

Antimicrobial Susceptibility Pattern of Gram-Negative Bacterial Isolates from Raw Chicken Meat Samples

Neha Gautam¹, Rojan Poudel¹, Binod Lekhak², Milan Kumar Upreti^{1*}

¹Department of Microbiology, GoldenGate International College, Wisdom Tower, Trikuti Colony, Old Baneshwor, Battisputali, Kathmandu

²Central Department of Microbiology, Tribhuvan University, Kritipur, Kathmandu

*Corresponding author: Milan Kumar Upreti, Department of Microbiology, Goldengate International College, Kthmandu, Nepal; Email: milanupreti@gmail.com

ABSTRACT

Objectives: This research aims to study the microbial quality of chicken meat available in retail shop of Kathmandu valley.

Methods: This study was conducted from June to December 2018 in three different districts of Kathmandu Valley. Samples were collected in sterile plastic bags, labeled properly and stored in an icebox and transported to the Food Microbiology laboratory of Golden Gate International College.

During sample preparation, 25 grams of each sample was taken and transferred to sterile flasks containing 225 ml of buffered peptone water. Potential pathogenic Gram-negative bacteria were isolated by using respective selective media and identified by biochemical test. Antibiotic susceptibility profile of isolates was carried out by Kirby-Bauer disc diffusion method according to CLSI 2017 guideline.

Results: Of total 81 chicken meat samples processed, 201 Gram negative bacteria were isolated. *E. coli* (100%) was the dominant Gram-negative isolates, followed by *Citrobacter* spp (62.96%), *Pseudomonas* spp (40.74%), *Proteus* spp (19.75%), *Salmonella* spp (16.04%) and *Klebsiella* spp (8.64%) respectively. No any multidrug isolates were detected.

Conclusion: The study showed that the raw chicken meat samples of Kathmandu valley was highly contaminated with Gram negative potential pathogenic bacteria. Antimicrobial resistance pattern shown by the isolates may indicates that there is not overuse of drug in animals and the less chance of risk of increasing antimicrobial resistance.

Key words: Chicken meat, Antimicrobial susceptibility test, Gram negative bacteria

INTRODUCTION

Poultry meat is the combination of muscle tissue, attached skin, connective tissue, and edible organs, comprising about two-thirds of the total meat production in the world (Ruban et al. 2010). Chicken meat is considered as a healthy food, due to high protein content and reduced fat content, as well as a higher proportion of polyunsaturated fatty acids (PUFA), when compared to other species meats (Riovanto et al. 2012). In Asian countries, chicken meat was periodically included in the diets of consumers in

the past. However, with the rapid economic growth and globalization of the food industry, the amount of meat production and consumption has grown rapidly in recent years (Nam et al. 2010).

Due to its high percentages of nitrogenous compounds of various degrees of complexity, high moisture, abundant supply of minerals, accessory growth factors and some fermentable carbohydrates (glycogen) of a suitable pH, meat are regarded as a perfect culture medium for most of the microorganisms (Holck et al. 2017). The hygiene often fails during slaughtering,

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scalding, evisceration, plucking, bleeding, washing, and rinsing, and increase the health risk associated with the consumption of this meat (Coulibaly et al. 2010). Similarly, animals can also become infected from water or food contaminated with wastes of human or animal origin or with human carrier workers (Da Silva et al. 2011). One of the possible ways of entry of various microbes could be the handling of meat and meat products by adopting improper hygienic measures during handling and processing (Kiranmayi et al. 2011). It has been pointed out that during slaughter, dressing, and cutting, microorganisms come chiefly from the exterior of the animal and its intestinal tract but more are added from knives, cloths, air, workers, carts, boxes, and equipment in general (Bhandare et al. 2007). *Escherichia*, *Enterobacter*, *Klebsiella*, *Proteus*, *Citrobacter*, *Serratia*, *Salmonella*, *Shigella* and *Yersinia*, are among the most important causes bacterial infections in humans (Jarzab et al. 2011).

Antibiotics are often used for therapy of infected humans and animals as well as for prophylaxis and growth promotion of food producing animals. Many findings suggest that inadequate selection and abuse of antimicrobial may lead to resistance in various bacteria and make the treatment of bacterial infections more difficult (Kolar et al. 2001).

