



Original Article

Journal of Food Safety and Hygiene

Journal homepage: <http://jfsh.tums.ac.ir>



Bacteriological quality of meat and hygiene practice among meat handlers in Kathmandu, Nepal

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ARTICLE INFO

Article history:

Received 22 Apr. 2023

Received in revised form

19 Jun. 2023

Accepted 27 Jun. 2023

Keywords:

Butchers shop;

Meat;

Hygiene practices;

Mean bacterial count;

Antibiotic resistance

ABSTRACT

We investigated bacterial growth from raw meats and items used during meat handling, and hygiene practices followed by meat handlers at butcher shops in Kathmandu. A cross-sectional study was conducted; a total of 200 swab samples were collected from 121 butcher shops. A mean bacterial count was performed, and bacterial identification and antibiotic susceptibility test were performed. A face-to-face interview was conducted to evaluate the hygiene practices. Out of 200 samples, 90.5% showed bacterial growth. All Buffalo meat samples and more than 90.0% of Goat and Pork meat samples showed bacterial growth, and only 60.0% of knife swabs showed bacterial growth. *Staphylococcus aureus* was the predominant isolate followed by *Escherichia coli*, *Salmonella spp.*, *Citrobacter spp.*, *Klebsiella spp.*, and *Proteus spp.* Mean bacterial count in Buffalo meat (6.43 log cfu/cm²) was the highest followed by Pork meat (5.26 log cfu/cm²) and Goat meat (5.04 log cfu/cm²). A total of 456 bacteria were isolated, of which 24.3% were multi-drug resistant. Out of 136 *S. aureus* isolated, 10.2% were Methicillin Resistant *S. aureus*. A statistically significant difference was noted in carcass handling during hand injury, before and after knives and chopping block cleaning, use of fly and rodent controllers, and clean water supply. Hygiene practices depicted a significant relation with the mean bacterial count, whilst no significant relation with hand hygiene practice after the use of restrooms and use of gloves. Bacterial growth in meat and butcher items is at an alarming rate in spite of having good hygiene practices and nearly a quarter of isolates are multi-drug resistant.

Citation: Saud B, Amatya N, Yadav RK, Paudel G, Adhikari S, Shrestha V, et al. **Bacteriological quality of meat and hygiene practice among meat handlers in Kathmandu, Nepal.** J food safe & hyg 2023; 9(2): 108-119

1. Introduction

Food-borne diseases are a major public health concern as they encompass a wide range of illnesses and inflict

significant morbidity and mortality rate along with socio-economic burdens (1-3).

The amount of zoonotic food-borne ailments is higher in developing countries; approximately 33.0% of the

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population has experienced food-borne illness in developing countries annually (4,5).

Consumption of pathogenic microorganism-contaminated meat causes food-borne diseases (6). In Nepal, annually 548,000 tons of meat is produced which is worth around 275 billion Nepalese currency (7). It is a staple food that reaches every household due to its high protein, essential fatty acids, vitamins, and minerals (8) which also serves as an excellent medium for the proliferation of microorganisms. Furthermore, several factors such as inadequate hygiene, environmental temperature, gas, water, and humidity also enhance the proliferation of microbes in meat (9). Bacterial infections such as Salmonellosis, Campylobacteriosis, *E. coli enteritis*, Yersiniosis, and Listeriosis are associated with the consumption of contaminated food (10). Generally, *Clostridium spp.*, *Escherichia coli*, *Klebsiella spp.*, *Citrobacter spp.*, *S. aureus*, *Salmonella spp.*, and *Proteus spp.*, are commonly found in raw meat (11,12). The national rate of enteric infection was 18.8 cases per 1000 between 2009 and 2014 in Nepal (13). Butcher houses have been found sub-standard and unsanitary in Nepal (14). Similarly, a study in Iran has noted that the chemical and microbiological levels of meat products did not match the national standards (15). Also, a survey recorded that 20.0% of butchers did not have training, 50.0% of shops were unregistered, and 52.0% had a lack of cold storage facilities in Nepal (16). Even though there is a lack of sophisticated slaughterhouses and proper slaughter hygiene, it is customary in Nepal and other parts of the world to consume raw and partially cooked meat (17-20).

