

Detection of Antibodies Against Zoonotic Food Borne Pathogens in Sera of Food Handlers

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Abstract: Diseases spread through food still remain common and a persistent problem. Microbiological examination of 185 meat samples collected from 13 restaurants located at Cairo and Giza Governorates revealed that 16.75% were infected. The commonest bacterial isolates were non typhoidal *Salmonella* (41.93%) and *E. coli* (32.25%) and 60% of the *E. coli* meat isolates were hemolytic. Blood samples were collected from 63 food handlers (complaining of symptom suggestive of gastroenteritis) with the aim of detecting antibodies in their sera against the prepared whole cell protein antigens of the commonest zoonotic bacterial isolates using ELISA and enzyme linked immunotransfer blot (EITB). ELISA results showed that 17.46 and 22.22% of the examined 63 food handler's sera were positive for the hemolytic *E. coli* and non typhoidal *Salmonella* whole cell protein antigens, respectively. Immunoblot fingerprinting of *E. coli* whole cell protein antigens reacted with its positive human and rabbit hyper immune sera displayed immunoreactive bands at 75.00, 70.36, 48.87, 45.15, 25.83 and 14.965, and 75.00, 70.36, 45.15, 43.00, 25.83 and 14.965 KDa and those for non typhoidal *Salmonella* at 138.48, 70.36, 46.36, 25.83, 20.91 and 14.00, and 138.48, 56.04, 46.36, 25.83, 20.91 and 14.00 KDa, respectively. So, it may be suggested that 48.87 and 70.00 KDa is the specific band of human infection with *E. coli* and non typhoidal *Salmonella*, respectively and the analysis of sera may provide information on the prior history of infections (with the target pathogens) of the donors. It is recommended that health education of food handlers is a necessary step to prevent food borne diseases.

Key words: Food handlers • *E. coli* • Non typhoidal *Salmonella* • ELISA • EITB

INTRODUCTION

Many high risk pathogens that cause diseases in humans are transmitted through various food items. Due to increased morbidity and mortality leading to time lost in the work place and decreased reproductivity, food borne diseases across the world cost billions of dollars annually [1]. It was estimated that 3.2 million children die annually as a result of diarrheal diseases while hundreds of millions more suffer from frequent episodes of diarrhea and impaired nutritional status [2].

Meat is an important source of protein and a valuable commodity in resource poor communities. In many developing countries, lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses of meat as well as invaluable by-products from animal carcasses. Slaughtering places are frequently contaminated and may not be protected against dogs, rodents and insects. Meat products coming from such conditions are often deteriorated due to

bacterial infection or contamination, which may cause food poisoning or diseases in consumers [3].

Food handlers play a major role in ensuring food safety throughout the chain of producing, processing, storage and preparation. Mishandling and disregard for hygiene measures by handlers may result in food contamination which of course will have non desirable attendant consequences [4].

Common pathogenic bacteria that may be causes of food borne diseases include strains of *Salmonella* and *Escherichia coli* (*E. coli*) [5]. Non-typhoidal salmonellosis is a major cause of food borne illness. *Salmonella* caused more outbreaks and cases of food poisoning than all other bacteria between 1993 and 1997 [6]. During 2003, *Salmonella* infections were responsible for 30% of 23,250 notifications of food borne diseases in Australia [7]

E. coli may cause acute enteritis of human and young animals including, piglets, calves and lambs. Acute enteritis in human subjects of all ages occurs mainly in the

tropics including traveler's diarrhea; a dysentery-like disease and hemorrhagic colitis or bloody diarrhea [8]. Over the last two decades, bacterial infections caused by enterohemorrhagic *E. coli* have emerged [9]. A multi-state outbreak of *E. coli* O157:H7 infections have been recorded [10].

One of the inherent difficulties in the detection of food pathogens is that they are generally present in very low numbers. These microbes may be lost among a background of indigenous microflora, and substances in foods themselves may hinder recovery [5]. Moreover, traditional methods of identification of food borne pathogens, which cause disease in humans, are time-consuming and laborious [11, 12].

