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Microbial Quality Assessment of Broiler Chicken Meat and Evaluation of Antibiotic Susceptibility Profile of Isolates from Retail Outlets of Vellore, Tamilnadu, India.

Ayan Modak, Vivek Kumar, and KV Bhaskara Rao*

Molecular and Microbiology Laboratory, Environmental Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore, (T.N), India.

ABSTRACT

This study was conducted to examine the availability of microbial contamination in retail meat available in Vellore, Tamil Nadu, India. Raw broiler chicken samples (5 numbers) were collected from the local market from Vellore and analyzed for microbiological contamination. Isolation of microbial cultures was performed by serial dilution and spread plate method on Mackonkey agar, Salmonella-Shigella agar, and Blood agar. Identification of the isolates was performed based on morphological, microscopic and biochemical characters. A total of 23 bacterial isolates were isolated and identified as *Salmonella* sp., *Escherichia coli, Shigella* sp., *Klebsiella* sp., *Staphylococcus* sp., *Micrococcus* sp., and *Proteus* sp. Among these 23 isolates, 19 (83%) bacterial isolates are pathogenic in nature and responsible for several diseases. Theses isolates were screened for their drug resistance pattern towards ampicillin, penicillin, streptomycin, vancomycin, cephotaxim, bacitracin, chloramphenicol, erythromycin, ciprofloxacin and rifampicin. Antibiotic Susceptibility test was performed by agar disc diffusion method on Muller Hinton (MH) agar. All the Salmonella sp. isolates were found to be resistant towards to ampicillin, penicillin, streptomycin, ciprofloxacin and bacitracin. Other isolates also exhibited high resistance towards the drugs used in the study. Food-borne pathogens found in retail shops could be sources for horizontal contamination of chicken. Data from the present study confirm the circulation of antibiotic resistant and pathogens in raw chicken and its environment in retail shops in Vellore, which could play a role in the spread of antimicrobial resistance amongst food-borne bacteria.

Keywords: microbial contamination, chicken meat, antibiotic resistance, antibiotic Susceptibility

Corresponding author

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INTRODUCTION

A major part of human diet is occupied by meat as it is an excellent source of protein. However, it also serves as a potential growth medium for many harmful microorganisms as being highly susceptible to microbial contamination. Such contamination could results in economic losses and substantial public health damage [1]. Food-borne pathogens are the leading causes of illness and death and are also responsible for millions of cases of infectious gastrointestinal diseases [2]. According to a report from WHO, 1997 [3], scores of food-borne diseases are due to the consumption of microorganism infected meat by the humans. A major enteric pathogen, Salmonella has frequently been isolated from the *abattoir environment* as well as gastrointestinal tract of animals, particularly form poultry of frames and wild [4, 5]. Such distribution has been reported globally with a 47.7 and 35.5% incidence of Salmonella in retail chicken of two states of Australia [6]. Source of such pathogens may be the animal themselves otherwise from outside, including the surroundings where the animals are kept or even can results from unhygienic conductance during slaughtering and processing [7] and such meat is considered to be poor of quality [8].

A lot of vital pathogens are havened in raw meat ranging from *Salmonella spp*., *Campylobacter jejuni/coli*, *Yersinia enterocolitica*, *E. coli*, *S. aureus* and even to *Listeria monocytogenes* in some extents, posing a high threat of food borne illness to human consumers without proper pathogen management and handling of the raw meat [9]. As already reported in other studies many of these contaminating microbes are common agents of food borne infections counting *Campylobacter jejuni, Clostridium botulinum, Clostridium perfringes, Escherichia coli 0157:H7, Salmonella, Streptococcus A, Listeria monocytogens, Shigella, Staphylococcus aureus, Vibrio cholera, Vibrio vunificus* etc. [10].

The tropical climate of India and south-east Asia has contributed more to such microbial invasions due to hot and humid conditions, ideal for their growth and multiplication and thus increasing the total aerobic counts on meat [8]. As reported by previous studies meat samples of retail outlets, particularly of chicken meat has a significantly high proportion of microbial contamination with special emphasized of *E.coli* and *S.aureus* [11]. In another study from Nigeria, the Aerobic plate counts showed a high level of contamination in the retail chicken and turkey with 33 and 43.4% of the samples contaminated with Salmonella and *E. coli* respectively [12].