In recent decades, antibiotics have been used arbitrarily and irrationally in food supplements for livestock contributing to the emergence of antibiotic resistance in bacteria. In Nepal, previous studies have found that more than 30.0% of chicken and buffalo meats were contaminated with multi-drug resistant (MDR) bacteria (21,22). Additionally, meat samples collected from Nepal contained Methicillin Resistant *Staphylococcus aureus* (MRSA) (23). A significant number of the population have been consuming meat therefore they are at the risk of meat-borne related illness (24). Therefore, the purpose of this research was to investigate the bacteriological quality of raw meats, knives, chopping blocks, and hygiene-related practices followed by butchers in Kathmandu, Nepal.

2. Materials and Methods

2.1. Study design, study area, and sampling technique

A cross-sectional study was conducted in Kathmandu from June 2021 to July 2022. A total of 200 meat samples were collected from 121 local butcher shops adopting a simple random sampling technique. Sampling was performed using sterile cotton swabs immersed in sterile peptone water and swab samples were obtained from carcasses of Buffalo (50), Goat (50), and Pork (50) and chopping block (25), and knives (25) from an area of 5×10 cm² aseptically. Collected swabs were placed in sterile wide-mouthed containers and transported in a vaccine carrier (2-8°C) within one hour of collection and processed in the Department of Medical Laboratory Technology, Janamaitri Foundation Institute of Health Sciences, Lalitpur. Bacterial isolation, identification, antibiotic susceptibility testing, and total aerobic plate count were performed. Selected

human pathogenic bacteria (*E. coli*, *Klebsiella spp.*, *Salmonella spp.*, *Proteus spp.*, *Citrobacter spp.*, and *S. aureus*) were tested.

2.2. Bacterial isolation and characterization

Initially, swab samples were transferred into a test tube containing sterile 9 mL buffered peptone water. Then, samples were inoculated on Blood Agar (Hi-Media), Mac-Conkey Agar (Hi-Media), Mannitol Salt Agar (Hi-Media), Cystine Lactose Electrolyte Deficient Agar (Hi-Media), Salmonella-Shigella Agar (Hi-Media) and Nutrient Agar (Hi-Media). All batches of each media were quality checked for growth and colony characteristics before use. Bacteria were then identified based on morphological characteristics, gram staining, and biochemical tests as per the Clinical and Laboratory Standards Institute guidelines (25).

2.3. Total aerobic plate count

A ten-fold dilution of the sample was prepared using 1 mL of sample solution and 9 mL of buffered peptone water. From each dilution, 0.1 mL sample and 20 mL of sterile agar were poured meticulously onto a Petri dish at 48°C. Control plates were used to check the quality of the media. Solidified plates were incubated at 37°C for 24–48 h. The number of colonies on each plate was counted whereas Petri dishes having colonies number between 30–300 were only considered for total aerobic plate count (19). The mean bacterial count was reported as log cfu/cm². Finally, the mean value of each sampling unit was calculated with a maximum limit of bacterial load that is acceptable with an aerobic plate count of ≤ 3.0 log cfu/cm² as per standard guidelines by the European Union (26).

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed on

Muller Hinton Agar (Hi-Media, India) by the Kirby-Bauer disc diffusion method (25). Commercially available following antibiotic disc (Hi-media, India): Amoxicillin (10 µg), Ceftazidime (30 µg), Ciprofloxacin (5 µg), Cotrimoxazole (1.25 µg), Erythromycin (15 µg), Gentamicin (10 µg), Nitrofurantoin (300 µg), Nalidixic acid (30 µg) and Tetracycline (30 µg) were used. For the detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA), *S. aureus* with an inhibitory zone of diameter ≤ 21 mm around Cefoxitin (30 µg) disc was claimed as the MRSA strain. Furthermore, multi-drug resistance was determined for those isolates that acquired non-susceptibility to at least one agent in three or more antimicrobial categories (27). *E. coli* American Type Culture Collection (ATCC) 25922 and *S. aureus* ATCC 25923 were used as reference organisms for quality control in antimicrobial susceptibility testing.

