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Research Article

Microbiological Evaluation of Raw Meat Products Available in Local Markets of Karachi, Pakistan

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Abstract: Contaminated food products have been reported to be responsible for numerous food borne diseases all around the world. Microbial contaminants have been shown to be present in a wide variety of food products, especially in raw meat. For this reason, their isolation and detection in food is crucial for the safety of public health. The purpose of this research was to evaluate the microbiological quality of different meat samples including chicken, mutton and beef. Thirty (30) meat samples were purchased from different local meat retailer shops in Karachi. These samples were analyzed for their total aerobic count, total coliform count, fecal coliforms and Salmonellae according to standard methods. Examination of meat samples revealed that almost all samples were unfit for human consumption due to the presence of high aerobic count, coliforms, fecal coliforms and *Salmonella* spp. The average aerobic count log10cfu/g of chicken, mutton and beef samples was 6.67, 6.38 and 7.05 respectively. Out of 30 samples, 29 were heavily contaminated with coliforms and among them 26 were positive for fecal coliforms. The results also showed that 13 out of 30 meat samples were positive for *Salmonella* using conventional and PCR methods. The microbiological quality of meat was associated with handling and processing in unhygienic conditions. It was concluded that the food industry and regulatory authorities concerning food safety should take better control measures to improve food hygiene and prevent the contamination of food to maintain public health status and also control the rate of incidence of food borne diseases.

Keywords: Raw meat, total aerobic count, coliforms, *Salmonella*

1. INTRODUCTION

Food borne diseases are the major cause of mortality and infections especially in the developing countries. A variety of pathogenic microorganisms including bacteria, viruses, protozoans, parasites are involved in number of severe outbreaks worldwide. According to an estimate, 600 million food-related infections occurred in 2010 alone with 420,000 deaths [1]. In 2013, 818 food borne outbreaks were reported in USA, which resulted in 13,360 illnesses, 1,062 hospitalizations along with 16 deaths, and 14 foods recalls [2]. Outbreaks caused by *Salmonella*; a food borne pathogen, increased 39% from 2012 (113) to 2013 (157). Outbreak associated hospitalizations caused by *Salmonella* spp. increased 38% from 2012 (454) to 2013 (628) [2]. In addition, several reports related to contaminated food products being imported or exported have further complex the situation of food safety and public health worldwide. The global economy links local markets to international markets on an unparalleled scale, which results in unrestricted transportation of contaminated food involving numerous countries in Asia, Europe, and Latin America [3].

Meat and meat products are among the most important edible commodities originating from cattle, poultry and fishes. They serve as an ideal medium for the growth of many organisms due to increased water activity, favorable pH and higher concentrations of proteins, minerals, growth factors, fermentable carbohydrates, etc.

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Contaminated raw meat is one of the main sources of food-borne illnesses since it has nutrients and conditions fit for bacterial contaminations [4]. These contaminated food products play a huge role in spreading food borne diseases to the consumers across the world. This fact poses a common as well as a life-threatening problem for millions of people all around the world.

Human food borne infections and especially *Salmonella* infections due to the consumption of chicken meat and other poultry products have increased dramatically around the globe. *Salmonella* and *Staph aureus* are on the top of the list in terms of food poisoning and infections [5]. Most of the human salmonellosis cases have been related to broilers chicken meat [6]. These harmful bacteria can grow in cooked and raw meat, fish and dairy products. Similar to *Salmonella* contaminations in meat, *E. coli* is also one of the bacteria that can be a major cause of food poisoning. *E. coli* which can contaminate meat products is also classified in the group of coliforms and fecal coliforms which are commonly used as bacterial indicators of sanitary quality of foods and water. Such food pathogens can easily contaminate food and spread food borne diseases.

This situation doesn't only affect people's health and well-being, but it also has many economical drawbacks [7]. For this reason, food products are being scrutinized intensively for microbiological contamination, especially during export/import or marketing across the boundaries. Consequently, the food industry is also facing economic disadvantages like rejection of consignments, loss of products, product recall, marred product prestige, etc. [8]. So, the purpose of this study was to microbiologically evaluate different meat samples of various meat retailer shops in Karachi to determine their meat hygienic quality.

