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Prevalence Rate and Antibiotic Susceptibility Pattern of Salmonella Spp. Isolated From Broiler Chicken Collected from Different Market Areas of Sylhet Region Bangladesh

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Abstract: Salmonellosis still remains as one of the major threat for poultry industry as well as public health in Bangladesh. Poultry is regarded as one of the most common reservoirs of *Salmonella* and contamination of poultry products can occur during any stage of poultry production. The current study was designed to identify Salmonella prevalence rate in broiler chickens and the antimicrobial susceptibility pattern of *Salmonella spp.* isolated from broiler chicken guts. A total of 320 bacterial colonies were isolated from broiler guts collected from 5 different markets of Sylhet city, Bangladesh, during January and February of the year 2017. The samples were subjected for isolation and identification of *Salmonella* spp.and through a series of morphological and biochemical observations, 14.37% of the isolates were identified as *Salmonella.* Antimicrobial sensitivity test was done against 8 commonly found antibiotics in chicken carcasses. *Salmonella* strains were found to be completely resistant against Ampicillin (100%). The frequency of antibiotic resistance ranked in the following order: Erythromycin (97.82%), Tetracycline (84.78%), Polymyxin B (73.91%), Co-trimoxazole (58.7%), Neomycin (13.04%) and Ciprofloxacin (6.52%). However, Salmonella isolates showed considerably greater sensitivity against Gentamycin (95.64%) and Ciprofloxacin (80.43%). Multidrug resistance was found to be present in all the isolates which is alarming for poultry business and consumer as well.

Keywords: Antibiotic resistance, Salmonella, Broiler

INTRODUCTION

Food borne salmonellosis is a major health issue throughout the world. 85% of total non-typhoidal salmonella infections are transmitted through food materials resulting in thousands of annual death and posing a worldwide risk to human health [1, 2]. Moreover, absence of effective immunoprophylactic measures has made avian salmonellosis a strong impediment for development of poultry industries [3]. Many of the poultry farms are currently experiencing substantial financial losses due to high mortality and reduced reproduction associated with infectious salmonellosis [4]. Salmonella outbreaks due to physical contact between human and animal has made difficult to establish the origin of infection and world health organization (WHO) and Food and Agriculture Organization (FAO) repeatedly identified salmonella infections as most common and significant form of zoonosis since 1950 [5, 6].

Fresh and processed poultry products have frequently been declared as the main source of foodborne human salmonellosis. In recent years, increased exposure towards pathogen associated with higher poultry consumption for mitigating the ever increasing food demand has been attributed to significant deterioration in diseases outbreaks worldwide [7, 8]. In spite of introducing better management technologies and hygienic practices in all stages of production accompanied by advanced improvements in public sanitation, Salmonella infection still remains a persistent major threat to poultry industry as well as human health [9].

Number of salmonellosis incidences in multiple geographical locations together with its economic losses estimated for human health and poultry sectors is growing awareness to prevent its transmission. Prevention of *Salmonella* infection in poultry is regarded as an essential first step in reducing *Salmonella* outbreaks in humans [10].

Invasive nature of salmonella infections requires antimicrobial therapy to shorten illness and to prevent adverse complications [11, 12]. Drug resistance in foodborne enteric bacterial population is an inevitable consequence of using antimicrobial agents in a regular basis for food producing animals [13]. Although a number of antimicrobial agents were effective against *Salmonella* before the 1990s, resistance against

ampicillin, chloramphenicol, and trimethoprim-sulfamethaxozole have been incessantly reported within last 15 years [14, 15]. Indiscriminate use of antibiotics to treat infectious diseases and its extensive use as growth promoters in poultry production are recognized worldwide as the most significant driving force for developing antimicrobial resistance [16, 17]. Higher rate of resistance is limiting the use of conventional antibiotics and resistance against newer antimicrobial agents is aggravating the situation [18].

