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Butcher-house related factors, Antibiogram signatures, and Bacteriological quality of raw meat, sold in Adama town, Ethiopia

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Highpoints

- Facility related quality of raw meat sold in Adama town, Ethiopia.
- Bacteriological assessment of raw meat sold in Adama town, Ethiopia.
- Observed meat contamination with resistant bacteria pathogens warranta the design and implementation of more effective intervention

Abstract:

Background: Foodborne diseases, mostly of animal sources (contaminated raw meat), remain an important local and global public health challenge worsened by the widespread preparation and consumption of raw meat under poor hygiene settings in Ethiopia**.** The study was designed to determine the Butcher-house related factors, Antibiogram signatures, and Bacteriological quality, of raw meat, sold in Adama town, Ethiopia, in 2019.

Methods: In this cross-sectional study, 112 consenting recruited meat handlers working in the butcher houses of Adama town were given interviewer administered pretested, structured questionnaires to assess their knowledge and practices on meat handling. Pooled raw meat cuts from hanging display for sale were processed and the quality of raw meat was determined using a serial dilution of total aerobic plate count, Total coliform, and total staphylococci count. The Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility patterns of the isolated bacteria and the statistical significance of the findings was calculated accordingly. Descriptive statistics was computed and frequency distribution tables were used to describe most

of the findings. All bacterial counts were normalized to colony forming unit/gram and converted into Log10 values. 95% confidences interval (CI) were used to determine factors independently associated with bacteriological quality of raw meat.

Results: Three-fourth (¾th) of collected raw meat yielded an unacceptable bacterial load of total aerobic plate count based on Gulf Standard. The average contamination was 5.89±0.86, 4.27±0.73, 2.77±1.37, 3.02±1.54 log colony-forming unit per gram for total aerobic plate count, total coliform count, fecal coliform count, and total Staphylococcus aureus count respectively. 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 the bacteriological quality of raw meat. The proportion of meat samples that were positive for Salmonella, Staphylococcus aureus, and shigella were 9.8%, 36%, 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%). Staphylococcus aureus was resistant to Ampicillin (86%), Amoxicillin/ Clavunilic(70%), ciprofloxacin,(84%) whereas Shigella expressed resistance to Ampicillin (50%) and was 100% sensitive to the rest antibiotics used.

Conclusion: Meat hygiene training for handlers at the Bucher-house significantly impacted the bacteriological quality of meat studied. Salmonella species, Staphylococcal species and shigella significantly resisted major groups of antibiotics (penicillins, quinolones) used in this study. 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 the hygienic quality of meat for consumers.

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Introduction:

Meat is the major source of protein and valuable qualities of vitamins for most people in many parts of the world(1). Raw meat safety and quality can be estimated with the use of organoleptic, physical, chemical, and microbiological process

hygiene criteria(2). 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(3). Microbial contamination of meat is a major cause of food poisoning and foodborne illnesses worldwide(4). An estimated 9.4 million illnesses are caused by 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 products recalls(5).

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 due to: *E. coli* O157 can cause bloody diarrhea and hemolytic uremic syndrome, Salmonella can cause systemic salmonellosis, *S. aureus* is responsible for causing food poisoning and Shigella can cause dysentery (6). Typically, the meat of healthy animals is sterile; however, contamination may occur due to poor infrastructural facilities in slaughterhouses, unhygienic vending operations and poor handling of carcasses attribute to the high bacterial load in meat(7).

In 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 (8). Other 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(9). In 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 this 98.9%, 26% samples were satisfactory,49 % samples were within an acceptable level and 25% exceeded the acceptable level(10).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*. In 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. In a similar study from North Africa, Morocco 23.8% of meat samples from butcher shops were above the recommended value set by WHO/FAO(11).

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(12). According to Guideline levels for determining the microbial quality of ready-to-eat food (Gulf Standards), the aerobic plate count of greater than 5cfu/gm has unsatisfactory quality standards and is also a hazard for consumption(13). Hygienic and quality control methods of meat and meat products, especially in food catering have been recommended in many countries. Without proper hygienic control, the environment in the butcher's area can act as an important source of bacterial contamination(14).

