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Multidrug Resistant *Salmonella* Isolated from Street Foods in Chittagong, Bangladesh

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MMH, SB, AAF and AI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MMH, SB, MA, TM and AI managed the analyses of the study. Authors AAF and MA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The oodles raising of zoonotic multi-drug resistance (MDR) *Salmonella* spp. during the last decade, especially in developing countries by repeated challenges resulting from increased and indiscriminate use of antimicrobials in food animals, fish and crop production, and human treatments is one of the dismal issues and might have a dire consequence in near future. The nascent MDR *Salmonella* may also find their way to commonly available street foods in Bangladesh. Therefore, it is imperative to find out the existence of MDR *Salmonella* in street foods of Bangladesh.
Study Design: We conducted a cross-sectional study to interrogate the prevalence of *Salmonella* spp. in street food items and the antimicrobial resistance pattern of isolated *Salmonella* spp.

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Place and duration of Study: The study was conducted from January to June 2016 in 5 street side markets (Agrabad, Colnel Hat, Alonkar Bazar, Bohderhat Bazar and Riazuddin Bazar) of Chittagong City Corporation (CCC) area of Bangladesh.
Methodology: Standard microbiological methods were used for isolation and identification of *Salmonella* spp. from selected street foods. The antibiotic susceptibility tests were conducted by using disc diffusion method with commercially available 11 antimicrobials which are frequently used for medical and veterinary practices in Bangladesh.
Results: Prevalence of *Salmonella* spp. were varied from 60% to 78% among the street food items. The study revealed MDR *Salmonella* (resistance up to 6 of 11 tested antimicrobials) from each of the food items tested. Concerning the degree of resistance, among the isolated *Salmonella*, the highest resistances (100%) were detected for Ampicillin and Amoxicillin and lowest for Pefloxacin (around 13%). Moreover, the degree of resistance of *Salmonella* to antimicrobials also varied among the various street food items.
Conclusion: The existence of MDR *Salmonella* notably a high rate in the street foods cues poor hygiene in street food production and it is a major threat for the advent of foodborne zoonoses.

Keywords: Antimicrobial; prevalence; resistance; street foods; Salmonella spp.

1. INTRODUCTION

Street foods are defined as a variety of ready-to-eat foods and beverages prepared and sold by vendors in streets and other public places for immediate consumption [1]. Microorganisms contamination of street foods has become a major public health concern globally [2,3]. Foodborne diseases are among the most widespread global public health problems of recent times, and their implication for health and economy is being increasingly recognized [4,5]. Among these pathogens, *Salmonella* are considered the most prevalent foodborne pathogens worldwide and has long been recognized as an important zoonotic pathogen of economic significance in animals and humans, predominantly in the developing countries [6]. The important route of transmission of *Salmonella* organism from animals to man is via food products of animal origin which may be contaminated at the source or during handling [7]. Infections through *Salmonella* throughout the world by food have increased [8]. Street foods in particular continue to be identified as leading food sources for human Salmonellosis [9]. Infections caused by eating foods contaminated with *Salmonella* spp. has important implication on public health worldwide [10]. The majority of human infections caused by *Salmonella* is related to the ingestion of contaminated foods such as poultry, beef, pork, egg, milk, cheese, seafood, fruit, juices and vegetables [11,12,13]. Worldwide *Salmonella* is a significant food and water-borne zoonotic pathogens [14]. In developing countries like Bangladesh antimicrobial resistance occur due to an increased and indiscriminate use of antibiotics in food animals, environments and human [6,15]. Throughout the previous era, multi-drug resistance of *Salmonella* spp. has increased in excessive amount [16]. It is presumed that the extensive use of antibiotics, especially in livestock production, may have resulted in the increasing incidence of antibiotic resistance in food borne *Salmonella* spp. and other microorganisms [17]. Street foods in particular continue to be identified as leading food sources for human Salmonellosis [18]. It is not yet clear as to which route is most important for *Salmonella* to contaminate the foods, which may be contaminated with *Salmonella* by vertical transmission and/or horizontal transmission [19]. Very few studies were conducted on isolation and drug resistance in *Salmonella* spp. throughout the world from street foods. In Bangladesh, evaluation of microbiological prevalence and antimicrobial susceptibility in common street foods is also negligible. This study, therefore, aimed to investigate prevalence of *Salmonella* spp. in common street foods (Fuska, Sugarcane juice and Borhani) and antimicrobial resistance pattern of *Salmonella* isolates from these foods to commonly used antimicrobials in Bangladesh.

