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Research Article

Analysis of Raw Meat for Heavy Metals and Bacterial Contamination and Comparison of Antibiotic Susceptibility of Isolated Bacteria

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Abstract: The focus of the study was to analyze the commercially available meat for its heavy metal contents and bacterial contamination. The meat samples were collected from four commercial markets of Lahore, i.e., as Wafaqi Colony (Site I), Township (Site II), G-1 Market (Site III) and Zenith (Site IV), and analyzed for heavy metal [i.e., manganese (Mn), nickel (Ni), chromium (Cr), cadmium (Cd) and copper (Cu)] contents and bacterial contaminants (*E. coli, Pseudomonas sp., Bacillus sp.* and *Salmonella sp.)* Atomic absorption spectrophotometery was employed for the detection of the heavy metals and plate count method was used for the detection of bacterial contaminants. The Ni concentration in the Site II sample only and Cd concentration in all meat samples were found above the standard value and the concentration of other metals (Cu, Cr, and Mn) was less than the standard concentrations. Bacterial (*E. coli, Pseudomonas sp., Bacillus sp., Salmonella sp.* and *Staphylococcus sp.*) contamination was found in all meat samples; however, the number was a little lower in the Site IV samples. Statistical analysis was done, by one-way ANOVA using SPSS, to compare heavy metal contamination in the meat samples. The results showed distribution of heavy metals in all meat samples; there was significant difference of Ni concentration in the meat samples. The measure of antibiotic susceptibility showed that isolated species of bacteria were resistant to lincomycin, streptomycin, tertracyclin, ampicillin, amoxicillin and doxycyclin, but did not survive in the medium containing ofloxacin.

Keywords: Heavy metal, Bacterial contamination, *E. coli, Pseudomonas sp., Bacillus sp., Salmonella sp., Staphylococcus sp.*

1. INTRODUCTION

Heavy metals are called trace metals when these are present in low concentration. Heavy metals are of two types, essential and non-essential heavy metals. When trace metals (Fe, Mn, Zn, and Cu) are found in body in small concentration, they are essential for the existence and survival of the living organisms. The trace metals can be found naturally in the rocks, water and soil bodies. Presence of heavy metals can cause vey toxic and harmful effects on the exposed plants, animals and human beings [1]. The combustion of fossil fuels, the utilization of antiseptics and disinfectants, the exhausted batteries, poor agricultural practices and disposing of industrial waste are the big sources for the entrance of heavy metals in the ecosystem. Trace heavy metals affect the quality of water, production of agriculture and health of human beings [2]. Industrial evolution has played an important role in causing the heavy metal pollution [3].

Most of the countries of world are facing the heavy metal pollution. Usually, heavy metals such as cadmium and chromium are accumulated in liver and kidney. Lead is a toxic heavy metal and can have the capacity to make strong bonding with enzymes, having sulfhydryl groups and disturbs the normal function of enzymes. Lead affects the

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blood, nervous, genital, urinary and gastric system and in experimental animals, it can cause carcinogenesis, mutagenesis and teratogenesis. Lead toxicity can cause the headache, learning disabilities, brain damages and hearing problems as well. Cadmium is another toxic heavy metal which causes the high blood pressure, mutations and prostate cancer. Other metals like iron, copper, zinc and manganese are important in physiological functions and act as co-factor of enzymes but the presence of these metals in excessive amount can also have the toxic effects.

Meat is represented as the ecosystem for the growth of many pathogenic organisms including *Brochothrix thermosphacta, Pseudomonas sp., Carnobacterium sp., Enterobacteriaceae, Lactobacillus sp. and Leuconostoc sp.* These organisms are responsible for the spoilage of refrigerated meat and meat products [4, 5, 6, 7, 8]. Meat is a good medium for the growth of these species [9, 10, 11]. *Listeria monocytogenes, E. coli O157:H7, Campylobacter jejuni and Yersinia enterocolitica* are recognized as foodborne pathogens spreading the foodborne diseases [12, 13, 14, 15]. Many food industries use *E.coli* and Total Coliform bacteria as indicators for the presence of pathogenic microbes [16].

More than three lac and seventy thousand animals are slaughtered annually in Pakistan [17]. Mostly organisms are transferred to the consumers through improper sanitary handling and lack of awareness regarding food safety. So the proper management of animal husbandry and meat processing is required for the safety of health and for maintaining the quality of meat and meat products for long time period.

