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Cross-sectional studies on microbial quality and antibiotic resistance patterns of isolated microorganisms from raw chicken retailed in Tripura

Seuli Saha Roy

Abstract
At present, in Tripura, there is no organized poultry slaughterhouse and people follow the traditional methods for slaughtering of poultry. The improper hygiene and sanitary measures followed in these traditional chicken shops favour easy invasion of microorganism in chicken. The present study was undertaken to assess the bacteriological quality of chicken and hygienic aspects of chicken vendor shops and the antibacterial resistance pattern of the isolated bacteria, in order to recommend ways to minimize public health hazards. A total of 110 randomly cut pieces of chicken, 110 water samples used for washing the carcasses, and swab samples from cutting knife, butchers' hands and chicken cutting wooden surface were collected from 110 retail shops of Tripura. The samples were subjected to total viable count (TVC), total coliform count (TCC) and total *staphylococcus* count (TSC). The isolation and identification of organisms of public health significance was done and antibiogram of the isolated microbes was also performed. The overall mean TVC, TCC and TSC in chicken samples were 7.59±0.070, 5.5±0.076 and 4.27±0.039 $\log_{10}$cfu/g, respectively. The water samples had mean values for TVC, TCC and TSC as 11.06±0.036, 9.2±0.026, 6.12±0.050 $\log_{10}$cfu/ml, respectively. The values of TVC, TCC and TSC of swab samples collected from cutting knife, butchers' hands and wood surface also indicated high contamination and act as a source of cross contamination to chicken. The chicken samples were predominantly contaminated with *E. coli* (68.18%), *Staphylococcus aureus* (39.09%), *Proteus* spp. (35.83%) and *Salmonella* spp. (16.36%). Most of the isolates were resistant to more than one group of antibiotics such as enrofloxacin, chloramphenicol, oxytetracycline, ampicillin, cefotaxime/clavulanic acid, amikacin and methicillin, whereas, majority of isolates were susceptible to gentamicin. The present study emphasized that the microbial quality of raw chicken was poor. The presence of antibiotic resistant pathogens in chicken is a potential threat to the public health.

Keywords: Antibiotic resistance, cross contamination, TVC, public health, environment, *Salmonella* spp.

Introduction
Antibiotic resistant food borne zoonotic bacteria has become a great threat to human health. Chicken is the cheapest and popular source of protein consumed worldwide including the developing countries. In India, hot and humid weather conditions prevailing in most parts of the country for a longer duration of the year favor the growth of bacteria in chicken. Also, improper storage facilities lead to spoilage of chicken and pose a threat to the consumers. Public health important bacteria like *Campylobacter* spp, *E. coli*, *Salmonella* spp., *S. aureus*, *Clostridium* spp., *Listeria* spp., etc. have frequently been isolated from raw chicken as well as cases of food borne diseases outbreaks. The increasing demand for chicken is leading to inadequate hygienic chicken production. During the process of conversion of muscle into meat, microbial contamination of poultry carcass surface is practically unavoidable (Mawia *et al.*, 2012) [13]. Internal organs such as large intestines, cloaca etc. contains public health significant microorganisms and during the defeathering and evisceration stages of the slaughter process, the carcass becomes contaminated with microorganisms. Chicken by-product cuisines are also widely consumed because of their special taste and low cost. The by-products viz. gizzards, liver, heart etc. were found naturally contaminated with *E. coli*, *Salmonella* spp., *Campylobacter* spp. (Silva *et al.*, 2011) [18]. It is known that more than 60% of all antibiotics produced worldwide are utilized in animal production system both as preventive and therapeutic purposes. A large variety of antimicrobials are also used in poultry farming in most of the countries (Sahoo *et al.*, 2010) [17] and in human medicine these antimicrobials are essentially required (WHO 2017) [21].
The indiscriminate use of such essential antimicrobials in animal production including poultry

farming beyond the preventive level to fulfill the supply and demand gap in market is likely to accelerate the development of antibiotic resistance in pathogenic bacteria as well as in commensal organisms. This is a major concern as reflected in treatment failures, economic losses and might act as a source of resistant gene pool for transmission to humans leading to an alarming threat to the public health (Mehdi *et al*., 2018; Abebe *et al*., 2020) [14, 1].