Despite there is no microbiological standard and monitoring system for retail raw meat

products in Ethiopia the desire for eating raw meat is at its highest and the way it is eaten is sometimes dreadful. On the other hand food consumers in Ethiopia suffer from food-borne bacterial illnesses. (15). The people of Adama and its surrounding are well known for their raw meat consumption**,** However, there is no or limited information available on the microbiological quality of raw meat in these highly populous communities.

Objective:

The study was conducted to assess the Butchers-house related factors, Antibiogram signatures, and Bacteriological quality, of raw meat, sold in Adama town, Ethiopia

Methods:

In this cross-sectional study, 112 consenting recruited meat handlers working in the butcher houses of Adama town were given interviewer administered pretested, structured questionnaires to assess their knowledge and practices on meat handling. The knowledge, practices, and the general hygienic and sanitation of the beef selling environments were evaluated through observation, questionnaire. The microbial evaluation of the raw beef sample was conducted at Adama public health research and referral laboratory center.

Sample Collection, Preparation, and Processing

A total of 112 raw beef samples from all butcher houses which were working during the study period were collected. Twenty-five grams of raw meat was weighed and placed in 225 ml sterile 0.1% buffered peptone water. The ground meat and diluent were thoroughly vortexes 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) was transferred to a tube containing 9 ml of normal saline diluent to make 10^{-2} dilutions. 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 aureus* count (TSC) in raw meat by using Plate Count (PC) agar, MacConkey (MC) agar and Mannitol salt agar media to find out the bacteriological quality of meat in these retail outlets in the Municipality.

Enumeration of aerobic plate count

To determine total viable counts in raw meat samples conventional SPC method was used. Tenfold serial dilution up to 10^{-6} was made from the homogenized sample. One mL from each serial dilution (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 between30-300 were counted using colony counter (TT20, Technol, and Technol, USA) and the average count was calculated and expressed as CFU/gm. After determining TAPC by counting each visible colony of bacteria, the Quality of each raw meat sample was judged based on Guideline levels for determining microbial Quality of ready-to-eat food. Meat samples of TAPC <5log10CFU/gm were acceptable and > 5log10CFU/gm were unacceptable.

Enumeration of Total coliforms and fecal coliforms

For the TCC and FCC 0.1ml of each dilution from $10^{-1,}$ $10^{-2,}$ and 10^{-3} was transferred and spread on triplicate on MacConkey agar (Oxoid, England). Then plates were

incubated at 37 °C and 44ºC for 24 hours for TCC and FCC counts respectively. Enumeration of the TCC and FC (typical pink colonies resulting from the fermentation of lactose).

Isolation of Staphylococcus aureus, Salmonella, and Shigella.

For Staphylococci aureus count, mannitol Salt Agar (MSA, OXOID) 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 aureus*(golden yellow colonies shining and convex), diameter 1.0-1.5 mm after 24 hours' incubation were isolated, purified, and tested for catalase and coagulase-positive as a confirmatory test

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 cystine 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 24 h(16). 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 Oxoid, UK.

Culture Media quality control

Qualities of culture media were 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 was also checked using strains kept for quality checking at APHRRLC. To exclude lab contaminants and check whether the media and diluent were completely sterilized, a representative number of a plate with media and broth without the test sample were incubated at 37 °C for 48 hours. If any growth is observed on control media, this batch will be discarded and another media will be replaced. Gram staining reagents were also checked for the 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, *S. flexineri* ATCC 12022, and *S. Typhimurium* ATCC 14028 were used as quality control reference strains.

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility tests were performed using the modified Kirby-Bauer disk diffusion technique(17). Bacterial suspension turbidity was adjusted to 0.5McFarland standard. A sterile swab stick was immersed into bacterial suspension and spread on the surface of Muller-Hinton agar Commercially available antibiotic disks Amoxicillin/clavulanic 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 Oxoid, the UK were used. Antimicrobial agents were selected based on clinical significance, local treatment protocol, and literature data search. The results were interpreted using Clinical Laboratory Standards Institute (CLSI) guideline. *E. coli* (ATCC 25922) was used as a quality control organism for the Antimicrobial susceptibility testing(18).

