Bacteriological Quality of Raw Meat, Antibiotic Susceptibility Pattern of Bacterial Isolation and Associated Risk Factors Among Butcher Houses of Adama Town, Oromia, Ethiopia, 2020

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Abstract

Background

Foodborne diseases continue to be an important public health problem globally and most of the food safety hazards are caused by foods of animal sources. It is also reported that contaminated raw meat has been identified as one of the main sources of foodborne illnesses. In Ethiopia, the widespread habit of raw meat consumption, lack of compliance with standard quality and sanitation protocols is a potential cause for foodborne illnesses**.** The purpose of this study was to assess bacteriological quality, antibiotic susceptibility pattern of bacterial isolation of raw meat and associated factors among butcher houses of Adama town, Ethiopia, 2019.

Results

Three-fourth (¾th) of collected raw meat was unacceptable bacterial load of Total Aerobic Plate count based on Gulf Standard. The average contamination was (5.89±0.86) log Colony Forming Unit per gram for total aerobic plate count. Raw meat collected from meat handlers who trained on meat hygiene (AOR=5.8, 95%CI:(1.99-17.34), collecting money (AOR= 0.14,95%CI (0.04-0.43) were associated with bacteriological quality of raw meat. Whereas, proportion of meat samples that were positive for Salmonella and shigella were (9.8% and 2.67%) respectively. The resistance of Salmonella was most frequently observed to Ampicillin (100%), Amoxicillin/ Clavunilic (54.5%), Tetracycline (36.3%) Trimethoprim-sulfamethoxazole (18.2%). Shigella expressed resistance to Ampicillin (50%) and 100% sensitive to the rest antibiotics used.

Conclusion

Bacterial logarithmic mean values were beyond the acceptable standard indication of poor hygiene, making it a potential source of food-borne infection. Therefore, stringent inspection, regular supervision, training and hygienic practices should be introduced to enhance hygienic quality meat for consumers.

Background

Meat refers to animal tissue used as a food mostly skeletal muscles and associated fat. It may also refer to organs including lungs, livers, brains, bone marrow, kidney and a variety of other internal organs (1,2). Meat is the major source of protein and valuable qualities of vitamins for most people in many parts of the world(3). Raw meat safety and quality can be estimated with the use of organoleptic, physical, chemical and microbiological process hygiene criteria(5).Furthermore testing against microbiological hygiene criteria provides a way of measuring how well the operator has controlled the production processes to minimize and control contamination(6). Microbial contamination of meat is a major cause of food poisoning and foodborne illnesses worldwide(7). An estimated 9.4 million illnesses are caused from foodborne diseases by known pathogens each year in the United States. Expectedly, according to the Centers for Disease Control and Prevention, in 2016, there were a reported 839 cases of foodborne disease outbreaks that resulted in 14,972 illnesses, 794 hospitalizations, 17 deaths, and 18 food product recalls(8). The most important food-borne bacteria transmitted through meat include Salmonella, Shigella, *Staphylococcus aureus*, *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Clostridium perfringens*, *Yersinia enterocolitica* and *Aeromonas hydrophila*.These bacteria usually cause self-limiting gastroenteritis however, invasive diseases and various complexities also may occur. *E. coli* can cause bloody diarrhea and hemolytic uremic syndrome, Salmonella can cause systemic salmonellosis, *S. aureus* is responsible for causing food poisoning, Shigella can cause dysentery and Vibrio can cause cholera if undercooked meat is consumed(9).Human illnesses caused by *Campylobacte*r are associated particularly with the consumption of chicken, Verotoxin toxin *E.coli* and salmonella are associated with beef and *Y. enterocolitica* are associated with pork(10). A study on the bacteriological quality of raw meat conducted in the united kingdom and Saudi Arabia revealed Tota Aerobic plate count was 6.11 log10CFU/gm and 6 log10CFU/gm in the United Kingdom and Saudi Arabia respectively (34).

Another study from France on raw pork meat that the contaminations were log-normally distributed with Enterobacteriaceae mean log counts ranging from 0.6 to 2.2 log10 CFU cm^{-2} and Pseudomonas log counts ranging from 1.1 to 4.4 log10 CFU cm^{-2}(35). In a cross-sectional study on 50 beef samples from the slaughter unit in Aizawland and revealed the total aerobic plate count of 6.13±0.09 log10cfu/g and 12% positive for *E. coli*(40).In 68 raw meat samples from butcher shops collected in Iran, reported total aerobic plate count that varied from 10^3 to 2.6×10^6CFU/g, Coliforms varied from <10^1 to 2.4×10^4CFU/g, *E. coli* varied from <10^1 to 3×10^2CFU/g and *S. aureus* 5×10^2CFU/g(41). A study conducted on *S. aureus*, *E. coli* and Salmonella from raw meat at abattoirs and butcher shops in different areas of the Lahore city,in Pakistan 51% of samples had Aerobic plate count more than acceptable value (42).Another studies from Asia, eastern Nepal show 84%,68%34% Coliforms,*S.aureu* and Salmonella species were isolated respectively from raw meat sold in retail shops(43).A study conducted on raw meat Sold in Sylhet Sadar, in Bangladesh Tota Aerobic count of the samples ranged between 2.5 × 10^5 to 2.25 × 10^5cfu/g and 28% were above acceptable value(44). A survey conducted on bacteriological quality and safety of raw beef from selected outlets in Windhoek,Namibia the overall prevalence of total plate count on the beef samples was 98.9%.Of these 98.9%, 26% samples were satisfactory,49 % samples were within acceptable level and 25% exceeded the acceptable level(12).In a study to investigate the microbiological quality and safety of beef from East Java Province, Indonesia 32.5% of the samples were contaminated with *E. coli* and 20.0% with *S. aureus*. The mean values of TAPC and *S. aureus* were 4.15 logCFU/g and1.39 logCFU/g respectively.About 29.66% of meat samples were found to exceed the maximum limit of TAPC based on the Food Safety and Standards regulation of India(28). In a study on the bacteriological quality of raw meat in Lafia metropolis,Nigeria shows mean Aerobic plate count,total coliform count and total staphylococcal count were 1.94×10^7cfu/g, 2.63×10^5cfu/g and 5.38×10^7cfu/g respectively(45). Another study on the assessment of the microbial quality of locally Produced Beef in Bolgatanga,Ghana 80% of the samples had a total aerobic count of more than 5log CFU/g.Similar study from North Africa, Morocco 23.8% of meat samples from butcher shops were above the recommended value set by WHO/FAO(20).