Not only the presence and population load of such pathogens in meat are the matter of concern but the fact that these food pathogens are acquiring more resistance to newer antibiotics day by day is actually the devastating threat we are facing now and should be more worried of. A common resistance to ampicillin has already been observed in Nigeria, Africa along with the following streptomycin, cephalexin, gentamycin being in the same path [13], with 90% resistance against tetracycline is of high alarm [14]. More importantly in a recent finding with Salmonella isolates from chicken meat showed 93% resistance to tetracycline and 100% to augmentin and amoxicillin, whilst it was 100% for both augmentin and amoxicillin in *E.coli* [12].

As chicken and chicken products are the major cause of food borne infections and the most important link between food producing animals and the humans, we investigated five samples of chicken and for the presense of the food borne pathogens and analyzed the isolates for antibiotic resistance. Since drug resistances in food-borne pathogens have harassed therapeutically intervention in humans, antibiotic resistances in food-borne microbes have become a public health issue. In this study we have isolated and characterized the pathogenic microorganisms from the chicken samples collected from the local market of Vellore, TN, India. Microbial isolates were also screened for their drug resistance profile toward ten common antibiotics.

MATERIALS AND METHODS

Chemicals

Mackonkey agar, Blood agar base, Salmonella Shigella agar, Muller-Hinton agar, Peptone, MR-VP Broth, Simmons-Citrate agar, Starch agar, Triple Iron Sugar (TSI) agar, H_2O_2, Oxidase discs were purchased from Hi-Media Chemicals, Mumbai, India.

Sample Collection

Samples were collected aseptically in air tight polythene bags from various chicken slaughtering shops in the local markets of Vellore, Vellore district, Tamil Nadu. The samples were bought to the Bioscience Lab, VIT University, Vellore, Tamil Nadu, India.

Isolation of microorganism

Isolation was performed by serial dilution and spread plate method on Mackonkey agar, Blood agar and Salmonella Shigella agar. The sample was grounded aseptically with sterilized mortar and pestle. 1 gram of this sample was serially diluted up to 10^{-6} in sterilized distilled water. 0.1ml of sample from 10^{-4}, 10^{-5} and 10^{-6} were transferred aseptically in Mackonkey agar, Blood agar and Salmonella -Shigella agar plates using micro pipettes. The plates were incubated at 37^{0}C for 24 hours. The bacterial isolates were purified by repeated sub-culturing on the respective media. The purified cultures were maintained in refrigerator (4° C) for the further use.

Identification of the isolates

Identification was performed on the basis of morphology, microscopic characteristics and biochemical characteristics.

Morphology

The incubated plates were observed for the bacterial growth and the colony morphology and were recorded with respect to the size, shape, color and appearance.

Microscopic Characteristics

The microscopic characteristics identification was performed by Gram's staining.

Biochemical Characteristics

The various biochemical tests were performed such as Catalase, Oxidase, Indole, MR, VP, Citrate, Starch Hydrolysis and Triple sugar iron test for the characterization of the isolates.

Antibiotic Susceptibility Pattern

Antibiotics used

The antibiotics included ampicillin (10 mcg/disc), Penicillin G, Streptomycin, Vancomycin, Cephotaxime, Chloramphenicol (30 mcg/disc), Ciprofloxacin (5 mcg/disc), Erythromycin, Bacitracin and Rifampicin (5mcg/disc).

Antimicrobial assay

Isolate organisms and were screened for their sensitivity towards ten standard antibiotics. Drug sensitivity test was performed by agar disc diffusion method on Muller Hinton agar (MHA) plates. Bacterial isolates were inoculated in to nutrient broth for 8 hours. Isolates were seeded on Mueller Hinton agar plates by using sterilize cotton swabs. The standard antibiotic discs were placed on the agar surface using a sterilize forceps. Plates were incubated at 37°C for 24 hours. Plates were observed for zone of inhibition.