2.5. Questionnaires for hygiene practice

A total of 10 questionnaires were administered to butchers by face-to-face interview to determine the practices related to personal and product hygiene. All the tools were developed with the help of an extensive literature review (28–30) and consultation with experts. The drafted questionnaires were pre-tested on 12 meat handlers (about 10% of the population size) in Lalitpur district, Nepal. The questions were prepared in both English and Nepali languages.

2.6. Statistical analysis

Statistical Package for the Social Sciences (IBM Corp., USA) version 21 was employed for encoding and analyzing the data. Univariate analysis was performed for central tendencies like Mean, Median, and Standard Deviation (SD). Bivariate and multivariate analysis and a Chi-square test were used to identify the

association and the strength of the relationship between bacterial isolation and hygiene practice related to person and product. Also, T-test was used to determine the relation between hygiene practices and mean bacterial count. P-value of less than 0.05 was considered significant.

2.7. Ethical approval

Ethical approval was taken from the Nepal Health Research Council (NHRC), Ramshah Path, Kathmandu, Nepal with reference number 1692 and protocol registration number 897/2019.

3. Results

Out of 200 samples, 181 (90.5%) showed bacterial growth. All buffalo meat samples showed bacterial growth followed by pork meat (97.5%) and goat meat (95.0%). Also, 80.0% of chopping block showed growth, and the least number (60.0%) of bacteria were isolated from the knives. The highest mean bacterial count was observed in buffalo meat followed by goat meat, pork meat, knives and chopping blocks (Table 1).

Out of 10^5 *E. coli* isolates, 20 were resistant to Tetracycline, 17 to Nalidixic acid, and 12 to Amoxicillin. Likewise, *Salmonella spp.* was mainly resistant to Tetracycline and Nalidixic acid, followed by Amoxicillin and Ceftazidime. Out of 58 *Citrobacter spp.*, 28 were resistant to Amoxicillin and 7 to Tetracycline. Also, out of 136 *S. aureus*, 134 were Amoxycillin resistant, 98 were Ceftazidime resistant, 43 were Tetracycline resistant and 12 were Erythromycin resistant (Table 2).

Furthermore, there were 14 MRSA isolates found and 48 isolates of *S. aureus* found to be MDR followed by 21

were *E. coli*, 18 were *Proteus spp.*, 15 were *Salmonella spp.*, 5 were *Citrobacter spp.*, and 4 were *Klebsiella spp.*

Regarding hygiene, overall butchers maintained their own and meat shop's hygiene properly with a statistically significant relation ($p < 0.05$), except for practices like handling carcasses even while having hand injuries and unclean water supply in shops. Butchers who washed their hands prior to meat handling showed comparatively fewer isolates (42.0%) than those who did not (58.0%) however, there was no significant relation seen among them ($p > 0.05$).

No significant relation was observed between hand washing with soap water every time after using the restroom and isolates. In addition, this study revealed that those who have practiced handling meat while having hand injury had three times higher odds of bacterial contamination (OR=3.52, CI=1.28-9.68) than those who did not ($p < 0.05$). Similarly, a significant relation ($p < 0.05$) was seen in those who cleaned knives and chopping blocks before and after use, those who use fly and rodent controllers, and having a clean water supply in the shop. In addition, comparing hygiene practices with mean bacterial count ($\log \text{ cfu/cm}^2$), overall hygiene practices depicted a significant relation ($p < 0.05$) whilst no significant relation was seen with hand hygiene practice after the use of restroom and use of gloves by butchers.