A similar study done on microbial assessment of retail beef meat in Meknes city,Morocco,the average contamination was 6.33 for TAPC,5.50 for TCC; 5.41 for FCC and 1.68 for *S.aereus* count in log CFU/g(20).A study done in Morogoro,Tanzania and Kenya meat handlers who receive training have good

hygienic practice than those counterparts(55,56).

A cross-sectional study conducted in the Federal Capital Territory of Nigeria showed knowledge and practice were influenced by previous training, whereas food handlers who had worked for long years had better practices of food hygiene(57). A study conducted in Mexico, Latin America on the prevalence and antibiotic susceptibility pattern of Salmonella on raw meat showed high levels of resistance to ampicillin (66.7% of isolates), tetracycline (61.3%), and chloramphenicol (64.5%) and low levels of resistance to cefotaxime (0%), gentamicin (3.2%), and kanamycin (4.3%) (26). A study conducted in the Wa Municipality of Ghana on Prevalence of Salmonella species isolated from raw meat and liver of cattle highly resistant to teicoplanin (96.77%) but susceptible to chloramphenicol (100%), ciprofloxacin (100%), tetracycline (100%), suphamethoxazole/ trimethoprim (100%), amoxicillin/clavulanic acid (93.55%), ceftriaxone (93.55%) and gentamicin (83.87%)(66).Another study in Addis Ababa, Ethiopia shows about 25% of Salmonella species were found resistant to ampicillin. Besides, 9% of Salmonella species and 2% of *E. coli* O157:H7 isolates were found to be resistant to ceftriaxone(15). A study done in Addis Ababa, Gullele sub-city *E. coli* isolates were observed to be the most resistant to penicillin (60%) followed by Amoxicillin (40%) and Ampicillin (40%) and none of the isolates were resistance for chloramphenicol. All isolates of *Salmonella* (100%) were resistant to penicillin and Vancomycin. And also 66.67% of the isolates were resistant to Ampicillin. None of the isolates were resistant to Ciprofloxacin(67).A study conducted in Jimma,Ethiopia on microbial flora and food borne pathogens on raw meat revealed Shigella was 100% resistant to co-trimoxazole and tetracycline whereas from the two Salmonella isolates one was susceptible against all 12 tested antimicrobials, while the other to all the 11 except cephalexin(68). A cross-sectional study done on prevalence of Salmonella in raw meat in dukem town Ethiopia, All the isolates (100%) were sensitive to Kanamycin where as 94.4%, 88.9% and 83.3% of the isolates were found to be sensitive to Sufisoxazole, Tetracycline and Nalidixic acid, respectively(69). In Ethiopia, there are a number of reports on foods including meat to have high incidence of bacteria(15),nonetheless, there are limited reports on the health implication of food borne diseases from raw meat that are displayed in open-air for sales.Thus,the objective of this study is to assess bacteriological quality of raw meat in order to assess the risk to public health, specifically via the enumeration of microbiological process hygiene criteria.

Results:

A total of 112 study participants were involved, making a response rate of 112/119(94%).The mean age of the participants was (32.83±8.31) years (table 1).

Table 1

Variables	Frequency		%	Mean ±SD	Range
Age					
	≤20	12	10.7		
	21-30	32	28.6	32.830±8.3	17-54
	31-40	47	42		
	41-50	20	17.9		
	>51	1	0.9		
Marital status					
	Married	76	67.9		
	Single	30	26.8		
	Others*	6	5.4		
Religion					
	Orthodox	71	63.4		
	Muslim	22	19.6		
	Protestant	19	17		
Level of education					
	Illiterate	3	2.7		
	Primary	56	50		
	Secondary	37	33		
	Diploma	10	8.9		
	Degree	6	5.4		
Working experience					
	<5	29	25.9		2-20
	5-10	32	28.6	9±4.543	
	>10	51	45.5		
Meat safety training					
	Yes	45	40.2		
	No	67	59.8		
Medical check up					
	Yes	36	32.1		
	No	76	67.9		

*divorced, widowed

Sixty nine (61.6%) of butcher shops wall and ceiling made of Ceramic. None of meat handlers weared hand glove. Eighty five point seven percent of meat handlers did not wear head cover. Moreover, sixty four (57%) of the butcher shops have no cashiers and they collect money while handling meat (Table 3).

