Antimicrobial Resistance Profile of *Salmonella* spp. Isolated from Raw Beef Meat Samples Collected from Karachi, Pakistan

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ABSTRACT

In order to isolate and characterize the antimicrobial profile of *Salmonella* spp. beef meat samples were collected from the local butcher shops (n=75) of Karachi, Pakistan. Three types of meat samples were collected viz., muscles, lymph nodes and minced meat (n=25 each). The samples were packaged aseptically and transported to laboratory within 4-6 hs of collection in cooling conditions. The samples were examined for *Salmonella* contamination; isolation and identification of *Salmonella* serovars was done using the standard procedure, while antimicrobial profile was illuminated using the disk diffusion method. The results indicated that 21.34% beef samples exhibited the *Salmonella* contamination. The statistical analysis exhibited a significantly higher ($p< 0.05$) prevalence rate in minced beef (28%) and lymph nodes (24%) as compared to the muscles (12%). The highest antimicrobial resistance was recorded against the antibiotics of penicillin group (88.5 to 93.7%), followed by the aminoglycosides (68.4 to 85.5%), tetracycline (73.9 to 78.6%), and quinolones (59.6 to 63.1%). While lowest resistance level was observed against the antibiotics of cephalosporin group (32.1 to 52.6%). Among 13 isolates of *S. Pullorum*, a highest number (10 isolates) showed resistance against neomycin, kanamycin and tetracycline. A total of 76.9% (10/13) isolates were recorded as multidrug resistant (MDR) resistant. Maximum number of *S.* Gallinarum (9/10), *S.* Typhi (12/14), *S.* Cholerasuis (8/10) and *S.* Enteritidis (13/15) isolates were exhibited resistance against ampicillin antibiotic. A 78.6% isolates of *S.* Typhi were recognized as MDR isolates. While, 80% isolates of *S.* Gallinarum, *S.* Cholerasuis and *S.* Enteritidis were observed as MDR isolates. From these results it could be concluded that meat sold at butcher shops of Karachi have significant level of *Salmonella* contamination. The *Salmonella* isolates exhibited highest antimicrobial resistance against the antibiotics of penicillin group, and have lowest resistance against cephalosporins. Among all isolates, 76 to 80% were exhibited as MDR organisms.

INTRODUCTION

Beef in minced and pulverized form is a foremost route of transmission of foodborne pathogens in humans that propagates salmonellosis as well. In 2014-15, three large outbreaks of salmonellosis has been reported on the globe (CDC, 2018). The study has suggested the strong role of cattle in *Salmonella* spread via ground beef products (Bosilevac *et al*., 2009).

Salmonella infection has been related with most of food items particularly the consumption of beef and beef products that have linked with many severe outbreaks (Abebe *et al*., 2014). There are above 2000 various *Salmonella* serotypes, and all are recognized as pathogenic to humans (D'Aoust, 1997). Nevertheless, comparatively a few serotypes are related with bovines, including *Salmonella* enteric sub spp. Enterica serotype Dublin (*S.* Dublin) and *S. enterica* sub spp. Enteric serotype. *Salmonella* species are the important causes of acute gastroenteritis in several countries and salmonellosis remains a vital public health issue throughout the globe, mainly in the emerging parts of the world (Rotimi *et al*., 2008). In growing countries, there are more than a billion cases of gastroenteritis and up to 5 million deaths yearly (Gould and Russell, 2003).

For treating salmonellosis the choice of the drug

ought to be based on sensitivity testing of the causal strain every time (Ansari *et al*., 2014). The non-judicial use of antibiotics in farm animals were recognized as a main cause of development of antibiotic-resistance organisms that lead to a major public health concerns. The antibiotic residues could be passed along the food chain which ultimately makes the microorganism in the food chain resistant to them (Ariffin *et al*., 2019; Manoj *et al*., 2017). Food-producing animals are the chief reservoir for zoonotic organisms and the widespread use of antimicrobials as growth promoters and/or healing agents in animals cause the progression of antibiotic-resistant strains (Thung *et al*., 2016).

