E. coli* was attempted from heart blood, pericardial and peritoneal fluids, liver, lungs, and kidney. All survivors were sacrificed on the 5th day post inoculation and all gross pathological lesions, if any, were recorded.

Ligated rabbit gut loop technique

The ligated rabbit gut loop technique was performed to determine the enteropathogenicity of *E. coli* in rabbits. The test was performed following a modified version of the method of Joshi and Kahlon (5). The animals were observed for 24 hrs and were then sacrificed after 24 hrs of operation. Strains which caused full distension with tightening of the test loops were designated as highly enteropathogenic (EP) and assigned grade 4+. Those which caused full distension with loosened loops were noted as moderately enteropathogenic and graded 3+, those with mild EP were graded as 1+, those which caused no distension were recorded as non-EP (–). After grading, each loop was opened lengthwise and fluid volume was measured. The volume (ml)/length (cm)

Table I. *Antimicrobial sensitivity test of* Escherichia coli *serogroups obtained from raw poultry meat samples collected in West Bengal (India).*

Serial No.	Antimicrobial agents	No. of strains	Resistant		Intermediate		Sensitive	
			No.	%	No.	%	No.	%
1.	Amikacin	13	0	0	1	7.69	12	92.31
2.	Cefixime	13	12	92.31	0	0	1	7.69
3.	Chloramphenicol	13	0	0	0	0	13	100
4.	Ciprofloxacin	13	2	15.38	2	15.38	9	69.23
5.	Erythromycin	13	8	61.54	3	23.08	2	15.38
6.	Gentamicin	13	2	15.38	0	0	11	84.62
7.	Kanamycin	13	1	7.69	8	61.54	4	30.77
8.	Methicillin	13	8	61.54	5	38.46	0	0
9.	Novobiocin	13	13	100	0	0	0	0
10.	Oxytetracycline	13	11	84.62	0	0	2	15.38
11.	Sulphafurazole	13	12	92.31	0	0	1	7.69
12.	Vancomycin	13	12	92.31	0	0	1	7.69

ratio was called mean index of loop. The total viable count of *E. coli* content in each loop was measured by pour plate methods and finally the lesion or inflammation present on intestinal surface of loops were also recorded.

In chicks

One day old chicks were procured from a poultry farm, in Mohanpur, West Bengal, India and the pathogenicity test to detect the virulence of *E. coli* strains on the chicks was performed following the method proposed by Savov (14), Dho and Lafont (4). The chicks were randomly divided into groups, each group being comprised of 6 chicks. Representative *E. coli* strains from each selected serogroup were cultured in nutrient broth and 0.2 ml of fresh broth culture containing 10^7 viable organisms in the case of *E. coli* were inoculated intraperitoneally into each group. The control group was inoculated with 0.2 ml of sterile nutrient broth by intraperitoneal route. All the chicks were observed for 3 days. The post-mortem examination was done in freshly dead chicks and all the gross pathological changes in internal organs were recorded.

Antibiogram

The drug sensitivity of different serotypes to different antibiotics was carried out as recommended in Bauer *et al.* (1) and has been presented in Table I.

Results and Discussion

A number of different microorganisms are recognised to cause foodborne illness (15). Among these, the importance of *E. coli* organisms as potential pathogens causing food poisoning needs to be emphasised. In the present study, meat samples from poultry birds, collected from different retail shops have been found to be contaminated with different strains of *E. coli*, which may cause infection to the consumers as well as to those involved in the meat processing chain.

Out of 83 poultry meat samples, 33 (39.76%) samples were positive for *E. coli*. This finding is consistent with those of Stanescu *et al.* (16) who reported that 37.1% of broiler carcass samples were contaminated with *E. coli*. Of the 30 samples of poultry meat collected from the local Mohanpur market, 13 (43.33%) samples yielded *E. coli* strains, while 20 samples collected from Jaguli, 17 samples from Kalibazar and 16 samples from Belgachia yielded 7 (35.00%), 8 (47.06%) and 5 (43.75%) *E. coli* strains, respectively. Thus, the present investigation shows the contamination of poultry meat with *E. coli* strains and highlights the possibility that this may be due to the breach of proper sanitary precautions before and during slaughtering.

All the 33 isolates of *E. coli* were serotyped in the National *Salmonella* and *Escherichia* Centre, in Kasauli, Himachal Pradesh. Out of 33 isolates of *E. coli*, 18 could be typed, 9 could not be typed and 6 were classified as rough strains (Table II). Table II shows that out of 13 different 'O' serogroups of *E. coli*, O120 serogroup comprising of 3 (9.09%) isolates was the most prevalent in the present study. O6, O81 and O100 serogrpups follow, (N=2; 6.06%) as well as serogroups O3, O25, O32, O73, O101, O104, O107, O109 and O158 (one strain each; 3.03%).