Data analysis

The data were entered in to Microsoft excel spread sheet and coded, categorized, sorted and cleaned. Following coded the data was analyzed using Statistical Package for Social Sciences (SPSS 25). 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. With Odds ratio of 95% confidence intervals variables p-value less than 0.25 in binary logistic regression analysis were entered to multiple logistic regression to determine factors independently associated with bacteriological quality of raw meat.

3. 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: Sociodemographic characteristics of participants

S/N	Demographic Characteristics				
1	**Age**	No (%)	**4**	**Education**	No (%)
	≤20	12(10.7)		Illiterate	3 (2.7)
	21-30	32(28.6)		Primary	56 (50.0)
	31-40	47 (42.0)		Secondary	37 (33)
	41-50	20 (17.9)		Diploma	10 (8.9)
	>51	1(0.9)		Degree	6 (5.4)
2	**Marital status**		**5**	**Working experience**	
	Married	76(67.9)		<5	29(25.9)
	Single	30(26.8)		5-10	32(28.6)
	Others	6(5.4)		>10	51(45.5)
3	**Religion**		**6**	**Meat safety training**	
	Othodox	71 (63.4)		Yes	45 (40.2)
	Muslim	22 (19.6)		No	67(59.8)
	Protestants	19(17.0)	**7**	**Medical Check up**	
				Yes	36 (32.1)
				No	76(67.9)

Sixty-nine (61.6%) of butcher shops' walls and ceilings are made of Ceramic. None of the meat handlers wore hand gloves. About Eighty-five percent (%) of meat handlers did not wear a

headcover. Moreover, sixty-four (57%) of the butcher shops have no cashiers and they collect money while handling meat (Table 2).

Table: 2: Hygiene related behavioral characteristics of meat handlers

S/N	Table: 2: Hygiene related behavioral characteristics of meat handlers	Yes - No (%)	No – No (%)
1	Butcher shop wall and ceilings free of dust and spider web	42 (37.5)	70 (62.5)
2	Meat handlers wear the white coat	61 (54.5)	51 (45.5)
3	Meat handlers wear headcover	16 (14.3)	96 (85.7)
4	Meat handlers wear the glove	0 (0.0)	112 (100)
5	Handling money while selling meat	64 (57)	48 (43)
6	Wear Jewelers	44 (39.3)	68 (60.7)
7	Butcher shop wall and ceilings made of		
a	Ceramic	69 (61.6)	
b	Concrete	31 (27.7)	
c	Others*	12 (10.7)	

Meat handlers and meat hygiene knowledge

About 98(87.5%) (95% CI=81.3,92.9)of respondents have satisfactory knowledge above the cut of point 10 (≥ 67% accuracy). The overall knowledge level of respondents about personal hygiene, cross-contamination, and transmission of foodborne diseases is summarized in (table 3).

Table 3: Knowledge of meat handling hygiene practices

	Statements on meat handling practices	Right No %	Wrong	Do not know
1	Improper handling of meat could pose health hazards to consumers?	112(100)	0	0
2	Insects and pests could be a source of contamination to meat?	101(90.2)	8(7.1)	3(2.67)
3	Regular handwashing during meat processing reduces contamination rate?	109(97.3)	3(2.67)	0
4	Glove use while handling meat reduces the rate of contamination?	53(47.3)	42(37.5)	17(15.2)
5	Washing and disinfection of butchery utensils reduce contamination rate?	110(98)	2(2)	0
6	Microbes are in the skin, nose, and mouth of healthy people?	64(57)	23(20.5)	25(22.3)
7	People with open skin injury, gastroenteritis, and ear or throat diseases should not be allowed to handle the meat?	88(78.6)	24(21.4)	
8	The health status of meat handlers should be checked before employment?	62(55.3)	11(9.8)	39(34.8)
9	Meat handlers with wounds or injuries on their hands must not touch or handle the meat?	21(18.8)	28(25)	63(56.2)
10	The regular rotation of disinfectants for cleaning reduces the risk of meat contamination from working surfaces and cutting material?	110(98)	2(2)	0
11	Know the diarrheal disease can be transmitted by food?	94(83.9)	6(5.4)	12(10.7)
12	Contaminated raw meats transmits food-borne pathogens to humans	99(88.3)	2(2)	11(9.8)
13	High temperature or freezing is a safe method to destroy bacteria?	108((96.4)	0(0)	4(3.6)
14	Eating and drinking in the workplace increase the risk of meat contamination	74(66)	20(17.9)	18(16.1)
15	Cross-contamination is when microorganisms from a slice of 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 3. Meat handlers and meat hygiene knowledge

