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RESEARCH ARTICLE OPEN ACCESS

Screening, Isolation, Identification and Antibiogram Study of Enterobacteriaceae in Ready to Cook, Chilled Food Products in Tiruchirappalli, India

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Abstract

The present study was concentrated to screen some members in Enterobacteriaceae family from chilled meat products procured from different retail shops in Tiruchirappalli, Tamilnadu. A total of six varieties of ready to cook chilled food products with five samples in each were randomly purchased from departmental stores, retailer meat shops and local vendors of Tiruchirappalli. Out of 30 ready to cook, chilled food products screened for the presence of Enterobacteriaceae, 28 found to be positive for Enterobacteriaceae. A total of 36 bacterial strains were selected at random and identified. Only 11 isolates were finally confirmed as Enterobacteriaceae and this was shared by *Escherichia coli* (*E. coli*), *Citrobacter spp.*, *Salmonella spp.*, *Serratia spp.* and *Proteus spp.* Among these Proteus spp. (23.3%) was found predominant in all the samples. Antibiogram study revealed that 54.5% isolates were susceptible to each of Ofloxacin and Ciprofloxacin followed by Ampicillin (45.5%), Chloramphenicol (27.3%) and Gentamycin (18.2%). A high percentage of 54.5% isolates were found to be multidrug resistance (resistant to 3 or more antibiotics). *E. coli* and *Proteus spp.* isolated from mixed vegetables and beef respectively, were exhibited 100% resistant to Penicillin, Ciprofloxacin, Chloramphenicol, Ofloxacin, Ampicillin and Gentamycin. The study revealed poor sanitation and cross-contamination in food processing area which resulted in the enhancement of enteropathogenic bacteria which are, known to cause foodborne illnesses. Also, the multidrug resistance noticed in the present study may be linked to the use of antibiotics in cattle rearing which constitute a serious threat to public health.

Keywords: Ready to cook, Enterobacteriaceae, Antibiogram, Sanitation, Foodborne illness, Cross-contamination

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INTRODUCTION

The emergence of multidrug resistant bacteria has been reported worldwide and it is a major public health concern[1]. The most important gram-negative bacteria found in the intestinal tract belong to the family Enterobacteriaceae is the challengeable contaminants in raw and cooked food products[2]. Coliforms are indicators of faecal contamination in food[3]. Enterobacteriaceae in ready to cook food products causes foodborne illness associated with mild to severe diarrhoea due to gastrointestinal disorders and clinical manifestations in human[4]. Enterobacteriaceae such as *E. coli* and *Salmonella* species are most predominant in some meat products[5,6]. *E. coli* are considered as a sepathogenic, non-pathogenic and commensal organism found in the intestinal region of both animal and human[7]. *Salmonella* is the predominant foodborne pathogen causing salmonellosis due to the consumption of undercooked meat and eggs[8].

Worldwide, 2.2 million adults and children under five years of age die of diarrhoeal disease every year from foodborne and waterborne illnesses. In developing countries, the major cause of foodborne infections is the cross-contamination of meat and raw vegetables by polluted water, soil, air and unhygienic handling. The major factors which affect the quality of perishable foods are microbes present within the animal before slaughtering, enzymatic changes and external sources during processing, microbial accumulation after slaughtering within the tissues, time and temperature of storage, handling at the distribution center[9]. In general, the rate of deterioration is higher in seafoods because of decay organisms present in their gastrointestinal system which rapidly multiply after death[10]. Nowadays, due to lifestyle modernization and raised standard of living, the consumption of ready to cook fresh slaughtered chilled meat, seafoods and vegetable products have increased in India[11,12]. Therefore, the present study was planned to determine the presence of members of Enterobacteriaceae and to study the antimicrobial resistance of isolated gram-negative bacteria in different ready to cook foods obtained in Tiruchirappalli, Tamilnadu.

MATERIALS AND METHODS

The isolation of members of Enterobacteriaceae was carried out as per The United States – Food and Drug Administration (US-FDA) bacteriological manual 8th edition with slight modification[13].