Dissemination of multi drug resistant Salmonella through the food chain has serious public health implications as potential therapeutic failure has reportedly been indicated to be associated with this scenario [19]. Moreover, the transfer of multidrug resistant enteric pathogens through food chain in between poultry and human is establishing a reservoir of resistant genes [20]. The emergence and rapid worldwide distribution of the multiple drug-resistant Salmonella are severe global threats for both animal agriculture and human health as once antimicrobial resistance has been incorporated into an ecosystem, resistance can spread and persist without continuous selection pressure from antimicrobial agents [21].

In developing countries, subsistent household farming is common practice which in turns render a larger fraction of population in the danger of transmitting resistant organism during close contact [22]. Furthermore, absence of religious obstacles in consuming poultry meat is increasing extending the commercial poultry market throughout the world [23]. Dissemination of resistant bacterial population through contaminated undercooked meat and fecal material released from the gut carcasses after slaughtering is a major public health concern as Salmonella is repeatedly being identified as the most incriminated pathogen for bacterial food poisoning [24].

Though there are lots of reports regarding Salmonella prevalence rate and its resistance against different therapeutically important antibiotic throughout the world, but still, there is no such comprehensive reports based on Bangladesh. The present study was designed to evaluate the antibiotic susceptibility pattern of Salmonella strains against eight antibiotics which are frequently administered for both human and poultry.

MATERIALS AND METHOD

Collecting and storing gut samples

In this study 10 pieces of poultry gut were collected from five different markets of Sylhet city. From each market, two gut samples were collected from two different far houses. The fresh gut was taken immediately after slaughtering and carried in a sterile zipbag to avoid contaminations. Immediately after collection, the samples were aseptically transferred to Microbiology, Fermentation and Environmental Biotechnology laboratory of Department of Genetic Engineering and Biotechnology, SUST, Bangladesh and stored in freezer.

Sample preparation for Salmonella isolation

About two inches of digestive tract containing part of jejunum and part of ileum was cut using sterile scissor. Excised contents was flushed in 50ml sterile water, then agitated with a glass rod. The water was used for further isolation.

Salmonella isolation

Each of the prepared samples was used for isolation of Salmonella. Each sample was inoculated into nutrient broth and kept 48 hours at 37℃ for enrichment of bacterial population. Enriched broth was subsequently spreaded in Salmonella-Shigella (SS) agar, a selective medium used for salmonella isolation. Black and white colonies from SS-agar plates were selected for further studies.

Biochemical Characterization

Biochemical tests were conducted with all the suspected isolates. Employed biochemical tests were methyl-red test, VP test and Indole test. Oxidase, catalase and urease tests were also carried out. For biochemical tests standard procedures were used. Salmonella colonies were identified using the result of biochemical tests and Salmonella isolates were subjected to antimicrobial susceptibility test.

Antimicrobial Susceptibility Test

Susceptibility of Salmonella isolates to different antibacterial agents was determined in vitro by employing a Kirby-Bauer method demonstrated by Bauer et al., 1996 [25]. The procedure involved measuring the diameter of the zone of inhibition that results from diffusion of antimicrobial agent into the medium surrounding the disc. The 0.5 McFarland standard isolates were inoculated onto Mueller Hinton agar plates. Eight commercially available antimicrobial discs (Hi-Media Laboratories Pvt. Limited, Mumbai) were used for the test. The followings are the antimicrobial agents tested against Salmoella isolates (**Table 1**).

Table 1: Antimicrobial agents and their disc concentrations

Antimicrobial agents	Disc concentrations in micro gram (µg)
Amplicillin(AMP)	25
Erythromycin	25
Ciprofloxacin (CIP)	5
Co-Trimoxazale(COT)	25
Neomycin	30
Gentamicin (GEN)	10
Tetracycline(TE)	30
Polymixin B	300units

RESULTS

Salmonella Isolation

All the black and white colonies from the SS agar were selected as Salmonella suspect and subjected to biochemical characterization for confirmation. The suspected colonies which were positive for indole and catalase tests and negative for urease and oxidase tests were selected for further examinations. After biochemical characterization, suspected colonies were confirmed as Salmonella by motility test using motility indole urease (MIU) media.