Meat handlers and meat hygienic practice

It was found that only 45(40.2%) (95 CI 32.1, 49.1) of respondents have good meat hygienic practice above the cut of point (≥ 70% accuracy) (table 4).

Table 4 Meat safety practices

	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 your hands after handling waste/garbage?	112	0
5	Do you wash your hands after using the toilet?	112	0
6	Do you wear a grown while working?	72	40
7	Do you wear a 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 sanitizer when washing service utensils (knives, & hooks,)?	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 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 workplace?	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 from 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 2.77log10cfu/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). 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 the presence of Shigella and Salmonella species. 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%) and 43/112 samples were shown to be positive for the presence of Shigella and S.aures respectively.

Table 5: Antibiogram signature pattern of isolates

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)
Salmonella	S I R	- 2(18.2%) 9(81.8%)	5(45.45%) - 6(54.5%)	11(81.8%) - 2(18.2%)	7(63.6%) - 4(36.3%)	11(100%) - -	11(100% - -	11(100%) - -	11(100%) - -
Shigella	S I R	1(50%) - 1(50%)	2(100%) - -	2(100%) - -	2(100%) - -	2(100%) - -	2(100%) - -	2(100%) - -	2(100%) - -
S. aureus	S I R	6(14%) - 37(86%)	13(30%) - 30(70%)	29(67%) - 14(33%)	38(88%) - 5(12%)	7(16%) - 36(84%)	43(100%) - -	43(100%) - -	32(74%) - 11(26%)

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

Summary of bivariate and multivariable logistic regression model analysis

In bivariate analysis, training of meat handlers, practices such as wearing the 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 the multivariable logistic regression model. However, in the 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. Summary of bivariate and multivariable logistic regression model analysis

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

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

Discussions:

Overall study participants (59.8%) (95% CI: 50,69) of meat handlers had not been trained on safe meat handling and personal hygiene. Similar to a study done in Mekelle, Ethiopia, where 58.4% of meat handlers had not taken pieces of training related to personal hygiene and meat handling(7). Even though the 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 meat handlers contaminating meat with microorganisms(19). Handling meat and money with the same unwashed hands is one source 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 in cross-contamination of meat with microbes. In a similar study in Mekelle, Ethiopia 91.7% of the meat handlers collect money while serving meat(7). Since the purpose of wearing overalls is to protect both the food products and the meat handler from cross-contamination, overalls should be suitable to wear over other clothing; however, this study showed that 81% of butchery did not wear a gown.

According to a compliance study based on the gulf, standard raw meat in this classifies 85/112(75.89%) (95% CI: 67.9,83.9) of meat have unacceptable bacteriological quality(20). Comparable findings were also obtained in meat retail shops of Meknes City, Morocco, which reported a total aerobic plate count of 67% in beef produced and marketed with unacceptable quality(21). It is higher than in a study done in Sylhet Sadar, Bangladesh in which 28% of meat was of unacceptable quality(22). However, it is lower than the study done in Bahir in which all samples or hundred percent unacceptable(16). According to the food and agricultural organization Total aerobic plate counts exceeding 5.0 log10 on fresh meat are not acceptable and alarm signals on meat hygiene(23).