Table. 2

Variable	Frequency		Percentage (%)
Butcher shop wall and ceilings made of			
	Ceramic	69	61.6
	Concrete	31	27.7
	Others*	12	10.7
Butcher shop wall and ceilings free of dusts and spider web			
	Yes	42	37.5
	No	70	62.5
Meat handlers wear white coat			
	Yes	61	54.5
	No	51	45.5
Meat handlers wear head cover			
	Yes	16	14.3
	No	96	85.7
Meat handlers wear glove			
	Yes	-	-
	No	112	100
Handling money while selling meat			
	Yes	64	57
	No	48	43
Wear Jewelers			
	Yes	44	39.3
	No	68	60.7

*Earthen materials, Aluminum

Meat handlers and meat hygiene knowledge

Overall knowledge level of respondents about personal hygiene, cross-contamination and transmission of food borne diseases summarized in table 3.

Table. 3

	Statements on meat hanling practices	Write No %	Wrong	Do not know
1	Improper handling of meat could pose health hazards to consumers?	112(100)	0	0
2	D o you know insects and pests could be a source of contamination to meat?	101(90.2)	8(7.1)	3(2.67)
3	Do you know regular washing of hands during meat processing reduces risk of meat contamination?	109(97.3)	3(2.67)	0
4	Do you know using gloves while handling meat reduces the risk of meat contamination?	53(47.3)	42(37.5)	17(15.2)
5	Do you know washing and disinfection of butchery utensils reduces the risk of meat contamination?	110(98)	2(2)	0
6	Do you know microbes be in the skin, nose and mouth of health people?	64(57)	23(20.5)	25(22.3)
7	Do you know people with open skin injury, gastroenteritis, and ear or throat diseases should not be allowed to handle meat?	88(78.6)	24(21.4)	
8	Do you know the health status of meat handlers should be checked before employment?	62(55.3)	11(9.8)	39(34.8)
9	Do you know meat handlers with wounds or injuries on their hands must not touch or handle meat?	21(18.8)	28(25)	63(56.2)
10	Do you know regular rotation of disinfectants for cleaning reduces the risk of meat contamination from working surfaces and cutting material?	110(98)	2(2)	0
11	Do you know diarrheal disease can be transmitted by food?	94(83.9)	6(5.4)	12(10.7)
12	Do you know contaminated raw meats transmit food borne pathogens to humans?	99(88.3)	2(2)	11(9.8)
13	Do you know high temperature or freezing is a safe method to destroy bacteria?	108((96.4)	0(0)	4(3.6)
14	Do you know eating and drinking in the work place increase the risk of meat contamination	74(66)	20(17.9)	18(16.1)
15	Do you know cross contamination is when microorganisms from a contaminated meat are transferred by the meat handler's hands or utensils to another?	100(89.3)	7(6.3)	5(4.5)
	Total	87.5	11.8	0.7

Table. 4

	Meat safety practices questions	Responses No (%)	
		Yes	No
1	Do you wash your hands before and after handling meat?	109	3
2	Do you use gloves while handling meat?	0	112
3	Do you smoke inside meat processing areas?	0	112
4	Do you wash hands after handling waste/garbage?	112	0
5	Do you wash hands after using toilet?	112	0
6	Do you wear a gawon while working?	72	40
7	Do you wear hair cover while working?	21	91
8	Do you frequently clean the meat storage area before storing new products?	88	24
9	Do you use the sanitizer when washing service utensils (knives, hooks and cutting boards)?	99	13
10	Do you replace knives or sterilize them after meat processing?	59	53
11	Do you remove your gown when using toilets?	108	4
12	Do you remove your personal stuffs such as rings, watch while processing meat?	74	38
13	Do you handle/process meat while you are ill?	55	57
14	Do you collect money while handling meat?	52	60
15	Do you eat or drink at your work place?	66	46
16	Do you wash your hand after sneezing or coughing?	60	52
17	Do you process meat when you have cuts, wounds, injuries on your hands?	58	54

Bacteriological quality of the raw meat:

Raw meat samples collected form butcher shops during the study period 85/112 (75.89%) have unacceptable bacteriological quality based on gulf standard. The Enumeration of the TAPC ranged between 3.70log10cfu/g to 7.43log10cfu/g with an average count of 5.89log10cfu/g. Enumeration of TCC ranged 2.73log10cfu/g - 5.76log10cfu/g with an average of 4.27log10cfu/g, whereas FCC and TSAC had mean of 3.16cfu/g and 3.02cfu/g respectively.

Table. 5

Microbial Indicators	Minimum count(log10cfu/g	Maximum count(log10cfu/g)	Mean±SD	Gulf Standards Maxpermissible count(log10cfu/g)
TAPC	3.70	7.43	5.89±0.864	5 log10cfu/g
TCC	2.77	6.67	4.27±0.73	4 log10cfu/g
FCC	0	5.67	2.77±1.37	3 log10cfu/g
TSAC	0	5.91	3.02±1.54	3 log10cfu/g

In bivariate analysis, training of meat handlers, practices such as wearing white coat, head cover, collecting money and washing using sanitizer were significantly associated (p-value less than 0.25) with overall bacteriological quality of raw meat and moved to multivariable logistic regression model. However in multivariable logistic regression model training and collecting money while handling meat were significantly associated (p-value less than 0.05) with bacteriological quality of raw meat in the butcher shops.(Table 6).