In Tripura, majority of population are non-vegetarian and there are no taboos for consumption of meat. Chicken eating is quite popular in Tripura along with consumption of fish. At present, there is no organized slaughter house in Tripura. The poultry birds are slaughtered in traditional method and chicken are retailed in open shops. There is no provision of potable water supply in the chicken shops. Butchers are habituated to use single bucket of water for all purposes including washing carcasses, butchers' hands, cutting knife, utensils and wooden chopping block etc. The improper hygiene and sanitary measures followed in these traditional chicken shops favour easy invasion of microorganism in chicken. The contamination of poultry carcass is occurred mainly through enteric pathogens during slaughter. There are no scientifically published data on microbial quality of chicken as well as antimicrobial resistance patterns of microbes isolated from chicken in Tripura. Keeping these in view, the present study was undertaken to assess the bacteriological quality of chicken and hygienic aspects of chicken vendor shops as well as the antibacterial resistance pattern of the isolated bacteria in order to recommend ways to minimize the public health hazard.

Materials and Methods

A cross-sectional study was carried out through random sampling from vendor chicken shops in order to obtain the detail scenario of the ongoing practices followed in the retailed chicken shops during 2016-2017. At first, scientific information on the level of hygienic status of butchers and sanitary practices followed during slaughter as well as while processing of carcasses were recorded through planned questionnaire.

A total of 110 raw chicken samples, 110 water samples used in chicken vender shops for washing the chicken carcass and butchers' hands, and swab samples from cutting knife (110), butchers' hands (110), chicken cutting wooden block surface (110) were collected from retail market (110) of Tripura. Around 50g random cut pieces of chicken samples were collected aseptically in a sterile sample pouch from different retail shops. A sterile sample container was used to collect 100 ml water samples on each occasion. Sterile swab (HiMedia) moistened in peptone water were used to collect all swab samples from different sources. After collection, all the samples were brought to the laboratory maintaining cold chain and also processed immediately. The tubes containing swabs samples were vortexed for 45 sec to maintain uniformity in distribution of microorganisms. After homogenization, all the chicken samples were serially diluted (up to 10^{-6}) in peptone water and all the water samples were prepared for inoculation by serial dilution.

The standard spread plate technique was followed using 10^{-4} and 10^{-5} dilutions for enumeration of total viable count (TVC), total coliform count (TCC) and total *staphylococcus* count (TSC). 0.1 ml sample from each source was inoculated in the plate count agar, MacConkey agar and Mannitol salt agar, and the plates were incubated at 37°C for 24 h. After that, colony counter was used to count the colonies (30 to 300 nos.) on each plate. The total count so obtained was multiplied with the number of colonies in a particular dilution with the dilution factor and expressed in mean $\log_{10}$ colony forming unit (CFU). The microbiological data were expressed in $\log_{10}$cfu/g, $\log_{10}$cfu/ml and $\log_{10}$cfu/cm^2 in case of chicken, water and swab samples, respectively. The bacteria of public health importance were identified on the basis of colony characteristics, Gram's staining, motility test, growth in selective media (EMB and XLT4) and by appropriate conventional biochemical tests.

Antibiotic resistance pattern was determined by disc diffusion method using a wide range of commonly used antibiotics (Bauer *et al*., 1996) [4]. The antibiotic discs (HiMedia Laboratories, Mumbai, India) selected for the present study were methicillin (MET-5 mcg), gentamicin (GEN-10mcg), cefotaxime/clavulanic acid (CEC-30/10 mcg), amoxicillin (AMX-30 mcg), ampicillin (AMP-10 mcg), oxytetracycline (OT-30 mcg), ciprofloxacin (CIP-30 µg), erythromycin (E-15mcg), enrofloxacin (EX-10 mcg), streptomycin (S-25 mcg), amikacin (A-30 mcg) and chloramphenicol (CH-25 mcg).

