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Prevalence of multi drug resistant *salmonella sp* from meat samples of Hyderabad city, India

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Abstract

Food is important need of survival for humankind. Food infections and etiological agents associated food is a serious threat. In the current study involved with Salmonella sp isolation and screening from retail meat shops and evaluation of antibiotic susceptibility. A total of 287 samples meat (goat meat-147 and chicken meat-140) were collected from retail meat shops of Hyderabad city, India. Samples were processed as per the ISO 6579:2002 and Salmonella isolates were identified based on the standard biochemical and cultural characterization. The serotyping was carried out by Kauffmann-White scheme for species level identification. Evaluation of antibiotic susceptibility test to various antibiotics as per CLSI guidelines and MIC of Ceftazidime and Ciprofloxacin was determined. Out of 287, 56 samples were positive for Salmonella with an incidence rate of 19.57%. The highest incidence of 22% was recorded in chicken meat samples and the goat meat samples had incidence rate of 16.32% (n=24). Based on serotyping, 87.5% (n=49), and 12.5% (n=7) isolates were identified as S. typhimurium and S. enteridis. A total of 85.7 % (n=48) of the 56 Salmonella isolates are multidrug resistant (MDR) and subsequently, ESβL (extended spectrum β-lactamase) producing Salmonella strains were 7.1% (n=04). Out of 48 Ciprofloxacin Resistant Salmonella sp (CRSs), 06 isolates have shown maximum MIC range of 256 µg – 512µg/ml. Further, 12 out of 20 Ceftazidime Resistant Salmonella sp (CzRS) isolates assessed for MIC to Ceftazidime showed substantially high MIC values of 64-128µg/ml. In the current study Salmonella sp from poultry and animal meat samples were isolated and described characteristic studies. The results emphasize the issue of food safety and highlights violation of food safety regulations. The emergence of MDR among Salmonella sp is an alarming crisis could lead to treatment failure.

Keywords: *Salmonella, MDR, ESBL, CRSs,* CzRS, MIC.

Introduction

Salmonella is recognized as a major cause of food related illnesses among human beings and other warm blooded animals. Annually worldwide reported cases of typhoid fever are more than 16 million and cases of gastroenteritis are about 1.3 billion and 3 million deaths[1]. The general symptoms of salmonellosis are fever, diarrhea, abdominal cramps, nausea, vomiting, chills, and prostration. Sometimes, fluid and electrolytes loss may causes serious to treat the infection and can be fatal, especially to the sick, infants, and the elderly[3,4]. Non-typhoid *Salmonella* infections are primarily reported from a variety of foods most commonly consumed[5] such as meat, chicken, egg, vegetables (either raw or cooked foods) or consumption of contaminated foods or water[6,7]. A distinctive feature of *Salmonella* is its wide host range and predominantly inhabiting the gastrointestinal tracts of animals. Consumption of contaminated cooked or raw foods and drinks via oral route and secondarily, contact from carriers such as maids, domestic or wild animals and food handlers are the major modes of infection occurs[8].

In India, salmonellosis (Enterocolitis) is endemic in many parts and is causing substantial economic loss every year, even then its significance as possible zoonosis is less emphasized[2].

Since the last two decades, major threat in public health and therapeutic crisis across the world is emergence of antibiotic-resistant *Salmonella*[9]. Presently, numerous therapeutic failures have been reported with increasing incidences of antimicrobial resistance among *Salmonella* species[10]. Over usage of antimicrobial drugs in order to preventing and treating diseases in animal food agriculture, poultry production could lead to emergence of multi drug resistance in *salmonella* strains[11,12]. Currently, *β*-lactam group of antibiotics are majorly prescribed to treat the bacterial infections, specifically to treat infections caused by Enterobacteriaceae. The production of *β*-lactamases contributes to drug resistance to *β*-lactam antibiotics such as the cephalosporins, monobactams, penicillins, and carbapenems. Several gram-negative bacteria possess naturally occurring, chromosomal mediated *β*–lactamases but *β*-lactamase encoded by plasmid borne genes confers the resistance to penicillins[13,14].

In the current study, we evaluate the prevalence of *Salmonella* in goat and chicken meat samples from Hyderabad City, India and their antibiotic resistance patterns.

