Research in
Agriculture, Livestock and Fisheries
ISSN : P-2409-0603, E-2409-9325
An Open Access Peer-Reviewed International Journal

Article Code: 0332/2021/RALF
Article Type: Research Article

Res. Agric. Livest. Fish.
Vol. 8, No. 1, April 2021: 135-143.

DETECTION AND ANTIBIOGRAM OF DIFFERENT BACTERIAL AGENTS FROM MARKET GOAT MEAT

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ARTICLE INFO

Received
18 February, 2021

Revised
27 March, 2021

Accepted
29 March, 2021

Online
May, 2021

Key words:
Bacteria
Goat meat
Antibiogram

A B S T R A C T

The study was conducted to detect various pathogenic as well as spoilage bacteria present in goat meats and also their antibiogram. Different body regions such as brisket, neck and thigh, supplied at different places like Krishi market, Mohakhali kacha bazar, and Charulata market within Dhaka City Corporation area, were taken and the bacteria were isolated. The isolated bacteria were identified on the basis of cultural, morphological, and biochemical characteristics. The isolated bacterial agents were: *Staphylococcus* spp., *Escherichia coli*, *Bacillus* spp., *Micrococcus* spp., *Streptococcus* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Salmonella* spp., and *Pseudomonas* spp. Antibiotic susceptibility test using disc diffusion method revealed that most of the isolates were found to be more or less resistant to tetracycline, ampicillin and erythromycin indicating that these may be a threat to consumer's health. It is emphasized that the present sanitary conditions, as revealed through this study, of meat processing need to be improved so as to minimize microbial contamination. Suggestions are made to introduce Longitudinally Integrated Safety Assurance (LISA) system and Good Manufacturing Practices (GMP) principles.

To cite this article: Hassan M. K., L. Jahan, P. Sultana, A. Hasan, and M. P. Siddique, 2021. Detection and antibiogram of different bacterial agents from market goat meat. Res. Agric. Livest. Fish., 8 (1): 135-143.

INTRODUCTION

Goat, as far as known, was probably the first domesticated animal (Morand and Boyazoglu, 1999). At present, the world's goat population is one billion which is lower compared to cattle (1.5 billion) and sheep (1.2 billion) (FAOSTAT, 2016). However, Asia contributes 54.4% of the world's goat population (Mazhangara *et al*., 2019). Goat is one of the most favored and widely farmed meat animals in Asian countries (Mazhangara *et al*., 2019). The approximate number of goats is 56.08 million in Bangladesh (FAOSTAT, 2016). Among different red meats, goat meat has occupied a suitable and sustainable place in the diet as a source of animal protein, due to its unique taste, no religious barrier, sometimes tradition, and most importantly availability (Das and Saikia, 2017). World's total goat meat supply was 3659 thousand MT in 1999, where Bangladesh alone supplied 127 thousand MT (FAO, 2001). Though meat plays a very vital role in keeping the human body strong in order to provide energy, health and vigor (Das and Saikia, 2017), meat has been considered as an important vehicle for the transmission of foodborne pathogens (Zhao *et al*., 2001). Several meat borne epidemics have been reported throughout the recent years and considered as one of the global public health concerns (Komba *et al*., 2012).

Bacterial flora of raw goat meat is obviously heterogeneous (Carrizosa *et al*., 2017), which may be harmful for human and may cause spoilage and may be used as indicator organisms (Bantawa *et al*., 2018). Many researchers have isolated and identified heterogeneous types of microflora from raw goat meat. The bacterial species isolated from goat meat include *Proteus* spp. *Staphylococcus aureus, Escherichia coli, Bacillus* spp*. Streptococcus* sp*p. Enterbacter aerogenes, Pseudomaonas aruginosa, Rhizopus* spp. (Eze and Ivuoma, 2012; Das and Saikia, 2017; Bantawa *et al*., 2018). Members of the genus *Salmonella, Yersinia enterocolitica*, *Clostridium perfringens* and *Campylobacter jejuni* cause food poisoning. *Clostridium botulinum*, *Staphylococcus aureus*, *Bacillus cereus* etc. cause meat-borne intoxication chiefly (Haque *et al*., 2008). The meat of healthy animals usually do not harbor microbial population, however, microbial contamination occurs from various external sources at any steps of meat processing and during raw meat transportation and marketing (Ercolini *et al*., 2006). Besides, many researchers also studied the *in-vitro* drug sensitivity of the bacterial isolates (Sharma *et al.,* 1995; Mukhopadhyay *et al.,* 1998). The transmission of antimicrobial resistance from the animals to humans may occur in various ways, with the direct oral route being the most common, such as meat consumption, and direct contact with the antimicrobial resistant microbes during raw meat processing (Lerma et al., 2014).

