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Original Article

Occurrence, Antimicrobial Resistance Pattern and Plasmid Profile of *Salmonella spp*. Isolated from Raw Beef Meat in Dhaka City

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Salmonella **is motile enterobacterium that causes diseases like typhoid fever, paratyphoid fever and food borne illnesses.** ***Salmonella*** **infections are due to the consumption of contaminated foods. The objective of the present study was to observe the sanitary and hygienic conditions of the retail areas and to isolate pathogenic** ***Salmonella spp*** **from raw beef, to observe antimicrobial sensitivity pattern of** ***Salmonella spp*** **from the raw beef. A total of forty (40) raw beef samples were collected from the sixteen different butcher shops of the four different markets namely Bazaar-1, Bazaar-2, Bazaar-3 and Bazaar-4 of Dhaka City to determine the presence of the significant enteric pathogen Salmonella. From the colony characteristic and biochemical properties, it was found that out of forty samples Salmonella were found in thirty four (85%) samples. The prevalence of drug resistance bacteria were also assayed by antibiotic susceptibility testing. Among thirty four isolates, nearly 6% (2), 32% (11), 76% (26), 6%(2) and 32% (12) isolates showed resistance to Nitrofurantoin, Nalidixic acid, Ampicillin , Gentamycin and Trimethoprim-sulfamethoxazole respectively. Almost 85%(29), 62%(21), 24% (8), 91% (31), 91% (31)and 68% (23) isolates showed sensitivity to Nitrofurantoin, Nalidixic acid, Ampicillin,Ciprofloxacin,Gentamycin and Trimethoprim-sulfamethoxazole respectively Some isolates also showed intermediate in sensitivity reaction for example 3% (1), 6% (2), 3% (1) and 9% (3) isolates showed intermediate in sensitivity reaction to Nitrofurantoin, Ampicillin, Ciprofloxacin, Gentamycin and Nitrofurantoin respectively. Twenty four isolates were investigated for the plasmid profile analysis where thirteen isolates (about 54%) were found to carry plasmids of different sizes ranging from small (2.3 kb) to large sizes (>10kb and also greater than the chromosomal DNA). The presence of large plasmids may be a possible source of drug resistance. The results of this study demonstrate the unhygienic quality of beef meat and their risk of contracting food borne infections to the consumers.**

Keywords: **Beef, Drug resistance, Hygienic, Prevalence, Plasmids, Retail markets, Sanitary, Slaughter.**

Introduction

There are several transmission routes for salmonellosis, but the majority of human infections are derived from the consumption of contaminated food, especially of animal origin Salmonellosis is the most frequently encountered food-borne bacterial disease in the world and an important public health concern[1]. *Salmonella* is estimated as an annual infectious rate of 21.6 million and approximate death rate of 600000 with the highest percentage in Africa and Asia[2]. A survey performed by the WHO[3] in Europe indicated that 25% of the food-borne outbreaks could be traced back to recontamination. The most important factors contributing to the presence of pathogens in processing food were insufficient hygiene (1.6%), cross-contamination (3.6%), processing and storage in inadequate rooms (4.25%), contaminated equipment (5.7%), and contamination by personnel (9.2%). Sources of microbial contamination in fresh meat have been documented[4, 5].

Meat and meat products are considered as an excellent source of high quality animal protein, vitamins especially B complex, and certain minerals, especially iron[6]. Contamination of raw meat is one of the main sources of food borne illnesses[7]. Like any other raw meat, raw beef may be contaminated during production, processing, storage and marketing with biological agents that may be hazardous to human health. A variety of sources, including air, water, soil, feces, feed, hides, intestines, lymph nodes, processing equipment, utensil and human, contribute to the microbial contamination of the sterile muscle of healthy animals during slaughter, fabrication, and further processing and handling[8]. *Salmonella* is one of the microorganisms most frequently associated with food-born outbreaks of illness. Meat products in general and poultry, in particular, are the most common sources of food poisoning by *Salmonella*[9].

In recent years, there has been increasing concern regarding the worldwide occurrence of multidrug resistant strains of a number of pathogenic bacteria including *Salmonella* in foods. The extensive use of antibiotics for therapeutic or preventive purposes in veterinary medicine and as growth promoters in

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animal feed has contributed to the occurrence of resistant bacteria in animals, including zoonotic pathogens, which can be transmitted to humans via food chain[10, 11]. The incidence of *Salmonella* strains resistant to various antimicrobial agents has increased over the last years, resulting in higher morbidity and mortality rates and higher overall treatment costs[12]. Several studies have shown that antimicrobial drug resistance seen in salmonellosis is a consequence of using antimicrobial drugs in food-producing animals[13].

