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Bacteriological Quality Assessment of Raw Beef Sold in Sylhet Sadar

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Abstract

This study was conducted to assess the bacteriological quality of fresh raw beef sold in different markets of Sylhet Sadar. A total of seventy five (75) fresh raw beef samples were randomly collected from seven major markets (Shibgonj, Mirabazar, Kazitula, Ambarkhana, Madina market, Bandar bazar and Sheikh ghat) of Sylhet Sadar. Total Viable Count of the samples ranged between 2.5×10^5 to 2.25×10^8cfu/g. Acceptability for consumption of these samples as satisfactory, acceptable and rejected were 40, 32 and 28%, respectively. A total of 115 bacterial isolates of 5 genera were identified including gram negative *Escherichia coli* [15(10%)], *Salmonella* spp. [20(13.33%)], *Klebsiella* spp. [30(20%)], *Enterobacter* spp. [10(6.67%)] and gram positive *Staphylococcus* spp. [40(26.67%)]. The presence of these organisms in fresh meat from conventional beef is alarming.

Keywords: Total viable count, conventional beef, public health hazard, food borne infection, intoxication

1. Introduction

Meat from mature cattle is known as "Beef". Beef is a good source of various nutrients specially protein, fat, phosphorus, enzyme, water etc. Meat is one of the most perishable food and its composition is ideal for the growth of a wide range of spoilage bacteria (Mayr *et al*., 2003). It also reported that fresh raw meat like beef have been implicated for a number of meat borne infections and intoxications in several countries (Mukhopadhyay *et al*., 2009). This is because both pathogenic and non-pathogenic organisms live in the gastro-intestinal tract of cattle which can be transferred onto the meat under faulty and poor processing conditions.

Fresh meat becomes contaminated with microorganisms during various processing stages up to consumer uptake. Contaminated raw meat is one of the main sources of food-borne illness (Bhandare *et al.*, 2007). Some of these bacteria may include pathogens. These are the food poisoning microorganisms causing food borne infection and intoxication or spoilage bacteria causing discoloration, bad odors and slime on meat surfaces. If the bacteria on meat include pathogens like *E. coli*, *Salmonella*, *Staphylococcus* etc. there could be a risk to human health. Members of the gram negative bacteria e.g. *E. coli* are widely distributed in the environment and are the major sources for food contamination. The possible sources of these bacteria are skin of the animal, the equipments used for each operation, clothes and hands of personnel and the physical facilities themselves (Tesfay *et al.*, 2014). It is difficult to set microbiological criteria that would indicate when

the food risk is unacceptable. This is because of improper sampling.

Another problem is that there are no clear food safety objectives (FSO) for pathogens on fresh meat. An FSO is the maximum level of a food safety hazard in a food that can be considered acceptable for consumer protection. Even if microbiological testing reliably indicates the level of contamination by pathogens (and this may be possible if enough samples are tested) it is difficult to assess the level at which the pathogen is an unacceptable food safety risk. Food safety is better assured through the application of Hazard Analysis Critical Control Point (HACCP) principles and good manufacturing practice (GMP) (FAO Corporate Document Repository, 2007).

Public health is the most uttering concern of today's world. Beef, a delicious food is widely used for public consumption. However, beef processing in most of the city markets is carried out under unhygienic environment and it is important to know the bacteriological quality of those meet for ensuring better food safety. Hence, this study was undertaken to assess the bacteriological quality of raw beef which focuses the very common food poisoning microorganisms causing public health hazard.

2. Materials and Methods

2.1. Sample collection

Seventy five samples of fresh cattle meat (beef) were randomly purchased from 7 major markets (Shibgonj, Mirabazar, kazitula, Ambarkhana, Madina market, Bandar bazar and Sheikh ghat) of Sylhet Sadar. These were collected from different portions of carcasses. During the study period of October, 2011 to December, 2011, the samples were collected twice from each market. The samples were aseptically collected in different clean polyethylene bags and were transferred immediately to the laboratory for bacteriological quality assessment as described in FAO Corporate Document Repository (2007).

