Multi-drug Resistant *Staphylococcus aureus* and *Escherichia coli* in Marketed Raw Meat in Kathmandu Valley

Deepa Neupane[1], Joshna Thapa[1*], Sushil Neupane[1], Namrata Dulal[1], Sushma Kathet[1], Sudeep K.C[2], Santosh Khanal[1], and Janak Raj Dhungana[1]

[1]*Department of Microbiology, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu 44600, Nepal.*
[2]*Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu.*

***CORRESPONDING AUTHOR:**

Joshna Thapa
Email: zthapa99@gmail.com

ISSN : 2382-5359(Online), 1994-1412(Print)

DOI :
https://doi.org/10.3126/njst.v21i2.67160

Date of Submission: Mar 15, 2023

Date of Acceptance: Jun 22, 2023

ABSTRACT

Antimicrobial resistance is a critical worldwide issue that affects both humans and animals. Antimicrobial resistance in microorganisms has been associated to the indiscriminate use of antibiotics in livestock and poultry. Bacterial antimicrobial resistance is witnessing a rapid rise in both human and animal health sector. Still antimicrobial resistance patterns in Nepal are poorly documented, with only a few studies on bacterial prevalence and antimicrobial resistance pattern have been done. This study characterized and provided antimicrobial resistance status of bacteria from raw meat sold in Kathmandu valley. This study was conducted with an objective to find the prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* and *Escherichia coli* from raw meat sold in Kathmandu valley. The cross-sectional study was conducted from February to April 2022 at Nepalese Farming Institute, Dillibazar, Kathmandu. A total of 100 raw meat samples, chicken (n= 60), pork (n=20) and buff (n=20) were collected from different retail shops. The meat samples were minced and enriched in M-Staph broth and MacConkey broth at 37°C for 24 h. The suspension was sub-cultured on Mannitol salt agar and MacConkey agar. The bacterial isolates were identified on the basis of colony morphology, Gram's staining reaction and biochemical tests. Antibiotic susceptibility pattern of bacterial isolates were determined by modified Kirby-Bauer disc diffusion method according to Clinical & Laboratory Standards Institute (CLSI) guidelines. Out of 100 meat samples, 162 bacterial isolates were detected, where *E. coli* 51.85% (n=84) was the most prevalent bacteria followed by *S. aureus* 48.15% (n=78). Majority of *E. coli* isolates (63.09%) were resistant to tetracycline and most of *S. aureus* isolates (37.1%) were resistant to gentamicin. Multi-drug resistance was higher in *E. coli* (51.1%) than *S. aureus* (23.07%). Therefore controlled use of antibiotics in all sectors should be implemented and proper policy and regulations should be formulated on meat handling.

Keywords: *Escherichia coli*, Meat, Multi-Drug Resistance (MDR), *Staphylococcus aureus*

1. Introduction

Meat is one of the most important, nutrient-dense, and energy-dense natural foods consumed by humans to meet their daily nutritional needs. It is very important in keeping a healthy and balanced diet, which is necessary for achieving optimal human growth and development (Ahmad *et al*. 2018). Meat is susceptible to contamination at any phases, from primary manufacture to consumption (farm-to-fork). Contaminated meat is one of the most common causes of food-borne illnesses and death, as pathogens enter the body through ingestion (Bersisa *et al*. 2019).

Food-borne diseases have consistently been the world's leading source of sickness and death. Food-borne infections are becoming more well-known as they have an impact on both health and the economy. Poultry and other meats are one of the most important carriers for bacteria that cause disease (Bantawa *et al*. 2019). Nepal lacks proper policy and regulations for the use of antibiotics in poultry and agriculture which leads us to tackle with these bacteria immune of antibiotics. Multidrug resistant bacteria are far greater threat to humanity than they are considered.

Antibiotic resistance has been linked to the widespread use of non-therapeutic antibiotics in animals, according to growing evidence (Shrestha *et al*. 2017). Multidrug resistance (MDR) refers to bacteria's ability to withstand multiple kinds of antibiotics (three or more classes) that are structurally diverse and have various molecular targets. Misuse of antibiotics can result in bacterial resistance to antibiotics which raise the burden of chronic disease and increases health-care costs. Humans are exposed to resistant bacteria through direct contact with animals, exposure to animal waste, eating of raw meat, and contact with meat surfaces (Marshall & Levy 2011).