2. MATERIALS AND METHOD

2.1. Sample Collection

A total of 30 minced beef, mutton and chicken meat samples were collected from local markets of Karachi-Pakistan during August to October 2015. A minimum sample size of 100g was taken and mixed carefully for 5 to 10 minutes. Homogenized samples were kept in air tight polyethylene bags and stored at 4°C till further analysis. The samples were analyzed for total aerobic count, total coliform count, fecal coliforms. The method described in bacteriological analytical manual was employed to perform above tests. *Salmonella* detection was also done using polymerase chain reaction (PCR) [9, 10].

2.2. Total Aerobic Count

Briefly, 10g of each sample was aseptically weighed and diluted in 90ml of sterile saline to achieve 1:10 dilution. Samples were thoroughly mixed by blending in a blender jar and serially diluted further to dilution $1:10^4$ or $1:10^5$ in sterile saline. One ml of each dilution was added into sterile petri plates. A portion of 15-20 ml sterile molten nutrient agar (Oxoid, UK) was added immediately and allowed to settle evenly by slightly rotating plates clockwise and anticlockwise. Medium was allowed to solidify and incubated at 35-37°C for 24-48 hours. After incubation, colonies were counted and colony forming unit/gram (CFU/g) was calculated. Data was expressed as mean and standard deviation. Statistical analysis was performed by one factor analysis of variance (ANOVA) and least significant difference method for comparison.

2.3. Total Coliform Count by Most Probable Number (MPN) Method

To enumerate total coliforms, dilution preparations described in above section were used. A portion of 1ml from each dilution i.e. 1:10, 1:100 and 1:1000 was inoculated into three sets of 9ml sterile MacConkey broth (Oxoid, UK) tubes each containing Durham's tubes. The tubes were incubated at 35-37°C for 24-48 hours. MacConkey broth tubes were examined for gas and color change of broth from violet to yellow or effervescence when tubes are gently agitated. Most probable number (MPN) of coliforms was calculated based on the proportion of confirmed gassing MacConkey tubes for 3 consecutive dilutions.

2.4. MPN - Confirmation Test for Fecal Coliforms and *E. coli*

From each positive MacConkey broth tube from

coliform count, a loopful was transferred to a tube of EC broth (Oxoid, UK). EC tubes were incubated for 24-48 hours at 45.5°C. EC tubes were examined for gas production. Results from this test were used to confirm the presence of fecal coliform/*E. coli*.

2.5. *Salmonella* Detection by Polymerase Chain Reaction

A method described earlier was used to detect *Salmonella* in meat samples [8]. Briefly, 10g of meat sample was weighed aseptically and added in 90 sterile lactose broths (Oxoid, UK). The flask was incubated at 37° C for 24 hours. After incubation, 2ml was taken in a sterile eppendorf tube and centrifuged at 5000 rpm for 10 to 15 minutes. Supernatant was discarded and pellet was re-suspended in 1ml of nuclease free water and vortex. The Thermal lysis was performed at 95°C for 10 minutes in a water bath. This cell lysate was used as a template for PCR. The thermal cycling was carried out using specific conditions and primers [8]. Briefly, a 20 µL reaction mixture was prepared containing 2 µL of cell lysate, 10 µL of 2X GoTaq Green Mastermix (Promega, USA), 0.5 µL of each primer and the volume was made up with nuclease free water. All PCR tubes were placed in a thermal cycler (Bio-Rad, USA) and PCR was started by initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 90 seconds, annealing at 62°C for 60 seconds and extension 72°C for 60 seconds. The reaction was completed by a final 7 min extension at 72°C. The amplified PCR products were resolved on agarose gel (Merck, Germany) with ethidium bromide (Sigma, USA) staining. Final products were visualized with UV transilluminator and photographs were taken for records. The primers used for this study amplified 389bp region of *invA* gene of *Salmonella*. The sequences of the primers were forward 5'-GCTGCGCGCGAACGGCGAAG-3' and reverse 5'-TCCCGGCAGAGTTCCCATT-3'.