The present work was a preliminary study for fingerprinting of the commonest zoonotic meat bacterial isolates in sera of food handlers using enzyme linked immunosorbent assay (ELISA) and enzyme linked immunotransfer blot (EITB) to assess the knowledge and practice of food hygiene by food handlers in restaurants.

MATERIALS AND METHODS

Serum Samples: Blood samples were collected from sixty-three food handlers (complaining of symptoms suggestive of gastroenteritis) from 13 restaurants randomly selected from Cairo and Giza governorates (Egypt) and interviewed by using structured questionnaire. There was a predominantly poor level for knowledge of food hygiene; the practice of storing and reheating leftovers was low and there was a very low frequency of hand washing. Inspection of food handlers showed a low level of personal hygiene. Serum samples were separated (by centrifugation at 2000 rpm for 10 min) and stored at -20°C till used.

Meat Examination: One hundred and eighty-five raw meat samples (90 poultry, 75 buffalo and 20 cows) were collected from the 13 restaurants under study. They were inoculated onto nutrient agar, MacConkey agar and blood agar and incubated at 37°C for 48 hr. They were also inoculated on Löwenstein-Jensen agar and incubated at 37°C for up to 8 weeks. Suspected colonies appearing on the different media were identified according to [13] and [14] for *Mycobacteria*. The recovered *Salmonella* isolates were identified serologically using the diagnostic polyvalent and monovalent antisera according to [15].

Preparation of Whole Cell Protein Antigens: Whole cell protein antigens of the isolated and identified hemolytic

E. coli and non typhoidal *Salmonella* were prepared [16]. The isolates were grown overnight at 37°C in brain heart infusion (BHI) broth and growth was harvested by centrifugation at 8000 rpm for 5 min at 4°C. Cells were washed twice in normal saline and the washed pellet was resuspended in 100 µL of the sample buffer [0.06M Tris HCl (pH 6.8), 5% β-mercapto-ethanol, 10% Glycerol, 2% Sodium dodecyl sulfate (SDS), 0.001% Bromophenol blue]. The suspension was boiled for 5 min. Protein concentration was determined according to [17].

Preparation of Rabbit Hyper Immune Sera Against the Prepared Antigens: Two white New-Zealand rabbits were immunized with 40 µg protein per rabbit of the each of the whole cell protein antigens of the hemolytic *E. coli* and non typhoidal *Salmonella* isolates emulsified in equal volumes of Freund's complete adjuvant, and two rabbits were kept as a control. Two weeks later, three booster injections in Freund's incomplete adjuvant were given with one week interval [18]. Serum samples were collected 4 days after the last booster injection.

Analysis of Antigens and Antibodies

Enzyme Linked Immunosorbent Assay (ELISA): ELISA was carried out according to [19]. The optimal antigen (whole cell protein antigens of the hemolytic *E. coli* and non typhoidal *Salmonella*) concentration, antibody and conjugate dilutions were chosen after preliminary checker board titration. In the present study, the optimum conditions were 40 µg mL⁻¹ coating buffer antigen concentration, 1:100 serum dilutions, and 1:1000 Horse radish peroxidase- labeled anti-human IgG (Sigma Co.) as conjugate and 1 mg *p*-nitrophenyl phosphatase dissolved in 1 ml substrate buffer as substrate. The absorbance of the colored reaction was read within 30 min at 405 nm using a titertek multiskan ELISA reader. All incubation steps were carried out at 37°C in a moist chamber. The positive threshold value was determined to be two-fold the mean cut-off value of negative sera.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE): SDS-PAGE was performed as described by [20]. The gel cast comprised 12% resolving and 4% stacking gels with applied 10 µg well⁻¹ of the whole cell protein antigens of *E. coli* and non typhoidal *Salmonella* strains. Mini-protein II Dual slab cell (Bio-Rad Labs, Richmond, CA) was used. The fractionated antigens were visualized by Commassie staining. Analysis of the separated bands was performed by soft ware analysis (Gel Proanalyser).

