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Bacteriological quality of poultry meat in Nepal

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Abstract
Poultry meat is one of the important sources of protein in under-developed countries like Nepal. In Nepal poultry contributes 4% of Gross Domestic Product. Bacteriological quality of poultry meat have been assessed by various researchers at a different time. To estimate the bacteriological load, TVC (Total viable Count), TCC (Total Coliform Count), Staphylococcal count etc. are used and for qualitative study different parameters like colony characteristics, biochemical reactions, enzymatic reactions, and antigenic characteristics are studied. Study of various research papers reveal that there is the high amount of bacterial load in terms of TVC, TCC and staphylococcal count and presence of different zoonotic bacteria like *E. coli, Salmonella, Staphylococcal aureus, Campylobactor spp.* Bacterial isolates also have shown the Antimicrobial Resistance(AMR) pattern for different commercially used antibiotics like Amoxicillin, Tetracyclines, and others. Presence of High bacterial load and AMR bacteria in poultry meat had led to potential health issues. The rules, regulations, guidelines and standards related to poultry meat production, processing and selling are not implemented effectively. To ensure hygienic poultry meat, effective implementation of rules, regulations, and guidelines should be made and regular microbiological surveillance of poultry meat should be done.

Keywords: Antimicrobial resistance, bacteriological characteristics, Poultry meat, Nepal, zoonosis

1. Introduction
The term meat refers to the flesh, skeletal muscle and any attached connective tissue or fat excluding bone and bone marrow. Meat is an ideal medium for the growth of bacteria because it contains essential nutrient like water, vitamin, minerals, essential fatty acids &essential amino acids (Ahmad *et al*., 2018) [2]. Fluctuance in storage temperature and mishandling of carcass during evisceration, processing, canning or packaging leads for the spoilage of meat by various bacteria (Russel, 2009) [40]. The contaminated meat products lead for deterioration of quality of meat in terms of biological, physical and chemical means (Kim *et al*., 2016) [22]. Foodborne infection are a major cause of death in the least developed countries and among them, meat-borne is most common. The different pathogenic bacteria which are contained in meat like *Staphylococcus aureus, salmonella sps, coliform* can cause a variety of diseases like typhoid, dysentery, enteritis, bloody diarrhea and others (Griffin, 2018) [19].
1.1 The poultry sector in Nepal: The livestock sector contributes 13% of the total Gross Domestic Product (GDP) in Nepal (Tutorials, 2014) [44]. About 4% of GDP is contributed by the poultry sector alone in Nepal. The annual growth rate of poultry production is about 17-18% (Nepal Agritech, 2018) [33] Poultry sector provides most of the meat demands with 1,50,000 kg/day of meat (Nepal Agritech, 2018) [33]. With this huge poultry industry, Nepal suffers from the potential risks which are associated with unhygienic meat supply, risk of zoonotic diseases & AMR development.

2. Microbial contamination in Meat
Sources of food contamination may be primary, coming directly from an infected food animal or its secretion; or secondary resulting from contamination to the handling of food. Secondary infection may come from infected humans or live animals' carriers of pathogens, soil, equipment, excreta and hands, nasal discharges, contaminated wounds, contaminated water, insects or feed additives. Infected human may be the source of contamination at any point in the food chain but are more frequently implicated when preparing food for the table (Hubbert *et al*., 1996) [20].

2.1 Spoilage Microbes

Microbial growth is the main cause of the spoilage of meat. Different bacteria, fungi and yeast cause spoilage of meat (Gram *et al*., 2002) [18]. Growth of spoilage bacteria may lead to deterioration of meat quality as unwanted color, odor, texture, and hygienic aspects (Rougre, Tresse, & Zagrec, 2017) [39]. Different gram-positive and gram-negative bacteria spoil the meat over a period of time. The list of different microbes in poultry meat as follows in the table (Rougre, Tresse, & Zagrec, 2017) [39].