Nowadays, public awareness is in the increasing trends regarding the impact of foodborne zoonotic microorganisms from foods of animal origin (Zhao *et al*., 2001; Bantawa *et al*., 2018). In developing countries like Bangladesh, the whole art of slaughtering and meat processing is still theoretical and less practiced for the production of raw goat meat available at retail markets (Haque *et al*., 2008). Moreover, the slaughterhouse contamination with spoilage and/or pathogenic bacterial population is an unavoidable problem in most developing countries (Hamdan *et al*., 2019). Carcass dressing and evisceration are considered high-risk factors for microbial contamination of carcass (Ivanovic *et al*., 2014) and constitute the critical points of contamination (Abdalla *et al*., 2009).

The extent of microbial contamination and the communities of microbial flora directly reflect the hygienic standard of raw meat (Bantawa *et al*., 2018). No systematic approach to address the issue is available in Bangladesh, relating to the present study. Hence, the study was undertaken to isolate different kinds of public health important bacteria in meat tissues obtained from slaughter yards and meat stalls at late market hours and to find out the *in-vitro* antibiotic sensitivity pattern of the isolated and identified organisms.

MATERIALS AND METHODS

Sample collection and transportation

A total of 36 meat samples were collected equally from three slaughter yards and meat stalls, namely Krishi market, Mohakhali kacha bazar, and Charulata market, within the Dhaka City Corporation area, each containing 12 samples. In each sampling site, out of these 12 samples, four samples from the brisket region, four from the neck region, and four from the thigh region were taken. After collection, bacteriological analyses of the samples were performed in two major principal assessments. Firstly, isolation and identification of various bacteria contaminating the meat samples by using different cultural and biochemical tests and secondly, their degree of sensitivity to different antibiotics. Samples were collected aseptically in sterile containers and brought to the laboratory, using an ice-box, within the shortest possible time to study the occurrences of different microflora gaining access to meat. The samples include raw meat and swab samples from goat meat.

Raw goat meat samples

Raw meat samples of goat meat were collected directly from the carcass and the retail hanging display of butchers. A quantity of about 250 gm of meat cut samples was collected from the different regions of the carcass, such as brisket, neck, and thigh. An individual selection is minced, and a quantity of 50 gm was transferred into sterile containers containing 450 ml of 0.1% peptone water. A homogenized suspension was made in a sterile blender according to the recommendation of McLandsborough (2005).

Swab samples

Swab samples were taken, following the methods of Abdalla *et al*. (2009) and Hamdan *et al*. (2019). Cotton swabs were used in this study. The swabs were prepared using non-absorbent cotton wool which was moistened before use with sterile 0.1% peptone water. These were rubbed firmly over the surface using parallel strokes with slow rotation. The surface to be examined was swabbed twice using parallel strokes at right angles to the first strokes. The predetermined area to be swabbed was chosen, and a template of ten square centimeter area was placed over the predetermined area. Care was taken to swab the whole of the predetermined area. After swabbing, the swabs were transferred to the respective tubes containing 5 ml of sterile 0.1% peptone water. The swabs were agitated up and down in the tubes to assist the rinsing of bacteria from the surface of the swabs.

Cultural and biochemical examination of bacterial isolates

The cultural examination of meat samples for bacteriological analysis was done according to the standard method (McLandsborough, 2005). The examination followed a detailed study of colony characteristics, including the morphological and biochemical properties. To find out different microorganisms in meat, different kinds of bacterial colonies were isolated in pure culture.

Gram's staining and motility test

The Gram's staining method and motility test using the hanging drop method were done to provide basic information about the presumptive bacterial identification suggested by Cheesbrough (2006) and Winn and Koneman (2006).