Materials and methods

Collection of samples: 287 meat samples from retail shops of goat meat (n=147) and chicken (n=140) were collected

aseptically in sterile polythene bags from retail meat shops of Hyderabad city, India during October 2015 to May 2016. All Samples were transported and processed as per standard bacterial processing techniques.

Screening of *Salmonella Sp.* Isolates: The collected meat samples were further processed as per the ISO 6579:2002 protocol with slight modifications. Briefly, meat sample was homogenized with 225 ml of sterilized buffered peptone water (BPW) (Himedia, India) and kept for per-enrichment of bacteria. To increase the count of *salmonella,* the culture inoculums was transferred from BPW into tetrathionate broth (TT) (Himedia, India). Consequently, the bacterial culture was processed onto the selective media, Xylose lysine Deoxycholate (XLD) Agar and bismuth sulfite agar (BSA) (Himedia, India). For all biochemical and cultural techniques bacteria samples incubated at 37°C for 24h[15] (Figure-1, Figure-2).

Biochemical tests: *Salmonella* isolates were further subjected to IMViC, triple sugar iron (TSI), urease and other biochemical tests, as per protocols recommended for *Salmonella* screening[16] (Figure-3).

Serotyping: The serotyping was carried out according to the Kauffmann-White scheme by slide agglutination (Bio-Rad Laboratories, inc). Subsequently, somatic antigen was identified and the phase-1 flagellar antigen, if the second antigen was negative, expression of the second flagellar phase was detected by the phase inversion method[17].

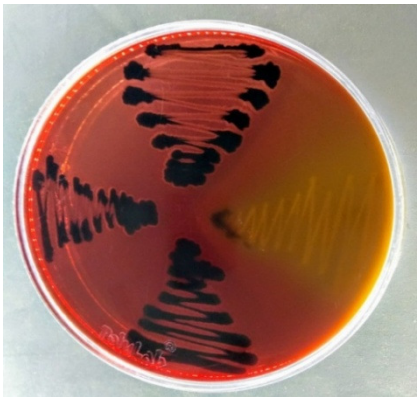

Figure-1: Growth on XLD agar.

Figure-2: Growth on BSA agar.

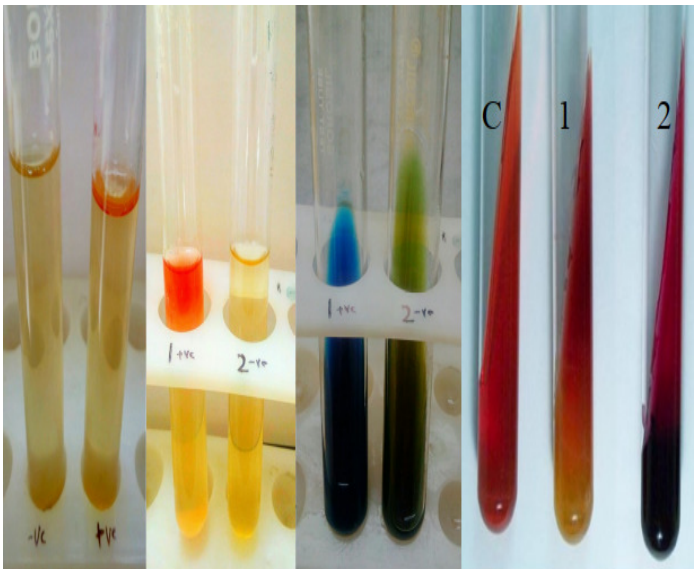

Figure-3: Biochemical tests, C= Control; 1= *S.typhi*; 2=*S.typhimurium.*

Antibiotic resistance studies: All the *Salmonella* isolates were tested for antibiotic susceptibility for various antibiotics by disk diffusion method as per the guidelines of Clinical Laboratory Standard Institute[18] (CLSI). A total of 17 antibiotics (Himedia Pvt.ltd, Mumbai, India) were selected for this study (Table-1). The results are interpreted based on the zone of inhibition by using standard Antibiogram scale.

Minimal Inhibitory Concentration (MIC): Further evaluation of MICs to Ceftazidime and Ciprofloxacin was carried out as per CLSI standards, 2012 by Agar Dilution and Broth Dilution Method. The Standard antibiotic solutions were prepared as per CLSI (CLSI document M07-A10 "Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically") with two-fold dilutions in the range of 2 µg/ml to 1mg/ml[19].