Table 1: Different spoilage microorganism in Meat

Gram-positive bacteria	Gram-negative bacteria	Fungi or Yeast
Staphylococcus	Pseudomonas	Cryptococcus neoformuis
Micrococcus	Actinobactor	Candida albicans
Bacillus	Psychrobactor	Candida
Brochothrix	Salmonella	
Listeria monocytogenes	Campylobacter	

2.2 Human pathogens and zoonosis

Amount of human pathogenesis is determined by the number of bacteria present in the raw poultry meat sample. The main zoonotic disease from poultry to human transmission are avian influenza, Newcastle disease, avian tuberculosis: *Mycobacterium avium*, ornithosis, *Chlamydophila psittaci*, Erysipelas: *Erysipelothrix rhusiopathiae, Salmonella, Campylobacter, Escherichia coli*. (Dale & Brown, 2013*)* [12]. the bacterial cause disease either by bacterial borne or releasing of pre-formed toxins in meat (Lacey, 1993) [23]. Sometimes a bacteria may produce infectious disease and can produce lethal toxins like streptococcus (Lacey, 1993) [23]. Thus, the importance of toxin formation is that toxins can act after bacteria had died and the effects of toxins may occur very rapidly after ingestion. In the case of foodborne disease, only a relatively small number of bacteria need for causing infection, meat serves as a transport medium rather than growth medium.

2.3 Food poisoning from Meat

Table 2: Sources of food poisoning from Meat

Organism	The main source of origin	Effects
Staphylococcus aureus	a) Nasal passages of animals and people, therefore, meat of head, and less prominent in other parts of the carcass. (b) Infected wounds, boils, etc. (c) Note that some staphylococci are salt-tolerant and may therefore survive in cured meats	Intoxication Severity: slight to moderate Onset: rapid 2-8 hrs
Salmonellae, Yersinia, Campylobacter	▪ Meat becomes contaminated at the carcass-dressing stage. ▪ Some people may be carrying salmonellae themselves without symptoms and may infect meat	(Infection) Severity: moderate to severe; fatal in extreme cases (e.g. small children, old people). Onset: slow, 12-24 h
Clostridium	Soil; therefore in dirt associated with animals Spices.	(Intoxication) Severity: *CI. perfringens* moderate; *CI. bofulinum* often fatal Onset: moderate or slow, 8-24 h or longer

***Source*:** (Handbook of meat product technology Ranken, 2000) [38].

2.4 Bacteria present in Poultry meat from Public Health significance

2.4.1 *Salmonella*: It is one of the most commonly encountered bacteria in poultry meat. Different species of salmonella are found as *S. pullorum, S. gallinarum, S. typhimurium, & S. Heidelberg,* among these four bacteria last two are importance of public health. (Lacey, 1993) [23] Salmonella, harbors in intestines of animals including humans and cause various illness like typhoid, diarrhea (Lacey, 1993) [23]. According to CDC estimation salmonella causes 1.2 million illness, 450 death in a year in the United States. Backyard poultry and incidence of human salmonella are also noticed in a report there are 768 hospitalized cases in 48 states out of which 2 are dead. In February 21, 2019, A total of 129 people infected with the outbreak strain of *Salmonella Infantis* were reported from 32 States. Twenty-five people were hospitalized. One death was reported from New York ("Outbreak of salmonella-CDC", 2019) [34].

2.4.2 *Staphylococcus aureus*: It is a most common bacteria present in poultry skin, feather and other parts of the chicken body. Methicllin Resistance Staphylococcus Aureus (MRSA) plays an important role in zoonosis and disease transmission. (MRSA CDC, 2019) [31] They act by producing toxins which cause disease. Staphylococcus poisoning is characterized by nausea, vomiting, diarrhea, and stomach cramps ("Staphylococcal Food", 2019) [42].