Enzyme Linked Immunotransfer Blot (EITB): The fractionated *E. coli* and non typhoidal *Salmonella* whole cell protein antigens were electrically transferred onto nitrocellulose (NC) membrane and immunoblotting was carried out as outlined by [21]. Sera diluted at 1:100, and Horse radish peroxidase- labeled anti-human IgG (Sigma Co.) diluted at 1:1000 in PBS-T and the chromogen BCIP/NBT substrate were used. The reaction was visualized by the naked eye.

RESULTS

Microbiological examination of the meat samples revealed that 31/ 185 (16.75%) were positive which were as follows; 22 poultry (11.89%), 4 buffalo (2.16%), and 5 cows (2.70%). The commonest bacterial isolates were non typhoidal *Salmonella* (*S. enteritidis* and *S. typhimurium*) (13/31, 41.93%) and *E. coli* (10/31, 32.25%). Six out of the 10 *E.coli* strains (60%) showed hemolysis on blood agar. Non typhoidal *Salmonella* isolates were as follows; poultry (10/31, 32.25%) and cows (3/31, 9.68%) and those of *E. coli* were poultry (5/31, 16.12%), buffalo (3/31, 9.68%), and cows (2/31, 6.45%).

ELISA results showed that 11 (17.46%) and 14 (22.22%) out of the 63 food handler's sera were positive for the hemolytic *E. coli* and non typhoidal *Salmonella* whole cell protein antigens, respectively.

The electrophoretic profile of the whole cell protein antigens of *E. coli* and non typhoidal *Salmonella* displayed 10 and 9 protein bands with molecular weight range 775.00-12.00 and 775.00- 11.47 KDa, respectively (Fig. 1).

The immunoblot fingerprinting of *E.coli* and non typhoidal *Salmonella* whole cell protein antigens reacted with positive human sera detected by ELISA for each antigen showed 6 for each, 75.00- 14.965 and 138.48-14.00 KDa, respectively (Fig. 2). Also, the immunoblot profiles of *E.coli* and non typhoidal *Salmonella* whole cell protein antigens reacted with its homologous rabbit hyper immune serum displayed 6 for each, 75.00- 14.965 and 138.48-14.00 KDa, respectively (Fig. 2).

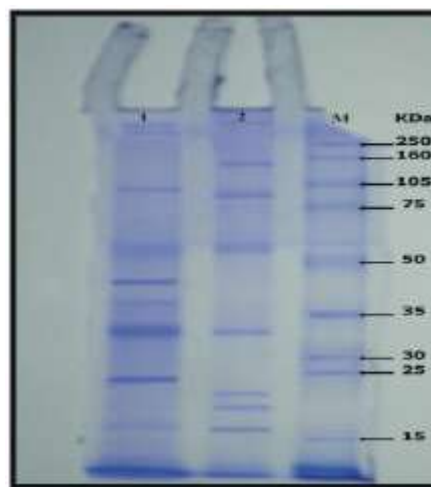


Fig. 1: SDS-PAGE of whole cell protein antigens of *E. coli* (lane 1) and non typhoidal *Salmonella* (lane 2). M: standard molecular weight marker

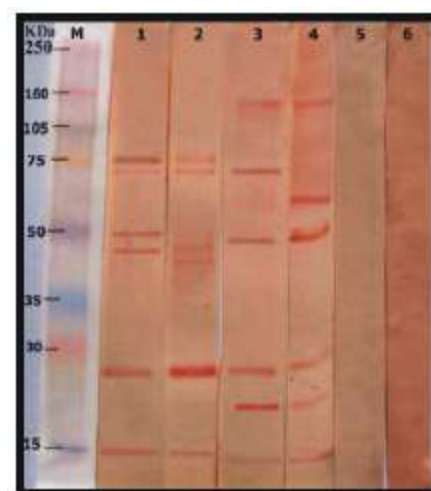


Fig. 2: Immunoblot of whole cell protein antigens of *E. coli* with positive human (lane 1), rabbit hyperimmune (lane 2) & negative human (lane 5) sera and non typhoidal *Salmonella* with positive human (lane 3), rabbit hyperimmune (lane 4) & negative human (lane 6) sera. M: standard molecular weight marker