Table 1. Prevalence of total isolates from the collected samples

Samples (N=200)	Mean bacterial count (log cfu/cm ²)	<i>E. coli</i> (n=105)	<i>Klebsiella</i> <i>spp.</i> (n=56)	<i>Salmonella</i> <i>spp.</i> (n=67)	<i>Proteus</i> <i>spp.</i> (n=31)	<i>Citrobacter</i> <i>spp.</i> (n=58)	<i>S. aureus</i> (n=136)
Buffalo meat (50)	6.43	32	16	25	9	18	28
Goat meat (50)	5.04	28	11	20	2	7	26
Pork meat (50)	5.26	23	15	17	16	22	24
Chopping blocks (25)	5.02	15	11	5	3	8	29
Knives swab (25)	5.0	7	3	0	1	3	29

Table 2. Antimicrobial drug resistance pattern of bacterial isolates

Antibiotics	<i>E. coli</i>	<i>Klebsiella spp.</i>	<i>Salmonella spp.</i>	<i>Proteus spp.</i>	<i>Citrobacter spp.</i>	<i>S. aureus</i>
Amoxicillin	12	18	10	19	28	134
Ciprofloxacin	2	3	0	1	1	0
Nitrofurantoin	1	0	6	4	0	0
Nalidixic acid	17	2	16	15	2	NT
Cotrimoxazole	9	2	2	21	3	3
Gentamicin	4	0	1	1	0	5
Ceftazidime	6	0	7	0	0	98
Tetracycline	20	2	16	11	7	43
Erythromycin	NT	NT	NT	NT	NT	12

Keys: NT- Not tested

Table 3. Practice related to hygiene among meat handlers

Variables	Response	Total Shops n=121 n (%)	Bacterial growth shops n=181 n (%)	No bacterial growth shops n=19 n (%)	OR (95% CI)	p-value	MBC (log CFU/cm ²) (n=121)	95% CI	p-value
Do you wash your hands before handling and processing?	Yes	111(91.7)	76 (42.0)	8 (42.1)	Ref.	0.99	3.07±1.23	1.96 -2.72	<0.0001*
	No	10 (8.2)	105 (58.0)	11 (57.9)	0.99 (0.38-2.59)		5.41±1.31		
Do you wash your hands with soap and water every time you use the restroom?	Yes	93(76.8)	80 (44.2)	9 (47.4)	Ref.	0.79	4.30±1.76	-0.39 -0.43	0.92
	No	28(23.1)	101 (55.8)	10 (52.6)	0.88 (0.34 - 2.27)		4.32±0.96		
Do you wear gloves during slaughter?	Yes	89 (73.5)	88 (48.6)	12 (63.2)	Ref.	0.23	4.49±1.21	-0.29- 0.47	0.64
	No	32 (26.4)	93 (51.4)	7 (36.8)	0.55 (0.21-1.47)		4.58±1.34		
Do you handle carcasses when you have injuries to your hands?	Yes	105 (86.7)	112 (61.9)	6 (31.6)	Ref.	0.01*	4.46±1.61	0.57-1.49	< 0.0001*
	No	16 (13.2)	69 (38.1)	13 (68.4)	3.52 (1.28-9.68)		5.49±1.39		
Do you use an apron during the process?	Yes	117 (96.6)	132 (72.9)	12 (63.2)	Ref.	0.37	4.48±2.10	0.35-1.71	0.0031*
	No	4 (3.3)	49 (27.1)	7 (36.8)	1.57 (0.59-4.22)		5.51±1.91		
Do you clean the slaughter area and equipment daily?	Yes	119 (98.3)	90 (49.7)	14 (73.7)	Ref.	0.05	3.85±1.89	1.11-2.15	<0.0001*
	No	2 (1.6)	91 (50.3)	5 (26.3)	0.35 (0.12-1.02)		5.48±1.67		
Do you clean knife and chopping block before and after use?	Yes	83 (86.5)	53 (29.3)	17 (89.5)	Ref.	0.001*	4.45±1.65	0.39 -1.27	0.0003*
	No	38 (31.4)	128 (70.7)	2 (10.5)	0.05 (0.01-0.22)		5.28±1.23		
Do you store meat in the freezer?	Yes	110 (90.9)	99 (54.7)	13 (68.4)	Ref.	0.26	4.45±1.56	0.54-1.74	0.0003*
	No	11 (9.1)	82 (45.3)	6 (31.6)	0.56 (0.20-1.53)		5.59±2.52		
Do you use fly and rodent controllers?	Yes	98(80.9)	45 (24.9)	11 (57.9)	Ref.	0.004*	4.41±1.62	0.43-1.27	<0.0001*
	No	23 (19.1)	136 (75.1)	8 (42.1)	0.24 (0.09-0.64)		5.26±1.09		
Do you have a clean water supply in the shop?	Yes	56 (46.2)	99 (54.7)	16 (84.2)	4.42 (1.24-15.69)		4.45±1.75	0.63 -1.53	< 0.0001*
	No	65 (53.8)	82 (45.3)	3 (15.8)	Ref.	0.02*	5.53±1.23		