Fresh cultures of isolates were used for antibiotic sensitivity test (AST). After that a sterile cotton swab was dipped into the inoculum and the soaked swab was rotated firmly against the upper inside wall of the tube to remove the excess medium. Then the entire surface of the Muller Hinhton agar plate was spread properly with the swab. The predetermined sets of antimicrobial discs were dispensed aseptically onto the surface of the inoculated plate. Each disc was pressed down to ensure complete contact with the agar surface and was incubated at 37 °C for 24 to 48 h. Diameter of the zones of inhibition was measured with a ruler (HiMedia) and interpreted as per Clinical and Laboratory Standard Institute guidelines (CLSI, 2016) [6].

Results and Discussion

A total of 110 butchers were interviewed and it was observed that none of them had received any professional training regarding slaughter of poultry, cutting and handling practices of chicken, and also, they did not perform their personal health checkup regularly. Traditional method of slaughter was used and after deskinning and evisceration butchers were habituated to deep the carcass in a bucket of water which was used for washing of several carcasses. Most of the butchers did not use any protective clothes and head cover. They were also used to handle money with bare hands while retailing the chicken to the consumers. In addition, there were no provisions for prevention of insects, dust etc. coming into the chicken shop as well as no running water supply facilities in the retail chicken shops (Table 1). The results of comparable studies showed that the level of food handling practice was not satisfactory among meat handlers and responsible for the potential health risk to the consumers. They also observed that butchers act as the main vehicles of microbial contamination of fresh chicken and may also be asymptomatic carriers of food-borne pathogens (Yenealem *et al*., 2020) [22].

The microbial quality of chicken was determined by TVC, TCC and TSC. The mean values for TVC, TCC and TSC were 7.59±0.070, 5.5±0.076 and 4.27±0.039 log10 cfu/g, respectively (Table 2). Although the microbial load of the chicken samples was high but below the spoilage limit. Vaidya *et al*., (2016) [19] and Kumar *et al*., (2020) [12] also observed significantly higher numbers of TVC in different parts of chicken samples collected from retail shops of different states of India. The total aerobic bacteria count 6.39 log CFU/g, 5.96 log CFU/g and 7.24 log CFU/g were estimated from raw chicken collected from different markets by other researchers (Bhandari *et al*., 2013; Faruque *et al*., 2019; Mpundu *et al*., 2019) [5, 9, 15]. In contrast, less coliform counts (4.97 log CFU/g) and higher coliform counts (6.5 log CFU/g) were reported from similar studies conducted by Kumar *et al*., (2012) [11] and Bhandari *et al*., (2013) [5]. The total Staphylococci count in the present study was 4.27±0.039 log_{10}cfu/g which was higher than 3.7 log CFU/g and 4.07 log CFU/g reported from chicken samples in north east and Kolkata. This is mainly due to lack of awareness and poor hygienic practices adopted by the butchers. *E. coli* count in poultry can be reduced by controlling cross contamination, continuing sanitary practices and maintaining appropriate temperature of carcass. Hygienic food production is necessary to ensure safeguarding of public health (Althaus *et al*., 2017) [3]. The water samples collected from retail chicken shops were highly contaminated and the mean values for TVC, TCC and TSC were 11.06±0.036, 9.2±0.026, 6.12±0.050 log_{10}cfu/ml, respectively (Table 2). There were no running water facilities in the retail shops and butchers usually collected water in a bucket and repeatedly use the same water for washing of carcass as well as washing of their hands and knife. Therefore, the water utilized in retail chicken shops played a significance role in determining the bacterial contamination of the carcass. Coliforms are used as an indicator organism of water quality and potential faecal contamination of water occurred due to unhygienic practices adopted by workers or environment of the processing plant (Wabeck, 2002) [20]. The higher mean value of TVC, TCC and TSC in water of retail chicken shops indicated the repeated use of contaminated water during washing of carcass as well as butchers' hands and knives. Therefore, it is very essential to have a provision of clean water supply in retail poultry shops in order to reduce the contamination which is a major public health concern.