RESULTS AND DISCUSSION

Being the leading cause of illness and death worldwide, food-borne pathogens find their sources majorly in contaminated raw chicken specifically in Indian subcontinent. Among the 23 potential bacterial pathogens isolated form 5 samples in this study, encompassing 43% *Salmonella* sp., 17% *E. coli* sp., 8.7 % *Shigella* sp., 8.7 % *Staphylococcus* sp., 4.3% Klebsiella sp. were characterized biochemically with comparatively

inferior presence of *Micrococcus* sp., *Proteus* sp. (Table 1.). However the predominance of *Salmonella* sp. here was significantly higher than some other recent finding by other investigator in India who fined 6.7 to 23.7% prevalence of Salmonella in chicken carcasses [15, 16]. Presence of Salmonella in more than 25 g of poultry meat is regarded as unsafe for human consumption and moreover poultry meat should be totally free of *E.coli* contamination before it can be considered fit for human consumption [17]. In this study both Salmonella and *E.coli* are the most prevalent organisms in the chicken samples obtained and are not safe for human consumption according to the recommended limits by foreign food agencies.

Such microbial incursions are mostly results of unhygienic handling and practices during meat processing, particularly in developing countries [18]. Number of researchers reported that such microbial contaminations are due to casual habits of sneezing and coughing of the meat handlers and processors [19, 20]. Overcrowding, poverty, inadequate sanitary conditions, and poor general hygiene are typical cause, why food-borne infections are on rise in public [7].

Observations showed heavy bacteriological load carried by chicken carcasses. The presence of a high number of viable bacteria increases the chances of chicken spoilage [21] and may cause several foods borne illness to the consumers. Results indicated the predominance of Gram- negative organisms such as *Salmonella* sp.*, Shigella* sp.*,* and *E. coli* as reported by other groups [22].

The overall antibiotic susceptibility profile demonstrates prevalence of a high resistance pattern among the most pathogenic isolates against Bacitracin, Streptomycin, Ampicillin, Penicillin and Erythromycin. Resistance against other antibiotics such as Vancomycin, Cephotaxim, Ciprofloxacin and Rifampicin was observed 82.6%, 73.91%, 65.21% and 69.56% respectively, which were also at a level of concern. The least resistance was shown against Ciprofloxacin with a 47.8% of the total isolates (Figure 1).