Salmonella isolates of animal-food have been recognized as most resistant organisms to numerous antibiotics, predominantly resistant to third-generation cephalosporins and fluoroquinolones, and were considered as an emerging problem worldwide (WHO, 2014). The root causes and spread routes of *Salmonella* in underdeveloped countries are not well elucidated due to the lack of coordinated research and epidemiological studies (Käferstein, 2003). Therefore, current study was envisioned to investigate the occurrence and antibiogram of *Salmonella* spp. in raw beef meat samples collected from butcher shops and supermarkets of Karachi, Pakistan. Antimicrobial susceptibility testing was done on the bacterial isolates to know the effective antimicrobials and the frequency of multidrug resistant (MDR) organisms in our food supply.

MATERIALS AND METHODS

Sample collection

A total of 75 raw beef meat samples were collected hygienically from butcher shops situated in clean and covered markets of Karachi city. Three types of meat samples were collected *viz*., muscles, lymph nodes and minced meat (n=25 each). The samples were collected randomly from various carcass sites, packed aseptically into polythene bags and transported within 4-6 h under refrigeration (4°C) to the laboratory for further processing. For isolation, confirmation, and serotyping of *Salmonella* standard methods were adopted (Popoff *et al*., 2001).

Isolation and identification of Salmonella

Raw beef meat samples (25 g) were minced into fine pieces using sterile scissors. Then samples were pre-enriched into sterile 0.1% peptone water (1/9, w/v) (Oxoid Ltd., UK) for 6 h at 37°C. After this, 0.1ml of incubated peptone water was inoculated on selective agar *i.e., Salmonella*-Shigella agar (SS agar) using streak plate method for isolation of *Salmonella* spp. (Yousafzai *et al*., 2019). Black centered colonies were selected and picked using sterilized wire loop and sub cultured on brilliant green agar (BGA) as well as *Salmonella*-shigella agar in order to purify the organism (Cowman, 1974). The presumptive identification of isolated bacteria was done by various tests including colonial morphology, Grams staining, Motility, Oxidase, Catalase, Indoxyl Acetate Hydrolysis, Triple Sugar Iron, Urease, Simmon's Citrate, Coagulase, Methyl Red and Voges Proskauer tests according to Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1994) and others (Cowman, 1974). The media were prepared according to manufacturers' instruction (Oxoid, Ltd. UK).

Antibiotic susceptibility profiling

Antibiotics (Oxoid Ltd., UK) belongs to various antimicrobial groups were used to test susceptibility of the *Salmonella* species through disc diffusion assay following the Clinical Laboratory Standard Institute (CLSI, 2014) protocol. Cut-off point guidelines from the CLSI M100 chart 2A was used to categorize strains into "resistant", "intermediate" or "susceptible" (CLSI, 2014). Several colonies of the test bacteria with the same characteristics were hand-picked up with the help of a wire-loop and mixed with nutrient broth. After overnight culture, the turbidity of the inoculum suspension was compared with 0.5 McFarland standard. A drop of the suspension was placed on the sensitivity testing plate. The bacterial material (drop) was spread throughout the surface plate with the help of dry cotton swab. The sensitivity plates were allowed to dry for few minutes. Then the different antibiotic discs were placed and slightly pressed on the sensitivity plate with a uniform distance. The plates were allowed to dry for 30 min and then placed at incubator for 24 h at 37°C. After 24 h incubation, the inhibition zones were observed and measured from the disc center to the degree of the zone (Ariffin *et al*., 2019). Isolates exhibiting resistance against three or more different classes of antibiotics were regarded as multidrug resistant (MDR) strains (Kamboh *et al*., 2018). All tests were run in triplicates.

Statistical analysis

All the data was entered into a computer database using the Microsoft Excel (Microsoft Inc., USA) spread sheets. Difference between the incidences of *Salmonella* in various samples were compared by Fisher's exact test at $p < 0.05$ via JMP statistical package software (version 5.0.1.a, SAS Institute Inc., Cary, NC).

RESULTS

Number and percentage incidence of Salmonella *in raw beef meat samples*

As shown in Table I, the incidence of *Salmonella* spp. in muscle, minced beef and lymph node samples of raw beef meat taken from butcher shops was recorded as 21.34%. Out of 75 samples, sixteen (16) were found positive. The statistical analysis exhibited a significantly higher ($p < 0.05$) prevalence rate in minced beef (28%) and lymph nodes (24%) as compared to the muscles (12%).