The mean values of TVC of swab samples collected from cutting knife, butchers' hands and chicken cutting wood surface were 5.69±0.047, 5.46±0.042 and 6.15±0.054 $log_{10}cfu/cm^2$, respectively. On the other hand, the TCC and TSC values of swab sample of cutting knives collected from retail chicken shop swab samples were 3.65±0.050 and 4.36±0.040 $log_{10}cfu/cm^2$. Whereas, the TCC and TSC values were 2.06±0.036 and 4.95±0.047 $log_{10}cfu/cm^2$ for butchers' hands swab and, 4.75±0.050 and 5.86±0.048 $log_{10}cfu/cm^2$ for chicken cutting wooden block swab, respectively (Table 2).

The wooden block surface was repeatedly utilized for cutting and chopping of chicken and the blood and drip might have served as an ideal medium for growth of microbes on wooden block. Furthermore, hot and humid weather is also providing ambient conditions for multiplication of microbes, thus increasing the microbial load (Bhandari *et al.*, 2013) [5]. Similarly, higher level of contamination of meat contact surfaces like hand and knife were also reported from abattoir and meat shops in Mumbai and Kolkata. Cutting of meat using contaminated knives significantly increased the level of microbial load and also incorporate pathogens in meat (Mpundu *et al*., 2019) [15].

In the present study, the higher mean value of TVC, TCC and TSC recorded from the swab samples from knife, butchers' hand and wooden block indicated the unhygienic practices followed by the butchers. This might be due to the practice of traditional methods of slaughtering and processing of chicken by repeated use of single knife and also use of same water for washing of the carcass, knives, as well as their hands owing to lack of basic facilities in the meat shops. The butchers of the retail chicken shops had no regards for their personal hygiene and also had no scientific knowledge on sanitation. Further, they were not using detergent or sanitizer regularly for cleaning purposes. So, higher microbial loads in retail chicken shops could be due to minimum hygienic practices that were followed compared to the abattoirs (Darshana *et al*., 2014) [7].

Public health significant bacteria isolated from chicken samples exhibited occurrences of *Salmonella* spp. (16.36%), *E. coli* (68.18%), Proteus spp. (35.83%) and *S. aureus* (39.09%). These organisms were also isolated from chicken samples from different parts of India such as Kolkata, Maharashtra, Hyderabad, Assam, Mizoram etc. (Faruque *et al*., 2019) [9]. After slaughter, improper storage of carcasses led to the multiplication of pathogenic bacteria within the short period of time and exceeded the acceptable levels. Significantly higher percentage of isolates of pathogenic bacteria in chicken clearly indicated that the external sources of contamination occurred through butchers' hands, cutting knives, wood cutting surface and water used during washing of carcass (Zehra *et al*., 2019) [23]. Presence of *Salmonella* spp. in chicken pointed to the poor hygienic conditions adopted by the butchers during slaughtering and processing of carcasses. The intestinal contents which are the main sources of enteric pathogens may spill out during evisceration and contaminate the water as well as whole carcass/muscle (Faruque *et al*., 2019) [9]. Food born salmonellosis is one of the major public health concerns worldwide and the majority of food borne infections occurred by consumption of contaminated chicken (Peixe *et al.*, 2015) [16]. *Staphylococcus aureus* is a commensal organism of human skin and causes minor to severe infections including food poisoning. Presence of *Staphylococcus aureus* in chicken indicated unhygienic conditions and cross contamination between surrounding environment, and personal contact. Generally, chicken becomes contaminated with *S. aureus* when an infected person does cough, sneezing, talking or breathing during slaughter and processing (Wabeck, 2002) [20].