Poor personal hygiene is prime cause of food borne illness. Hand washing is the crucial measure to prevent propagation of diseases and to cut back the transmission of bacteria among individuals and to food as well (Lambrechts et al. 2014). Resistant bacteria are transmitted to human through direct contact with animal, by exposure to animal manure, through consumption of uncooked meat, and through contact with meat surfaces (Marshall and Levy 2011). The expansion of antibiotic resistance in bacteria is also an emerging public health hazard due to the compromised efficacy in the treatment of infectious diseases (Helmy et al. 2017).

MATERIALS AND METHODS

Study was conducted from June to December 2018. Random, purposive sampling was conducted for the collection of sample to study. A total of 81 raw chicken meat samples were collected from different retail meat shops at Kathmandu, Lalitpur and Bhaktapur districts. Samples were collected in sterile plastic bags. Samples collected from different butcher shops at Lalitpur,

Kathmandu and Bhaktapur districts were labeled properly and stored in an icebox until delivery and transported to the Food Microbiology laboratory of Golden Gate International College. Raw chicken meat was collected from different meat shops and studied. Packaged, cold stored or chemically treated meat was not collected (Da Silva et al. 2013).

During sample preparation, 25 gram of each sample was taken and transferred to sterile flasks containing 225 ml of buffered peptone water. Samples were being homogenized using a meat grinder under aseptic condition and kept in rotatory shaker at 80 rpm for 30 minutes (Shrestha et al. 2017).

For the enrichment, 1ml of the sample from the flask was suspended in 9ml of Selenite Broth and incubated for 24 hours at 37°C (Shrestha et al. 2017). Loopful of sample from Selenite broth was streaked on Xylose Lysine Deoxycholate (XLD) agar plates, plates were then incubated at 37°C for 24 hours (Da Silva et al. 2013).

Plates were examined for presence of typical *Salmonella* colonies. *Salmonella* colonies may appear pink colonies with black center (Shrestha et al. 2017). Isolated colony was taken and streaked in Nutrient agar and incubated for 24 hours at 37°C. Isolated colony from NA was transferred to Triple Sugar Iron Agar (TSIA) and was incubated at 37°C for 24 hours for the confirmation of *Salmonella* (Shrestha et al. 2017). For the enrichment, 1ml of the sample from the flask was suspended in 9ml of Nutrient Broth and incubated for 24 hours at 37 °C (Shrestha et al. 2017). Loopful of sample from Nutrient Broth was streaked on M-endo agar. The plates were incubated at 37 °C for 24 hours. Colonies exhibiting metallic sheen on M-endo agar was sub cultured on NA to obtain pure culture. Pure cultures were confirmed to be *E. coli* by biochemical tests (IMViC, TSI, O/F and Urease) (Da Silva et al. 2013).

Isolation and identification of *Pseudomonas* spp. was done by streaking loopful sample from NA to cetrimide agar plate. Colonies showing yellowish green color was tested for conformation of *Pseudomonas* (Estepa et al. 2015).

Colonies showing pink color in Mac conkey agar plate were conformed as Gram negative isolates and species were confirmed by following CLSI guidelines 2017.

Testing for antibiotic sensitivity was done by the

Modified Kirby-Bauer Disc diffusion method by using Muller Hilton Agar. The bacterial suspensions made in normal saline were compared with 0.5 McFarland solutions and swabbed on MHA plate using sterile cotton swab. Antibiotic disc were then placed on the swabbed MHA plate. After overnight incubation at 37°C the zone of inhibition was observed around the antibiotic disc. Using the CLSI guidelines, the

susceptibility or resistance of the organism to each drug tested was determined. For each drug, the recording sheet whether the zone size is susceptible (S) or resistant (R) based on the interpretation chart was indicated. The results of the Kirby-Bauer disk diffusion susceptibility test were reported only as susceptible or resistant (CLSI 2017). Statistical analysis was done by using SPSS version 16.

RESULTS

Of the total of 201 potential bacterial pathogens isolates, meat samples from the Kathmandu district show the highest contamination and samples from the Lalitpur least as shown in Table 1.