3. RESULTS AND DISCUSSION

In order to evaluate the microbiological quality of meat, 30 meat samples from different local meat retailer shops were examined for the detection of total aerobic count, coliforms, fecal coliforms and *Salmonella.* Meat samples were selected for this study because they are reported to frequently harbor various enteric organisms. All 30 meat samples were analyzed to evaluate the microbiological quality of meat. The overall total aerobic count of samples was very high ranging from 5.88 to 7.39 log10 cfu/g with a mean value 6.70 ± 0.45 log10 cfu/g (Fig. 1). The beef samples were shown to be contaminated with maximum bacterial load followed by chicken and mutton respectively (Fig. 2). The log10 cfu/gm was found to be between 5.88 to 7.23 in chicken samples, 6.00 to 7.24 in mutton samples and 6.57 to 7.39 in beef samples. According to various food authorities and regulatory organizations such as GCC standardization organization (GSO), Gulf technical regulations, European Union standards and British meat processors association, the aerobic plate count (APC) of raw meat should be below 10^6 cfu/g [11-13]. In this study, except for a few, all samples were unfit for human consumption. The higher aerobic count in meat indicated that sanitary measures during handling, manufacturing process, and packaging were neglected and also low quality of meat was used. The variations in total aerobic count in meat samples might be due to the contamination from equipment or the environment.

Similarly, the total coliform counts of nearly all the samples were very high and in most of the cases exceeding 1100cfu/g. All the beef samples were contaminated with coliforms and 9 out of 10 samples had >1100cfu/g coliform count. However, two of the beef samples were negative for fecal coliforms. In case of chicken samples, 9 out of 10 samples were positive for both coliforms and fecal coliforms. As far as mutton samples were concerned, all mutton samples were heavily contaminated with coliforms and only 1 sample was found negative for fecal coliforms. The results of total coliform count and fecal coliforms are summarized in table 1. It was quite discouraging that only one sample out of thirty was negative for coliforms and only four for fecal coliforms. These indicator organisms clearly showed that these meat samples were contaminated with fecal pollution and may transmit variety of bacterial and viral diseases.

Polymerase chain reaction (PCR) was used to detect *Salmonella* spp. in 30 raw meat samples. PCR primers directed for *invA* gene were used to

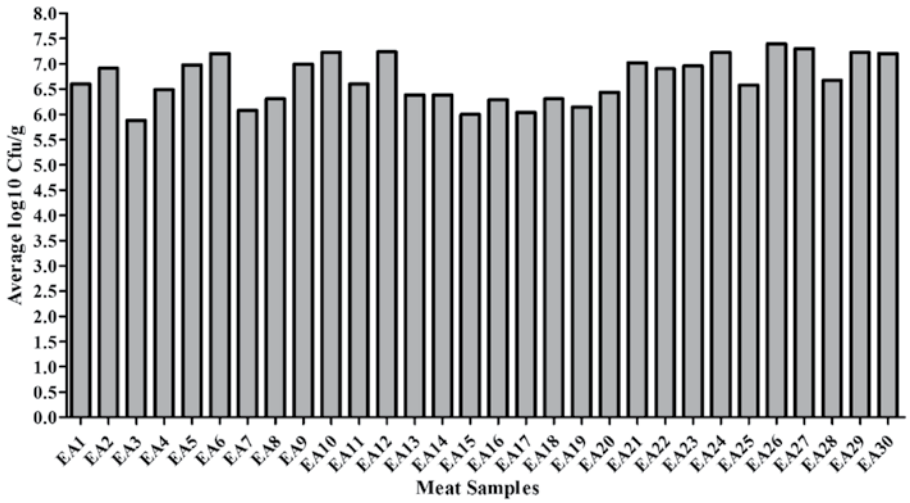

Fig. 1. Total aerobic count of 30 meat samples. Mean log10 colony forming unit (cfu)/ gram was calculated for each sample.