Media preparation

All the media used in the study were prepared according to the manufacturer's instructions.

Sample Collection

A total of six different chilled ready to cook food products with five samples in each such as raw meat samples (chicken, pork and beef meat), seafood samples (fish and prawns) and mixed vegetables were purchased at random from local vendors, departmental stores and local meat retailers located in Tiruchirappalli, Tamilnadu. The samples were transported immediately to the laboratory for microbial evaluation under the packaging condition provided by the retailers and stored under same condition until analysis.

Sample Preparation

Twenty-five grams of all chilled ready to cook samples were finely chopped with a sterile knife and transferred to 225 ml of 10x Phosphate Buffer Solution (PBS) and incubated for 24 h at 37°C for pre-enrichment. The enrichment process was done by transferring 1 ml of pre-enriched broth to 9 ml of Lactose broth, Selenite F broth and Tetrathionate broth (Himedia, Mumbai) and incubated at 37°C for 24 h. A loop full of each enriched broth was streaked on to MacConkey agar plates and Xylose Lysine Deoxycholate (XLD) agar plates (Himedia, Mumbai) in triplicates. The probable morphologically distinct colonies were picked up and streaked on to nutrient agar media (Himedia, Mumbai) and incubated at 37°C for 24 h. Then the pure colonies were subjected to various biochemical analysis for identification.

Identification of Isolated Bacteria

The isolated bacteria were identified using colony morphology and biochemical tests[14,15]. *E. coli* colonies were further re-streaked on Eosin Methylene Blue agar (EMB) (Himedia, Mumbai) and incubated at 37°C for 24 h for reconfirmation. Colonies with a green metallic sheen were confirmed as *E. coli.* Black colonies on XLD agar plates were further tested with urease broth. The urease positive was confirmed to be *Proteus* sp. and *Salmonella* sp. was confirmed by the H_2S formation in Triple Sugar Iron (TSI) agar

and negative urease tests. The production of red prodigiosin pigment in nutrient agar confirmed the presence of *Serratia* sp. *Citrobacter* sp. was confirmed by colony morphology and other biochemical tests such as Indole, Methyl Red, Voges-Proskauer, Citrate (IMViC), Lysin Iron Agar (LIA), Motility Indole Ornithine (MIO), Oxidase, Catalase, Extracellular enzymatic activities test such as Amylase, Protease and Lipase. Oxidative Fermentative test using OF media and carbohydrate fermentation test using Phenol Red Broth (PRB) (Himedia, Mumbai) were performed with glucose and sucrose.

Antibiotic susceptibility test

The antibiotic sensitivity test was performed against isolated colonies by Kirby-Bauer disc diffusion method as described by CLSI standard guidelines[16]. The antibiotics used were Penicillin (P, 10 units), Ciprofloxacin (CIP, 5 mcg), Gentamycin (GEN, 10 mcg), Ofloxacin (OF, 5 mcg), Chloramphenicol (C, 30 mcg) and Ampicillin (AMP, 10 mcg). The 24 h fresh culture of isolated gram-negative bacteria was streaked on to Nutrient Agar (NA) plates and incubated at 37°C for 18-20 h. The isolated single colony of all bacterial cultures were inoculated into the nutrient broth and incubated for 4 h at 37°C until the appearance of moderate

Table 1. Different members of Enterobacteriaceae family isolated from ready to cook chilled food samples

Samples	Total no. of samples	Total no. of positive samples	Total no. of suspected isolates	Total no. of typical isolates	*E. coli*	*Citrobacter sp.*	*Proteus sp.*	*Salmonella sp.*	*Serratia sp.*
Mixed veg	5	3	3	1	1	0	0	0	0
Chicken	5	5	6	2	0	0	1	1	0
Beef	5	5	10	3	0	1	1	0	1
Pork	5	5	5	3	0	0	3	0	0
Fish	5	5	6	1	0	0	1	0	0
Prawn	5	5	6	1	0	0	1	0	0
Total	30	28	36	11 (36.6%)	1 (3.3%)	1 (3.3%)	7 (23.3%)	1 (3.3%)	1 (3.3%)