The objectives of this study were to analyze the commercially available meat for its heavy metal contents and bacterial contamination. The comparison was also made between the selected sites to find the most contaminated and least contaminated sampling site. Antibiotics resistance of isolated bacteria was measured by growing bacteria in a medium exposed to various antibiotics.

2. MATERIALS AND METHODS

This study has been conducted to detect the presence of heavy metals and bacterial species in commercial meat collected from different markets of Lahore.

2.1. Site Selection

The samples were collected from four different commercial markets of Lahore. These four sites were Wafaqi Colony (Site I), Township (Site II), G-1 Market (Site III) and Zenith (Site IV). The samples were collected for the detection of heavy metals and bacteria in them.

2.2. Pretreatment of Samples for Heavy Metal Detection

The meat samples collected from different markets of Lahore were washed with distilled water to remove the dust particles on samples and to get rid of excessive blood. These meat samples were kept in oven for drying purpose at 80^0C for 48 hours.

2.3. Heavy Metals Analysis

The wet digestion method was performed by adding conc. HNO_3 in to the meat samples. The 0.5g of meat samples and 5mL of conc.HNO_3 were taken in to the digestion flask. Hot plates were used for digestion at 80-90^{o}C and raised to 150 ^{o}C. More acid was added up to 3-5mL until clear solution was obtained. The samples were cooled at room temperature and filtered through filter assembly and the volume was raised up to 25 mL with the help of distilled water. The blank sample was also prepared. Atomic Absorption Spectrophotometer was used for the detection of heavy metals presence in the meat samples (FAAS, Shimadzu AA-7000F).

2.4 Statistical Analysis

A one way ANOVA was performed to measure the difference between the concentrations of individual heavy metals in the meat samples collected from different markets of Lahore.

2.5. Preparation of Meat Sample Homogenate

Twelve grams of meat was weighed from each meat samples collected from different sites and

dissolved them in 100 mL of BPW. The orbital shaker was used to make the homogenate of meat samples for 15-20 minutes. Desired dilutions were prepared. The Petri dishes were sterilized in autoclave and the nutrient agar was poured in to the Petri dishes. The meat inoculums were taken from each dilution and poured in the nutrient agar. The sample was spread over the medium in uniform manner and allowed to solidify properly. The Petri dishes were kept in an incubator at 32^0C for 48 hours.

2.6. Detection and Identification of Salmonella

The meat homogenate was transferred in to the 500mL sterilized bottle and placed it in incubator for 15-20 hours at 37^0C. Ten milliliters of sample from sterilized bottle was taken and transferred in to tetra-thionate broth and other 10 mL was transferred to the selective medium and placed in incubator for 2 days at 42-43^{o}C. The Petri plate were taken and sterilized. The Salmonella/Shigella agar was poured and streaked carefully and plates were kept in incubator for 24 hours and examined the presence of *Salmonella*.

2.7. Detection of Bacillus sp.

For the identification of the *Bacillus sp.*, MacConkey agar was prepared by adding 5g agar in 100mL distilled water. The Petri plates were sterilized and inoculated with meat sample through streaking method and placed in incubator for 24 hours. The colonies appeared and confirmed the presence of *Bacillus sp*.

2.8. Detection of *E. coli*

To identify the presence of *E.coli,* Eosine Methylene Blue (EMB) agar was prepared by adding 5g of agar in 100mL of distilled water. The Petri plates were sterilized in autoclave for 15 minutes at 121^{o}C and meat sample was inoculated by streaking on the Petri dishes. The Petri dishes were placed in incubator for 24 hours. The growth of colonies on the Petri plates indicated the presence of *E. coli.*

2.9. Detection of Pseudomonas sp.

Pseudomonas agar was prepared by adding 5 g agar in 100 mL distilled water for *Pseudomonas sp.* identification. After washing the meat sample was transferred to the Petri dishes and streaked. The *Pseudomonas sp.* grew on the agar and confirmed its presence in meat samples.

2.10. Detection of Total Coliform Bacteria by Dilution Formation

The test tubes were taken and filled with 5mL of EC broth. The test tubes were covered with cotton and aluminum foil and kept in autoclave for 15 minutes at 121^0C. The test tubes were cooled at room temperature. Five dilutions of 0.1mL sample, 5 dilutions of 1mL sample and 5 dilutions of 10mL sample were made by pouring samples in the sterilized test tubes containing media except 1 test tube serving as blank. The test tubes were kept in incubator for 2 days. After the incubation period these were compared with blank to count the total coliform MPN/kg [18].