The objective of the present study was to observe the sanitary and hygienic conditions of the retail areas and to isolate pathogenic *Salmonella spp* from raw beef, to observe antimicrobial sensitivity pattern of *Salmonella spp* from the raw beef.

Materials and Methods

Sample collection

From May 2011 to December 2011, forty raw beef samples were collected from four different retail markets of Dhaka City namely Bazaar-1, Bazaar-2, Bazaar-3 and Bazaar-4. At each time of collection, precaution was taken to minimize cross-contamination of samples. Samples were aseptically collected in sterile bottles according to the standard procedures and transported to the Food Microbiology laboratory of Institute of Food Science and Technology (IFST), BCSIR, Dhaka in an insulated box with ice to maintain a temperature ranging from 4ºC to 6ºC[14]. Samples were stored in ice for up to 6h from the time of collection for transport and subsequent analysis in the laboratory.

Isolation and characterization of Salmonella spp isolates

Twenty five gram raw beef sample was taken in 225 ml sterile lactose broth (Oxoid, England) aseptically and incubated for 24 hours at 37°C for enrichment. After incubation, 0.1 ml of the broth was added to 10 ml of selenite cystine broth (Himedia, India) and incubated overnight at 37°C. One loopful of enrichment medium was then streaked onto the selective and differential medium namely Bismuth Sulfate Agar (BSA) (Himedia, India) and Xylose Lysine Deoxycholate agar (Himedia, India) and incubated for 18-24 hours at 37°C. Presumptive isolated culture strains were obtained and subcultured into the nutrient agar media subsequently preserved in nutrient broth with 30% sterile glycerol at -20°C for further studies. The shape and type of Gram reaction are microscopically studied using 18 hour culture from agar plate. The biochemical tests involved Simmon's Citrate Slant, Motility Indole Urease (MIU), Methyl Red (MR), Voges Proskauer (VP), kligler iron agar (Oxoid, England) test was done. Identification of isolates obtained in pure culture was based on Gram staining, biochemical characteristics and growth pattern on selective and differential media and; according to the procedures recommended in the Bergey's Manual of Determinative Bacteriology [15, 16].

Antimicrobial susceptibility test

All isolated strains were tested for antibiotic resistance by the standard agar disc diffusion method (17) on Mueller-Hinton agar (OXOID, England) using commercial discs (OXOID, England). The following antibiotics with the disc strength in parentheses were used: ampicillin (10ìg), gentamicin (10ìg), trimethoprim-sulphamethoxazole (25ìg), ciprofloxacin (5ìg), nalidixic acid (30ìg), and nitrofurantoin (300ìg). A control strain of reference *Escherichia coli* PDK-9 was included each plate. Antimicrobial breakpoints and interpretation were taken from the CLSI standards[18].

Plasmid DNA Profiling

The selected pure culture of bacterial isolate (single colony) was grown overnight in Luria-Burtoni (LB) broth at 37^0C giving aeration by the orbital shaker. A quantity of 1.5 ml overnight culture was taken out in Eppendorf tubes for plasmid DNA extraction. The plasmid DNA from *Salmonella spp* isolates was extracted through Mini alkaline lysis[19].

Reference Marker

The plasmid DNA extracted from *Salmonella spp* isolates were compared to known molecular weight standards (1kb DNA ladder).

Agarose Gel Electrophoresis of Plasmid DNA

The agar was prepared incorporated with ethidium bromide and electrophoresis was done by horizontal gel apparatus[20].

Results and Discussion

Identification of the isolates

Forty suspected *Salmonella spp* colonies have been isolated from the samples analyzed from Bismuth Sulfate Agar (BSA) and Xylose Lysine Deoxycholate agar. The isolates were purified by restreaking further on Nutrient agar and incubated for 18-24 hour at 37ºC; the isolates were preserved in 30% sterile glycerol at -20°C. The shape and type of Gram reaction are microscopically studied using 18 hour culture from agar plate. The biochemical tests involved Simmon's Citrate Slant, Motility Indole Urease (MIU), Methyl Red (MR), Voges Proskauer (VP), kligler iron agar (Oxoid, England) test was done. In KIA test some species produced H_2S while others produced gases other than H_2S. Also in Simmons citrate test, some isolates showed positive while others showed negative result in Simmons citrate test (Table 1). This result agrees with the document of the procedures recommended in the Bergey's Manual of Determinative Bacteriology.