Table 1: Percentage occurrence of bacterial pathogens in Kathmandu valley

Districts	Number	Percentage
Kathmandu	84	41.79
Bhaktapur	61	30.34
Lalitpur	56	27.86

Among 81 samples examined from three different districts, Gram negative bacterial isolates identified were *E. coli*, *Salmonella* spp, *Proteus* spp, *Citrobacter* spp, *Pseudomonas* spp, and *Klebsiella* spp. respectively. Among them, *E. coli* was isolated from all the samples 81 (100%), *Salmonella* spp. from 13 (16.04%) samples, *Pseudomonas* spp. from 33 (40.74%) samples, *Proteus*

spp. from 16 (19.75%) samples, *Klebsiella* spp. from 7 (8.64%) samples and *Citrobacter* spp. from 51 (62.96%) samples as shown in Table 2. Among the 27 meat samples collected from each district, *E. coli* was isolated from all the samples, whereas *Klebsiella* was not isolated from sample of Bhaktapur and *salmonella* from sample of Lalitpur district.

Table 2: Percentage occurrence of the isolated bacteria from chicken sample

Bacteria	No. of isolates	Percentage
<i>E. coli</i>	81	100.00
<i>Salmonella</i> ssp	13	16.04
<i>Pseudomonas</i> ssp	33	40.74
<i>Proteus</i> ssp	16	19.75
<i>Klebsiella</i> ssp	7	8.64
<i>Citrobacter</i> ssp	51	62.96

Antibiotics susceptibility testing showed all isolates of *E. coli* were resistant to Ampicillin and sensitive to

Amikacin, Gentamicin, Ceftriaxone and Cotrimoxazole (Table 3).

Table 3: Sensitivity pattern of *E. coli* (n=81)

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	-	81(100)
Ciprofloxacin	78(96.29)	3(3.71)
Gentamicin	81(100)	-
Chloramphenicol	80(98.76)	1(1.24)
Tetracycline	9(11.11)	72(88.89)
Cotrimoxazole	81(100)	-
Amikacin	81(100)	-
Ceftriaxone	81(100)	-

All the isolates of *Salmonella* spp. isolates were sensitive to Cotrimoxazole, Ciprofloxacin, Amikacin and

Ceftriaxone and resistant to Ampicillin (Table 4).

Table 4: Sensitivity pattern of *Salmonella* spp. (n=13)

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	-	13(100)
Ciprofloxacin	13(100)	-
Gentamicin	11(84.62)	2(15.38)
Chloramphenicol	9(69.23)	4(30.77)
Tetracycline	3(23.07)	10(76.93)
Cotrimoxazole	13(100)	-
Amikacin	13(100)	-
Ceftriaxone	13(100)	-

All the isolates of *Pseudomonas* spp. isolates were subjected to AST and found sensitive to Gentamicin, Ciprofloxacin, Amikacin, Ceftriaxone and Cotrimoxazole and resistant to Ampicillin (table 5).

Table 5: Sensitivity pattern of *Pseudomonas* spp. (n=33)

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	-	33(100)
Ciprofloxacin	33(100)	-
Gentamicin	33(100)	-
Chloramphenicol	8(24.24)	25(75.76)
Tetracycline	10(30.30)	23(69.70)
Cotrimoxazole	22(66.67)	11(33.33)
Amikacin	33(100)	-
Ceftriaxone	33(100)	-

All the isolates of *Citrobacter* spp isolates were found to be sensitive to Chloramphenicol, Ciprofloxacin, Amikacin, Gentamicin and Ceftriaxone. All the isolates of *Proteus* spp. were sensitive to Gentamicin, Cotrimoxazole, Amikacin and Ceftriaxone and resistant to Tetracycline. All the isolates of *Klebsiella* spp were sensitive to Amikacin and Ceftriaxone. No any MDR isolates were identified.

DISCUSSION

Out of 81 samples collected from different meat shops, a total of 201 potential pathogenic Gram negative bacterial isolates were detected among which, all samples showed growth with multiple isolates. Similar result were reported from other studies conducted in North East India, *E. coli* (98%), *Citrobacter* (52%), and *Salmonella* (20%) (Sharma 2012).