Table 2: Number of *Salmonella spp*. isolates from various sample sources

Source	Total number of bacterial isolates	Number of *Salmonella* sp.	Prevalence rate of *Salmonella* sp.
Modina Market	35	4	11.42%
Noya Bazar	25	2	8%
Rikabi Bazar	179	33	18.43%
Subid Bazar	27	7	29.89%
Tuker Bazar	54	0	0%
Total	320	46	14.375%

Antimicrobial Susceptibility Test

Susceptibility of isolated *Salmonella* sp. to different antibiotics was determined by Kirby-Bauer disc-diffusion technique as specified by the National Committee for Clinical Laboratory Standards (CLSI-2007).

Fig-1: Antibiotic resistance pattern of *Salmonella* showing resistance and sensitivity against commonly used anti biotics

Table 3: Antimicrobial susceptibility pattern of *Salmonella* isolates.

Name of Antibiotics	Resistant	Intermediate	Sensitive
Ampicillin(AMP)	100%	0%	0%
Ciprofloxacin(CIP)	6.52%	13.05%	80.43%
Co-trimoxazole(COT)	58.7%	21.67%	19.63%
Erythromycin(E)	97.82%	2.18%	0%
Gentamicin(GEN)	0%	4.36%	95.64%
Neomycin(N)	13.05%	58.69%	28.26%
Tetracycline(TE)	84.78%	4.35%	10.87%
Polymyxin B(PB)	73.91%	-	26.09%

DISCUSSION

Over the last few decades, continuous increase in Salmonella prevalence rate in poultry has gained considerable scientific attention as poultry has repeatedly been indicated as one of the most common Salmonella carrier leading foodborne illness in human and contamination of poultry products by Salmonella is not restricted to any particular stage of poultry production [26]. Moreover, Salmonellosis accounts for 2.8 billion cases of gastroenteritis annually and severe worldwide economic loss associated with infections in human and food producing animals [2]. Though, poultry industry is evolving and emerging as one of the world largest economy, fowl salmonellosis is incessantly rampant in different areas of the world as a strong impediment for the economy as well as the future development of poultry sector.

In our study, 320 bacterial suspects were isolated from 10 different poultry gut samples, collected from 5 different market places of Sylhet city. After initial selection in Salmonella-Shigella agar medium, a series of biochemical and motility tests were performed to identify Salmonella. Out of 320 suspects, 46 isolates were confirmed as Salmonella with prevalence rate of 14.38% which closely matched with the 12.7% prevalence percentage estimated in China by Zhao et al., 2016 [27]. But, the Salmonella occurrence rate in our study was much lower than 60% prevalence rate found in Portugal by Maharjan et al., 2006 [28]. Opposed to overall percentage, individual market area had differential Salmonella prevalence rate ranging from 0% to 29.90% at maximum. Fluctuations in the Salmonella occurrence may be due to sample type and size as well as the large variations in the hygienic conditions prevailing in the commercial areas. The season during which the study was conducted can be considered as another factor as fluctuations in Salmonella prevalence rates with seasonal variations were observed by Maharjan et al., 2006 [28].

Advancements in globalization and technologies for production and therapeutic applications of antimicrobial agents have greatly improved healthcare both in the veterinary and public settings. Nonetheless, irrational use of antibiotics has considerably facilitated the emergence of antimicrobial resistance [29].

The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections. Avian Salmonella was found to be resistant against many antimicrobials agent; tetracycline, oxytetracycline, penicillin, aminoglycosides, sulpha-drugs and fluoroquinolones [30, 31, 32]. With the emergence of multidrug resistant strains over time, available therapeutic options have already been limited and narrowed down to currently sensitive antibiotics.

There is a good chance for potential therapeutic failure in near future since the bacteria will eventually acquire resistance against currently effective antimicrobial agents due to their extensive and indiscriminate uses [33].