2.2. Culturing, enumeration and isolation of bacteria

All the chemicals and reagents used were of analytical grade, obtained from Hi-media Laboratories Pvt. Limited, India. Media used in this study included: Nutrient Agar (NA) and Peptone Water (PW) as general and enriched media. Other media with selective and differential characteristics used were Violet Red Bile Agar (VRBA), Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB), Salmonella-Shigella (SS) Agar, Brilliant Green Agar (BGA), Blood Agar (BA), Mac Conkey Agar (MCA) etc. All media were prepared according to the manufacturer's specification and sterilized at 121 °C and 15 lb pressure for 15 min. Total viable aerobic bacteria count was performed on Nutrient Agar. For this - Meat sample (10 gram meat+ 90 ml sterile distilled water) were homogenized in a sterile blender (first dilution). One ml from first dilution (10^1) was transferred to second test tube (test tube contains 9 ml. of sterile distilled water) (2^{nd} dilution or 10^2) so on up to the 6^{th} dilution.

Then, inoculation of sample was done. Inoculation of sample was done by pipetting 1 ml from 3^{rd} dilution and was transferred to the sterile petridish, also from the 4^{th} dilution to another sterile petridish up to the 6^{th} dilution. The inoculation was followed by the pour plate method, where the sample was first put into the petri dish and 15 ml agar (liquefied in a water bath at 44-46 °C) were poured into the plate afterwards. Agar and sample were thoroughly mixed by rotating the petri dish. After that, incubation for 24 hours at 37 °C and counting of normal plates of 25-250 colonies were carried out. The counts for each plate were expressed as colony forming unit of the suspension (cfu/g). Discrete colonies were sub cultured into differential and selective media aseptically to obtain pure cultures of the isolates. Pure isolates of the resulting growth were then stored at 4 °C.

2.3. Identification of bacterial isolates

Colonies identifiable as discrete on the selective media were carefully examined macroscopically

for cultural characteristics such as the shape, color, size and consistency. Bacterial isolates were characterized based on microscopic appearance, colonial morphology and Gram's staining reactions as well as appropriate biochemical tests *i.e.* Lysine Iron Agar (LIA) test, Triple Sugar Iron (TSI) test, Indole production test, Methyl Red (MR) test, Voges-Proskauer (VP) test, Citrate utilization test, Catalase test and Carbohydrate fermentation test as described by Buxton and Fraser (1977), Cheesbrough (1985) and Carter *et al*. (1995) were carried out. The isolates were identified by comparing their characteristics with those of known taxa, as described by Bergey's Manual for Determinative Bacteriology (Buchanan and Gribbons, 1974). Data were analysed statistically using the general linear model procedure.

3. Results and Discussion

3.1. Total viable count (TVC)

To obtain total counts, plates containing well isolated colonies were selected and plates containing overgrowth colonies were avoided. Area basis TVC findings are presented in Table 1. Total Viable Count of collected fresh beef ranged between 2.5×10^5 to 2.25×10^8 cfu/g. Minimum bacterial load *i.e.* 2.5×10^5 cfu/g was recorded at Kazitula and Bandar bazar. The collected meat samples of Mirabazar area had maximum bacterial load *i.e.* 2.25×10^8 cfu/g. Mean microbial count of seven markets ranged between 1.6×10^7 to 4.23×10^7 cfu/g.

Fresh beef containing organisms less than 0.5 million/gm are considered satisfactory for consumption, more than 0.5 million/gm but less than 2 million/gm are acceptable and more than 2 million/gm are rejected i.e. not good for public consumption (Rahman, 2007). Based on this data, area basis quality percentages of collected meat are presented in Table 2. Overall meat quality percentages based on TVC is illustrated in Figure 1. Beef of Madina market (50%), Mirabazar (44.44%) and Bandar bazar (43.75%) were of good quality. Raw beef of these markets is satisfactory for public consumption. Total bacterial load into meat of Shibgonj (37.5%) and Sheikh ghat (33.33%) area possess acceptable limit. Meat of these areas is conditionally approved and for this investigation is needed. Meat collected from Ambarkhana (41.67%) and Kazitula (37.5%) region is not good for public consumption *i.e.* rejected. On the basis of microbiological standards of raw beef, 40% of test samples were satisfactory for consumption, 32% were acceptable and 28% were rejected *i.e.* unacceptable for public consumption.