In this study all the samples showed presence of *E. coli*, which is very high as compared to previous study (4.8%) by Shrestha et al. 2017 and Thanigaivel and Anandhan 2015 (70%). The reason behind this huge difference could be due to the use of contaminated water during slaughtering, washing and in other handling processes, and also due to *E. coli* being common inhabitants of animal and human guts (Cabral 2010). As this study does not focus on the water quality of the water used in

the meat shops and the parameters on the water quality are not studied either. Total of 62.96% of occurrence of *Citrobacter* spp was seen, which is less as compared to result presented from other studies, (Shrestha et al. 2017) i.e. 44.7% and (Gwida 2014) in Egypt which is 13.3%. The higher percentage of *C. freundii* presence in foods can be ascribed to secondary contamination (Ryan et al. 2004; Raphael and Riley 2017).

Thirteen (16.04%) isolates were detected as *Salmonella* spp which is much lower than the result presented in other similar studies from Egypt, Azez (2013) i.e. 44% and Nigeria i.e 33.33% (Adeyanju and Ishola 2014).

About 41 % (33) isolates were *Pseudomonas* spp. which is much higher than 1.9% reported (Shrestha et al. 2017) in Bharatpur, Nepal and 10% (Thanigaivel and Anandhan 2015) in India but somewhat similar to 46.66% found in Dharan (Bantawa et al. 2018). *P. aeruginosa* is one of the most relevant opportunistic human pathogens, although there are also reports that show clinical cases caused by environmental *Pseudomonas*, such as *P. mendocina* and *P. fulva* (Nseir et al. 2011). Differences in prevalence rates from this study to another might be attributed to the unhygienic processing and poor sanitation of meat shops. It showed that meat retailers were found to be unaware of the basic requirements of

basic guidelines regarding meat (Almuzara et al. 2010 and Seok et al. 2010).

Percentage of *Proteus* spp. isolates were more similar to study by Shrestha et al. (2017) but lower than study by Al-Mutairi (2011) from Saudi Arabia. *Proteus* spp. is considered as an indicator of contamination of meat during any of the processing, handling, storage stages. If the optimal condition for the isolated *Proteus* were existed, typical cases of food poisoning, urinary infection and other *Proteus* related human illnesses could happen due to rapid proliferation of the pathogen (Al-Mutairi 2011).

Klebsiella spp had least occurrence as compared to other isolates i.e.7 (8.64%), which is very less as compared to the result shown (Kim et al. 2005) in Oklahoma which is 30% but similar to Al-Mutairi 2011 (10.66%). *Klebsiella* spp is a colonizing opportunistic pathogen of humans and animals, and a common contaminant of retail meat (Kim et al. 2005). In animals, *Klebsiella* spp. causes disease in cows, horses, and other animals (Bersisa et al. 2019 and Ewers et al. 2014). In humans, *Klebsiella* spp. frequently colonizes the gut and sporadically causes extra intestinal infections (Podschn and Ullmann 1998).

All *E. coli* isolates were resistance to Ampicillin, 88.89%, 3.71% and 1.24% were resistant to Tetracycline, Ciprofloxacin and Chloramphenicol respectively. All of isolates were sensitive to Cotrimoxazole, Amikacin, Gentamicin and Ceftriaxone followed by 98.76%, 96.29% and 11.11% sensitivity to Chloramphenicol, Ciprofloxacin and Tetracycline respectively. In the study carried at Nigeria 83% of *E. coli* isolates were resistant to Tetracycline, 89% resistant to Cotrimoxazole and 28.3% resistant to Gentamicin (Adeyanju and Ishola 2014). Thanigaivel et al. 2015 in India reported 26% resistivity of *E. coli* to Tetracycline. Somda et al. 2018 in Burkina Faso, reported 100% sensitivity to Ciprofloxacin, Gentamicin, and Chloramphenicol. However, resistance was observed with Ceftriaxone (10.71%), Ampicillin (42.86%), and tetracycline (64.3%).

A potential health hazard to consumers can be expected from resistant bacteria. If the organism is resistant to antibiotics, then initial treatment may be ineffective both in man and animals and an alternative treatment need to be applied (cotterill et al. 1977).