In the 21st century, antimicrobial resistance is the global health concern and is a top health challenge, with bacterial infections increasingly failing to antibiotics therapies. Burden of antimicrobial resistant bacteria affects the economy and health of people in both developed and developing countries. Therefore, in this study, we aim to investigate the presence of foodborne illness causing microorganisms in raw meat, along with their antibiotic resistance pattern. We expect the dissemination of our results would lead to improved hygiene among the meat vendors and updated policy and guidelines for the use of antimicrobial drugs.

2. Materials and Methods

2.1 Study site and study period

The research work was conducted in the microbiology laboratory, Nepalese Farming Institute, Kathmandu from February to April 2022.

2.2 Sample collection

In the study, 60 chicken meat (20 each from Kathmandu, Bhaktapur and Lalitpur), 20 pork and 20 buff meat (each 10 samples from Kathmandu, 5 from Bhaktapur and 5 from Lalitpur) were collected from 100 different retail vendors. The samples were collected in sterile vial and were transported to the laboratory while maintaining cold chain (4 °C).

2.3 Culture of specimens

The meat samples were minced and enriched in MacConkey broth and M-Staph broth and were incubated at 37 °C for 24 hours. After incubation, the suspensions were sub-cultured in Mannitol salt agar and MacConkey agar and were incubated 37 °C for 24 hours. The bacterial colonies were sub-cultured in Nutrient Agar (NA) on the following day.

2.4 Identification of bacterial isolates

The colony morphology on Mannitol salt agar and MacConkey agar were noted. The isolated colonies were subjected to Gram staining and different biochemical tests were performed for bacterial identification.

2.5 Antibiotic susceptibility test

Antibiotic susceptibility test of *S. aureus* and *E. coli* isolates was performed by modified Kirby-Bauer disc diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI 2021). Bacterial suspensions were prepared as per guidelines from the overnight grown culture in NA. The turbidity of the suspension was matched with McFarland Standard 0.5 and lawn culture of test bacterium was prepared on Mueller Hinton Agar (MHA). The antibiotic discs were placed on MHA plates with sterile forceps and the plates were incubated at 37 °C for 24 hours. After incubation, diameter of zone of inhibition was measured, and the results were interpreted as sensitive, intermediate, or resistant. Antibiotics used in susceptibility test were Ciprofloxacin (5 mcg), Co-trimoxazole (25 mcg), Ceftazidime (30 mcg), Cefotaxime (30mcg), Piperacillin-Tazobactam (100/10 mcg), Cefepime (30 mcg), Chloramphenicol (30 mcg), Tetracycline (30 mcg), Imipenem (10 mcg), Ampicillin (10 mcg), Amikacin (30 mcg), Gentamicin (10 mcg), Aztreonam (30 mcg) for *E. coli* and Linezolid (30 mcg), Cotrimoxazole (25 mcg), Tetracycline (30 mcg), Cefoxitin (30 mcg), Gentamicin (10 mcg), Clindamycin (2 mcg), Ciprofloxacin (5 mcg), Erythromycin (15 mcg), Penicillin (10 mcg). *S. aureus* isolates, which were resistant to Cefoxitin (30 mcg), were considered as methicillin resistant. Clindamycin (2 mcg) and Erythromycin (15 mcg) antibiotics were used to detect inducible Clindamycin resistance in *S. aureus* by D-zone test confirmation.

2.6 Data Analysis

The data were entered in Microsoft Excel and frequency, distribution were analyzed accordingly.

3. Results

All buff meat samples were contaminated with *E. coli* followed by 49 (81.66%) from chicken whereas 42 (70%) *S. aureus* were recovered from chicken meat followed by buff and pork, each with 90% (Table 1).