Coagulase test

This test is usually used to differentiate *Staphylococcus aureus,* which produces the enzyme coagulase, from *S. epidermidis* and *S. saprophyticus* which do not produce coagulase. Coagulase test was performed by mixing 0.5 ml undiluted rabbit plasma separately in two different test tubes containing an equal volume of 24 hours old staphylococcal broth culture and incubated at 37^0C for 4 hours. The tubes were examined after 2-4 hours for detecting the presence of clots of plasma, and the result was recorded according to the method described by Harley (2005). The negative tubes were left at room temperature overnight and then re-examined.

Catalase test

This test was used to differentiate catalase-producing bacteria, such as *Staphylococci*, from non-catalase-producing bacteria such as *Streptococci*. An amount of 2-3 ml of 3% hydrogen peroxide solution was poured into a test tube. A freshly and fully grown test organism was immersed in the solution using a sterile glass rod. The bubbles of oxygen were released, which indicated the positive result. The culture was less than 24 hours old and was grown on blood agar (Winn and Koneman, 2006).

Hemolytic activity

Several isolates of bacteria were inoculated into BA and incubated at 37^0C for 24 hours to determine their hemolytic property. The colony developed on the BA was examined for various types of hemolysis. Hemolytic patterns of the bacteria were categorized according to the kinds of hemolysis they produced on BA, and this was made as per the recommendation of Cheesbrough (2006) and was listed as (1) alpha ($\propto$) hemolysis: a zone of greenish discoloration around the colony manifested by partial hemolysis, (2) beta (β) hemolysis: complete clear zone of hemolysis around the colony and (3) gamma (γ) hemolysis: no detectable hemolysis.

Oxidation-Fermentation test

This test was used to differentiate those organisms that oxidize carbohydrates (aerobic utilization) from those that ferment carbohydrates (anaerobic utilization). The test organisms were inoculated into two test tubes of either tryptone or peptone agar medium containing glucose and the indicator bromothymol blue. One tube was sealed with a layer of liquid paraffin to exclude oxygen. The change of the color of the medium from green to yellow was indicative of a positive test (Winn and Koneman, 2006).

Oxidase test

It was used to identify *Pseudomonas*, *Vibrio,* and *Pasteurella* spp. that produce oxidase enzymes. For this purpose, a piece of filter paper was placed in a clean petri dish, and 2-3 drops of freshly prepared oxidase reagents were added. A colony of the test organism was then smeared on the filter paper by a glass rod. The development of a blue-purple color within a few seconds was considered a positive test (Winn and Koneman, 2006).

IMViC test for enteric bacteria

This test is useful in classifying the enteric bacteria, that is Gram-negative rod and oxidase-negative bacteria. In this test, Motility Indole Urea medium (MIU), Bacto MR-VP medium, and Koser's citrate medium were used. An amount of 5 ml of MIU medium was inoculated with a smooth colony of the test organism using a sterile straight wire. Indole paper strip was placed in the neck of the MIU tube. The tube was closed with a stopper and incubated at 35-37^{o} C overnight. In the Indole positive case, the strip became red, and in the urease-positive case, the medium turned into a reddish color. The motile bacteria produced turbidity throughout the medium (Harley, 2005). An amount of 2 ml of MR-VP broth was taken in test tubes. In two of the test tubes, test organisms were inoculated, and these were incubated at 35^{0} C for 48 hours. After incubation, in one of the tubes, five drops of MR solution were added, and the color was observed. To the other tube 0.6 ml of VP reagent 1 (creatine) and 0.2 ml VP reagent 2 (40% w/v sodium hydroxide) were added. The tubes were gently shaken with the caps off to expose the medium to oxygen to oxidize the acetoine. The tubes were allowed to stand for 15-30 min. The development of pink to red color was indicative of a positive VP test. An amount of 3-5 ml of sterile Koser's citrate medium was inoculated with a broth culture of the test organisms and incubated at 35-37^{0} C for up to 4 days, checking daily for growth. Turbidity and change in color of the indicator showed growth of the organisms in the medium from light green to blue due to the alkaline reaction following citrate utilization.