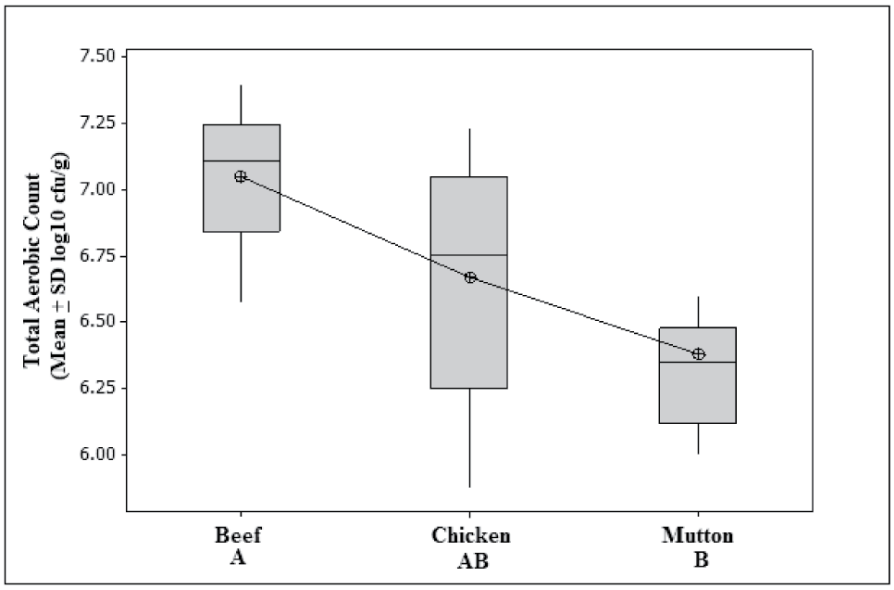

Fig. 2. Mean ± SD log10 cfu/g total aerobic count of each type of meat sample. Mean values bearing different letters for different meat types differ significantly ($P<0.05$).

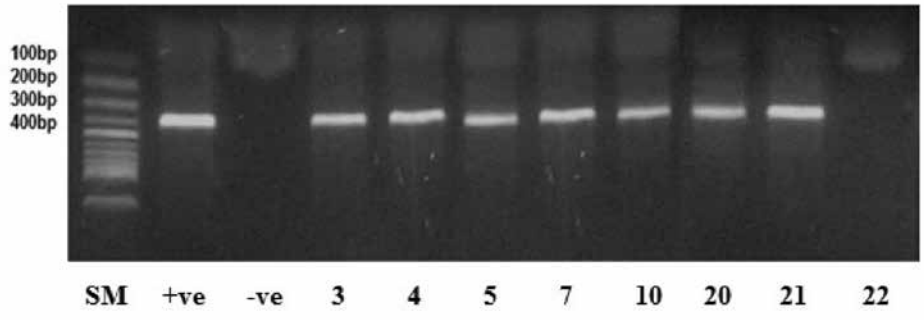

Fig. 3. Agarose gel electrophoresis of amplified *invA* gene sequence. The positive control was the lysate of *Salmonella typhimurium* ATCC 14028 and negative control was PCR master mix only without any sample.

Table 1. Total coliform count, fecal coliforms and *Salmonella* in meat samples.

Sample code	Sample	MPN/g	Fecal Coliforms	*Salmonella*	Sample Code	Sample	MPN/g	Fecal Coliforms	*Salmonella*
EA 1	Chicken	>1100	Positive	Positive	**EA 16**	Mutton	>1100	Positive	Negative
EA 2	Chicken	<3	Negative	Negative	**EA 17**	Mutton	>1100	Positive	Positive
EA 3	Chicken	>1100	Positive	Positive	**EA 18**	Mutton	1100	Positive	Negative
EA 4	Chicken	>1100	Positive	Positive	**EA 19**	Mutton	1100	Positive	Negative
EA 5	Chicken	>1100	Positive	Positive	**EA 20**	Mutton	>1100	Positive	Positive
EA 6	Chicken	>1100	Positive	Negative	**EA 21**	Beef	>1100	Negative	Positive
EA 7	Chicken	>1100	Positive	Positive	**EA 22**	Beef	>1100	Negative	Negative
EA 8	Chicken	>1100	Positive	Negative	**EA 23**	Beef	>1100	Positive	Negative
EA 9	Chicken	>1100	Positive	Negative	**EA 24**	Beef	>1100	Positive	Negative
EA 10	Chicken	>1100	Positive	Positive	**EA 25**	Beef	>1100	Positive	Negative
EA 11	Mutton	>1100	Positive	Negative	**EA 26**	Beef	>1100	Positive	Positive
EA 12	Mutton	>1100	Negative	Negative	**EA 27**	Beef	>1100	Positive	Positive
EA 13	Mutton	1100	Positive	Negative	**EA 28**	Beef	>1100	Positive	Positive
EA 14	Mutton	>1100	Positive	Negative	**EA 29**	Beef	150	Positive	Negative
EA 15	Mutton	1100	Positive	Negative	**EA 30**	Beef	>1100	Positive	Positive