Antimicrobial resistance percentage of bacterial isolates of Salmonella

The results regarding antimicrobial resistance in bacterial isolates of *Salmonella* isolated from raw meat have been presented in Table II. The highest antimicrobial resistance was recorded against the antibiotics of penicillin group (88.5 to 93.7%), followed by the aminoglycosides (68.4 to 85.5%), tetracycline (73.9 to 78.6%), and quinolones (59.6 to 63.1%). While lowest resistance level was observed against the antibiotics of cephalosporin group (32.1 to 52.6%).

Table I.- Incidence of *Salmonella* spp. in raw beef meat samples sold at butcher shops of Karachi.

Sample type	Positive samples (n)	Total samples (n)	Percentage (%)
Muscles	3	25	12
Minced beef	7	25	28*
Lymph nodes	6	25	24*
Total	16	75	21.34

*Statistically higher as compared to muscle at P < 0.05.

Table II.- Percentage of *Salmonella* isolated from raw meat susceptible, intermediate and resistant to various antibiotics.

Class / group of antibiotics	Antibiotics	Disc potency (µg)	Susceptible (%)	Intermediate (%)	Resistant (%)
Aminoglycosides	Gentamycin	10	21.00	10.58	68.42
	Neomycin	30	09.21	06.58	84.21
	Kanamycin	30	08.33	06.17	85.50
Cephalosporins	Cefixime	05	31.68	15.69	52.63
	Cefoxitin	30	38.79	13.84	47.37
	Cefepime	30	44.05	23.84	32.11
Quinolones	Ciprofloxacin	05	24.26	12.58	63.16
	Enrofloxacin	05	27.33	13.04	59.63
Tetracycline	Tetracycline	30	17.42	03.90	78.68
	Oxytetracycline	30	14.37	11.68	73.95
Penicillin	Ampicillin	10	06.25	00.00	93.75
	Amoxicillin	30	09.15	02.27	88.58

Table III.- Antimicrobial resistance profile of *Salmonella* spp. isolated from raw meat.

Serovars	No. of isolates	No. of isolates resistant to each individual antibiotic												No. of isolates resistant to multiple antibiotic				Total No. (%) of MDR isolates
		Gen	Neo	Kan	Cfm	Cfn	Cfp	Cip	Enr	Tet	Oxy	Amp	Amo	0-1	2-3	4-5	>5	
S. Pullorum	13	9	10	10	7	6	4	8	7	10	9	12	9	3	4	3	3	10 (76.9)
S. Gallinarum	10	7	6	5	5	4	3	6	6	7	7	9	7	2	2	2	4	8 (80.0)
S. Typhi	14	10	11	11	7	6	5	9	8	10	10	12	11	3	3	3	5	11 (78.6)
S. Choleraesuis	10	7	6	5	4	3	3	6	6	7	7	8	7	2	3	2	3	8 (80.0)
S. Enteritidis	15	10	11	12	8	7	5	9	8	11	10	13	12	3	4	4	4	12 (80.0)
Total	62	43	44	43	31	26	20	38	35	45	43	54	46	13	16	14	19	47 (79.1)

Gen, Gentamycin; Neo, Neomycin; Kan, Kanamycin; Cfm, Cefixime; Cfn, Cefoxitin; Cfp, Cefepime; Cip, Ciprofloxacin; Enr, Enrofloxacin; Tet, Tetracycline; Oxy, Oxytetracycline; Amp, Ampicillin; Amo, Amoxicillin.

Antimicrobial resistance profile of Salmonella

Antimicrobial resistance profile of *Salmonella* isolates recovered from raw beef meat samples have been presented in Table III. Among 13 isolates of *S.* Pullorum, a highest number (10 isolates) showed resistance against neomycin, kanamycin and tetracycline. A total of 76.9% (10/13) isolates were recorded as MDR resistant. Maximum number of *S.* Gallinarum (9/10), *S.* Typhi (12/14), *S.* Cholerasuis (8/10) and *S.* Enteritidis (13/15) isolates were exhibited resistance against ampicillin antibiotic. A 78.6% isolates of *S.* Typhi were recognized as MDR isolates. While, 80% isolates of *S.* Gallinarum, *S.* Cholerasuis and *S.* Enteritidis were observed as MDR isolates.