Table 1: Results of bacteriological examination of 185 meat samples

Meat samples	Total samples	Positive samples	(%) of positive samples	Non typhoidal <i>Salmonella</i>	(%) of positive samples	<i>E. coli</i>	(%) of positive samples
Poultry	90	22	11.89	10	32.25	5	16.12
Buffalo	75	4	2.16	---	---	3	9.68
Cows	20	5	2.70	3	9.68	2	6.45
Total	185	31	16.75	13	41.93	10	32.25

DISCUSSION

The fact that food safety is given a low priority in the health care systems of many countries despite an increase in food borne diseases may be due to a lack of reliable quantitative data on incidence of disease. In many developing countries, regulations concerning meat inspection and/or control are inadequate or non-existent allowing consumers to be exposed to pathogens including zoonotic pathogens.

In the present study, the isolation rate of enteric pathogens from 185 meat samples was 16.75% and the infection rate of poultry samples was 11.89%. [8] recorded lower isolation rate (12%) of enteric pathogens from 820 uncooked food samples. On the other hand, higher infection rate of poultry was found by [22] who reported that nearly all (96-98%) of the examined chicken samples were infected. In the present study, the isolation rate of non typhoidal *Salmonella* and *E. coli* from cows and buffalo were 9.68 and 6.45%, and 0 and 9.68%, respectively. [23] recorded isolation rate of salmonellae of 19% from carcass samples of cattle. While, [24], [25] and [26] reported isolation rate of *Salmonella* (6%), *E. coli* and *Salmonella* spp. in cattle (1.25%) and *Salmonella* from raw buffalo meat (13.5%), respectively.

ELISA results showed that the seroprevalence rates of the hemolytic *E. coli* and non typhoidal *Salmonella* enteropathogens among the examined food handlers were 17.46 and 22.22%, respectively (average 19.84%). [24] reported prevalence rate of enteropathogens among non-Jordanian and Jordanian food handlers as 48.0% and 12.3%, respectively (average 30.15%).

The electrophoretic profile of the whole cell protein antigen of *E. coli* displayed 10 protein bands with molecular weight range 775.00-12.00 KDa. While, [27] reported that SDS-PAGE of whole-cell protein extracts of *E. coli* strains obtained from milk samples of dairy cows suffering from subclinical mastitis produced patterns containing 26 to 35 discrete bands with molecular weights of 6500-200.000 Kda.

The whole cell protein electrophoretic patterns of *E. coli* and non typhoidal *Salmonella* displayed 4 common bands at 775.00, 55.29, 33.13 and 16.23 KDa. Non typhoidal *Salmonella* whole cell protein SDS-PAGE profile showed a 20.91 KDa protein band which is not present in that of *E. coli*. [28] compared the protein profiles of selected *Salmonella* serovars with *E. coli* to identify genus specific protein(s) for *Salmonella* and stated that a protein of molecular weight 20.89 KDa was found in all *Salmonella* serovars, but not in *E. coli* suggesting its genus specific attribute.

The immunoblot fingerprinting of *E. coli* whole cell protein antigen reacted with its positive human and homologous rabbit hyperimmune sera showed immunoreactive bands at 75.00, 70.36, 48.87, 45.15, 25.83 and 14.965, and 75.00, 70.36, 45.15, 43.00, 25.83 and 14.965 KDa, respectively. Also, Immunoreactivity of non typhoidal *Salmonella* whole cell protein antigen with its positive human and homologous rabbit hyper immune sera displayed polypeptide bands at 138.48, 70.00, 46.36, 25.83, 20.91 and 14.00, and 138.48, 56.04, 46.36, 25.83, 20.91 and 14.00 KDa, respectively. So, it could be suggested that 48.87 and 70.00 KDa is the specific band of human infection with *E. coli* and non typhoidal *Salmonella*, respectively

In conclusion, results of this study showed high percentage of meat contamination with important pathogenic zoonotic strains. Also, analysis of sera of the food handlers indicated previous infection of them with the target pathogens. Several strategies are to be recommended for improvement of animal slaughtering and meat inspection and planning health education intervention programs for food handlers.

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