The results of the antibacterial resistance pattern revealed that the isolates were resistant towards one or more groups of antibiotics, frequently used for preventive as well as therapeutic purposes. Isolated *Salmonella* spp. Exhibited resistance to enrofloxacin (55.56%), ampicillin (38.89%), oxytetracycline (27.78%), amikacin (22.22%), cefotaxime/clavulanic acid (38.89%) and, 33.33% isolates were resistant to amoxicillin, ciprofloxacin, erythromycin, streptomycin and chloramphenicol. *E. coli* isolates were highly resistant to enrofloxacin (70.67%), amikacin (61.33%), erythromycin and chloramphenicol (52.00%), oxytetracycline (49.33%), amoxicillin (42.67%), Ciprofloxacin (37.33%), and streptomycin, ampicillin and cefotaxime/clavulanic acid (46.67%). Isolated *Proteus* spp. exhibited resistant enrofloxacin (54.17%), cefotaxime/clavulanic acid (45.83%), streptomycin (39.53%), Ciprofloxacin (33.33%), ampicillin and amikacin (25.00%), oxytetracycline (20.83%) and amoxicillin, erythromycin and chloramphenicol (29.17%). *Staphylococcus aureus* isolates were resistant to methicillin (83.72%), amoxicillin and amikacin (62.79%), ampicillin (60.47%), enrofloxacin (58.14%), oxytetracycline (55.81%), streptomycin (39.53%), Ciprofloxacin (37.21%) and, cefotaxime/clavulanic acid, erythromycin and chloramphenicol (48.84%). Most of the isolated organisms were susceptible to gentamicin (Table 3).

Antimicrobial resistance of isolated bacteria may be attributed to diverse sources including the natural resistance of species to certain antibiotics and probable transfer of antimicrobial resistance among species. Acquired resistance to antimicrobial drugs is more prevalent in diarrhoeagenic *E. coli* and other pathogens. The results of the present study agree with the findings of earlier researchers. *Salmonella* spp. was also isolated from poultry and 53.2% of isolates were showed multidrug resistance to three or more classes of antibiotics including streptomycin (89.2%), sulfonamides (72.4%), florfenicol (59.2%) and ampicillin (44.8%) (Faruque *et al*., 2019) [9]. Multi drug resistant (80-86%) and ESBL positive (33%) *E. coli* was also reported from chicken collected from different markets of India including Assam and Mizoram. They observed that the isolated *E. coli* developed resistance to broad spectrum antibiotics, like tetracycline, 80% to nalidixic acid, 76% to ampicillin (Kaushik *et al*., 2018; Debbarma *et al*.,2022) [8, 10]. This study has clearly revealed the risk factors associated with consumption or handling of marketed poultry products and also highlighted the matter of excessive administration of antibiotics in poultry farming practices. Isolation of multidrug resistant organisms from poultry indicated that poultry production environment might have acted as reservoirs of antibiotic resistance bacterial genes which may spread from livestock production farms to human through manure and contaminated water (Adelowo *et al*., 2014) [2]. *S. aureus* isolated from different meat samples also developed phenotypic resistance, highest to penicillin (90.97%) followed by ciprofloxacin (61.80%), tetracycline (45.14%) and erythromycin (11.11%) in Punjab (Faruque *et al*., 2019; Zehra *et al*., 2019) [9, 23].

Table 1: Key findings of survey on hygienic practices observed by butchers in different retail chicken Shops in Tripura (n=110)