2. MATERIALS AND METHODS

2.1 Study Design and Sampling Area

A cross-sectional study was conducted from January to June 2016 in 5 street side markets (Agrabad, Colnel Hat, Alonkar Bazar, Bohderhat Bazar and Riazuddin Bazar) of Chittagong City Corporation (CCC) area of Bangladesh. These places are the hot spots of street food trading.

2.2 Sample Collection and Preservation

Among the various street foods, we considered only 3 Bangladeshi traditional street food items: *(i) Fuska,* a fried food prepared mostly from flower, eggs and various spices; (*ii) Sugarcane juice*, a drink prepared from the trunk of mature sugarcane by pressure extraction and *(iii) Borhani*, a drink prepared from milk card with incorporation of rock salt and spices. A total of 143 samples of various street foods (Fuska surface water: 55, Sugarcane juice: 58 and Borhani: 30) were collected from 5 aforementioned street markets. All the samples were collected in sterile vials containing 6 ml amines transport media (Oxoid) and transported to the Poultry Research and Training Center (PRTC) laboratory, Chittagong Veterinary and Animal Sciences University (CVASU) using an insulated ice cool box.

2.3 *Salmonella* Isolation and Identification Procedures

A previously described protocol [20] was used for this study for the isolation and identification of *Salmonella*. Briefly, 1ml of food samples were transferred into 10 ml Mannitol Selenite Broth (Oxoid) and incubated at 37°C for 18 hours. After incubation, a loop full of broth was streaked on Xylose Lysine Deoxycholate medium and incubated at 37°C for 24 hours. Colonies with black centers were considered presumptive *Salmonella* spp. Presumptive colonies were grown on blood agar and the *Salmonella* was confirmed based on cultural properties and biochemical tests (Urease: Negative, Oxidase: Negative and Catalase: Positive).

2.4 Selection of Antimicrobials for Antimicrobial Susceptibility Testing

In the present investigation, the *Salmonella* isolates were tested whether they are resistant or not to antimicrobials by using commonly used antimicrobial (Ampicillin, Amoxicillin, Ciprofloxacin, Enrofloxacin, Pefloxacin, Colistin sulphate, Oxytetracycline, Tetracycline, Azithromycin, Erythromycin, Ceftriaxone) in Bangladesh.

2.5 Anti-microbial Susceptibility Test

An antimicrobial susceptibility test was done by disk diffusion method as described by Clinical and Laboratory Standards Institute (CLSI) [21]. In this method, Mueller Hinton agar plates were prepared as per instructions provided by the manufacturer. McFarland 0.5 turbidity standards were prepared as the standard guidelines described by the CLSI. After swabbing the pure *Salmonella* suspension with cotton swab, selected antibiotic disks were placed on the surface of the plate at equidistance. The plates were then kept at 4°C for 1-2 hours for proper diffusion of antibiotics and incubated for 24 hours at 37°C. The zone of inhibition was observed for antibiotic sensitivity or resistant, and zone diameter was measured. The sizes of zones of inhibition were interpreted by referring to zone diameter interpretive standards from NCCLS 2000 [21] and the isolates were considered as sensitive, intermediately sensitive, or resistant to these tested antimicrobials according to the standard [21].

2.6 Data Analysis

Field and laboratory data were stored and then cleaned in the MS Excel-2007 program before exporting to STATA/IC-13 for analysis. Descriptive analysis was performed to know the frequency and distribution of *Salmonella* and antibiotic resistance pattern. Chi-square test was performed to compare the frequencies between groups.

3. RESULTS AND DISCUSSION

3.1 Realm of *Salmonella* in Street Foods

We first looked for the existence of *Salmonella* based on cultural properties and biochemical test among the collected food samples and expressed them in frequencies and percentages (Table 1).

We found that, considering the categories of food item, the highest prevalence was found in sugar cane juice (77.58%) and lowest (60.00%) in borhani. Giving consideration to sites of sample collection, the prevalence was highest (80%) in Alonker Bazar and lowest (66.67%) in Agrabad. Neither types of food item nor the sites of sample collection were varied significantly ($p>0.2$) in terms of prevalence of *Salmonella*.