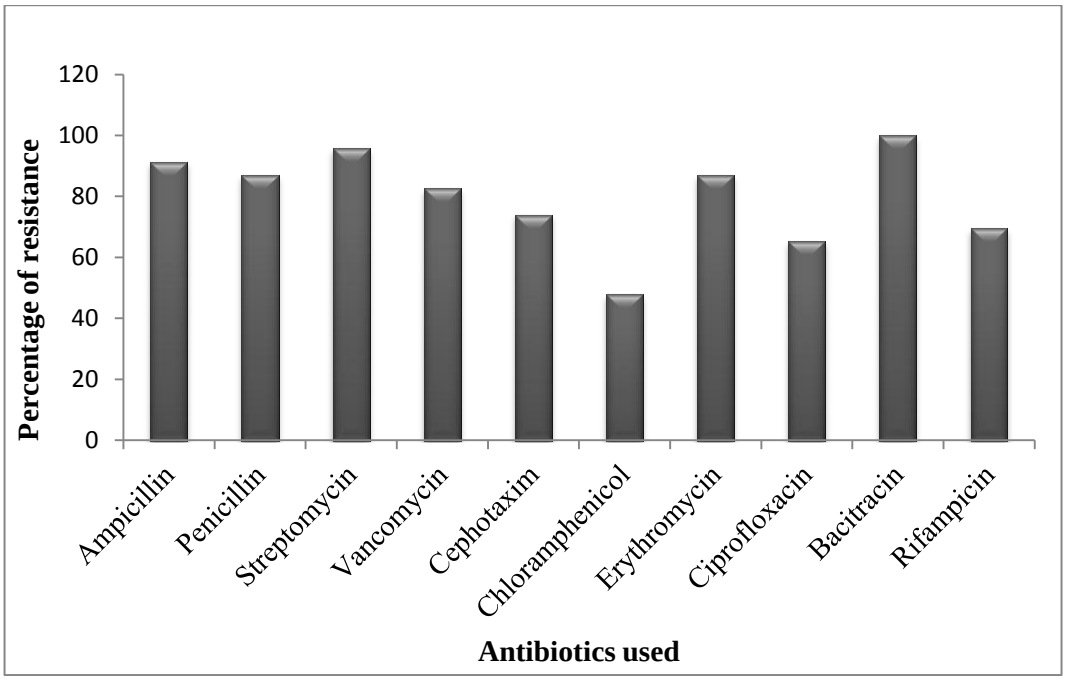

Figure 1: Overall microbial resistance against the antibiotics

The rate of antibiotic resistance among commonly isolated bacteria was given in Table 2. The antibiotic susceptibility profile showed the prevalence of ampicillin, penicillin G, streptomycin, followed by vancomycin resistance against all potential isolates. Most of the isolates are characterized as *Salmonella* sp. which shows a high spectrum of resistance against a number of antibiotics including ampicillin, penicillin, streptomycin, ciprofloxacin and bacitracin being at a resistance level of 100%. *E.coli* also shows a relatively high degree of resistance with 100% against a majority of antibiotics except a few such as chloramphenicol and ciprofloxacin. Such findings are also supported by another study results internationally which also reported a higher degree of resistance in *E.coli* against ciprofloxacin, more particularly in isolates from broilers than other

meat sources like pigs [12]. However, a high antibiotic resistance in E.coli is of great concern worldwide as it acquires such resistance much faster than other conventional bacteria and thus used as an indicator bacterium of food contamination [23]. *Staphylococcus* sp. and *Proteus* sp. being isolated at lower populations observed to exhibit a greater resistance profile. On the other hands, *Micrococcus* sp.*, Shigella* sp. shows a lesser degree of resistance comparatively, although isolated in a lesser numbers.

Enteric fevers causing *S. typhi* and *S. paratyphi* A have had a fascinating evolution [10]. Till 1987 low level resistance was seen in them but the infections could be treated with the three front line drugs ampicillin and chloramphenicol [24]. In the present study antibiotic resistance pattern of *Salmonella* sp. isolates from chicken showed a 100% resistance against ampicillin and ciprofloxacin and 50% against chloramphenicol, which have been some of the antibiotics of choice for the treatment against *Salmonella* sp. in human. The results more or less coincide with the recent finding of some other researchers in India who find a similar 100% resistant profile to Ampicillin, Penicillin, Vancomycin among the Salmonella isolates in their study and a moderate resistance against Ciprofloxacin which was warningly to a absolute resistant in the present study [15]. All of the *Salmonella* sp. isolates were multidrug-resistant and were also highly resistant to penicillin, streptomycin, erythromycin, and bacitracin. Many studies have suggested that poultry products, especially chicken, could be the most common reservoir of *Salmonella* sp. [25-30]. The results showed that the prevalence of *Salmonella* sp. contamination and drug resistance in them in chicken meat samples purchased from traditional marketplaces was high. As could be derived from the above discussion Salmonella and *E.coli* are the most prevalent and highly resistant contaminants indicating a fast and hazardous spread of resistance among these bacteria specifically which is also concurring with other studies worldwide [12].

Even though, usually occurrence of Vancomycin resistance in *Staphylococcus* sp. was observed at a considerably low level [10], however, the present study showed an appearance of high resistance against Vancomycin among the *Staphylococcus* sp. isolates suggesting the reckless use of the antibiotic may alter the scenario. This fact coupled with the emergence of CAMRSA would give rise to serious clinical problems with global increase in antibiotic resistant among the organisms [9, 31].

Resistance of bacterial isolates and the rate of concurrence of such resistance against a number of available antibiotics were observed. The problem may be attributed to a number of possible sources, including the natural resistance of species to certain antibiotics [32] possible transfer of antibiotic resistance among species, and the use of sub-therapeutic doses of antibiotics in animal feeds to improve animal productivity, which could also select for resistant strains [24]. However, no control strains were used for antibiotic susceptibility profiles, which can be considered as limitation of the study to reach valid conclusion.

The presence of bacterial pathogens in chicken-processing equipment and associated surfaces may contribute to the contamination of chicken [7]. Many studies suggested that use of low-level, non-therapeutic, antibiotic feed supplements may contribute to selection of antibiotic-resistant bacterial populations in the environment and animals [33]. Many antibiotics such as Bacitracin, are used as growth-promoting antibiotics, and their doses are lower than therapeutic one. Inappropriate uses of these growth-promoting antibiotics contribute largely to the emergence of antibiotic resistant bacteria [34]. The inadvertent use of antibiotics for long duration or in suboptimal doses leads to emergence of new resistant strains and thus should be considered based on their recent antibiogram profile only; as use of antibiotics based on past reports may not be responsive always leading to development of resistant strains [35]. Besides researchers suggested that a short withdrawal period of antimicrobial used, from treated chickens before sending to slaughter house may impose high risk to public health and therefore recommended for an extensive antimicrobial removal time [23]. The findings of the present study regarding the prevalence of multidrug resistant Salmonella in retail chicken samples is also substantiate other recent studies in India [35, 36].