Keys: n=Number; MBC=Mean Bacterial Count; Ref.-Reference;*- significant values where p-value is less than 0.05

4. Discussion

Globally, food-borne associated hospitalization causes out-of-pocket health expenses. In this study, 90.5% of samples showed selected bacterial growth. However, similar studies from Pakistan (31), India (32), and Nepal (33) had low bacterial prevalence than ours. In contrast, the high prevalence was reported in Banepa and Dhulikhel, Nepal (34). Likewise, a study from Bhaktapur, Nepal noted 90.0% of buffalo and chicken meat were contaminated with human pathogenic bacteria (21). A study from Ghana reported beef meat contaminated with human pathogenic and coliform bacteria (35).

There are more than 200 zoonotic diseases that transmit from animal to human (36). *Listeria monocytogenes*, *Salmonella spp.*, *Yersinia enterocolitica*, *S. aureus*, *E. coli*, *Campylobacter jejuni*, *Clostridium perfringes*, and *Aeromonas hydrophila* are most commonly linked with meat-borne infections in human (33). A study in Ethiopia revealed that *E. coli* (65.0%) had the highest preponderance followed by *S. aureus* (59.0%) with *Shigella spp.* (4.3%) being the least one. MRSA among isolated *S. aureus* is 51.0% (37). On the contrary, in the current study, *S. aureus* was the most predominant bacteria followed by *E. coli*, *Salmonella spp.*, *Citrobacter spp.*, *Klebsiella spp.*, and *Proteus spp.* The highest number of bacteria was in buffalo meat followed by pork meat and goat meat whereas the least isolates from knives. The least frequency of bacteria in knives might be due to frequent cleaning and washing practice by butchers and metal and steel knives less suitable for bacteria growth than meat and wet, porous, and wooden blocks. Also, the mean bacterial count was higher in Buffalo meat (6.43 log cfu/cm²) followed by

pork (5.26 log cfu/cm²) and goat meat (5.04 log cfu/cm²).

A study from Ethiopia (19) depicted that 37.5% of the meat exceeded the limit set by the European Union (≤ 3.0 log/cm²) (26). Overall, the mean bacterial count was higher than the limit set by the European Union (26). A similar study from Mumbai, India showed a total viable count of 5.80 ± 0.17 log cfu/cm² (38). We have noted mean bacterial count in chopping blocks samples was 5.02 log cfu/cm², which was lower than reports from Tanzania (39) and Pakistan (31). Wooden blocks are rough and porous which also plays a pivotal medium for harboring microorganisms also cleaning with tap water is not efficient to remove microorganisms (40).

In this study, *E. coli* was mainly resistant to Tetracycline, Nalidixic acid, and Amoxicillin. *Salmonella spp.* was mainly resistant to Tetracycline and Nalidixic acid. *Citrobacter spp.*, resistant to Amoxicillin and Tetracycline. *S. aureus* resistant to Amoxycillin, Ceftazidime and Tetracycline. Likewise, 24.3% of isolates were multi-drug resistant and 10.2% of isolates were *S. aureus* methicillin-resistant. A similar study from Nepal found 32.7% of multi-drug resistant bacterial isolates mainly resistant to Amoxicillin, Tetracycline, Cotrimoxazole, and Nalidixic acid (21). In a study conducted on fresh carcasses, 71.4% of *Salmonella* isolates were resistant to two or more antibiotics; mainly resistance to Tetracycline and Erythromycin (41). Additionally, a study from China reported 19.7% contamination of retail meat with the highest in pork meat (37.3%) and whilst highest resistance for antibiotic Tetracycline, followed by Ampicillin (42). Antibiotic resistance developed in