amplify 389bp PCR product (Fig. 3). The primers and conditions described earlier were used to perform *Salmonella* PCR [8]. It was found that 13 out 30 tested samples were positive for *Salmonella* spp. (Table 1). *Salmonella* was more prevalent in chicken samples as 6 out of 10 samples were positive followed by 5 out of 10 in beef and only 2 positive samples of mutton (Fig. 4). It is apparent that the prevalence of *Salmonella* in chicken was higher as compared to mutton and beef and that the total percentage of positive meat samples among the 30 meat samples tested was found to be 43.33% which is a significant value to consider food hygiene. Raw meat samples which were positive for *Salmonella* in PCR were also confirmed by conventional detection methods and it was found that all the samples which were positive in PCR were also positive in cultural and biochemical identification (data not shown). The specific primers used in this study for PCR, were an amplified segment of around 389 base pairs present on *InvA* gene. This specific gene was selected due to the fact that it has been reported in all of the *Salmonella* serovars except some conflicting reports for *S. pullorum* and *S. arizonae* [14-16].

This study revealed that fresh meat products available in local markets are seriously contaminated with variety of microorganisms. The presence of higher number of organisms makes meat more prone to spoilage and may serve as a tool for the transmission of pathogenic strains. The diseases of gastrointestinal tract are very common in this part of the world and they are mainly transmitted through contaminated food and water. It is largely due to improper handling, unhygienic conditions, lack of awareness and ignorance of regulatory authorities. Several studies in Pakistan have been conducted to see the microbiological quality of meat and meat products. In one such study 84% meat samples were found to be contaminated with variety of enteric organisms including some of the obligate pathogens [17]. Similar results were obtained in another study, when it was observed that chicken meat samples were heavily contaminated with coliforms and total bacterial count in Lahore, Pakistan [18].

4. CONCLUSIONS

In conclusion, high level of contamination was observed in all types of meat samples. Samples failed to meet any of the criteria made by different regulatory bodies of food and food products. The high level of contamination and presence of pathogens indicate the unhygienic handling of meat during slaughtering, processing and storing at retailers' shop. These contaminated food items are routinely involved in several outbreaks of different infections and intoxications. So, it is a need of

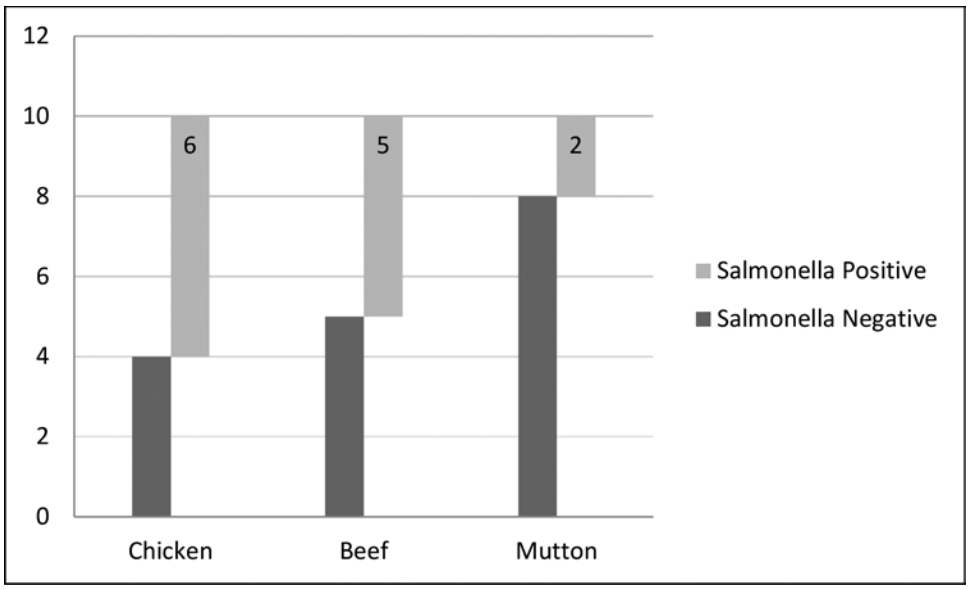

Fig. 4. The prevalence of *Salmonella* in raw meat samples.

time to improve hygienic conditions and prevent the chances of biological contamination. The concerned regulatory authorities should also take action in order to control and manage the system of aseptic handling and processing of meat and meat products.

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