Antimicrobial susceptibility test of the Salmonella isolates were conducted against eight commonly prescribed antibiotics. The used antibiotics were Ampicillin (AMP), Polymixin B (PMB), Ciprofloxacin (CIP), Co-trimoxazole (COT), Erythromycin (ERT), Gentamycin (GEN), Neomycin (NEO), Tetracycline (TE). Most of the *Salmonella* isolates showed resistance against the first line antibiotics that are commonly prescribed by veterinary physicians. Multidrug resistance was present within all of the Salmonella isolates which is a clear indication of limited therapeutic option [32]

Variations in ampicillin resistance were indicated in many studies. According to recent reports 15% to 73% Salmonella resistance against ampicillin were found [21, 29]. In our experiment, ampicillin was found to be most ineffective therapeutic agent as all the isolates were completely resistant against it.

80% and 100% Salmonella resistance against erythromycin were reported by Phagoo et al., 2015 and Cardoso et al., 2006 respectively [26, 35]. Our result demonstrated a slight deviation from absolute erythromycin resistance where over 97% of the total isolates were resistant against erythromycin.

Similarly, a significant resistance percentage was observed against co-trimoxazole, tetracycline and polymixin B. Over 50% of the total isolates were resistant against Co-trimoxazole. 84% of Salmonella resistance against tetracycline in our experiment was much higher than 46% resistance against same antimicrobial agent, reported by Bai et al., 2015 [34]. Though the result didn't agree with absolute tetracycline resistance reported by Phagoo et. al., 2015, it nearly corresponded with 91% of Salmonella resistance estimated by Ziech et al., 2016 [26, 36]. Moreover, opposed to complete resistance of Salmonella isolates against polymixin B, indicated in many studies [37, 38], nearly 74% of the isolates were resistant to polymixin B.

The higher degree of resistance can be attributed to the inadequate dose, extensive use and sub-active concentration of the drug used in poultry farms as reported by Davis et al., 1994 [39]. Furthermore, widespread use of antibiotics in medical, veterinary, agricultural and aquacultural settings as prophylactic measures and growth promoters have resulted in resistance to a large spectrum of antibiotics leading to the proliferation of antibiotic resistant genes in the horizontal gene pool [40].

Gentamycin and ciprofloxacin are considered as effective therapeutic agent against Salmonella infections till date as lower resistance and higher sensitivity of Salmonella against these antibiotics were repeatedly being reported. 100% susceptibility against ciprofloxacin and over 50% susceptibility against gentamycin were reported by Singh et al., 2010. Moreover, many of the recent studies revealed complete susceptibility of Salmonella strains against both of these antimicrobial agents [41, 42]. Our experiment also established the same fact as all of the Salmonella isolates of our experiment were susceptible to gentacymcin whereas the susceptibility for ciprofloxacin was slightly reduced to 80%.

Only 13.04% Salmonella resistance was observed against neomycin. But, it can't be referred as a therapeutic choice as nearly 60% of total isolates were in between resistant and susceptible for neomycin.

The indiscriminate use of antimicrobials in livestock farming have resulted in incessantly growing greater resistant Salmonella strains which have been transmitted to humans via the food chain and thus have been the major cause of drug resistance in humans. Serious health consequences together with potential therapeutic failures and economic disasters are associated with multidrug resistance of the infectious agents [43].

CONCLUSION

In conclusion, bacterial resistance against antibiotics greatly interferes with the effectiveness of control strategies against infectious diseases. Therefore, good hygiene practices and the controlled use of Antibiotics is necessary to prevent the spread of antibiotic resistant strains of Salmonella. This current study has revealed that Gentamycin is the most effective antimicrobial therapeutic agent against Salmonella and Ampicillin was found to be most ineffective drug as Salmonella strains exhibited absolute resistance against it. The gradual increase in resistance percentage of Salmonella against commonly administered antibiotics poses a great threat to human health as well as poultry sector and implies that the indiscriminate use of antimicrobial agents should be monitored in order to control emergence and subsequent spread of resistant Salmonella pathogen.

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