bacteria from food supplements, during disease control, and treatment of livestock. Generally, a wide range of antibiotic regimens is used for the prevention and treatment of enzootic diseases. For instance, Ceftiofur, Tetracyclines, Tiamulin, Lincomycin, Enrofloxacin, Penicillins, Enrofloxacin, Macrolides, etc. are commonly used antibiotics in veterinary medicine (43). Thus, the residues of such antibiotics after treatment remain in them resulting in a higher risk of developing antimicrobial resistance. This, in turn, may have detrimental effects on human health while consuming them by increasing morbidity rate through low-dose exposure and most prominently enhancing antibiotic resistance (44). However, rational use of antibiotics, identifying sources of contaminants, improving hygiene, vaccination, and improving food safety could minimize sources of infection and drug resistance (45). It is expected that the number of antimicrobial agents use in livestock reaches 105,500 tons by 2030 globally (46).

Related to positive and negative responses to hygiene practices among meat handlers, there was significant relation ($p < 0.005$) recorded in responses to "Do you handle carcasses when you have injuries to your hands?", "Do you clean knife and chopping block before and after use?", "Do you use fly and rodent controllers?" and "Do you have a clean water supply in the shop?". In Pakistan, a study revealed that butchers had poor hygiene conditions and a lack of knowledge about disinfection (31). Poor handling and hygienic practices cause cross-contamination and re-contamination of bacteria in meat (47). We have found that those who had practice handling caresses while having hand injury had three times higher odds

(OR=3.52, CI=1.28-9.68) than those who did not. Similarly, a significant relation ($p < 0.05$) was seen in those who cleaned knives and chopping blocks before and after use, and those who use fly and rodent controllers and had a clean water supply. Generally, uneducated/untrained meat handlers, those working without protective clothes and following unhygienic processes increase the risk of cross-contamination (48). In Ethiopia, around three-quarters of meat handlers did not wash their hand with soap after visiting the toilet and did not wear mouth masks during handling carcasses (49). Furthermore, the partial implementation of national standards by factories resulted in the delivery of poor-quality of meat products to consumers (15). In Nepal, there is a substantial lack of periodic medical examinations among butchers; therefore, this allowed them to work while having purulent wounds (50) and poor hygiene practices (51). In Kathmandu, it is noted that meat hygiene practice by meat handlers was not satisfactory (52). Therefore, a high bacterial count was seen due to unhygienic practices in shops (48). Poor hygiene and sanitation contribute prominently to the transmission of deadly zoonotic diseases. In addition, the lack of appropriate specialized infrastructure and insufficient awareness related to meat handling and processing exacerbate meat microbial load and inadequate hygiene. Only 14.3% of slaughterhouses have maintained good environmental sanitation in Nepal (14). Moreover, contamination of raw meat during processing via knives, chopping blocks, benches, floor, contaminated water, and ambient temperature account for the rapid amplification of pathogens. It has been observed that bacterial growth increased by 1.9 times higher in the

evening in comparison to the morning also open-type shops have been found higher bacterial growth than closed-type shops (40). Therefore, strict implementation of food policies, periodic inspection by concerned authorities, vaccination, judicious use of antimicrobials, and training the butchers for safe and hygienic butchery may reduce the morbidity and mortality associated with food-borne diseases.

5. Conclusion

In conclusion, bacterial count in meat and butcher items is higher than the standard guidelines and nearly a quarter of isolates are MDR. Albeit, most of the hygiene-related standards are maintained, lack of proper practices concerned with the usage of uncleaned chopping boards, unavailability of clean and safe water, keeping meat in the open air, and handling meat during hand infection are major culprits for meat contamination. Proper execution of food hygiene practices and regular inspection by the concerned authority at regular intervals in slaughters and butchers' shops will contribute substantially to decreasing the risk of food-borne illnesses.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Acknowledgment

This study was supported by the Janamaitri Foundation Institute of Health Sciences, Hattiban, Lalitpur, Nepal.

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