The 13 different serogroups of *E. coli* were tested for their pathogenicity in Swiss albino mice. Among these, 5 (38.46%) serogroups (O3, O6, O25, O73, O120) were highly pathogenic, 4 (30.77%)

Table II. *Distribution and frequency of serogroups of* Escherichia coli *isolates obtained from raw poultry meat in West Bengal (India).*

Sl No.	Serogroup	Frequency	Percentage
1.	03	1	3.03
2.	06	2	6.06
3.	025	1	3.03
4.	032	1	3.03
5.	073	1	3.03
6.	081	2	6.06
7.	0100	2	6.06
8.	0101	1	3.03
9.	0104	1	3.03
10.	0107	1	3.03
11.	0109	1	3.03
12.	0120	3	9.09
13.	0158	1	3.03
15.	Untypeable	9	27.27

serogroups (O32, O81, O109, O158) were moderately pathogenic, 2 (15.38%) serogroups (O101, O107) were least pathogenic and 2 (15.38%) serogroups (O100, O104) were non-pathogenic to mice. Most of the serogroups obtained in this investigation were reported in other studies, like Chachra and Katoch (2), Mukherjee *et al.* (8). Serogroups O100, O101, O104 were not reported from poultry in the relevant literature. It may be the case that such groups come from other livestock or other environmental source distant from poultry origin. *E. coli* serogroup O158 was found to be associated with gastroenteritis and diarrhoea in infants as well as adults (12). *E. coli* serogroups, O6 and O25, were found to be associated with infantile and travellers' diarrhoea (6). So, for what it may concern transmissibility, it is evident that there is every chance for meat handlers and consumers to be infected by the *E. coli*, particularly in those cases in which scanty higenic measures are maintained.

As for the enteropathogenicity, 3 of the 13 isolated *E. coli* strains were highly enteropathogenic, 4 were moderately enteropathogenic and 6 were non enteropathogenic. Serogroups O6, O25, O158 were highly enteropathogenic. Serogroups O3, O32, O73, O120 were moderately enteropathogenic strains. Serogroups O101, O109 were least entropathogenic and rated as 1+ whereas serogroups O81, O100, O104, O107 were non enteropathogenic with no distension. The gross lesion in the intestine varied from inflammation, oedema to haemorrhage. In the control loops inoculated with sterilised normal saline, lesions were absent.

The 13 'O' serogroups of *E. coli* were tested for their pathogenicity in 1 day old chicks. The results showed that 4 'O' serogroups (30.77%) (O6, O25, O73, O120) were highly pathogenic and 4 serogroups (30.77%) (O32, O101, O109, O158) were moderately pathogenic, while 3 serogroups (23.08%) (O3, O81, O107) were least pathogenic. Finally, 2 serogroups (15.38%) (O100, O104) were non-pathogenic. These results are consistent to some extent with previous findings described in Mukherjee *et al*. (8) and Orden *et al.* (9).

Thirteen 'O' serogroups of *E. coli* isolates were tested with 12 different antimicrobial agents. The results showed that *E. coli* isolates were 100% sensitive to chloramphenicol, followed by amikacin (92.31%), gentamicin (84.62%), ciprofloxacin (69.23%), erythromycin (15.38%), oxytetracycline (15.38%), cefixime (7.69%), sulphafurazole (7.69%) and vancomycin (7.69%). Intermediate sensitivity showed to kanamycin (61.54%) followed by methecillin (38.46%), erythromycin (23.08%), ciprofloxacin (15.38%) and amikacin (7.69%). For novobiocin, 100% of the isolates showed resistant followed by cefixime (92.31%), sulphafurazole (92.31%), vancomycin (92.31%), oxytetracycline (84.62%), erythromycin (61.54%), methicillin (61.54%), ciprofloxacin (15.38%), gentamicin (15.38%) and kanamycin (7.69%). Antimicrobial sensitivity test of *E. coli* strains has been presented in Table I. It is noteworthy that these findings were partially correlated to those reported in Sackey *et al*. (13) and Mishra *et al*. (7). The possible explanation for this resistance of *E. coli* against the antibiotics as found in the study could be attributed to the indiscriminate use of these antibiotics in poultry. This high resistance might be due to transmissible drug resistance and resistance might also develop due to mutational changes.

The present investigation sheds some light on high degree of prevalence of pathogenic *E. coli* strains in poultry meat. Considering the frequency of *E. coli* serogroups O6, O25, O158 which are important zoonotic pathogens, special attention need to be paid so to maintain strict hygienic measures in slaughterhouses and retail meat shops, in order to avoid the serious health risks for the meat handlers working in the meat shops and for the consumers.

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