Total of 13 isolates of *Salmonella* spp., 100% were sensitive to Ciprofloxacin, Cotrimoxazole, Amikacin,

and Ceftriaxone and 100% were resistant to Ampicillin. Only 84.62%, 69.23%, and 23.07% sensitivity were shown towards Gentamicin, Chloramphenicol and Tetracycline respectively. The study conducted in Egypt (Moawad et al. 2017) reported that 87% and 40% *Salmonella* spp were resistant to Ampicillin and Tetracycline, 53% sensitive to Chloramphenicol and 67% sensitive to Ciprofloxacin.

Similarly, all *Pseudomonas* spp. isolates were found sensitive towards Ciprofloxacin, Gentamicin, Amikacin and Ceftriaxone and completely resistant to Ampicillin. Among which 24.24%, 30.30% and 66.67% were sensitive to Chloramphenicol, Tetracycline and Cotrimoxazole respectively. According to result reported by Estepa et al. 2015 in Spain, *Pseudomonas* spp. showed 100% sensitivity to Amikacin, Gentamicin, Kanamicin, and Tobramycin.

Citrobacter spp. showed sensitivity towards many antibiotics such as, Ciprofloxacin, Gentamicin, Chloramphenicol, Amikacin and Ceftriaxone. Liu et al. 2017 in China reported that all *Citrobacter* spp. isolates were sensitive to Amikacin, 66.67% were resistant to Ampicillin, 16.67% resistivity to Ciprofloxacin, 5.56% resistivity to Gentamicin, 44.45% resistivity to Tetracycline and 27.78% resistivity to Cotrimoxazole.

All *Proteus* spp. isolates were found sensitive towards Gentamicin, Amikacin, Cotrimoxazole and Ceftriaxone and resistant to Tetracycline. Shrestha et al. 2017 in Bharatpur, Nepal reported 11.7% of *Proteus* spp. resistant to Ciprofloxacin.

All *Klebsiella* spp. isolates were found sensitive towards Amikacin and Ceftriaxone. They showed 14.29%, 85.71%, 71.43%, 42.86%, 57.14% and 71.43% sensitivity to Ampicillin, Ciprofloxacin, Gentamicin, Chloramphenicol, Tetracycline and Cotrimoxazole respectively. According to the report of Kim et al. 2005, the study conducted in Oklahoma, 100% of *Klebsiella* isolates were found resistant to Ampicillin, Tetracycline and also Ceftriaxone. Davis et al. (2015) also reported 100% resistivity towards Ampicillin and 100% sensitivity to Amikacin.

Antimicrobial are used even in the absence of illness to prevent diseases when animals are susceptible to infection (Turtura et al. 1990). In slaughterhouse, resistant strains from the gastrointestinal tract may infect chicken carcasses and, as a result, chicken meats are often related to antimicrobial-resistant

microorganisms (Reza et al. 2014). Therefore, these antimicrobial-resistant bacteria from poultry can infect humans directly and indirectly with food. Though rarely, these resistant bacteria may colonize in the human gastrointestinal tract and may also transfer resistance bacteria to human endogenous flora (Reza et al. 2014). However, the rate of MDR for all the isolates was nil whereas, 79.6% prevalence of MDR bacteria was found in chicken meat in Bharatpur (Shrestha et al. 2017).

Poor hygienic practices during slaughtering and marketing of meat are one of the major contributing factors for unsafe meat in Nepal (Joshi et al. 2003). Slaughtering animals in open and unhygienic places, use of dirty water during slaughtering process, and selling meat in open and non-refrigerated places are some of the unhygienic practices being used by the sellers (Sharma 2012).

CONCLUSION

Out of 81 samples, all the samples showed the presence of potential pathogenic bacteria with *E. coli* being the dominant. Antibigram studies of all isolates against 8 different antibiotics showed that majority of isolates were sensitive. And rate of MDR was nil for each isolate. Among the three districts, Lalitpur showed comparatively satisfactory hygienic condition in relative to Bhaktapur and Kathmandu. Slaughtering of animals in unhygienic place, not maintaining the good hygiene practices during cutting, handling, transportation results to the poor quality of the meat and furthermore, use of polluted water contaminate the meat with different microorganism which can be harmful for the health of the consumers and which can increase the risk of food-borne illness and epidemic diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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