TSI agar slant reaction

After isolation on the selective media, differential-screening media such as TSI agar was used to further categorization. Colonies were inoculated by stab into TSI agar slants. In TSI agar, if the organisms ferment only glucose, the tube will turn yellow in a few hours. The bacteria quickly exhausted the limited supply of glucose and started oxidizing amino acids for energy, giving off ammonia as an end product. Oxidation of amino acids increases the pH, and the indicator in the slanted portion of the tube turned back to red. The butt remained yellow. The organism in the TSI slant fermented lactose and/or sucrose so that the butt and the slant turn yellow and remained yellow for days due to the increased level of acid production. The appearance of bubbles in the agar indicates gas production. The test also shows whether H_2S has been produced due to the reduction of sulfur-containing compounds. H_2S reacts with the ferrous sulfate of the medium, producing ferric sulfide, a black precipitate (Cheesbrough, 2006).

Procedures for bacterial isolation and identification

The above-mentioned criteria were employed for the isolation and identification of bacteria from the meat and swab samples as suggested by McLandsborough (2005), Harley (2005), Cheesbrough (2006), and Winn and Koneman (2006).

Techniques for *Staphylococcus spp.*

Colonies of staphylococci are usually round, glistening, convex, smooth, and opaque. They are Gram-positive cocci arranged in clusters mostly. *Staphylococci* are differentiated from streptococci by catalase test. They are catalase positive. Beta (β) hemolysis was produced by most strains on BA. The coagulase test was performed for the identification of the pathogenic *Staphylococcus aureus*. Usually, non-pathogenic, coagulase-negative do not produce β-hemolysis (McLandsborough, 2005; Cheesbrough, 2006).

Techniques for *Streptococcus spp.*

These organisms were easily isolated and identified by their Gram-positive cocci morphological characteristics arranged in short or long chains when grown on SSB. The organisms produce small grayish round colonies on NA; some of the species produce hemolysis on BA. Only the enterococci can grow on MC broth. The hemolytic activity of *Streptococc*i was detected by growing them on BA. *Streptococci* were differentiated from *Staphylococci by* catalase test (McLandsborough, 2005; Winn and Koneman, 2006).

Techniques for *E. coli* and other coliforms

For the isolation and identification of coliform organisms, the samples were first inoculated to MC agar. The suspected colonies were inoculated on TSI agar slants. Acid slant, acid butt, no hydrogen sulfide, and no gas in butt were indicative of coliform. The organisms are oxidase negative. Lactose fermenting red colonies from the MC agar was subcultured on EMB agar. Colonies on EMB agar with metallic sheen were suspected as positive for E. coli and were confirmed by the IMViC test. E. coli is characterized by positive to Indole and MR tests and negative to VP and citrate tests. Enterobacter is negative to Indole and MR test, positive to VP and citrate test (McLandsborough, 2005).

Techniques for *Salmonella* and *Shigella* spp.

Processed samples were inoculated on MC agar and incubated at 37^0C for 24-48 hours. Lactose non-fermenter colorless colonies from MC agar were subcultured on SSA. Translucent, round, and colorless colonies on SSA were suspected to be Salmonella and/or Shigella, which were later confirmed by biochemical and the motility test. Organisms were Gram-negative rod and oxidase-negative (Winn and Koneman, 2006).

Techniques for *Bacillus* spp.

Diluted samples were inoculated on BA and incubated at 37^0C for 24-48 hours. The growth of Gram-positive large sporulated rod-shaped bacteria in chain form with large hemolytic colony indicated the presence of Bacillus (Winn and Koneman, 2006).

Maintenance of stock culture

The stock cultures of the bacterial isolates were prepared following the method described by Siddique *et al*. (2017). A pure colony was taken in nutrient broth and kept in a shaker incubator for 6 hours until turbidity appears for stock culture. 50% sterile buffered glycerin was made by mixing 50 parts pure glycerin and 50 parts PBS. Then the pellet bacteria, obtained by centrifugation at 2500 rpm for 20 minutes, were mixed with 50% sterile buffered glycerin in small cryovials and were preserved at -20°C.