Table 1: Distribution of bacterial isolates from different sampling sites

Samples	Sites	Culture positivity	
		E. coli N (%)	S. aureus N (%)
Chicken	Kathmandu	18 (90)	15 (75)
	Bhaktapur	14 (70)	15 (75)
	Lalitpur	17 (85)	12 (60)
Total		49 (81.66)	42 (70)
Buff	Kathmandu	5 (100)	5 (100)
	Bhaktapur	10 (100)	9 (90)
	Lalitpur	5 (100)	4 (80)
Total		20 (100)	18 (90)
Pork	Kathmandu	9 (90)	9 (90)
	Bhaktapur	3 (60)	4 (80)
	Lalitpur	3 (60)	18 (90)
Total		15 (75)	18 (90)
Grand Total		84 (84)	78 (78)

A total of 84 *E. coli* were isolated from various meat samples and all of them were sensitive to Imipenem, Aztreonam, Cefepime and Piperacillin-Tazobacatam. In case of chicken meat, 93.87% were resistant to Tetracycline followed by Ampicillin (77.55%) and Co-trimoxazole (75.51%). Similarly, in buff meat samples, 30% of bacterial isolates were resistant to Cefotaxime followed by Amikacin (15%). And, 40% of bacterial isolates from pork meat were resistant to Tetracycline and Co-trimoxazole (Table 2)

Out of total bacterial isolates, 78 of them were *S. aureus* and all of them were sensitive to Linezolid. 52.38% *S. aureus* isolated from chicken meat were resistant to Ciprofloxacin followed by Tetracycline with 47.61%. Similarly, in pork and buff meat samples, 27.77% and 37.17% of *S. aureus* were resistant to Gentamicin respectively. A total of nine *S. aureus* isolates were methicillin resistant and among them, five isolates were from chicken meat and two isolates each from (n=5) pork and buff meat. Out of 78 *S. aureus*, 12 (24.48%) isolates from chicken and one isolate from pork were found to be inducible clindamycin resistant. However, no any *S. aureus* isolates from buff meat were inducible clindamycin resistant (Table 3).

Table 2: Antibiotic resistance pattern of *E. coli*

Antibiotics	N (%) of resistant E. coli isolates from			
	Chicken	Buff	Pork	Total
Ciprofloxacin	16 (32.65)	0	1 (6.67)	17 (20.23)
Co-trimoxazole	37 (75.51)	1 (5)	6 (40)	44 (52.38)
Ceftazidime	3 (8.16)	1 (5)	0	4 (4.7)
Cefotaxime	6 (12.24)	6 (30)	1 (6.66)	13 (15.47)
Chloramphenicol	16 (32.65)	0	0	16 (19.04)
Tetracycline	46 (93.87)	1 (5)	6 (40.0)	53 (63.09)
Imipenem	0	0	0	0
Amikacin	3 (6.12)	3 (15)	1 (6.66)	7 (8.33)
Aztreonam	0	0	0	0
Gentamicin	9 (18.36)	0	1 (6.66)	10 (11.90)
Ampicillin	38 (77.55)	3 (15)	5 (33.33)	46 (54)
Cefepime	0	0	0	0
Piperacillin-Tazobactam	0	0	0	0

Table 3: Antibiotic resistance patterns of *S. aureus*

Antibiotics	N (%) of S. aureus isolates from			
	Chicken	Buff	Pork	Total
Cefoxitin	5 (11.90)	2 (11.11)	2 (11.11)	9 (11.5)
Linezolid	0	0	0	0
Co-trimoxazole	2 (4.76)	1 (5.55)	0	3 (3.84)
Tetracycline	20 (47.61)	0	1 (5.55)	21 (26.92)
Gentamicin	19 (45.23)	5 (27.77)	5 (27.77)	29 (37.17)
Clindamycin	3 (7.14)	0	0	3 (3.84)
Ciprofloxacin	22 (52.38)	3 (16.66)	2 (11.11)	27 (34.61)
Erythromycin	9 (21.42)	1 (5.55)	4 (22.22)	14 (17.94)

Among *E. coli* isolates, 51.19% were MDR and most of them were isolated from chicken meat. Similarly, 23.07% of *S. aureus* were MDR and most of them were contributed by chicken meat. However, the numbers of MDR isolates were lower in buff meat samples (Table 4).

