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MICROBIAL QUALITY OF PORK MEAT MARKETED IN DHARAN SUB-METROPOLITAN CITY, NEPAL

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ABSTRACT—This study aims to access the microbial quality of the pork meat marketed in Dharan sub-metropolitan city of Nepal. A cross-sectional study was conducted from April to October of 2019 among 7 established meat shop that sells pork meat in Dharan. Meat and swab samples from the hand of the butchers, chopping board, and knife were collected from all meat shop in triplicate manner. The average TPC in pork meat, chopping board, knives and butchers' hand of seven meat shop was found to be 181×105 cfu/g, 287×102, 49×102 and 274×102 cfu/cm2 respectively. The average total coliform in meat samples and swabs of the butchers hand, chopping board, knives were 176×101 cfu/cm2, 918×101 cfu/g and 133×101, 106×101 and respectively. All meat samples were found E. coli and S. aureus positive with four out of seven meat samples were detected salmonella positive, whereas six among seven samples were found positive with Shigella. From the data obtained it is inferred that the hygienic and sanitary conditions are not satisfactory to assure meat safety in Dharan.

Keywords— Pork meat, Hygiene, Meat safety, Dharan Nepal.

I. INTRODUCTION

Meat and meat products are the primary sourcs of many bacteria and responsible for food born infection and intoxications. Meat hygiene is the creation of conditions and implementation of measures necessary to ensure the safety and suitability of meat at all stages of the meat production chain (Chambers & Grandin, 2001). The microbiological profile of the carcass meat is highly influenced by the conditions under which animals are reared, slaughtered and processed. The composition of the microbial flora and extent of contamination occurs reflects the standard of hygiene in the slaughterhouse (Koutsoumanis & Sofos, 2004). Due to the large amount of exposed surface area, more readily accessible water, nutrients and greater oxygen penetration available, the retail cut may also result in higher microbial load, so retail cuts displayed are conducive to microbial growth and proliferation that contributes to meat spoilage (Adzitey et al., 2011).

Several studies have been issued outlining the potential for carcass contamination during de-hairing and evisceration operations (Adhikari et al., 2012; Gill & Bryant, 1993; Rivas et al., 2000). Such studies were focused on the listing of total aerobic and indicator organism counts from samples recovered from carcasses, a broad range of possible pathogens in the pig slaughter process, such as, *Salmonella spp., Listeria monocytogenes*, amongst others, can contaminate the surface of carcasses.

Dharan Sub-metropolitan (192.61km^2 area) situated on the foothills of Mahabharat range is the second-largest city in the eastern region of Nepal (Dharan sub-metropolitan city office). The consumption of pork meat and meat product is increasing day by day with the increase of population. According to Pig Entrepreneur Association Nepal, 2016 people here, consume 3850 kg of pork meat (approx. 55 pigs) per day, but hygienic standards of meat and meat shops

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are not satisfactory. At butcher shops around the city, meat contamination can occur due to different possible reasons like improper storage of meat, unclean utensils, unhygienic handling behaviours and possible cross-contamination from the surrounding.

Meat borne zoonotic diseases such as *Salmonellosis, E. coli* enteritis and food poisoning by *Clostridium, Staphylococcus*, etc. are the common problems faced by the consumers by consuming contaminated meat. The microbial aspects, shelf life and food safety of commercial retail pork meat in Dharan are little known. The hygienic condition of meat sold in Dharan sub-metropolitan city market is a very poor facility of the properly managed slaughterhouse, no practice of postmortem and antemortem inspection of meat. Meat sold in an unhygienic condition can pose threat to the health of the consumers. Such contamination of meat can result from contaminated working surfaces, equipment and the workers' hands used in the processing. Consumption of unhygienic meat causes food poisoning due to the entrance of pathogenic and food poisoning bacteria. The microbial count is most appropriately used to monitor hygiene which is lacking in Nepal. Thus meat safety in Dharan is a common concern for the regulatory authority and consumer point of view (Bantawa et al., 2018). This study have accessed the microbiological quality of pork meat marketed in Dharan and hygienic status of the meat shop.