Antibiotic susceptibility test of the isolated bacteria

To determine the sensitivity or resistance pattern antibiogram of different types of bacteria isolated from meat sample, antibiotic discs of ampicillin (AM, 10 µg), gentamicin (GM, 10 µg), erythromycin (EM, 15 µg), tetracycline (TC, 30 µg), ciprofloxacin (CIP, 5 µg), and nalidaxic acid (NA, 30 µg) were used. The antibiotic sensitivity test was done using Mueller-Hinton agar medium, based on the principle described by Bauer *et al*. (1966). The diameter of the zone of inhibition was calculated and interpreted according to the interpretative guideline of the Clinical and Laboratory Standard Institute, CLSI (2018).

RESULT AND DISCUSSION

Distribution of selected bacteria present in goat meat

Bacterial isolates, belonging to different genera, were isolated from raw meat samples, followed by identification through routine (conventional) bacteriological procedures, such as, cultural, morphological, and biochemical characteristics. The findings of the routine bacteriological procedures were then interpreted, based on the description from the previously published reports (McLandsborough, 2005; Harley, 2005; Cheesbrough, 2006; Winn and Koneman, 2006). It was found that most of the meat samples contained Staphylococci (about 98%) organisms. Next to the Staphylococci, *Escherichia coli* (55%) ranked second, and *Bacillus* spp. (50%), the third position. Other organisms isolated from meat samples were Micrococci, Streptococci, *Enterobacter spp., Proteus* spp., *Citrobacter* spp., *Salmonella* spp., and *Pseudomonas* spp. etc., and their percentages (%) of occurrence were 36, 28, 10, 25, 13, 6, and 10, respectively (Table 1). A total of 129 bacterial isolates were recovered from different meat samples.

Table 1. Percent distribution of bacteria contaminating goat meat

Suspected bacterial isolates		No. of samples		Percentage of occurrence	Percent distribution (%)
Genus	**Species**	**examined**	**isolated**		
Staphylococcus	*Staphylococcus aureus, S. epidermis*	50	49	98%	37.95
Micrococcus	*Micrococcus luteus, Micrococcus* spp.	50	18	36%	13.95
Streptococcus	*Streptococcus pyogenes*	25	7	28%	5.43
Bacillus	*Bacillus coagulans, B. cereus, B. subtilis*	30	15	50%	11.63
Escherichia	*Escherichia coli.*	36	19	55%	14.73
Enterobacter	*Enterobacter aerogenes*	30	3	10%	2.33
Proteus	*Proteus mirabilis, P. vulgaris, P. morganii*	40	10	25%	7.75
Citrobacter	*Citrobacter freundii*	30	4	13%	3.10
Salmonella	*Salmonella* spp.	36	2	6%	1.55
Pseudomonas	*P. aerogenosa*	20	2	10%	1.55

The percentage distribution of selected bacteria, present in goat meat, was also determined and is represented in Table 1. The highest percentage distribution was found for Staphylococci (36.95%), and lowest value was found for *Salmonella* and *Pseudomonas* spp., which was 1.55%. Eze and Ivuoma (2012) also reported the similar findings on detection of bacterial strains from goat meat samples sold in retail market of Abia State, Nigeria. Das and Saikia (2017) isolated and identified *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter* spp., and *E. coli* from the raw goat meat, available at the retail markets of Dibrugarh, India, and *Staphylococcus aureus* and *E. coli* were found in all samples. Tanganyika *et al*. (2017) isolated and identified *E. coli*, *Bacillus* spp., *Proteus* spp., and *Klebsiella* spp., from goat carcasses in Lilongwe, Malawi. Bacterial isolates found, in this study, are considered the most common meat-borne pathogens of serious public health concern (Bhandare *et al*., 2007). Moreover, among isolated bacteria, *Pseudomonas* spp. are responsible for meat-spoilage, causing gas production and changes in odor, color, and flavor (Arnaut-Rollier *et al*., 1999). The presence of an increased number of pathogenic bacteria in meat is of potential public health significance (Haque *et al*., 2008). All these organisms in meat foods should receive particular attention because their presence indicates public health hazard and give a warning signal for the possible occurrence of foodborne intoxication (Carrizosa *et al*., 2017).