3.2 Drug-resistance *Salmonella*

We, investigated the *Salmonella* positive samples, for the existence of drug resistance *Salmonella* by antimicrobial susceptibility test and the outcomes are presented as each category of food items (Table 2).

Table 1. Prevalence of *Salmonella* in different samples and sampling sites

Variables	Categories	Number of samples	Positive (%)	χ2-value	P-value
Samples	Fuska surface water	55	40 (72.72)	3.057	0.216
	Sugarcane juice	58	45 (77.58)		
	Borhani	30	18 (60.00)		
Sampling sites	Agrabad	30	20 (66.67)	1.502	0.826
	Colnel Hat	35	24 (68.57)		
	Alonkar Bazar	25	20 (80.00)		
	Bohderhat Bazar	31	23 (74.19)		
	Riazuddin Bazar	22	16 (72.72)		

Table 2. Antimicrobial resistance pattern of *Salmonella* isolates from fuska surface water, sugarcane juice and Borhani

Antibiotics	Fuska surface water				Sugarcane juice				Borhani			
	N	R (%)	I (%)	S(%)	N	R (%)	I (%)	S (%)	N	R (%)	I (%)	S (%)
Ampicillin	40	100	0	0	45	100	0	0	18	100	0	0
Amoxicillin	40	100	0	0	45	100	0	0	18	100	0	0
Ciprofloxacin	40	27.5	42.5	30	45	60	28.89	11.11	18	11.11	5.55	83.33
Enrofloxacin	40	60	37.5	2.5	45	51.11	48.89	0	18	38.88	5.55	55.55
Pefloxacin	40	12.5	35	52.5	45	40	42.22	17.78	18	38.88	5.55	55.55
Colistin sulphate	40	57.5	7.5	35	45	91.11	0	8.89	18	33.33	50	16.66
Oxytetracycline	40	62.5	17.5	20	45	86.67	13.33	0	18	100	0	0
Tetracycline	40	82.5	12.5	5	45	82.22	17.78	0	18	100	0	0
Azithromycin	40	95	5	0	45	84.44	15.55	0	18	100	0	0
Erythromycin	40	90	10	0	45	100	0	0	18	100	0	0
Ceftriaxone	40	70	30	0	45	62.22	26.67	11.11	18	0	0	100

N: Number of Salmonella positive isolates in each categories of food; R: Resistance; I: Intermediate and S: Sensitive.

The *Salmonella* isolates were found to exhibit a certain degree of resistant to all of the anti-microbials tested. In case of Fuska surface water, the resistance was highest (100%) for Ampicillin and Amoxicillin followed by Azithromycin (95%), Erythromycin (90%) and lowest in Pefloxacin (around 13%), and none of ani-microbials were 100% sensitive to *Salmonella*.

Considering the data on Sugarcane juice, the highest percentages of drug-resistance *Salmonella* (100%) were detected to Ampicillin, Amoxicillin and Erythromycin followed by Colistin Sulphate (around 92%), Oxytetracycline (approximately 87%), and lowest in Pefloxacin (40%).

In a view to Borhani, the highest rate of antimicrobial resistant *Salmonella* were found (100%) against Ampicillin, Amoxicillin, Oxytetracycline, Tetracycline, Azithromycin, and Erythromycin followed by Enrofloxacin and Pefloxacin (around 39%). The highest sensitive drugs against *Salmonella* isolates was Ceftriaxone (100%) followed by Ciprofloxacin (83%), Enrofloxacin and Pefloxacin (56%).

Foods are important part of the human health [22]. Consuming un-hygienic street foods has been associated with negative health impacts. Street foods if improperly handled can be a source of food-borne diseases such as Salmonellosis [5]. The aim of this study was to determine the prevalence of *Salmonella* spp. in street foods along with the prevalence and pattern of antimicrobial resistance of isolated *Salmonella* spp. against commonly used antimicrobials in selected areas of Chittagong City Corporation, Bangladesh. The results of the present study indicated that, a considerable prevalence of *Salmonella* in selected street foods and similar finding was reported in Vietnam [23]. The prevalence levels of *Salmonella* infection caused by eating contaminated foods reported in United Kingdom, was from zero to 7% [24,25] but scenario of prevalence in developing countries were much higher, this might be due to poor hygienic measurement in food production and processing. Salmonellosis can be controlled in