Materials and methods

This cross-sectional study was conducted from April to October of 2019 in Dharan Sub metropolitan city.

Meat sample collection

Pork meat samples were collected from seven popular sites of Dharan. Triplicate destructive meat samples were taken from each meat shops. About 250 g meat samples were collected in a sterile plastic bag and kept in an icebox until microbial analysis. The samples were collected at 7-9 AM for each analysis day. Samples were analyzed immediately at the microbiology Laboratory of Central Campus of Technology, Dharan, Sunsari , Nepal.

1) Sample collection from possible contaminating source

Swab samples from chopping board, knife and butchers' hands were collected from each shop using cotton wool swabs of 4 cm length and 1.5 cm thickness in triplicate manner. At first sterile swab was soaked in peptone solution then it was rubbed on the surfaces, performing three times upward and downward and zigzag movements. After the friction swabs were placed individually into coded tubes containing 10 ml of sterile peptone solution, and put in icebox cooler and taken to the laboratory (Harrigan & McCance, 1976).

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Microbial analysis

For microbial analysis, meat samples (25 g) were aseptically transferred into meat mincer (National meat grinder, Model-MK-G10N, Matsusiuta Electric Ind. Company Ltd.) and 225 ml of sterile distilled water was added in the same machine and homogeneous mixture of the sample was obtained. Swab samples in tubes were thoroughly mixed for 30 sec using vortex to make initial dilutions.

For each sample, 1:10 serial dilutions were made by using peptone water. Then serial dilution was made to 10^{-7} for each sample to get appropriate number of colony which ranges from 30 to 300. All of the analyses total plate count (TPC), total coliform count (TCC), *Staphylococcus aureus, E. coli, and Salmonella spp.*, and *Shigella spp.*, were performed following the method described by (Boone & Castenholz, 2001; Varadaraj, 1993). The triplicate plate cultures were prepared for all tests performed and all of the culture media used were supplied by Himedia, India. The confirmation of the microorganisms was carried out by observing the cultural characteristics, Gram staining, and biochemical tests as described by (Boone & Castenholz, 2001). Data obtained were tabulated and processed with Microsoft Office Excel 2007.

2) Study of the sanitary condition of meat shops and personal hygiene

To study the sanitary condition of meat shops and the personal hygiene of butchers, a semi-structured questionnaire was prepared. Thirty-seven pork meat shops were asked and observed for sanitary behaviour and facilities.

RESULTS AND DISCUSSION

3) Microbiological quality of pork meat

Samples of meat, swabs of the chopping board, knife and hands of the butchers were analyzed for enumeration of total plate count (TPC), total coliforms (TC), *E. coli*, and *Staphylococcus aureus*. Findings of microbiological counts were given in table 1 and 2.

The average TPC of pork meat, chopping board, knives and butchers' hand of seven meat shop was found to be 181×10^5 cfu/g, 287×10^2, 49×10^2 and 274×10^2 cfu/cm^2 respectively. With maximum 75×10^6 cfu/g and minimum 165×10^4 cfu/g. The average count of total coliform in meat samples, swabs of the chopping board, knives and hands of the butchers found to be 918×10^1 cfu/g, 133×10^1, 106×10^1 and 176×10^1 cfu/cm^2 respectively. All the analyzed meat samples were found to be contaminated by *E. coli* and *S. aureus.*

The result of the present study is in agreement with (Bantawa et al., 2018) that the contamination of pork meat could be due to non-potable water, unclean utensils, knives, unscientific slaughtering practices and, environmental contamination. Besides this lack of scientific methods of storage and due to lack of knowledge on microbial contamination, many types of microorganisms introduce into the meat. Further, this can be evidenced by the survey result.

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The average total plate count was found to be higher than the inspected German quality meat standards referred for cutting and packaging plants, which is less than 5×10^6/g. The value for average total coliform count of meat sample was also found beyond the standard of European Union for retail sale and further processing.