***In-vitro* antibiotic sensitivity test**

The results of *in-vitro* antibiotic sensitivity and resistant patterns of different organisms are presented in Table 2. All microorganisms (*Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Salmonella* spp., and *Proteus* spp.) tested were highly sensitive to antibiotics such as ciprofloxacin and gentamicin (about 100% susceptible). In the case of *Staphylococcus* spp., it was susceptible to ampicillin, nalidixic acid, erythromycin, and tetracycline at the rate of 90, 70, 10, and 100%, respectively. In the case of *Streptococcus* spp., all of them were sensitive to ampicillin (100%), but all were resistant to tetracycline. The sensitivities to erythromycin and nalidixic acid were 60 and 80%, respectively. In the case of *Escherichia coli,* 13% were sensitive to ampicillin, 62% to tetracycline, 75% to nalidixic acid, and 87% to erythromycin. Although all the *Salmonella* spp. were resistant to ampicillin and tetracycline, 50% of them were sensitive to erythromycin. About 20, 60, 80, and 100% of *Proteus* spp. were susceptible to ampicillin, tetracycline erythromycin, and nalidixic acid.

The antibiotic sensitivity test of the isolated *E. coli, Streptococci, Staphylococcus aureus, Salmonella,* and *Proteus* revealed that all these organisms were susceptible to the third generation of antibiotics like ciprofloxacin, gentamicin. In contrast, they were partially resistant to tetracycline, erythromycin, and ampicillin. Sharma *et al.* (1995) and Mukhopadhyay *et al.* (1998) reported similar evidences on the antibiogram profile of the isolated bacteria from goat meat. The antibiogram result of *Staphylococcus* spp. in this study is in agreement with the findings of Franca *et al*. (2012) and Rola *et al*. (2015). Similarly, antibiogram results of *Salmonella* spp., were supported by Andoh *et al*. (2017); findings of antibiogram of *E. coli* were in close agreement with Mansor *et al*. (2019) and Ismail and Abutarbush (2020). Haque *et al*. (2015) reported 100% resistance patterns of *Proteus* spp. against ciprofloxacin and nalidixic acid.

Table 2. *In-vitro* antibiotic sensitivity patterns of bacterial isolates

Isolated bacteria	No. of organisms tested	No of isolates sensitive to antibiotics											
		Ampicillin		Ciprofloxacin		Erythromycin		Gentamicin		Nalidixic acid		Tetracycline	
		No	%	No	%	No	%	No	%	No	%	No	%
Staphylococci	10	9	90	10	100	1	10	10	100	7	70	10	100
Streptococci	5	5	100	5	100	3	60	5	100	3	60	0	0
Escherichia coli	8	1	13	8	100	7	87	8	100	6	75	5	62
Salmonella spp.	2	0	0	2	100	1	50	2	100	1	50	0	0
Proteus spp.	5	1	20	5	100	4	80	5	100	5	100	3	60

The results of the present study reflect the fact that some microorganisms acquire resistant factors, which by way of bio-magnification ultimately reach the consumers and cause alarm to produce potential health hazards (Lerma *et al*., 2014). In addition, indiscriminate use of antibiotics for treating animals and environmental contamination with antibiotic residues, antimicrobial resistance genes, and antimicrobial resistant microorganisms may be responsible for acquiring resistant factors by microorganisms (Argudin *et al*., 2017). These endogenous organisms cause meat-mediated diseases to induce threat to consumers' health and bring forth risk in the integrity of consumers' protection.

CONCLUSIONS

The presence of pathogenic *Staphylococci*, *E. coli*, *Streptococcus*, and *Salmonella* revealed the fact that the meat sold in local markets may endanger public health. Some of the bacteria isolated from meat samples were resistant to conventionally used antibiotic like tetracycline, ampicillin, erythromycin, etc., which reflects the fact that wide and indiscriminate use of antibiotics in health management practices may threaten to build up risk factors and create resistant factors which through bio-magnification, this may reach the consumers to cause potential health hazards. Hygienic slaughtering and meat processing, scientifically approved transportation, storage, and marketing systems may be adopted to obtain wholesome, safe, and sound meat through the application of the Longitudinally Integrated Safety Assurance (LISA) system and Good Manufacturing Practices (GMP).

CONFLICT OF INTEREST

The authors decaled that there is no conflict of interest.

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