2.4.3 *Coliforms*: Diarrhea caused by enterotoxigenic *E. coli* is highly present in young children in developing countries and travelers. It spreads through the contaminated water and feed. (E. coli, 2019) [15] Extraintestinal pathogenic *E. coli* cause diverse infection in humans including meningitis, Urinary tract infections, and sepsis (Mellata, 2013) [30]. Many poultry and human pathogenic E. coli isolates tend to exhibit similar genomes, and colony backgrounds leading to potential zoonotic threat (Jakobsen *et al*., 2011) [21] Infections in humans from Antimicrobial resistance *E. coli* (G3CREC) caused from poultry lead to 21 death in the Netherlands. If we considered total G3CREC cases in Europe 56% is from the poultry industry it had led in 1,518 deaths and increase of 67,336 days of hospitalization (Collignon, 2013) [10].

2.4.4 *Listeria monocytogenes*: Listeria is gram-positive, Psychrotrophic, facultative anaerobic non-sporulating motile and small rods. This disease is rare and sporadic in poultry but occasionally causes septicemia and localized encephalitis in chickens. Human listeriosis is a worldwide phenomenon causing outbreaks in developing and developed Countries. (Dharma *et al*., 2013) [14] This disease ranks second after salmonellosis from foodborne infection particular affects young (Neonates) and immunocompromised people of old age (Dharma *et al*., 2013) [14] An estimated 1,600 people get listeriosis each year, and about 260 die (Listeria-CDC, 2019) [25]. Refrigeration doesn't inactive or kill bacteria so the process of cooking is of great importance. This disease manifests the condition of encephalitis or meningitis characterized by high temperature, stiffness of the neck, tremors, ataxia, incoordination, vomiting malaise, and pregnant abortion (Listeria-CDC, 2019) [25].

2.4.5 *Campylobacter*: These are gram-negative, curved's' shaped bacteria that are commonly occurring in the environment and the most common cause of foodborne disease. They are facultative anaerobes and found on the gut wall as commensals (Campylobacter, 2019) [7]. It is one of four global cause of human diarrhea. Major campylobacter isolates from humans are *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*. Clinical signs manifested by infection of this disease are of diarrhea (most commonly bloody), abdominal pain, fever, vomiting, cramps onset of these signs are seen 2 to 5 days of ingestion of infected meat(Campylobacter, 2019) [7]. During 2018, 3,030 cases of campylobacteriosis were reported, as foodborne but no source were identified (Campylobacter infection, 2018) [8].

3. Assessment of Bacteriological quality of meat

3.1 Qualitative assessment: In this procedure of bacteriological assessment of meat, it tends to identify the presence of the specific bacterial organism in Meat. It uses the different techniques of bacterial isolation, identification and serotyping. Main tools used in this process are of two types (Mead, & Mead, 2007) [29].

3.1.1 Cultural method which uses different biochemical test (e.g MR/VP, citrate utilization, TSI), Colony characteristics in different media (XLD for Salmonella, Mac-Conkey for *E-Coli*)

3.1.2 Rapid Methods: The cultural methods present difficulty by taking time to yield result and chances of human error is relatively high. There are two classes of this methods antibody detection (ELISA, Agglutination test & immunomagnetic separation) and gene detection methods. (Nucleic acid hybridization, PCR).

3.2 Quantitative assessment: Cultural techniques are widely used for the quantitative approach. TVC (Total Viable Count) & TCC (Total coliform count): This count illustrates the total number of living / multiplying bacteria present in the sample. This is done by plate culture in a specific media like nutrient agar or selective medium to restrict the growth of unwanted bacteria ("Counting bacteria", 2019) [11]. The total number of the visible colony is counted and represented after calculation of dilution ratio. Represented as Colony Forming Unit per gram or per milliliters or centimeter square.