Molecular typing of such isolates from chicken meat, and live chickens may allow us to trace the contamination origins and transmission routes for antibiotic resistance genes [37]. This study presents the contamination status of retail chicken and its surrounding environment as well as demonstrates the role of raw food as a reservoir of antibiotic resistance bacteria that can be transferred to humans, thereby constituting a health problem. The application of hygiene practices along the food chain and prudent use of antibiotics in animal husbandry are therefore essential to control further emergence of antibiotic resistance. It is essentially important to provide training to chicken and meat handlers regarding food safety.

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Table 1: Biochemical Characterization of the isolates

Test performed		Microbial isolates						
		B1a	B1b, B2c, B3b	1b, 2a	3, 4, 5, A1b, A2a, A2c, A3b, A3c, B2a, B2d	A1a, A2b, A3a, B2b	1a, B3c	B3a
Gram Staining		GPB	GPC	GPC	GNB	GNB	GNB	GNB
Catalase		+	_	+	+	+	+	+
Oxidase		_	_	_	_	_	_	_
Indole		+		_	_	+	+	_
MR		+		+	+	+	+	_
VP		_		_	_	_	_	+
Citrate		_		_	+	_	_	+
Starch Hydrolysis		_	_	_	_	_	_	_
TSI	Slant	Y(A)	R(NC)	R(NC)	R(NC)	Y(A)	R(NC)	Y(A)
	Butt	Y(AG)	R(NC)	Y(AG)	Y(AG)	Y(AG)	Y(AG)	Y(AG)
	H_2S Production	_	_	_	+	_	_	_
		Proteus sp.	*Micrococcus* sp.	*Staphylococcus* sp.	*Salmonella* sp.	*E. coli*	*Shigella* sp.	*Klebsiella* sp.

Y(A)- yellow, acid production, Y(AG)- yellow, acid and gas production, R(NC)- red, no change, (+) = positive, (-)= negative

Table 2: Antibiotic resistance profile of the Isolate

Antibiotics	Organisms													
	***Proteus* sp. (n=1)**		***Micrococcus* sp. (n=3)**		***Salmonella* sp. (n=10)**		***E.coli* (n=4)**		***Shigella* sp. (n=2)**		***Klebsiella* sp. (n=1)**		***Staphylococcus* sp. (n=2)**	
	n	**%**	**N**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**N**	**%**
Ampicillin	1	100	2	66.6	10	100	4	100	1	50	1	100	2	100
Penicillin	1	100	1	33.3	10	100	4	100	1	50	1	100	2	100
Streptomycin	1	100	3	100	10	100	4	100	1	50	1	100	2	100
Vancomycin	1	100	3	100	7	70	4	100	1	50	1	100	2	100
Cephotaxime	1	100	0	0	7	70	4	100	2	100	1	100	2	100
Chloramphenicol	1	100	0	0	5	50	2	50	1	50	0	0	2	100
Erythromycin	1	100	3	100	8	80	4	100	1	50	1	100	2	100
Ciprofloxacin	0	0	0	0	10	100	2	50	1	50	1	100	1	50
Bacitracin	1	100	3	100	10	100	4	100	2	100	1	100	2	100
Rifampicin	1	100	1	33.3	6	60	4	100	1	50	1	100	2	100

n = Number of isolates, %= percentage of drug resistance in a particular group of isolate

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CONCLUSION

Food-borne pathogens found in retail butcher shops could be sources for horizontal contamination of chicken. The data from the present study confirms the circulation of antibiotic resistant pathogens in raw chicken and its environment in retail shops, which could play a role in the spread of antimicrobial resistance amongst food-borne bacteria. Therefore it is important to ensure the practice of basic hygiene principles, which cover food safety procedures. It is especially important to provide training to meat handlers regarding food safety.

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