Fresh meat samples from local markets of Sylhet sadar yielded moderate growth of bacteria. The presence of *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp. and *Staphylococcus* spp. on meat parts could be attributed to the fact that meat is enriched with all nutrients required for the growth of bacteria in adequate quantity. Then, moderate total viable counts recorded in this study showed the bacterial diversity (differences in forms or species) in these markets, real condition of the market and the hygienic practice employed by meat sellers and butchers. This determined the variation of bacterial contamination.

Table 1. Area basis total viable count obtained from collected fresh beef

Area	Minimum count (cfu/g)	Maximum count (cfu/g)	Mean (cfu/g)
Shibgonj	2.6×10^5	1.15×10^8	1.64×10^7
Mirabazar	2.8×10^5	2.25×10^8	3.49×10^7
Kazitula	2.5×10^5	1.71×10^8	4.23×10^7
Ambarkhana	3.5×10^5	1.2×10^8	2.25×10^7
Madina market	3.7×10^5	1.75×10^7	3.74×10^6
Bandar bazar	2.5×10^5	8.4×10^7	8.6×10^6
Sheikhghat	3.1×10^5	9.7×10^7	1.6×10^7

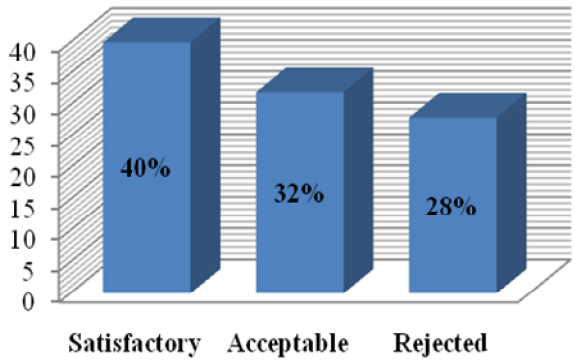

Figure 1. Quality of beef suitable for consumption based on total viable count

Table 2. Area basis meat quality percentage based on total viable count

Area	No. of Sample	Satisfactory/Passed for Consumption <.5 million/g)		Acceptable/Conditional Approval (>.5 million/g & <2million/g)		Rejected/ Condemned (>2 million/g)	
		No.	Percent	No.	Percent	No.	Percent
Shibgonj	8	3	37.5	3	37.5	2	25
Mirabazar	9	4	44.44	3	33.33	2	22.22
Kazitula	8	3	37.5	2	25	3	37.5
Ambarkhana	12	4	33.33	3	25	5	41.67
Madina market	10	5	50	3	30	2	20
Bandar bazar	16	7	43.75	6	37.5	3	18.75
Sheikh ghat	12	4	33.33	4	33.33	4	33.33

This is an indication of recontamination in food due to handling and hygienic techniques (Clarence *et al*., 2009). Similar values were reported by Yousuf *et al*. (2008) and Okonko *et al*. (2008c, d; 2009 a, b).

3.2. Isolation and identification

Characteristic growth of microorganisms into differential media indicated them as *E. coli*, *Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp. and *Staphylococcus* spp. Results of these tests are shown in Table 3. A total of 150 isolated colonies from NA agar were inoculated into VRBA and MSA (75 for each). One hundred and fifty colonies of VRBA and MSA were sub-cultured into differential media, performed different biochemical test. Presence of these isolated bacteria is graphically presented in Figure 2.

A total of 115 isolates comprising of 4 different genera of gram negative bacteria and 1 genus of gram positive bacteria were isolated in this study. This showed that different markets contributed equally to the microbial diversity. The bacteria isolates are identified as *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp. and *Staphylococcus* spp. by comparing their morphological and biochemical characteristics with standard reference organisms (Buchanan and Gribbons, 1974; Cheesbrough, 2003). Microorganisms isolated from fresh meat samples in this study have been earlier found in foods, environment

and other places and their pattern is similar to previous reports (Clarence *et al.*, 2009; Okonko *et al.*, 2008 a, b, c, d, 2009).

Agbeyegbe and Uraih (1982) study reported high prevalence rate of *Escherichia coli* in raw meat samples. Enabulele and Uraih (2009) reported *E. coli* prevalence rate to be 85.65% in a study with the fresh meat samples from abattoir and traditional open market each, recording 100% *E. coli* prevalence. Clarence *et al*. (2009) reported the presence of *S. aureus, E. coli, Bacillus* spp., *Enterobacter, Pseudomonas* and *Klebsiella* in meat pie.