It was revealed that the contamination of meat, cutting board, knife and butchers hand was also similar to (Lara et al., 2019). Due to the exposure of meat to multiple sources of microbial contamination, including contact with hide, viscera, mucous secretion, staff hands and clothes, water used for carcass washing and even air in the processing and storage environment. Large numbers of coliforms suggest insufficient washing, unsanitary handling and post-processing contamination of the polluted environment around the stores. Strong contamination of knives, chopping blocks and hands was demonstrated by microbiological examination. This directly represents the highly polluted and unhygienic condition of meat being sold in the sub-metropolitan city of Dharan's local market. The outcome means that customers are at greater risk of food poisoning.

Salmonella and _Shigella_ in pork meat, chopping board, knives and butchers' hands

Table 3 shows the presence or absence of *Salmonella* and *Shigella* in pork meat, chopping board, knives and hands of butchers. Four out of seven meat samples were detected *salmonella* positive, whereas six among seven samples were found positive with *Shigella.* Two chopping board samples, one knife sample and four butcher's hand swab samples were found to be *Salmonella positive*. Five chopping board samples, three knife samples and two hand samples were identified positive for *Shigella*.

There are several works which relate meats and surfaces bringing the presence of gram-negative microorganisms as well as to the present study (Adhikari et al., 2012; Bantawa et al., 2018; Karki, 1995; Lara et al., 2019). It can be concluded that the contamination of meat with *salmonella* and *shigella* was may be due to the use of non-potable water, contaminated hands of butchers and utensils. The pork meat may also get contaminated carcass, slaughtering environment, knives and other equipment (Borch et al., 1996; Warriner et al., 2002)

Due to lack of farm to fork food safety practices in Nepal, illness-causing bacteria like *Salmonella* and *Shigella* were found in most of the meat and swab samples of the chopping board, knife and hands of the butchers. The results directly reflects the highly polluted and unhygienic status of meat being sold on the local market of city. So Good Manufacturing and Good Hygiene Practice are the current demand in slaughtering, selling, production and marketing of pork meat in Dharan sub-metropolitan city.

4) Sanitary and hygienic status of the pork meat shop

From the survey among 37 pork meat shop, it was found that 85% of the meat sellers control the flies manually. 15% was found using chemical and physical means. 42.85 % of the butchers clean the

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shop every day, 57.14 % of the butchers clean their shop only 2 to 4 times a week. Likewise, it was found that 76.67 % of the meat handlers sanitize meat processing equipment before each use. More than half (57.14 %) of the shops used water, only 28.58 % used soap or detergent powder as the sanitizing agent and 14.28 % used cloth for the cleaning purpose.

Regarding the knowledge about zoonoses 85.71% butchers responded that they did not examine the animal for diseases before slaughtering the animal. 62.85% of the butchers were unaware of zoonoses while 37.14 % had the fair knowledge that meat was a prominent source of disease. It was found that 91.43 % denied having any idea about the Slaughterhouse and Meat Inspection Act. Only 5.71% of the butchers have a slaughterhouse facility.

The machinery used comes into contact with the surfaces of the animals during a slaughtering process. Cutting utensils such as knives, cutting blocks and the seller's hand are the key causes of cross-contamination of the meat when the animal is cut and served to the customer. Therefore, the cleanliness of the utensils, knives and other contact surfaces is equally responsible for the poor hygienic condition of the meat sold in Dharan metropolitan city. Regular improvements have been observed than previously reported by (Adhikari et al., 2012); Joshi and Olesen Hans (1999) but hygienic practices did not adequately ensure the meat safety. Similar observation on local meat shop of Dharan has also been reported by (Bhattarai et al., 2017).