Based on the biochemical and colony characteristics of isolates, fifteen samples (78.95%) of 19 samples collected from Bazaar-1, nine samples (90%) of 10 samples collected from bazaar-2, five samples (83.33%) of 6 samples collected from bazaar-3 and five samples (100%) of 5 samples collected from bazaar-4 were found to be contaminated with *Salmonella spp.* The overall

Table 1. *Salmonella spp isolation and percentage of positive samples*

Sampling site and no of samples	Change in broth, colony character and staining character			Change in biochemical properties			Occurrence%	
	Selenite broth (growth)	Grams staining (pink short rod and gram negative)	Colony on BSA(black colony with metallic sheen)	MR and mobility test	Indole VP and urease test	KIA test	Individual strategy	Overall
Bazaar-1(19)	++	++	++	++	- -	++	78.95	
Bazaar-2(10)	++	++	++	++	- -	++	90	85
Bazaar-3 (6)	++	++	++	++	- -	++	83.33	
Bazaar-4 (5)	++	++	++	++	- -	++	100	

occurrence of *Salmonella spp* in raw beef was found 85% (Figure 1). This result indices the serious occurrence of *Salmonella spp* in raw beef in Dhaka City. Studies in northern Thailand revealed 57% prevalence in meat at the market during 2002-2003[21], 14.5% prevalence in Kathmandu, Nepal[22], and 42.63% prevalence in Ho Chi Minh City, Vietnam[23]. Sero-prevalence of poultry *Salmonella* in Bangladesh has been reported to be 23.46%[24].

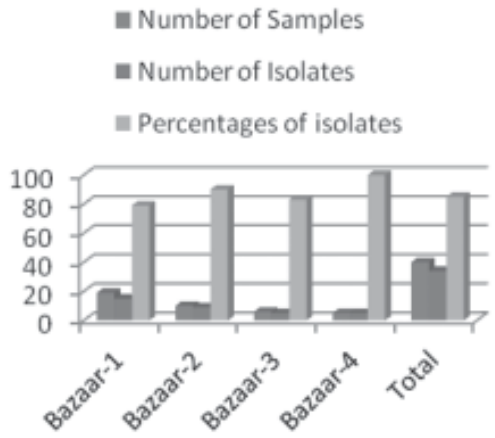

Figure 1: *The prevalence of Salmonella spp in raw beef samples from four local markets*

As observed in the course of study, the method of slaughtering of cattle and retailing system were found traditional which don't follow the rules and regulations of sanitary and hygienic conditions. Retail shop's environment, personal hygiene of process workers, hygienic conditions of slaughtered animal were found to be very poor which may be responsible for the microbial recontamination. *Salmonella spp*. may also be dispersed in dust and aerosols generated during the handling and processing of animals. Contamination in the environment can act as a source of infection to other animals[25].

Water and Equipment used were found to be unfit for beef processing and retailing. Dhaka WASA supplied water was found to be used in the meat processing. One study conducted in Dhaka City, it was found that Eighty nine percent of the tested supplied water samples of Dhaka city were found unsuitable for human consumption and also harbored fecal coli form bacteria eg *Escheriachia.coli* and *salmonella* spp[26].

Antibiotic Resistance Pattern

Drug resistant *Salmonella* emerge in response to antimicrobial usage in humans and in food animals and selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance. Multi-drug resistance to critically important antimicrobials is compounding the problem. There are reports of high prevalence of resistance in *Salmonella* isolates from countries such as Taiwan[27], India[28], The Netherlands[29], resistant isolates from France[30], Canada[31], and Ethiopia[32].

In this study it was found that around 5.88% (2), 32.35% (11), 76.47% (26), 5.88% (2) and 32.35% (12) isolates showed resistance to Nitrofurantoin, Nalidixic acid, Ampicillin , Gentamycin and Trimethoprim-sulfamethoxazole respectively. Also about 2.94 % (1), 5.88 % (2), 2.94% (1) and 8.82% (3) isolates showed intermediate in sensitivity reaction to Nitrofurantoin, Ampicillin, Ciprofloxacin, Gentamycin and Nitrofurantoin respectively. While about 85.29% (29), 61.76% (21), 23.53% (8), 91.18% (31), 91.18% (31)and 67.65% (23) isolates showed sensitivity to Nitrofurantoin, Nalidixic acid, Ampicillin, Ciprofloxacin, Gentamycin and Trimethoprim-sulfamethoxazole respectively (Figure 2).