2.11. Detection of Fecal Coliform Bacteria

Samples were analyzed for faecal coliform bacteria following the procedure mentioned in Standard Methods of American Public Health Association [18] and reported as MPN/kg.

2.12. Analysis of Antibiotics Susceptibility

For analysis of antibiotic resistance, filter paper disks impregnated with selected antibiotics were placed on the surface of growth medium containing bacterial isolates. Growth of bacteria around the disks impregnated with specific antibiotic was observed to check its susceptibility for that antibiotic.

3. RESULTS AND DISCUSSION

3.1. Heavy Metal Contamination in Meat

The samples were analyzed for individual heavy metals by Atomic Absorption Spectrophotometer and are shown in Fig. 1.

The concentration of manganese was not same for all samples. The highest concentration (0.0124 ppm) was found in the samples collected from Site II and in other three sites Mn was BDL (Below Detection Limit). Many previous studies have

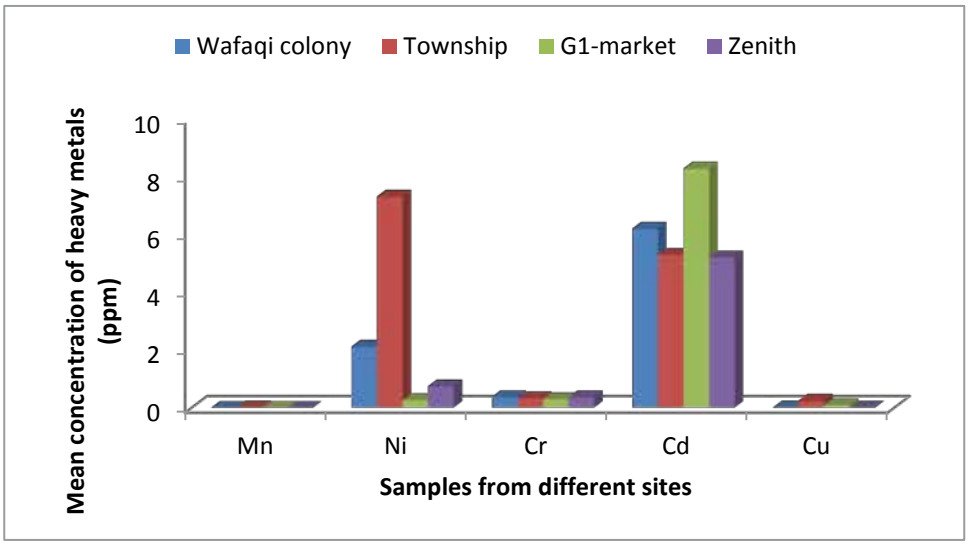

Fig. 1. Heavy metal concentrations in meat samples collected from different markets of Lahore.

been conducted to detect the presence of Mn in meat products. Cabrera et al. [19] analyzed the meat samples and found the Mn contents in meat (0.05-0.17mg/kg). Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for Mn is 2-5mg/kg [19]. The comparison of current study with standard values indicates the safe level of Mn in all samples of meat of selected sites. The concentration of nickel varies in all samples in order of Site II>Site I>Site IV> Site III. The highest concentration of Ni was found in Site II (7.32ppm), which was quite higher than standard value while other samples have showed the less concentration of nickel in comparison with standard value. According to Food and Agriculture Organization [20], the standard of nickel for food items is 5 mg/kg. Demirezen et al. [21] conducted the study to analyze the meat and meat products and find the contents of trace metals in meat. Nickel contents were present in a range of 8.2-24µg/g in the study conducted by Demirezen et al. [21]. The concentration of chromium also varied among all samples in order of Site I>Site IV>Site II>Site III The standard value of chromium is 2.3 mg/kg according to FAO [20]. The result indicates that all samples have less chromium concentration than the standard value. Bratakos et al. [22] found chromium contents in lamb meat (0.08–0.16µg/g) and chicken meat (0.11–0.21µg/g). In case of cadmium concentration, the highest concentration was found in Site III (8.29ppm). FAO has given the standard value of cadmium in food items which is 0.2 mg/kg [22]. Our results showed that the concentration of cadmium in all meat samples is quite high and the utilization of that meat was harmful for health. Farmer et al. [23] analyzed the horse meat and found the mean concentration of Cd to be at 128 mg/kg. The copper concentration in meat samples collected from different sites of Lahore. The copper concentration was highest in the Site II sample and results showed its concentration greater than 0.25 mg/kg in Site II sample and 0.1 mg/kg in Site III. The standard value of copper concentration in food items is 40 mg/kg and all samples have less copper contamination. Demirezen et al. [21] found copper concentration in meat samples (7.18–10.01µg/g). The copper contents in current study are lower than study conducted by Demirezen et al. [21], as well as recommended value of FAO [20].