Table 4: Distribution of multi-drug resistant bacterial isolates

Sources	Chicken		Buff		Pork		
Bacteria	Number of isolates	MDR N (%)	Number of isolates	MDR N (%)	Number of isolates	MDR N (%)	Total MDR N (%)
E. coli	49	36 (73.47)	20	2 (10)	15	5 (33.33)	43 (51.19)
S.aureus	42	17 (40.48)	18	0	18	1 (5.55)	18 (23.07)

4. Discussion

This study showed that the higher presence of *E. coli* was found in the buff sample followed by chicken and pork. Likewise, the higher presence of the *S. aureus* was found equally in both pork and buff followed by chicken (70%). This result contradicted with previous study conducted in Nepal, Washington DC and Nigeria. Study conducted in Nepal showed the prevalence of *E. coli* was found to be 66.6% and 40% in chicken and buffalo meat, respectively (Bantawa *et al*. 2018). Research in Washington DC, reported that out of 210 collected sample, *E. coli* was found to be 38.7% in chicken, 19% in beef and 16% in pork (Nathan & Cars 2014). A study performed in meats sample in Nigeria, reported that *S. aureus* was found to be 80% in chicken, 28% in beef & goat and 64% in pork (Zhao *et al*. 2001). This variation in prevalence may be due to factor of hygiene, sanitization, difference in the quality of sample, errors during sample processing, difference in the geographical conditions and the use of different techniques and interpretation guidelines.

In the present study, *E. coli* was the most commonly detected bacteria in comparisons to the S. aureus. A similar finding was reported from Kathmandu, where they found more than 80.0% of sample had coliform (Maharjan *et al*. 2006). Previous study conducted in Nepal showed the prevalence of *E. coli* was found to be 66.6% and 40.0% in chicken and buffalo meat, respectively (Bantawa *et al*. 2018). In this study, out of 84 isolates of *E. coli* from all three meat samples, 43 showed MDR pattern. In comparison to buff, pork and chicken meat, a higher number of MDR strains were found in chicken meat sample. From chicken meat, 74.47% MDR strains were isolated whereas 33.33% and 10% were found in pork and buff meat respectively. In poultry, it was reported that intestinal microflora changed into MDR, 77.4% from Saudi Arabia (Al-Ghamdi *et al*. 1999), 81.3% from households and small-scale farms in Vietnam (Nguyen *et al*. 2015). A study conducted in chicken breast sample in the United States showed 83.5% prevalence of *E. coli*, of which 38.9% isolates were MDR (Zhao *et al*. 2012). The variation on the rate of resistance can be related to the difference in time and place and depends on the amount of antibiotics used. Another reason for the difference in resistance rates might be a rapid change in antibiotic sensitivity patterns of bacteria within a short period.

S. aureus isolates, which were resistant to Cefoxitin (30 mcg), were considered as methicillin resistant. In our study, 9 MRSA strain were detected among which higher MRSA strain were found in chicken meat (n=5) whereas in pork and buff meat (n=2, each). MRSA has been reported in a variety of meats including raw chicken, pork, veal, beef and mutton. Our finding coincides with the MRSA results from different countries. (De Boer *et al*. 2009) reported prevalence of MRSA was highest in chicken (16.0%) followed by veal (15.2%), pork (10.7%) and beef (10.6%). Similar study performed on meat sample in Netherlands reported that out of 2217 sample of meat 264 (11.9%) of MRSA were isolated among which 16.0% and 10.7% were from chicken and pork meat respectively (van Loo *et al*. 2007). MRSA in chicken meat was found to be 20.0% in Bangladesh (Ali *et al*. 2017).

5. Conclusion

This research sheds light on the presence of multidrug-resistant *E. coli* and *S. aureus* in chicken, buffalo, and pork meat sold in the Kathmandu Valley. The findings highlight the serious threat to public health presented by these microorganisms in commercially available raw meat. Future research endeavors should delve deeper into the molecular mechanisms and genetic factors driving antimicrobial resistance in *E. coli* and *S. aureus* found in meat samples. Longitudinal studies across different seasons and regions can provide a more comprehensive understanding of the prevalence, distribution, and dynamics of drug-resistant bacteria, enabling evidence-based interventions to combat this pressing issue. These efforts will aid in effectively addressing potential threats. Additionally, conducting training and awareness programs is necessary to decrease the indiscriminate use of antimicrobials and thereby mitigate the development of drug resistance in poultry and livestock.