Most of the organisms found in this study are those commonly found in soil and water. *Staphylococcus* spp. (26.67%) was isolated in the present study as reported in all previous work mentioned above. The presence of *E. coli* (10%) and *Enterobacter* spp. (6.67%) in this fresh meat samples is an indication of fecal contamination of the meats. This might be due to possible contamination of fresh meats or meat products itself during sales or unhygienic handling of the meats right from slaughtering, butchering plants and processing or due to contamination from the skin, mouth, or nose of the handlers which can be introduced directly into foods by process line workers, with lesions caused by *S. aureus* on hands and arms coming into contact with the food or by coughing and sneezing (Sobukola *et al*., 2009; Okonko *et al*., 2008 a, b, c, d and 2009 a, b).

Table 3. Morphological and biochemical characteristics of isolated organisms

Parameters	Isolates				
	I.	II.	III.	IV.	V.
Gram's reaction	-	-	-	-	+
Cellular morphology	Straight rods	Rods	Rods	Rods	Round, arranged in grape like structures
Motility	+	+	-	+	-
Catalase test	+	+	+	-	+
Citrate test	-	+	+	-	
Indole test	+	-	-	+	-
MR test	+	+	-	+	+
VP test	-	-	+	-	+
Growth on TSI	Slant – Red, Butt - Yellow	Butt – Black	Butt & Slant – Yellow	Gas bubbles in butt & medium frequently split	N/A
Growth on LIA	Butt & Slant – Red	Butt – Yellow	Slant -Red	Butt & Slant – Red	N/A
Sugar fermentation					
Sucrose	A	A & G	A	A	A
Glucose	A & G	A & G	A & G	A & G	A
Dextrose	A & G	A & G	A & G	A & G	A
Mannitol	A	A	A	A	A
Maltose	A	A & G	A	A	A
Lactose	A	A & G	A	A	A
Isolated bacteria	*Escherichia coli*	*Salmonella* spp.	*Klebsiella* spp.	*Enterobacter* spp.	*Staphylococcus* spp.

N/A = Not applicable, (-) = No growth, (+) = Growth, A & G = Acid production and gas production and A = Acid production only and no gas production.

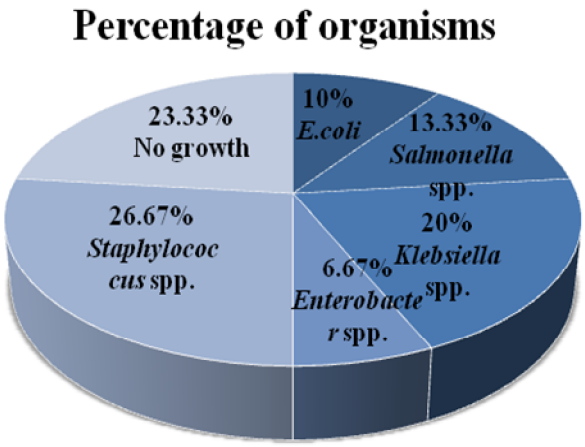

Figure 2. Graphical presentation of different isolated bacteria from the test beef samples

The isolation of *Enterobacter* spp. may be the result of poor environmental conditions such as dust and contamination of the water used during slaughtering, *Enterobacter* spp. are also being the inhabitants of dairy products (Talaro and Talaro, 2006). *Salmonella* spp. (13.33%), and another organism found in the meats are also a pathogenic organism of public health significance and concerns (Okonko *et al*., 2009 a, b). The isolation of *Salmonella* spp. (13.33%) in this study is of practical impact. This organism might have contaminated the meats as a result of handling by meat sellers.

4. Conclusions

Maximum microbial load of fresh beef was recorded in Mirabazar and minimum load was at Kazitula and Bandar bazar. On the basis of microbiological standards of raw beef, 40% of test samples were satisfactory for consumption, 32% were acceptable and 28% were rejected. Bacteriological quality assessment revealed that both gram positive and gram negative bacteria were common in fresh beef. It may be concluded from the above all findings that microbial load of fresh beef was in acceptable limit. Hence, isolated organisms have public health significance i.e. they are responsible for various food borne infection and intoxication. Precautionary management is therefore, deemed necessary.

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