The average TAPC was 5.89log CFU/g (95% CI: 5.7,6.1). The finding of this result is higher than East Java, Indonesia where the mean of TAPC was 4.158 CFU/g and Chennai city, India(4.78log10)(13,24). However, it is less than Addis Ababa, Ethiopia (6.44 log CFU/g) (25). The variations of bacterial load observed in different studies might be due to a 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 accepted than those who did not receive training (AOR=5.8,1.99-17.34). This is because the 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(26). Regarding collecting money, in the current study, raw meat which was collected from butcher shops in which meat handlers handle money while selling meat was 86% less likely to be acceptable than their counterpart (AOR=0.14,0.04-0.43). According to the WHO/FAO report, handling 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. Even though 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 log CFU/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)(27). 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)(28).

The data in the present study indicate 81(72.3%) of samples collected in the town showed contamination with fecal coliforms. The presence of fecal coliforms suggests fecal contamination which is normally associated with poor hygiene and faulty slaughtering. It also suggests the possibility of finding enteric pathogens such as salmonella, shigella, and others(21). 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)(13). However, it is Lower than the study done in Bahir dar, Ethiopia(16).

The highest number of *S.aureus* on meat indicates the presence of cross-contamination, which is usually related to human skin, hair, hand, and discharge from the nose, and clothing(29). Concerning the prevalence of pathogenic bacteria, Salmonella was detected in 11(9.8%) of analyzed samples. This finding revealed 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 the United State of America (6%). However, the result of this study was much lower than that found in Senegal (87%) and Bahir Dar (70%)(16,30).

This difference may have possibly arisen from the source of animals, types of samples, and sampling technique. The antimicrobial susceptibility profiles of Salmonella isolates revealed a higher rate of resistance against Ampicillin9(81.8%) and Amoxicillin6(54.5%). These findings are in agreement with (31), 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 gentamycin, ciprofloxacin, tetracycline, and ceftriaxone. This is in line with the study conducted in Ghana(32).In addition, 7(63.6%) and 9(81.8%) exhibited susceptibility to Tetracycline and Trimethoprim-sulfamethoxazole respectively.

This result is also in alignment with the study done in Gondar, Ethiopia. However, resistance to Ampicillin is much higher than in Bahir dar(23.8%) (16,33).In other words, Staphylococcus aureus was detected in 43(36%) of the collected sample which is higher than the study done in Jimma, However it is lower than the study done in Addis Ababa(34,35). In another way, the study revealed an overall Shigella prevalence of 2.67% which is higher than the study done in Jimma, Ehiopia but, lower than in Karachi, Pakistan, and Gondar (4,19,35), however, a similarly lower rate of isolation was reported from Ebony, Nigeria (36). 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, Ciprofloxacin, and ceftriaxone. However, a higher rate of resistance against ampicillin was observed in Gondar(19). Differences in the geographical location of the isolates or the emergency of drug-resistant strains could partially explain this discrepancy.

Conclusion: Meat hygiene training for handlers at the Bucher-house significantly impacted bacteriological quality of meat studied. Salmonella species, Staphylococcal species and shigella significantly resisted major groups of antibiotics (penicillin's, quinolones) used in this study. 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 the hygienic quality of meat for consumers. The following areas need big attention and concern for the future suggestion to Adama town Training of ablators enterprise on good meat handling practices, constant health inspection, avoidance of raw meat consumption, sustained surveillance on meat supply chain are recommended

Data and materials access:

The datasets examined during this study are available from both authors corresponding and other authors on request

Abbreviations:

AHMC: Adama hospital medical college; APHRRLC: Adama public health research and referral laboratory center; ASP: Antimicrobial susceptibility 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

Ethics issues

The informed consent form for study participants (to all participants the butchers) was obtained in written form and approved by the ethics committee as well 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 ease of the study process and permission. Oromia Health Bureau was writing a supporting letter to Adama Town Administration and then Adama Town Administration wrote a supporting letter to selected Butcher Houses in Adama Town. The purpose and benefit of the study along with their right to refuse were explained to all butchers available during the 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.

Consent for publication: There are no identifiable details on individual participants reported in the manuscript, so, consent to publish is not required. Not applicable.

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