Table. 6

Variables	Bacteriological quality of raw meat		COR (95%CI)	AOR (95%CI)	p-value
	Acceptable No (%)	Unacceptable No (%)			
Receive training	20(17.85)	25(22.3)	6.8(2.56-18.34)	5.8(1.99-17.34)	0.001*
Yes	7(6.25)	60(53.57)	1.00	1.00	
No					
Wear white coat	18(16%)	43(38.4)	1.9(0.78-4.88)	1.3(0.41-4.32)	0.6
Yes	9((8)	42(37.5)	1.00	1.00	
No					
Wearing head cover	7(6.25)	9(8)	2.95(0.98-8.91)	2.2(0.5-9.6)	0.26
Yes	20(17.85)	76(67.8)	1.00	1.00	
No					
Collect money	6(5.4)	58(51.78)	0.13(0.045-0.36)	0.14(0.04-0.43)	0.01*
Yes	21(18.75)	27(24)	1.00	1.00	
No					
Washing using sanitizer	26(23.2)	73(65)	4.21(0.5-34)	1.9(0.21-17.7)	0.54
Yes	1(0.9)	12(10.7)	1.00	1.00	
No					

**p-value<0.05, Crude odds ratio Adjusted odds ratio*

In this study, from a total of 112 samples,11(9.8%) of them were designated as positive for the presence of Salmonella species. Whereas only 3/112 (2.68%) sample was shown to be positive for presence of Shigella and Salmonella species

Figure. 2

Table. 7

Bacterial isolates	Patterns	Antimicrobial Agents							
		APX (10µg)	AMX\C (20\10µg)	SXT 12.5µg\23.75	TET (30µg)	CPX (5µg)	ERY (15µg)	GEN (10 µg)	CFX (30µg)
Salmonela	S	-	5(45.45%)	11(81.8%)	7(63.6%)	11(100%)	11(100%	11(100%)	11(100%)
	I	2(18.2%)	-	-	-	-	-	-	-
	R	9(81.8%)	6(54.5%)	2(18.2%)	4(36.3%)	-	-	-	-
Shigella	S	1(50%)	2(100%)	2(100%)	2(100%)	2(100%)	2(100%)	2(100%)	2(100%)
	I	-	-	-	-	-	-	-	-
	R	1(50%)	-	-	-	-	-	-	-

APX:ampicillin,AMX\C:amoxicillin\Clavunilic,SXT:trimethoprim,sulfamethoxazole,TET:tetracycline,CPX:ciprofloxacin,ERY:erythromycinGEN:gentamycin,cefx s:sensitive,I:intermediate,R:resistant.

Discussion

Overall study participants (59.8%)(95% CI: 50,69) of meat handlers had not taken training on safe meat handling and personal hygiene. Similar with study done in Mekelle, Ethiopia, where 58.4% of meat handlers had not taken trainings related to personal hygiene and meat handling(78). Even though regular medical examination is recommended for food handlers by WHO, In this study Seventy three (65.2.%) (95% CI: 57.1, 74.1) of meat handlers did not have evidence of medical certificate. This study confirms that although there exist personnel medical health requirements in Ethiopia there is very little attention given to their implementation and enforcement in a food enterprise like butcher shops. Therefore, there is a high possibility of the meat handlers contaminating meat with microorganisms(79).Handling of meat and money with the same unwashed hands is one sources of meat contamination. Results of this study revealed sixty four(57.1%)(95% CI:48.2,66.1) of the meat handlers handled money (papers/coins) which may result into cross contamination of meat with microbes. Similar study in Mekelle, Ethiopia 91.7% of the meat handlers collect money while serving meat(78).According to compliance study based on gulf standard raw meat in this classifies 85/112(75.89%) (95% CI: 67.9,83.9) of meat have unacceptable bacteriological quality(80).Comparable findings were also obtained in meat retail shops of Meknes City, Morocco, reported a total aerobic plate count of 67% in beef produced and marketed with unacceptable quality(81).It is higher than in study done in Sylhet Sadar, Bangladesh in which 28% of meat were un acceptable quality(44).However, it is lower than study done in bahir in which all samples or hundred percent unacceptable(11). According to food and agricultural organization Total aerobic plate counts exceeding 5.0 log10 on fresh meat are not acceptable and alarm signals on meat hygiene(16).