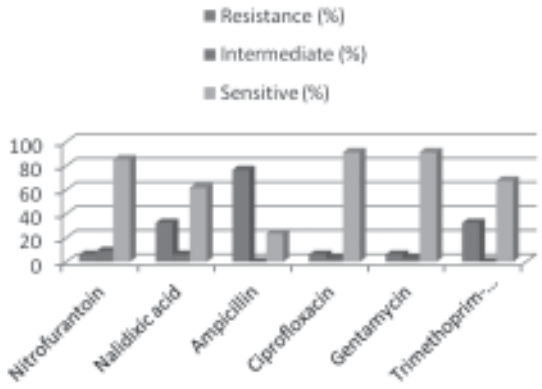

Figure 2: *The antibiotic sensitivity pattern of Salmonella spp in raw beef samples from four local markets*

This result agrees with the studies carried out in India, non-typhoidal *Salmonella* species, the typhoidal species and the avian isolates were found resistant to amoxicillin, nalidixic acid, cotrimoxazole, chloramphenicol, ciprofloxacin, sulphadiazine, sulphamethoxy pyridazine, neomycin, furazolidone, doxycycline, ampicillin, tetracycline, chlortetracycline, kanamycin, gentamicin, amikacin, ceftizoxime and ceftriaxone [33,34,35]. Also one of the studies indicated a rise in the antibiotic resistance in *Salmonella* Typhi [36]. In recent years, antibiotic resistance in *Salmonella* has assumed alarming proportions [37].

Plasmid Profile

Salmonella spp carry multiple plasmid profile which carries genes related to their pathogenicity. It is well proved by various studies.Plasmid profile analysis of 24 *Salmonella* isolates by agarose gel electrophoresis showed different plasmid bands occurring in various combinations. The size of these bands ranged from 1.5 to >10 kbs and the highest number of plasmid bands shown as 3 (Table 2 and Figure 3).

Table 2: *Plasmid profile and Antibiotic sensitivity patterns of Salmonella spp*

Isolates	Resistances to antibiotics	Number of plasmid band	Plasmid size in kb
1S	NAL,AM,T-S	1	1.1kb
2S	AM,T-S	1	One >10kb
3S	NAL,AM,T-S	3	3,6 and one >10kb
4S	AM,T-S	5	1.5,5,10 and two>10kb
5S	AM,T-S	3	1.3,5 and 10kb
6S	AM	6	1.5,5,7,10 and two >10kb
7S	AM,T-S	6	1.5,5,7,10 and two >10kb
17S	AM	2	5 and 10kb
18S	AM	2	6 and 7kb
19S	AM	3	4 and two >10kb
21S	AM	4	2.5, 6 and two>10kb
24S	-	3	7, 10 and >10kb
26S	-	2	10 and one >10kb

NB- NAL-Nalidixic acid, AM-Ampicillin, T-S-Trimethoprim-sulfamethoxazol

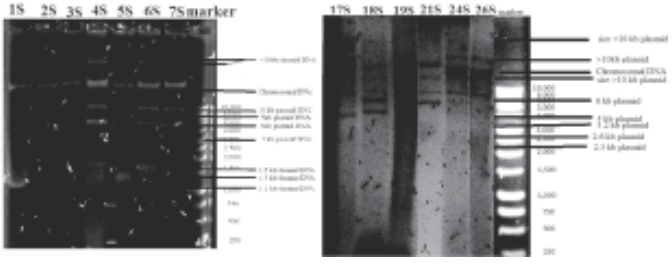

Figure 3: *Plasmid profile of 24 Salmonella isolates of raw beef samples analyzed by 0.8% agarose gel electrophoresis after staining with ethidium bromide and the DNA bands were visualized by UV-transilluminator.*

Plasmids are distributed at random in these strains and there was no notable correlation between antibiotic resistance and plasmid presence. In most of the cases, isolates having similar antibiotic sensitivity patterns had different plasmid patterns, implying that plasmid may not have link with the resistance. This supposition may be further supported by the finding that all the plasmid less isolates was resistant to one or more antibiotics.

Conclusion

The country like Bangladesh where the overall hygienic condition is not up to the mark and the chances of recontamination is also very high in retail environment. It is, therefore essential to know the frequency and distribution of *Salmonella spp* in raw beef and also to know about the resistance pattern and plasmid DNA profile of this organism.

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