foods of animal origin by several ways such as improved bio-security, vaccination, introduction of novel immune-potentiators etc. with limited use of antimicrobials [26]. An organism develops resistance against an antibiotic by repeated low dose exposure. Microorganisms that can be transmitted via foods might get exposure to low dose repeated antibiotic exposure from environmental contamination as most of the antimicrobials used in human and food-producing animal find their way to environment as final destination. The situation in developing countries like Bangladesh may be exaggerated by easy accessibility of antimicrobials at a cheaper price and their extensive use in food production system [27]. Thus, there is widespread availability and uncontrolled use of antibiotics poses the antimicrobial resistance in food products, which is the actual threat of public health [16]. 100% resistant Ampicillin and Amoxicillin were found in the present study almost similar (87-100%) resistance that was reported earlier in Bangladesh [14,28,29]. Ampicillin and Amoxicillin antibiotics resistant might have been due to use as growth promoters. Cross antimicrobial resistance cannot be ignored as it is evident in many earlier studies and causes higher resistance to Ampicillin and Amoxicillin [15,30,31].

The high resistance of Ampicillin and Amoxicillin is a great threat of public health. Resistance to Ciprofloxacin was recorded relatively higher proportions in present study. Ciprofloxacin is used for the treatment of Salmonellosis in humans [32,33]. Among Fluoroquinolones, resistance to Ciprofloxacin was found comparatively higher in the present study as compared to 35% resistance in USA [34] and 10.2-16.8% in Germany [35]. In present study higher resistant of Enrofloxacin were evident against the *Salmonella* isolates. In several investigations resistant to Enrofloxacin were found 14% [36] and 0.6-2% [37] in Australia that were comparatively lower than the current investigation. In the current study resistance to Pefloxacin was relatively lower. Similar type of result was found in Bangladesh in case of layer poultry *Salmonella* isolates [12]. It is less used for the treatment of Salmonellosis in humans and animals [38] that might be a cause of less resistance. The resistance pattern of *Salmonella* to Colistin sulphate was not high in the current study. Resistances to Colistin sulphate among street foods isolates are reported from Senegal [39] Mexico [40] and USA [26] were more or less similar to the current study result. Oxytetracycline and Tetracycline are most commonly used antibiotics in Bangladesh that might be the cause of higher resistant revealed in the present study and the results agreed with the earlier researchers of Bangladesh and India [28,41]. *Salmonella* was resistance to Azithromycin in the present study, similar result was found in several reports of Bangladesh. It could be due to frequent use of Azithromycin against different infectious diseases including Salmonellosis. High resistant to Enrofloxacin by *Salmonella* isolates was observed in this study and this result is consistent with many other previous studies in street foods in developing countries including Bangladesh. In the present study highest sensitive drugs against *Salmonella* isolates were found in Ceftriaxone similar result was found recently in Bangladesh [42,43]. It may be due to less exposure of this drug to the community.

All the isolates were resistant to Ampicillin, Amoxicillin, Oxytetracycline, Tetracycline, Erythromycin, and Azithromycin. This study has also confirmed the prevalence of varying drug resistance pattern among the *Salmonella* isolates. This may be due to the presence of more than one serovar of *Salmonella* in the various food items. A higher proportion of antibiotic resistance in *Salmonella enteritidis* has been reported from southern Brazil [44]. Increasing antibiotic resistance can limit the therapeutic options available to physicians for clinical cases that require antibiotic treatment. There is a need to find strategies to minimize the risk of spreading antimicrobial resistance among animal and human populations.

4. CONCLUSION

Salmonella is a well-known food borne threat in a tropical country like Bangladesh. The current study revealed a relatively greater prevalence of *Salmonella* among the street foods. Moreover, the *Salmonella* isolates from most of the food samples were multidrug resistant. The findings of the current study suggest that food born drug-resistant *Salmonella* is one of the major concerning issues in Bangladesh. The poor sanitation and handling of sewage could be a source of contamination. The excess utilization of antibiotics in the veterinary, human and fish practice might be the cause of increased resistance to different antibiotics. The valuable information of these research findings might be useful for awareness buildup among the common people, consumers and street food trader. Strict hygienic measures like- efficient hand cleaning,

cleaning of food contact surfaces and utensils might reduce *Salmonella* contamination to those street foods. In the view of drug-resistant *Salmonella,* obviously, it is not possible to stop the use of antibiotics, but a rational use may minimize the risk.

CONSENT

Written consent from the salesmen of mentioned products were taken before sample collection. No animal or human experiments were involved here.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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