The average TAPC was 5.89log CFU/g (95% CI: 5.7,6.1).Finding of this result is higher than East Java,Indonesia where mean of TAPC was 4.158 CFU/g and Chennai city, India(4.78log10)(28,82).However it is less than Addis Ababa,Ethiopia (6.44 log CFU/g) (31). The variations of bacterial load observed in different studies might be due to lack of good processing, handling practices,sampling and sanitary standard operating procedures of meat handlers.Raw meat collected from butchers who trained on meat safety hygiene was 5.8 times more likely to be acceptable than those who did not receive training(AOR=5.8,1.99-17.34).This is because training of food handlers about the basic concept and requirements of personal hygiene and its environment plays an important part in safeguarding the safety of products to consumers(83).Regarding collecting money,in the current study,raw meat which were collected from butcher SHOPS in which meat handlers handle money while selling meat was 86% less likely to be acceptable than their counter part(AOR=0.14,0.04-0.43).According to WHO/FAO report, handling of foods with bare hands result in cross contamination and high microbial load. Furthermore, WHO recommends food handlers should be educated, encouraged or supervised to stop their business promptly if at any time, they suffer from diarrhea, vomiting, fever, sore throat or have visibly skin lesions.Eventhough it is not independent predictor in this study,fifty percent of meat handlers had practice of working while they were ill. With regard to contamination by Total coliform, the average is 4.27 logCFU/g (95% CI: 4.1,4.4). This value is higher than that of commercial beef meat in Tanzania (4.13log CFU/g) and in India (2.07 log CFU/g) but lower than that found in Lafia metropolis, Nigeria(4.19)(45). Variations in total coliform counts among studies may be due to differences in storage conditions and season in which samples were collected. The average contamination of meat by Feacal coliform is 2.77log CFU/g, (95% CI: 5.7,6.1) it The result is lower than that of retail beef meat in Algeria (3.41 log CFU/g and higher than in beef meat of Namibi(1.70 log10CFU/g)(84).

The data in the present study indicate that 81(72.3%) of samples collected in the town showed contamination with faecal coliforms. Presence of faecal coliforms suggests faecal contamination which is normally associated with poor hygiene and faulty sloughtering.It also suggests the possibility of finding enteric pathogens such as salmonelll,shigella and others(81). The average contamination of meat by Staphylococcus aureus is 3.14logCFU/g, (95% CI: 2.9,3.3).This value is higher than that of commercial beef meat in Chennai city, India (2.07log10)(28).However, it is Lower than study done in Bahir dar, Ethiopia(11).The highest number of *S.aureus* on meat indicates the presence of cross-contamination, which usually related to human skin, hair, hand and discharge from nose, and clothing(61). Concerning the prevalence of pathogenic bacteria Salmonella was detected in 11(9.8%) of analyzed samples. This finding reveled that there was a considerable rate of contamination in the butcher SHOPS of Adama town, which potentially poses a risk of causing food-associated illness. The prevalence reported in the current study is higher than other reports such as in United State of America (6%).However, the result of this study was much lower than that found in Senegal (87%) and Bahir Dar (70%)(11,85).This difference possibly arises from the source of animals, types of samples, and sampling technique. With regard to the antimicrobial susceptibility profiles of Salmonella isolates revealed a higher rate of resistance against Ampicillin9(81.8%) and Amoxicillin6(54.5%).These findings is in agreement with (86),where salmonella was 100% resistant to Ampicillin. An intermediate resistance of 2(18.2%) was also found for Ampicillin. On the other hand, Interestingly all of the isolates were 100% susceptible to

gentamycine,ciprofloxacin,tetracycline and ceftraxione.This is in line with the study conducted in Ghana(66).In addition 7(63.6%) and 9(81.8%) exhibited susceptibility to Tetracycline and Trimethoprim-sulfamethoxazole respectively. This result is also in aligning with study done in Gondar, Ethiopia. However, resistance to Ampicillin is much higher than in Bahir dar(23.8%) (11,25).

In other way the study revealed an overall Shigella prevalence of 2.67% which is higher than study done in Jimma,Ehiopia but, lower than in Karachi,Pakistan and Gondar (7,68,79), however, similarly lower rate of isolation was reported from Ebony,Nigeria (1). The antimicrobial susceptibility profiles of the isolates revealed resistance against ampicillin 1(50%), but, all the isolates were sensitive to Amoxicillin, Tetracycline, and Trimethoprim-Sulfamethoxazole, Erythromycin gentamycin, Ciprofloxalin and ceftraxione. However, higher rate of resistance against ampicillin was observed in Gondar(79).Differences in the geographical location of the isolates or the emergency of drug resistant strains could partially explain this discrepancy.

Conclusion

Compliance study based on gulf standard 75.9% of raw meat collected from butcher shops have unacceptable bacteriological quality. Under observation more than half of meat handlers in the butcher shops handle money with their bare hands while processing of meat and serving of custom. The following areas are need big attention and concern for future suggestion to**:** Adama town ablators enterprise should get training on how to cope with good handling practices on the use of proper clothing such as hand gloves, head covers, clean white coat and dedicated cashier to collect money. Adama health bureau should regular be inspected butcher shops in the town. Owners of butcher shops should be dedicated cashier in order to collect money and consumers refrain from eating raw meat appropriately to avoid intoxication and infection due to microbes. Further investigation should be carried out to isolate and characterize the bacterial load of raw meat along with meat production chain.

Methods

The study designed to assess bacteriological quality, its associated factors and antibiotic susceptibility pattern of the isolated raw meat from butcher shops of Adama Town from October 1 to December 2019, Oromia Ethiopia.Study design was Cross-Sectional study design conducted from October 1 to December 30,2019.Source population was all the butcher shops in Adama town. Study population was all butcher shops in which cattle meat were sold. Study unit was butcher shops in which sample were actually collected. Inclusion criteria was Butcher shops which used to sell meat of cattle originally and exclusion criteria was Butcher shops in which goat's and sheep's meat sold; Butcher shops which closed and shifted their task during the study period and non-volunteer.