Sl. No	Questions asked / Observations	Frequency of response	Percentage of Response
Educational status of Butchers / workers			
1	Literate	105	95.45
2	Illiterate	5	4.55
Any scientific training taken for slaughtering and cutting of carcass			
1	Yes	0	0.00
2	No	110	100.00
Protective clothing used: Apron/ separate cloth			
1	Yes	28	25.45
2	No	82	74.55
Hair cover Used			
1	Yes	5	4.55
2	No	110	100.00
Stunning done before slaughter			
1	Yes	0	0.00
2	No	110	100.00
Carcass washing			
1	Dipping in a water Baquet	110	100.00
2	Water pouring	0	0.00
Running water facilities available in shops			
1	Yes	0	0.00
2	No	110	100.00
Water used during carcass washing: Changing of water after each carcass washing			
1	Yes	0	0.00
2	No	110	100.00
How butchers wash their hand?			
1	With only water	87	79.09
2	With soap	23	20.91
Handling money in chicken shop			
1	Butcher himself with contaminated hand	83	75.45
2	Another person/ cashier	37	33.64
Whether butchers wash their hands properly before taking any snakes or tea			
1	Yes	29	26.36
2	No	81	73.64
Whether butchers wash their hands properly before using their mobile phones			
1	Yes	21	19.09
2	No	89	80.91
Whether butchers wash the weighing balance regularly			
1	Apply detergent	26	23.64
2	Only water	84	76.36
Whether butchers wash the chicken cutting Wooden block with disinfectant regularly			
1	Yes	26	23.64
2	No	84	76.36
Whether butchers/ municipality or other authority regularly disinfect the area of market regularly			
1	Yes	59	53.64
2	No	51	46.36
Whether butchers wash properly the duster/ cloth used for drying/ cleaning their hands			
1	Regularly	45	40.91
2	Often	55	50.00
Use of jewelry material			
1	Yes	42	38.18
2	No	68	61.82

Table 2: Depicting the microbial loads in chickenand environmental samples collected from different retail shops

Sl. No.	Microbial contamination	TVC	TCC	TSC
1.	Chicken (log_{10} CFU/g)	7.59±0.070	5.5±0.076	4.27±0.039
2.	Water sample (log_{10}CFU/ml)	11.06±0.036	9.2±0.026	6.12±0.050
3.	Swabs from Cutting knife (log_{10} CFU/cm^2)	5.69±0.047	3.65±0.050	4.36±0.040
4.	butchers Hand swabs (log_{10} CFU/cm^2)	5.46±0.042	2.06±0.036	4.95±0.047
5.	Chickencutting wood surfaces swabs (log_{10} CFU/cm^2)	6.15±0.054	4.75±0.050	5.86±0.048

Table 3: Antibiotic resistant pattern of public health significant bacteria isolated from chicken and environmental samples collected from chicken retailed shop (%)

Sl. No.	Name of isolated bacteria	Name of antibiotic discs (mcg/disc)											
		Methicillin (5 mcg)	Gentamicin (10 mcg)	cefotaxime/clavulanic acid (CEC- 30/10 mcg)	Amoxycillin (30 mcg)	Ampicillin (10 mcg)	Oxytetracycline (30 mcg)	Ciprofloxacin (30 mcg)	Erythromycin (15 mcg)	Enrofloxacin (10 mcg)	Streptomycin (25 mcg)	Amikacin (10 mcg)	Chloramphenicol (25 mcg)
1.	*Salmonella* spp.(n=18)	16.67	11.11	38.89	33.33	38.89	27.78	33.33	33.33	55.56	33.33	22.22	33.33
2.	*E. coli* (n=75)	38.67	12.00	46.67	42.67	46.67	49.33	37.33	52.00	70.67	46.67	61.33	52.00
3.	*Proteus* spp. (n=24)	12.50	8.33	45.83	29.17	25.00	20.83	33.33	29.17	54.17	20.83	25.00	29.17
4.	*Staphylococcus aureus* (n=43)	83.72	9.30	48.84	62.79	60.47	55.81	37.21	48.84	58.14	39.53	62.79	48.84

Conclusions

The present study throws light on the unhygienic and improper sanitary conditions prevailing in the retail chicken shops in Tripura and its associated public health risks. It also underlines the prime importance for the improvement of basic facilities and scientific hands-on training for chicken handlers of Tripura on food safety and sanitation measures in order to enhance good safety practices through better understanding and positive attitude as a whole. The present study also demonstrated that the contamination of chicken should be prevented during handling, slaughtering and processing to protect the public health. The presence of antibiotic resistant *Salmonella* spp., *E. coli, Proteus* spp. and *Staphylococcus aureus* may cause potential threat to the public health. The antibiotic resistance patterns may lead towards the sensible use of antibiotics in poultry production chain. The outcomes of the study also demand for thorough surveillance and monitoring on usage of antibiotic both in animal husbandry and human.

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