4. The permissible: limits of TVC, TCC, and Staphylococcal counts in ready to eat foodstuffs are illustrated as following:

Table 3: Permissible limits of bacteriological loads

Microbial Group	Good	Acceptable	Unsatisfactory	Unacceptable And potentially dangerous
Total viable count	<10^4	10^4 - 10^6	>=10^6	N/A
Total coliform count	<10^2	10^2-10^4	>=10^4	N/A
S. aureus	<10^2	10^2-10^3	10^3- 10^4	>=10^4

5. Different bacterial loads from poultry meat sample at various places in Nepal

Table 4: Bacterial loads from poultry various places in Nepal

Place and sample size Count in mean±SE ($log_{10}cfu/gm$)	Year	Mean TVC (total viable count)	Mean TCC (Total coliform count)	Staphylococcus count	Bacteria isolated And the prevalence percentage	Source
1. Bhaktapur n=40	2018	8.31±0.23	5.42±0.27	4.39±0.40	*Salmonella* (75%)	(Lamichhane, 2018) [24]
2. Biratnagar n=40	2017 Jan-2018 July	8.5	-	-	*S. aureus (52.5)* *E.coli (62.5)* *Salmonella (42.5)* *Vibro (5)* *Pseudomonas (47.5)*	(Mahato, 2018) [27]
3. Kanchanpur n= 45	March 2014 to April 2014	10.17 ±0.1	-	-		(Bohara, 2017) [6]
4. Dharan n =15	Jan to June 2017	8.22l±0.14	8.13±0.13	-	*Salmonella(60)* *E.coli(66.6)* *Pseudomonas aeroguinosa(46.66)* *S. aureus(53.33)* But *NO vibro spp*	(Bantawa, Rai & Limbu, 2018) [3]
5. Lekhnath unnicipality n=271	2015	-	-	-	*Salmonella (10%)*	(Dhakal *et al*., 2016) [13]
6. Bharatpur n=26	2012	11.1±0.3	6.5±0.3	6.5±0.2	*Coliforms, Salmonella*	(Bhandari, Nepali, & Paudyal, 2013) [4]
7. Ratnagar n=9	2012	11.5±0.3	7.6±0.3	6.8±0.3	*Coliforms Salmonella*	(Bhandari, Nepali, & Paudyal, 2013) [4]
8. IAAS, Rampur n=4	2012	12.2±0.5	8.4±0.5	7.7±0.4	*Coliforms Salmonella*	(Bhandari, Nepali, & Paudyal, 2013) [4]
9. Katmandu local market n=55	2002 Nov-Dec	-	-	-	*Salmonella (14.5%)*	(Maharjan *et al*., 2006) [26]

***Source*:** (Gulf Standards and NSW Food Authority).

6. Antimicrobial Resistance bacteria in poultry meat of Nepal

The use of antibiotics in the poultry sector in Nepal is quite high. Prophylactic does of antibiotics has been banned in Nepal but lack of implementation of strict rules and regulation there is high use of antibiotics which leads to antimicrobial resistance (AMR) development (Acharaya & Wilson, 2019) [1]. No proper veterinary guidance, self-medication by poultry farmers, low regulation of antibiotic prescription in veterinary practice and high use of antibiotics (chlortetracycline (CTC), bacitracin methylene disalicylate, tylosine tartarate, lincomycin, neomycin, and doxycycline) in poultry feed poultry are a main contributing factor for the development of antimicrobial resistance. The ignorance of drug withdrawal period and increasing trend of finding antibiotic residue in poultry is also a factor for antimicrobial resistance (Acharaya & Wilson, 2019) [1]. The pattern of antimicrobial resistance (AMR) bacteria isolated from poultry meat in from various places of Nepal are as follows.

Table 5: Antibiotic resistance pattern of bacteria isolated from poultry meat at various places in Nepal.