Table 2. Antimicrobial susceptibility of isolated bacteria against different antibiotics

No.	Identified species	Name of the Antibiotics						Overall percentage of antibiotic resistance
		P	CIP	GEN	OF	C	AMP	
1	*E. coli*	R	R	R	R	R	R	100
2	*Citrobacter sp.*	R	S	S	S	I	R	33.3
3	*Proteus sp.*	R	S	R	S	R	R	66.6
4	*Proteus sp.*	R	R	R	R	I	S	66.6
5	*Proteus sp.*	R	R	R	S	I	S	50
6	*Proteus sp.*	R	S	R	S	I	S	33.3
7	*Proteus sp.*	R	S	I	S	I	R	33.3
8	*Proteus sp.*	R	R	R	R	R	R	100
9	*Proteus sp.*	R	S	R	I	S	S	33.3
10	*Salmonella sp.*	R	S	R	S	S	R	50
11	*Serratia sp.*	R	I	S	I	S	S	16.6

The overall Multidrug resistance was found to be 6 out of 11 isolates with 54.5%
(S-Susceptible, I-Intermediate, R-Resistant, P-Penicillin, CIP-Ciprofloxacin, GEN-Gentamycin, OF-Ofloxacin, C- Chloramphenicol, AMP-Ampicillin)

turbidity. Then the bacterial growth as measured by optical density (OD_{600}) and adjusted. With the sterile wooden swab sticks the bacterial culture was swabbed on 4 mm depth Muller Hinton agar (Himedia, Mumbai) and incubated for 24 h at 37°C with antibiotics discs.

RESULTS

In the present study, 30 ready to cook chilled raw food samples were collected and tested for the presence of members of Enterobacteriaceae bacteria. It was found that only 28 samples showed positive. Among 28 samples, 36 suspected strains were isolated and only 11 (36.6%) bacterial isolates were confirmed to be that of Enterobacteriaceae (Table 1). The biochemical tests revealed the presence of five pathogenic bacterial isolates viz. *E. coli*, *Citrobacter* sp., *Salmonella* sp., *Serratia* sp. and *Proteus* sp. *Proteus* sp. was the most predominant bacteria in all the chilled meat (chicken, beef, pork) and seafood products (fish, prawn). *Salmonella* sp. and *Proteus* sp. were recovered from ready to cook chicken sample. From ready to cook chilled beef sample, *Citrobacter* sp., *Serratia* sp. and *Proteus* sp. were isolated. It was found that minimally processed or unprocessed chilled ready to cook food samples were highly contaminated with Enterobacteriaceae. The antimicrobial susceptibility study indicated the resistance of all isolates to penicillin (100%) followed by 72.7% of the isolates to Gentamycin, 54.4% to Ampicillin, 36.4% to Ciprofloxacin, and 27.2% each to Ofloxacin and Chloramphenicol. The gram-negative bacteria of *E. coli* and *Proteus* sp. isolated from chilled ready to cook mixed vegetables and beef samples have shown 100% resistance to all the six antibiotics used in this study. 54.5% of bacterial isolates were found to be multidrug resistance (resistant to 3 or more antibiotics) (Table 2). All the isolated 5 species were found susceptible to Ofloxacin (54.5%), Ciprofloxacin (54.5%), Ampicillin (45.5%), Chloramphenicol (27.3%) and Gentamycin (18.2%). As a result, the isolates were highly susceptible to Ofloxacin and Ciprofloxacin (54.5%) and highly resistant to Penicillin (100%) followed by Ampicillin (54.5%) (Table 3).