Table. 8

Microbial groups(CFU/g)	Acceptable	Borderline	Unacceptable	Potentially hazardous
Tota aerobic plate count	<4log	4log_5log	>5log	NA
Total coliform count	<2log	2log__4log	≥4log	NA
Total fecal coliform count	<2log	2log__3log	≥13log	NA
S.aureus count	<2log	2log__3log	3log_4log	≥4log
Pathogens	___	___		Detected in 25 gram

Sample size and sampling technique

All 119 butcher shops which were working during study period were included in the study and simple random sampling method was employed to select meat handlers for interview.

Data collection tools

A pre-tested structured questionnaire initially developed in English and then translated in to local language translation expert and then translated back to English by another person to check its consistency. The questionnaire structured into three distinct parts including demographic information such as respondents' sex, age, years of experience, medical checkup and attending meat safety training. The second section of the questionnaire is about meat safety knowledge.

Questions on knowledge referred to mainly about their personal hygiene, cross contamination and temperature. It contains 15 close ended questions and each question has three optional answers ("Yes", "No" and "I do not know").The response was analyzed as categorical variables (right or wrong answer). A score of one was given to right answer and zero to the wrong and I do not know answer. The last section dealt with meat hygiene practices. The question comprises the issues of personal hygiene, hand washing practices, practices against food borne diseases and cross contamination. This section had 17 questions with two possible responses: "yes", and "no". Each correct practice reported scored one point(51).

Observational check list

Observational check list was developed after reviewing relevant literatures to assess the butcher shops' hygienic status and practice. The check list incorporated personal hygiene of meat handlers and hygienic conditions of the butcher shops' premises.

Data collection procedure

Face-to-face interview and the general sanitary condition of the butcher shops as well as the workers were observed. After finishing of questionnaires one hundred gram of raw meat sample was collected for laboratory investigation.

Data quality assurance

The data collectors were selected based on their educational background (two environmental health) and the selected data collectors were trained on the purpose and objective; benefit of the study, individual's right, informed consent and techniques of the interview for one day. Daily check-up of data completeness were made by the principal investigator.

The questionnaire was pre-tested on 5% of butcher shops in Olanciti town neighboring town 25km to Adama town before the study. The structured questionnaire was then rephrased in the light of the responses.

Statistical analysis

Before analysis, data were checked for completeness, consistencies and entered into computer using Epi info version 7.2.3.1software. Then the data was exported to SPSS version 25, coded, categorized, sorted and cleaned to facilitate analysis. Descriptive statistics was computed for the study variables and frequency distribution tables were used to describe most of the findings. All bacterial counts were normalized to CFU/g and converted into Log10 values. Mean and standard deviation were also computed. Variables with p-value less than 0.25 in binary logistic regression analysis were entered to binary multiple logistic regression using Enter methods to determine factors independently associated with bacteriological quality of raw meat. Odds ratio with their 95% confidence intervals were computed to identify the presence of association and statistical significance were declared if p value is < 0.05.All other assumptions of the analysis like normality of variables were checked. Odd Ratio was considered to assess the strength of association between dependent and independent variables.

Laboratory work:

One hundred gram of pooled raw meat cuts from leg area, limb area and flank area of hanging display for sale were collected from butcher SHOPS in a sterile zipped plastic bag in an icebox(70).The samples were collected in the morning (9:00am-10:00am), After labelling properly, they were keptin an ice box between 2-4ºC and were immediately transported to Adama public health research and referral laboratory centre in Adama town Oromia, Ethiopia.

The samples were analysed immediately upon arrival in the laboratory. From 100g of grinded and homogenized meat 25g was weighted and placed in 225 ml sterile 0.1% buffered peptone water. The grinded meat and diluent were thoroughly vortexed on a platform shaker for 5 minutes to wash off and dislodge any microbe that may be resident on the surface of the meat. The mixture was considered to be a 10^{-1} dilution. The mixture (1ml) were transferred to a tube containing 9 ml of normal saline diluent to make 10^{-2} dilution. Further dilutions were made by transferring 1 ml of the succeeding dilutions to the tubes containing 9 ml diluent up to 10^{-6}. After preparation, bacteriological analyses of the samples were performed to assess the selected microbial attributes such as Total aerobic plate count, Total coliform count,fecal coliform count and Total *staphylococcus aureous* count (TSC) in cattle meat by using Plate Count (PC) agar, MacConkey (MC) agar and Manitol salt agar(28).All the media used were from Hardy diagnostic, America.

Determining Total aerobic plate count and Judging Meat Quality

For the enumeration of total aerobic bacteria in raw meat samples conventional standard plate Count method was used. Tenfold serial dilution up to 10^{-6} was made from the homogenized sample. One mL from each serial dilutions(10^{-3},10^{-4},10^{-5} and10^{-6}) of the test sample was pipetted into sterile Petri dishes and then molten, cooled nutrient agar was added and incubated for 24h at 37°C.Plates with colonies lying between 30-300 were counted using colony counter (TT20,Techmel, USA) and the average count was calculated and expressed as logCFU/gm(11,71,72). After determining TAPC by counting each visible colony of bacteria, the Quality of each raw meat samples were judged based on Guideline levels for determining microbial Quality of ready-to eat food(Gulf Standards).Meat samples of TAPC <5log10CFU/gm were acceptable and > 5log10CFU/gm were unacceptable(28,73).