References

Ahmad, R. S., A. Imran, and M. B. Hussain, 2018. Nutritional composition of meat. Meat science and nutrition 61:10.5772
DOI: 10.5772/intechopen.77045

Al- Ghamdi, M. S., F. El- Morsy, Z. H. Al- Mustafa, M. Al- Ramadhan, and M. Hanif, 1999. Antibiotic resistance of Escherichia coli isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia. Tropical Medicine & International Health 4(4):278-283.
DOI: 10.1046/j.1365-3156.1999.00392.x
PMID:10320654

Ali, Y., M. A. Islam, N. H. Muzahid, M. O. F. Sikder, M. A. Hossain, and L. W. Marzan, 2017. Characterization, prevalence and antibiogram study of Staphylococcus aureus in poultry. Asian Pac. J. Trop. Biomed 7(3):253-256.
DOI: 10.1016/j.apjtb.2016.12.001

Bantawa, K., N. Sah, D. Limbu. P. Subba, and A. Ghimire, 2019. Antibiotic resistance patterns of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio* isolated from chicken, pork, buffalo and goat meat in eastern Nepal. BMC research notes 12(1). 1-6.
DOI: 10.1186/s13104-019-4798-7
PMID: 31752992 PMCID: PMC6873459

Bersisa, A., D. Tulu, and C. Negera, 2019. Investigation of bacteriological quality of meat from abattoir and butcher shops in Bishoftu, Central Ethiopia. Int. J. Microbiol. 2019.
DOI: 10.1155/2019/6416803
PMID: 31191657 PMCID: PMC6525944

De Boer, E., J. T. M. Zwartkruis-Nahuis, B. Wit, X. W. Huijsdens, A. J. De Neeling, T. Bosch, and A. E. Heuvelink, 2009. Prevalence of methicillin-resistant Staphylococcus aureus in meat. Int. J. Food. Microbiol 134(1-2):52-56.
DOI: 10.1016/j.ijfoodmicro.2008.12.007
PMID:19144432

Shrestha, A., A. M. Bajracharya, H. Subedi, R. S. Turha, S. Kafle, S. Sharma, and D. K. Chaudhary, 2017. Multi-drug resistance and extended spectrum beta lactamase producing Gram negative bacteria from chicken meat in Bharatpur Metropolitan, Nepal. BMC research notes 10(1):1-5.
DOI: 10.1186/s13104-017-2917-x
PMID: 29116010 PMCID: PMC5678746

Marshall, B. M., and S. B. Levy, 2011. Food animals and antimicrobials: impacts on human health. Clinical microbiology reviews 24(4):718-733.
DOI: 10.1128/CMR.00002-11
PMID:21976606 PMCID:PMC3194830

Maharjan, M., V. Joshi, D. D. Joshi, and P. Manandhar, 2006. Prevalence of Salmonella species in various raw meat samples of a local market in Kathmandu. Annals of the New York Academy of Sciences 1081(1):249-256.
DOI: 10.1196/annals.1373.031
PMID:17135520

Nathan, C., and O. Cars, 2014. Antibiotic resistance problems, progress, and prospects. N. Engl. J. Med 371(19):1761-1763.
DOI: 10.1056/NEJMp1408040
PMID:25271470

Nguyen, V. T., J. J. Carrique-Mas, T. H. Ngo, H. M. Ho, T. T. Ha, J. I. Campbell, and C. Schultsz, 2015. Prevalence and risk factors for carriage of antimicrobial-resistant Escherichia coli on household and small-scale chicken farms in the Mekong Delta of Vietnam. J. Antimicrob. Chemother 70(7):2144-2152.
DOI: 10.1093/jac/dkv053
PMID: 25755000 PMCID: PMC4472326

Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, and J. Meng, 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. Applied and Environmental Microbiology 67(12): 5431-5436.
DOI: 10.1128/AEM.67.12.5431-5436.2001
PMID:11722889 PMCID:PMC93326