3.2. Bacterial Contamination in Meat

Samples in triplicate were collected from all four sites for the determination of bacterial contents by streaking and inoculation on selected media. In the samples of Site I the *E.coli* and *Salmonella* were

seen by observing pink color colonies and reddish green colonies of *Bacillus sp.* were observed (Table 1). Previous studies were also conducted to isolate the bacterial species from meat and meat products. *E.coli* and *Salmonella sp.* were isolated by Nychas et al. [7]. For samples of Site II, the EMB agar and Salmonella/shigella agar gave positive results by confirming the presence of pink colonies. *E.coli, Bacillus*, *Pseudomona s*and *Salmonella species*were observed (Table 1). Mrema et al. [24] during their study isolated the different species of *Salmonella* and Barrera et al. [25] isolated the *E.coli* from meat samples. The comparison indicates that results are similar to the previous research results. The samples collected from Site IV, only EMB agar showed the pink colonies of *E.coli*, the reddish green colonies of *Bacillus species,* other selected media showed negative results and confirmed the presence of Staphylococcus *sp.* (Table 1). Ali et al. [26] isolated the *E.coli, Salmonella, Sheigella and Staphylococcus aureus* during their studies. Marty et al. [27] also isolated the *Staphylococcus species* during their study. The comparison of this study with above mentioned studies indicated the accurate results to some extent. In sample of Site III, there were pink colonies of *E.coli* and *Pseudomonas species*. There were *Bacillus sp.* and *Salmonella sp.* also observed (Table 1). Audenaert et al. [28] isolated *lactobacillus species* along with Lactic Acid Bacteria. Results of Audenaert et al. [28] also indicated the presence of *E.coli, Salmonella and Staphylococcus* in their results but the *Pseudomonas sp.* and *Bacillus sp.* were not isolated which indicates that these two bacterial species are found less frequently in meat and meat products.

3.3. Comparison of Total Coliform and Fecal Coliform in Meat Samples of all Sites

Samples in triplicate collected from all sites were analyzed to detect the presence of Coliform Bacteria and fecal Coliform. The number of bacteria in all samples was measured by MPN (Most Probable Number) method. The mean value of Site I samples (3.1×10^4), Site II and Site III samples (9.4×10^4) and Site IV samples (2.0×10^4) were recorded (Table 1). Odumeru et al. [16] also measured the Total Coliform Bacteria in ground beef during their studies. The mean values for Site I (0.1×10^4), for Site II (0.02×10^4), for Site IV (0.01×10^4) and for Site III (0.03×10^4) were measured (Table 2). The results showed the highest Fecal Coliform in Site I meat sample and lowest in Site IV meat samples. The number of Fecal Coliform varied from site to site in order of Site I>Site III >Site II>Site IV. Bhandare et al. [29] also found the Fecal Coliform in meat samples by using MPN method.

Table 1. Isolation of various bacteria by growing them at selected media in meat samples of all sites.

Source	EMB agar	Pseudomonas agar	MacConkey agar	Salmonella agar	Results
Site I	+	–	+	+	*E. coli* *Salmonella sp.* *Bacillus sp.*
Site II	+	+	+	+	*E.coli* *Bacillus sp.* *Salmonella sp.* *Pseudomonas sp.*
Site III	+	–	+	+	*E.coli* *Bacillus sp.* *Salmonella sp.*
Site IV	+	–	+	+	*E.coli* *Bacillus sp.* *Salmonella sp.*

Table 2. Determination of total Coliform and Fecal Coliform in meat samples collected from different sites.

Samples	Site I (MPN/kg)	Site II (MPN/kg)	Site III (MPN/kg)	Site IV (MPN/kg)
Total Coliform	3.1×10^4	9.4×10^4	9.4×10^4	2.0×10^4
Fecal Coliform	0.1×10^4	0.02×10^4	0.03×10^4	0.01×10^4

3.4. Statistical Analysis for the Heavy Metals in Meat Samples

For the comparison of heavy metals from different locations ANOVA was performed. The significant value is 0.05 by default. The concentration of heavy metals (Cu=0.496, Cr= 0.688, Cd=0.913 and Mn=0.441) showed the value greater than significant value (0.05) which means concentration of metals were same and could not differ among different sites. Whereas only Ni metal showed the significant value (0.023) which means that only the concentration of this metal varies significantly between the groups or selected sites.