Table 3. Percentage of isolated bacterial antibiogram profile

Antibiotics name	Sensitive	Inter-mediate	Resistant
Penicillin	0	0	100
Ciprofloxacin	54.5	9.1	36.4
Gentamycin	18.2	9.1	72.7
Ofloxacin	54.5	18.2	27.3
Chloram-phenicol	27.3	45.5	27.3
Ampicillin	45.5	0	54.5
Overall	33.3	13.7	53

DISCUSSION

A total of 11 strains were isolated from 28 ready to cook, chilled samples. The occurrence of high Enterobacteriaceae count indicated that there was poor sanitation during slaughtering, storage and hygienic condition inside the butcher's shop which became good sources of cross-contamination from knife, cloth and processing area. Vegetables might get contaminated from manure, water and handling. Hence, this study has clearly shown that the occurrence of different members of Enterobacteriaceae species were common in chilled vegetables and retail meat. In this study, *Proteus* sp. was present in all the chilled meat and sea food samples with an incidence of 23.3% and is the most predominant strain isolated from chicken, beef, pork, fish and prawn. The predominance of *Proteus* sp. might be due to cross-contamination by food handlers while removing intestinal content by hands. It is important to wash their hands after processing any food materials to prevent cross-contamination due to the rapid proliferation of the pathogen. In Tiruchirappalli, seafoods are transported from various places of Tamilnadu. The collected sea food samples were observed to be in broken packages. Also, it was suspected to be contaminated by water and the quality of ice used during transportation. *Proteus* sp. is considered as an indicator of meat contamination during processing. Many researchers have isolated *Proteus* sp. from different food samples[17-19]. In addition, *Proteus* sp. has been proved to cause urinary tract infection and diarrhoea[20]. *E. coli* contamination was prevalent among vegetables. The frequency of *E. coli* in vegetables was found to be high. This study revealed the presence of 3.3% of *E. coli* in mixed

vegetable samples. Here, the mixed vegetables are precut, packed, chilled and sold in retail markets for daily routine cooking purpose. The vegetables were processed by labors working in the shop. Presence of *E. coli* in vegetables indicated faecal contamination due to unhygienic practices of workers while chopping on the uncleaned floor with open air. A number of reports were published on *E. coli* contamination of ready to cook foods[2,3,10]. Presence of *Salmonella* sp. in fresh poultry has been reported worldwide[6,21,8,22]. This study has also confirmed the presence of *Salmonella* sp. (3.3%) in chicken sample. It was found that minimally processed ready to cook chicken samples (leg piece, lollypop, and wings) were contaminated with *Salmonella* sp. This bacterium occupies the second most foodborne pathogen causing illness. The miscellaneous bacteria of *Citrobacter* sp. and *Serratia* sp. constitute a public health hazard which causes diarrhoea and gastroenteritis. Our study revealed that the presence of these species in beef samples (3.3%). Many studies have reported the presence of *Serratia* sp. and *Citrobacter* sp. in different ready to cook food samples[5,23,4,24]. Overall, the poor sanitation in retail outlets and improper handling during storage and transportation increases the microbial contamination of foods and are difficult to control[25]. The occurrence of members of Enterobacteriaceae from ready to cook samples have shown 100% resistant to Penicillin followed by Gentamycin (72.7%) Ampicillin (54.5%). This is in complete agreement with the studies of Kilonzo-Nthenge et al. (2013)[26] who recorded 89% resistance to Penicillin and 65.8% resistance to Ampicillin. It indicated the indiscriminate use of antibiotics as a growth factor in poultry and cattle. Increasing emergence of horizontal gene transfer could disseminate the virulence factor to other strains of same species or different species, might also be the reason for developing multidrug resistance.

CONCLUSION

The study showed the presence of Enterobacteriaceae in all the collected samples of ready to cook, chilled food products. Personal hygiene, storage temperature maintenance and handling during production could have been the reason which led to the microbial contamination. The knowledge of thawing and storing chilled food should be made aware to the public. Also, improper cooking and storage temperature lead to foodborne illness. The regulated food laws should be followed by both local retailers and consumers. The emergence of multiple antibiotic resistance by the isolated organisms may be occurred due to horizontal gene transfer which has become a global threat to public health.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

KS designed the work, AJ performed the experiments, generated data and wrote the manuscript. KS read and approved the manuscript.

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None.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY

All the data generated during the study are included in this manuscript

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