DISCUSSION

Salmonella infection has been recognized as a major cause of food poisoning on the globe (Thanki *et al.*, 2019). Meat and meat products including beef meat is a principal source of *Salmonella* infection (Abebe *et al.*, 2014). In current study, 21.34% samples collected from butcher shops exhibited the *Salmonella* contamination that indicated that these shops do not operating in a clean and safe environment. It was also observed that meat shops were using same meat cutting equipment for all muscle and non-muscle carcass parts (legs, head *etc.*). Poor slaughtering and processing procedures at the abattoirs could also be the other hidden cause of this contamination. In a previous study, 13.5% buffalo meat samples collected from Kathmandu, Nepal exhibited the *Salmonella* contamination (Maharjan *et al.*, 2006) and reported the occurrence of *S.* Pullorum, *S.* Gallinarum, *S.* Typhi and *S.* Choleraesuis serovars in all meat samples. The prevalence reported in aforementioned study is quite little as compared to our study. Nevertheless, regional differences in food safety surveys are always being observed and have been linked to sanitation conditions (Qu *et al.*, 2017). While, in agreement to our study, the Mikanatha *et al.* (2010) reported a 22.2% prevalence of *Salmonella* spp. in chicken meat sold at retail outlets of central Pennsylvania.

Cefixime is known as drug of choice for human cases of salmonellosis (Shrestha *et al.*, 2016). In our study, unfortunately, more than 50% *Salmonella* isolates were found resistant against the cefixime. It remains highly debated whether or not these resistant strains of poultry may translate into increasing resistance of *Salmonella* causing human infections. However, it is widely established in previous literature that development of antimicrobial resistance of microbes of animal/food origin is directly proportional with the resistance of human pathogens (Kamboh *et al.*, 2018). According to CLSI (2014) recommendation, resistance level observed

for cephalosporin should be considered collectively for its' antimicrobial class. Hence, all antimicrobials of quinolones class would considered more than 63% resistant (as observed in our study) against *Salmonella* isolates of beef origin. This situation is of great concern as quinolones are commonly being used to treat *Salmonella* infections of human origin (Pokharel *et al.*, 2006).

Other antimicrobial classes screened in our study also exhibited a relatively higher level of resistance against *Salmonella* isolates (aminoglycosides 68.4 to 85.5%, tetracycline 73.9 to 78.6% and penicillin 88.5 to 93.7%). In our previous study, 96.3% *Salmonella* isolates of broiler chickens were recognized resistant against oxytetracycline, while 92.6% exhibited resistance against the penicillin group (Kamboh *et al.*, 2018). It is probably due to the frequent use of these antimicrobials in veterinary practice. Universal incidence of antimicrobial resistance in *Salmonella* spp. recovered from animal food is variable with percentages ranging from 30.7% to 100% (Ansari *et al.*, 2014; Kamboh *et al.*, 2018; Sharma *et al.*, 2019).

Among *Salmonella* isolates of beef meat recovered in this study 79.1% were declared as MDR. Among various serovars, 80% isolates of *S.* Gallinarum, *S.* Choleraesuis and *S.* Enteritidis were resistant to two or more antimicrobials; while 76.9% isolates of *S.* pullorum and 78.6% isolates of *S.* Typhi showed resistance to ≥2 antibiotics. This is a concern since the previous studies have reported the prevalence of multi-resistant *Salmonella* in animal food between 54.8% to 69.3% (Kamboh *et al.*, 2018; Mikanatha *et al.*, 2010). The high prevalence rates of MDR organisms in animal-origin food have been linked with antimicrobial agents such as tetracycline, penicillin and aminoglycoside which are been used frequently in animal husbandry (Ariffin *et al.*, 2019; Rehman *et al.*, 2019) and is therefore recognized as giant challenge in the veterinary and medical sciences to treat *Salmonella* infections (Thai *et al.*, 2012).

CONCLUSIONS

It was concluded that meat sold at butcher shops have significant level of *Salmonella* contamination. Minced meat and lymph nodes have more contamination as compared to the muscles. The *Salmonella* isolates exhibited highest antimicrobial resistance against the antibiotics of penicillin group, followed by the aminoglycosides, tetracycline, quinolones and cephalosporins. Among all isolates, 76 to 80% were exhibited as multidrug resistant organisms.

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Statement of conflict of interest

The authors declared no conflict of interest.

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