3.5. Antibiotics Susceptibility Profile

Antibiotic resistance in bacteria isolated from samples of selected sites is illustrated in Fig. 2. All the Bacterial strains were found highly resistant to Lincomycin, streptomycin, tertracyclin and ampicillin but were less resistant to amoxicillin and doxycyclin. Bacteria were not survived in medium containing ofloxacin therefore none of the bacteria were found resistant to Ofloxacin. However Pseudomonas was found more resistant to all antibiotics except ofloxacin, in comparison to other bacteria.

4. CONCLUSIONS

Lahore is a mega and overpopulated city which attracts the people due to many facilities found here. People of Lahore demand food products containing meat. The current study showed that quality of meat of different commercial markets of Lahore is not so good. Meat samples were collected from different markets (Site I, Site II,

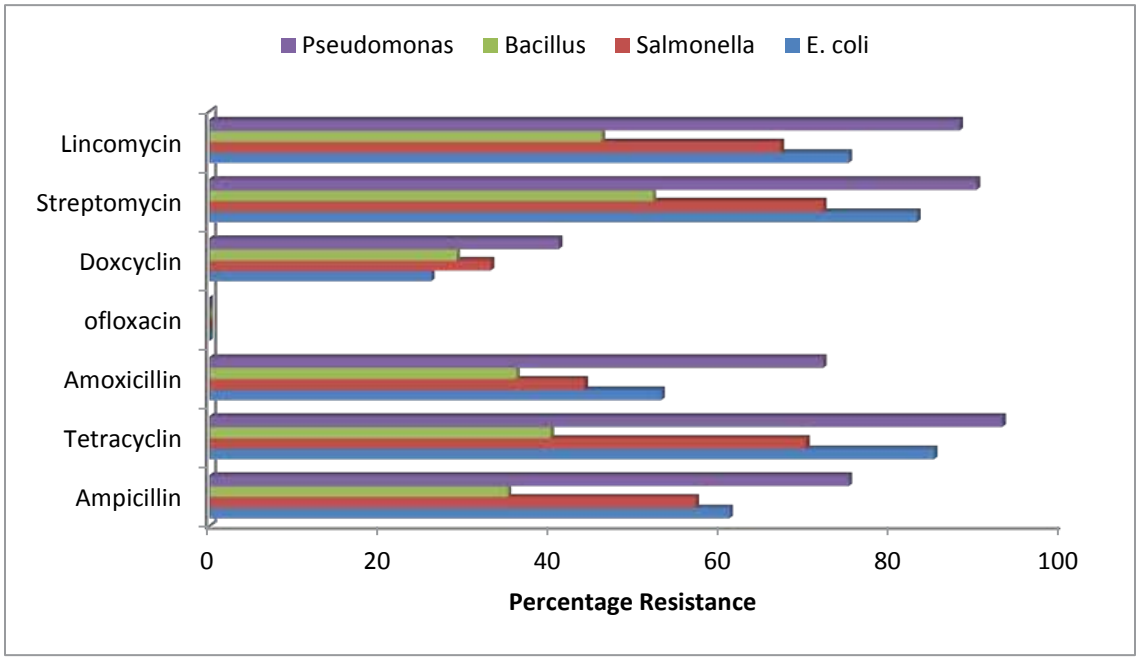

Fig. 2. Antibiotic resistance percentage isolated bacteria from meat samples.

Site IV and Site III) of Lahore to check the quality of meat. The meat samples were analyzed for heavy metals and bacterial contamination. Results showed the presence of Cu, Ni, Cr, Cd and Mn metals in all the samples but concentration was higher in Site II samples and less in Site IV samples. Concentration of Ni was found significantly higher in all sites in comparison to other heavy metals. The bacteria (*E.coli, Salmonella sp., Staphylococcus sp., Pseudomonas sp.* and *Bacillus sp*.) were also isolated from all the samples. Bacteria were found resistant to all antibiotics except ofloxacin. Results indicated that meat if not properly cooked can be harmful for human health and its long term use can cause heavy metals accumulation and toxicity in the body.

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