Conclusions

The study found that the sanitation status of the meat shop was insufficient. All analysed meat samples were found to contain higher microbial load. The pork meat, knife, board and butchers' hand, therefore, it can't be considered bacteriologically sound and safe. However, the outbreak of zoonotic diseases has not been recorded so far, that might be due to intense cooking practices in the locality. Unhygienic slaughtering and poor sanitation practices and detection of *Salmonella*, *Shigella* and *Staphylococcus aureus* demonstrate a potential health risk for the consumer, and a warning signal for the possible occurrence of foodborne intoxication. Thus, regular inspection, microbial assessment of fresh meat and other utensils, training on basic health, and hygienic practices are recommended to reduce and control the possible hazards.

Authors' contributions

Study design, sample collection, survey: DA, BP, DS. Laboratory experiments: BP, DS. Data analysis and preparing the manuscript and reviews: DS, DA, BP. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Table 1. TPC and TCC in pork meat (cfu/g), chopping board, knives and butchers hand (cfu/cm^2)

Shop	Total plate count (TPC)				Total coliform count (TCC)			
	Meat	Chopping board	Knives	Butchers hand	Meat	Chopping board	Knives	Butchers hand
A	165×10^4	12×10^2	6×10^3	68×10^3	76×10^2	7.1×10^2	2×10^3	38×10^2
B	75×10^6	71×10^2	25×10^2	42×10^3	24×10^2	5.2×10^2	2.5×10^2	25×10^2
C	49×10^5	58×10^3	18×10^2	31×10^3	9.1×10^2	35×10^2	3.6×10^2	4.8×10^2
D	5×10^6	21×10^2	45×10^2	81×10^2	42×10^2	1.2×10^2	6.1×10^2	6.8×10^2
E	79×10^5	45×10^3	62×10^2	32×10^3	53×10^2	7.8×10^2	5.8×10^2	4×10^3
F	33×10^5	91×10^2	91×10^2	21×10^2	39×10^2	2.5×10^2	35×10^2	1.8×10^2
G	29×10^6	79×10^3	46×10^2	86×10^2	4×10^4	31×10^2	1.2×10^2	6.5×10^2
Average	181×10^5	287×10^2	49×10^2	274×10^2	918×10^1	133×10^1	106×10^1	176×10^1

Table 2. E. *coli* and S. *aureus* in pork meat (cfu/g), chopping board, knives and hands of butchers (cfu/cm^2)

Shop	E. *coli*				S. *aureus*			
	Meat	Chopping board	Knives	hands of butchers	Meat	Chopping board	Knives	hands of butchers
A	52	ND	41	210	480	300	ND	ND
B	170	ND	32	620	45×10^2	150	ND	350
C	170	20	ND	350	68×10^2	200	250	750
D	160	30	120	ND	63×10^2	ND	ND	200
E	380	200	35	210	46×10^2	130	28	600
F	200	ND	150	ND	53×10^2	ND	280	ND
G	49×10^2	100	ND	ND	51×10^2	200	ND	ND
% Prevalence	100	42.85	28.57	42.85	100	28.57	57.14	42.85

Note. ND = Not detected

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Table 3. Prevalence of *Salmonella* and *Shigella* in pork meat, chopping board, knives and butchers hand

Shop	*Salmonella*				*Shigella*			
	Meat	Board	Knives	Hands	Meat	Board	Knives	Hands
A	ND	ND	ND	D	ND	ND	ND	ND
B	D	ND	ND	D	D	D	D	D
C	ND	D	ND	D	D	D	ND	ND
D	ND	ND	ND	ND	D	ND	ND	ND
E	D	ND	D	ND	D	D	ND	ND
F	D	ND	ND	ND	D	ND	ND	ND
G	D	D	ND	D	D	D	ND	D
% prevalence	57.14	28.57	14.28	57.14	85.71	57.14	14.28	28.57

Note. 'D' = Detected, 'ND' = Not detected, A=Zero point, B=Machhavaudi, C=Panbari, D=Hatara line , E=Railway, F=Bhotepul, G=Ganeshman chowk of Dharan metropolitan city, different location used for sample collection.