Location	Year	Organism	Resistance%	References
National Avian Laboratory n=63	2012	Various Microorganism	Tetracyclines (33.33) Ciprofloxacxin (20), Gentamicin (0), Amicacin (41.20), levofloxacin (17.70), cephalexin (0), ceftriaxone (0), Norfloxacin (33.33), Cotrimoxazole (75),	(NAL, 2012) [32]
Chitwan 7 hatcheries n= 140	2013	*E. coli*	Amoxicillin(93), Gentamycin (86), Enrofloxacin (50), Ciprofloxacin (36)	(Shah., 2013) [41]
Chitwan gizzard, liver breast muscle n=225	2015	*Salmonella*	Furazolidone (82.1), Tetracycline (32.1), Cephalexin (28.6), Cotrimoxazole (17.9), Enrofloxacin (14.3), Colistein (10.7),	(Ramdam, 2015) [37]
Chitwan: gizzard, liver breast muscle n=225	2015	*Staphylococcus*	Furazolidone (97.1), Tetracycline (10.4), Cephalexin (12.5), Cotrimoxazole (62.5), Enrofloxacin (54.2), Gentamycin (14.6)	(Ramdam, 2015) [37]
Chitwan; gizzard, liver breast muscle n=225	2015	*E. coli*	Furazolidone (98.1) Tetracyclins (88.9) Cephalexin (79.6)	(Ramdam, 2015) [37]
Bhaktapur Municipality n=70	2017 April to September	*E. coli* (33 isolates)	Amoxicillin (69.6) Tetracycline (60.6) Nalidixic Acid (54.4)	(Bhuvan *et al.*, 2019) [5]
Bhaktapur Municipality n=70	2017 April to September	*Klebesella spp* (24 isolates)	Amoxicilli (79.1) Tetracycline (41.6) Nalidixic Acid (33.3) Cotrimoxazole (29.1)	(Bhuvan *et al.*, 2019) [5]
Bhaktapur Municipality n=70	2017 April to September	*Ciratobactor spp* (11 isolates)	Amoxicillin (54.5) Tetracyclines (18.8)	(Bhuvan *et al.*, 2019) [5]
Bhaktapur Municipality n=70	2017 April to September	*Staphylococcus spp*	Amoxicillin (33.3) Teteracyclines (44.2) Azithromycin (22.2)	(Bhuvan *et al.*, 2019) [5]
Chitwan n=50	May 2016 to March 2017	*E. coli*	Ampicillin (98) Amikacin (16)	(Subedi *et al.*, 2018) [43]
Bhaktapur n=40	July 2018 to September 2018	*Salmonella*	Gentamycin (26.67) Ceftriazone (6.67) Ampicillin (100) Nalidixic acid (100) Ciprofloxacin (20) Tetracyclines (93.33) Chloramphenicol (90), Colistein (36.67)	(Lamichhane, 2018) [24]

Above data clearly shows that the antibiotic resistance pattern of various commonly found bacteria like *E. coli, staphylococcus. klebesella* are higher to common antibiotics that are found in the market. The data illustrates that antibiotics like Amoxicillin, Tetracyclines, furazolidones are losing their potency over time. These data clearly represent that there is an increasing trend of antibiotic resistance.

7. Recommendation

There are various methods to control the microbial contamination and spoilage organism in poultry meat:

7.1 Farms: Cleaning/sanitizing poultry houses after every flock has been shipped to the slaughterhouse. Animals should be sampled before slaughter and pathogen-free flocks run through production lines before infected flocks to reduce the incidence of cross-contamination. Transport cages should be cleaned and disinfected after every use.

7.2 Slaughter-House: The use of HACCP (Hazard analysis and critical control points) and GMP (Good manufacturing practices) in poultry processing and packing industry could reduce the risk of possible microbial Contaminations. Educating and training to slaughterhouse employee is also a possible way of reducing the microbiological load.

7.3 Rules and Regulations: There should be effective implementation of rules, regulations, acts, standards which are associated with the poultry industry. There should be regular monitoring of slaughterhouse, poultry meat processing plants and wholesalers/retailer meat shops. Surveillance on bacteria and pattern of antimicrobial resistance (AMR) in poultry meat should be made by different agencies like the Department of Food and quality control, public health offices and veterinary hospitals. Different awareness and training on the rational use of antibiotics should be provided.

8. Authors contribution

Rajesh Neupane has conceived the original paper and provided a rough draft. Krishna Kaphle checked the paper for consistency, corrected the language and checked all references for consistency and accuracy.

9. Conflict of interest statement

The authors declare that this review is conducted in the absence of any funding or support from any organization.

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