Enumeration of Total coliforms and fecal coliforms:

For the TCC and FCC 0.1ml of each of dilution from 10^{-1},10^{-2} and 10^{-3} was transferred

and spread on triplicate on MacConkey agar. Then plates were incubated at 37 °C and 44ºC for 24 hours for TCC and for FCC counts respectively. Enumeration of the TCC and FC (typical pink colonies resulting from the fermentation of lactose)(74). For Staphylococci aures count, mannitol Salt Agar (MSA, HARDY DIAGNOSTIC) was surface plated with 0.1ml of the homogenate from duplicates of 10^{-1}& 10^{-2}.The inoculum was evenly spread on the surface of the agar and allowed to dry for 15 min at room temperature. The plates were inverted and incubated for 24 to 48h at 37ºC.Typical colonies of *Staphylococci aures*(golden yellow colonies shining and convex) after 24 hours' incubation were isolated, purified and tested for catalase and coagulase positive as a confirmatory test(75).

Isolation of Salmonella and Shigella

For the isolation of Salmonella and shigella samples were pre-enriched in buffered peptone water (incubated aerobically at 37^0c for 24), followed by secondary enrichment in selenite cysteine broth(incubated aerobically at 37^0c for 24) and plated on to XLD incubated aerobically at 37^0c for 24 ,The suspected colonies were sub-cultured on the blood agar and incubated at 37 °C for 24hr. Further identification was made with triple sugar iron agar (TSI),urea broth, lysine iron agar (LIA),citrate broth and then incubated for 24 to 48 hours at 37^0C.All biochemical test reagents were obtained from Hardy diagnostic, America (11). Antimicrobial susceptibility tests were performed using the modified Kirby-Bauer disk diffusion technique(76).Bacterial suspension turbidity was adjusted to 0.5McFarland standard.

A sterile swab stick was immersed into bacterial suspension and spread on surface of Muller-Hinton agar Commercially available antibiotic disks Amoxicillin/clavunilic acid(20/10µg),ceftriaxone(30µg), erythromycin(15µg),trimethoprim-sulfamethoxazole(12.5/23.75µg),tetracycline(30µg), gentamycin(10µg),ampicillin(10µg) and ciprofloxacin (5µg); all were from Hardy diagnostic, America were used. Antimicrobial agents were selected based on clinical significance and literature data search. The results were interpreted using Clinical Laboratory Standards Institute (CLSI), 2019 guideline. *coli* (ATCC 25922) was used as quality control organism for the Antimicrobial susceptibility testing(77).

Culture Media quality control

Quality of culture media was maintained after checking its expiration date and preparation according to manufacturer instruction by sterilizing at 121 °C (15 lbs. sp) for 15 minutes. Sterility of culture media were also checked using strains kept for quality checking at APHRRLC.To exclude lab contaminants and check whether media and diluent completely sterilized, a representative number of a plate with media and broth without the test sample were incubated at 37 °C for 48hours. If any growth observed on control media, this batch will be discarded and another media will be replaced. Gram staining reagents were also checked for their expiry dates of each reagent, their storage condition, and checked with known quality control organisms (ATCC, American type culture collection Organism) before performing study samples.*S.aureus* ATCC 25923,*E.coli* ATCC 25922, Shigella flexineri ATCC 12022 and Salmonella typhimurium ATCC 14028 were used as quality control reference strains.

Abbreviations

AHMC: Adama hospital medical college;

APHRRLC: Adama public health research and referral laboratory center;

ASP: Antimicrobial susceptility pattern;

BPW: Buffered peptone water**:**

CFU: Colony forming unit;

EU: European Union:

FAO: Food and agricultural organization:

FBI: Food-borne illness;

HACCP: Hazard analysis critical control point;

MSA: Manitol salt agar;

NA: Nutrient agar;

NSS: Normal saline solution;

OR: Odd-ratio

ORHB: Oromia regional health bureau:

PCA: Plate count agar;

SPC: Standard plate count;

TAPC: Total aerobic plate count;

TCC: Total coliform count;

TSI: Triple sugar iron;

WHO: World Health Organization

Declarations

Availability of data and materials

The datasets examined during this study is available from the both authors corresponding and others authors on sensible inquiry.

Ethics approval and consent to participate

The consent form for study participants was obtained and approved by the ethics committee in written form at each stage of concerned sectors as follow: First, ethical clearance was obtained from Adama Hospital Medical College Institution Review Board (IRB). An official supporting letter was written by Adama Hospital Medical College to Oromia Regional Health Bureau for an ease of the study process and permission. Oromia Health Bureau was writing supporting letter to Adama Town Administration and then Adama Town Administration wrote supporting letter to selected Butcher shops in Adama Town. The purpose and benefit of the study along with their right to refuse were explained to all butchers available during data collection period. For those Potentially Hazardous Pathogens obtained during laboratory investigation, immediately re-inspect the butcher shops and take an action to solve the problem.

Availability of data and materials

The datasets examined during this study is available from the both authors corresponding and others authors on sensible inquiry.

Consent for publication

There is no identifiable details on individual participants reported in the manuscript, so, consent to publish is not required**.** Not applicable.

Competing Interests

We as the authors declare that they have no competing interests.

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Authors' information:

Aschalew Abebe, Godana Arero and Taklu Shiferaw contributed equally to this work.

Authors' contributions

AA proposed study, secured funding, collected data, performed the experiments and analyzed data and wrote results. GA Participated in advising, supervising overall process of project, and edited the manuscripts. TS participated in title selection, participated in advising, supervising overall process of project. All authors have read and approved the final version of the manuscript.

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Supplemental Information Note

The additional files mentioned at the end of the paper were omitted by the authors in this version of the submission.

Figures

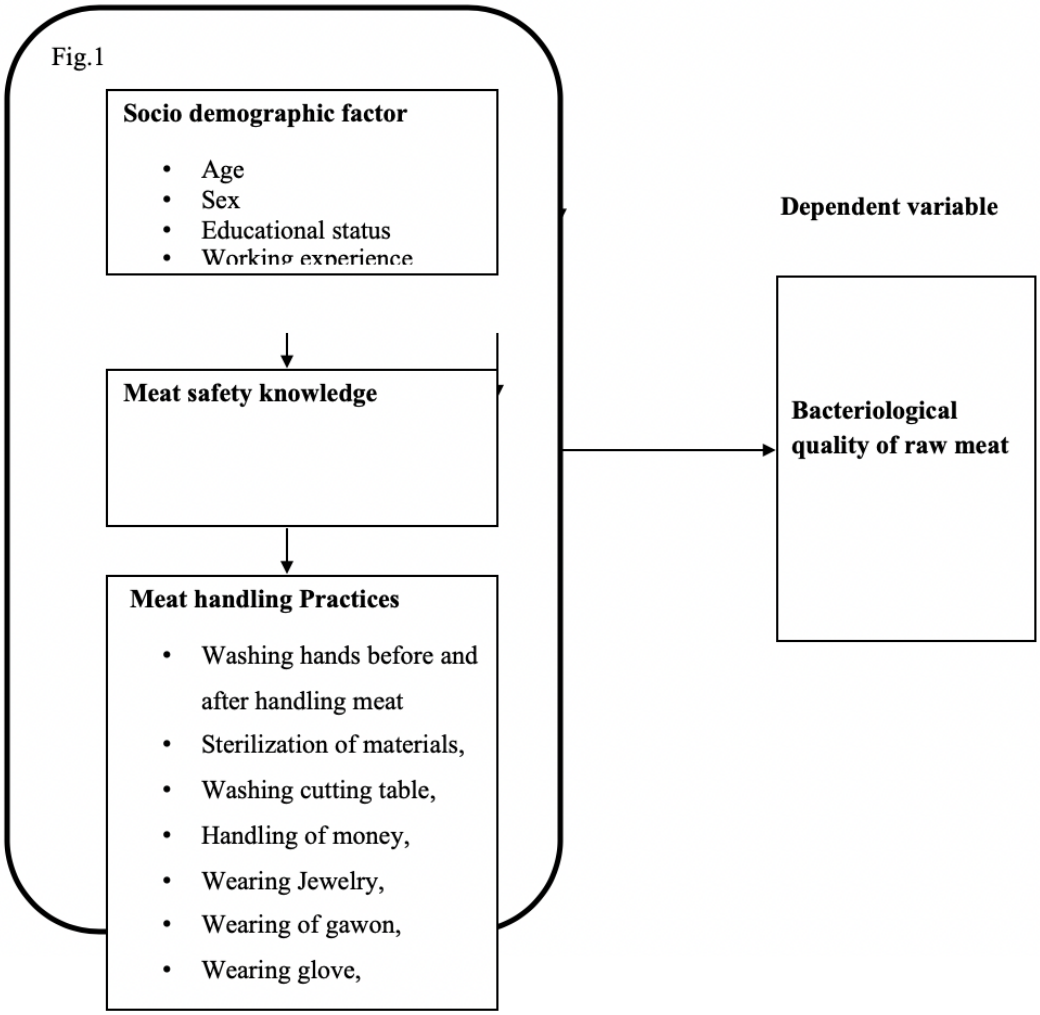

Figure 1

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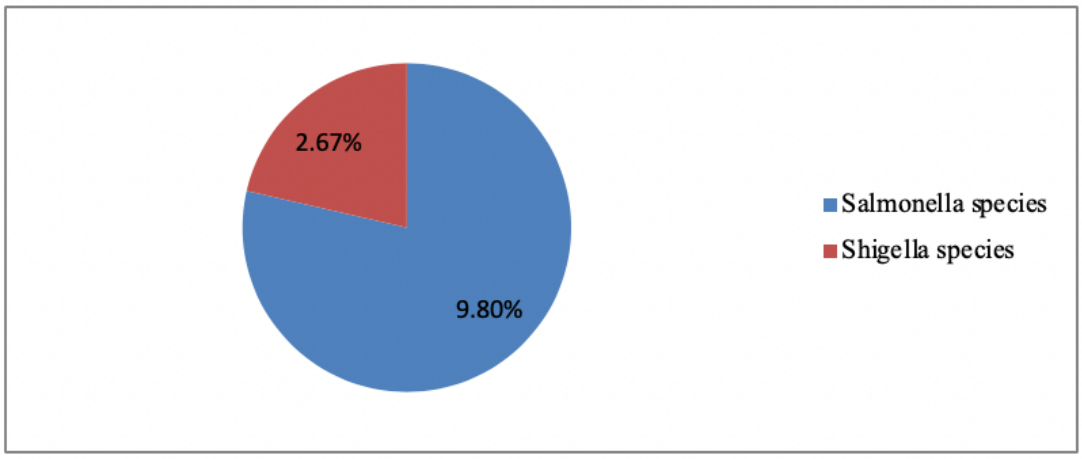

Figure 2

A caption was not provided in this version of the paper.