Results and discussion

Screening of *Salmonella sp:* *Salmonella* sp from raw food samples were confirmed (Table-2). Fifty six *Salmonella* were isolated, with an incidence rate of 19.57%. Among the chosen samples, highest incidence rate of 22% (n=32) was observed in chicken meat samples, comparatively it was less in goat meat samples with an incidence rate of 16.32% (n=24).

The serotyping confirmed that, 87.5% (n=49) of the isolates are *S. typhimurium* and 12.5% (n=7) strains were identified as a *S. enteridis.* Among the 32 *Salmonella* isolates from chicken meat, 90.6% (n=29) isolates are serotyped as *S. typhimurium* and only 9.4% (n=3) isolates are belonged *S. enteridis.*

Furthermore, amongst the 24 *Salmonella* isolates from goat meat, 83.3% (n=20) isolates belonged *S. typhimurium* and 16.66% (n=4) isolates to serotype *S. enteridis* (Table-3).

Table-1: antibiotics used for the evaluation of drug resistance in *Salmonella* isolates.

Group	Antibiotic	Concentration (mcg)
ß-Lactams	Ampicillin	10
	Azeotrenem	30
Cephalosporins	Cefexime	5
	Cefexime	30
	Ceftriaxone	30
	Ceftazidime	30
	Cefetoxime	30
Quinolones	Nalidixic acid	30
	Ciprofloxacin	5
	Levofloxacin	5
	Gatifloxacin	5
Cotrimoxizol	Cotrimoxizole	25
Aminoglycosides	Streptomycin	10
	Amikacin	30
Macroloides	Azithromycin	15
Tetracyclines	Tetracycline	30
Phenicols	Chloramphenicol	30

Table-2: Biochemical characteristics *Salmonella sp*

Biochemical Test	Reaction
Catalase	+
Oxidase	-
H_2S production (TSI)	+ (Red- Slant, Black-Butt positive for gas formation)
I	-
MR	+
Vi	+
C	+
Urease test	-

TSI=Triple sugar iron, MR=methyl red, VP=voges proskauer.

Table-3: Incidence of *Salmonella typhimurium* and *Salmonella enteridis* isolates from collected chicken and goat meat samples.

Samples	Percentage of *Salmonella* positive isolates	Serotyping	
Chicken Meat	22% (n=32)	*Salmonella typhimurium*	90.6% (n=29)
		Salmonella Enteridis	9.4% (n=3)
Goat Meat	16.32% (n=24)	*Salmonella typhimurium*	83.3% (n=20)
		Salmonella Enteridis	16.66% (n=4)

Antibiotic susceptibility: 17 antibiotics belonging to 8 groups were used for antibiotic susceptibility test to all the 56 Salmonella isolates. Among them, 06.06% (n=04) isolates were ampicillin resistant, but maximum resistance of 85.72 % (n=48) was observed against Aztreonem, 24.25% (n=12) isolates were Cefexime resistant, but in contrast 13.63% (n=4) resistance was observed to Ceftriaxone, additionally increased resistance of 35.71% (n=20) and 32.14% (n=18) to Ceftazidime and Cefetoxime respectively. Moreover, the antibiotic susceptibility profiling of *Salmonella* isolates to Quinolones and fluoroquinolones group of antibiotics significantly resulted in 28.57% (n=16) resistance to Nalidixic acid, and 85.72% (n=48) to Ciprofloxacin. In contrast all isolates (n=56) were sensitive to Levofloxacin, Gatifloxacin. Additionally and Cotrimoxizole. The maximum no of isolates were Aminoglycoside sensitive, only 7.15% (n=4) and 3.58% (n=2) showed resistance to Streptomycin and Amikacin respectively. Among the 56 *Salmonella* isolates 67.86% (n=38) have shown resistance to Azithromycin. Most of the isolates were sensitive to Tetracycline and Chloramphenicol, except few isolates, 14.29 and 7.15% respectively, showed resistance (Table-4).

Minimum Inhibitory Concentration (MIC): The MDR *Salmonella sp* have shown remarkable increase in the MICs of both Ceftazidime and Ciprofloxacin. Among the 48 Ciprofloxacin Resistant *Salmonella sp* (CRSs), 06 isolates have shown an MIC range of 256µg–512µg/ml, 18 of the isolates have shown MIC of 128-256µg/ml and further 24 isolates have shown the moderate MIC valves of 32-64µg/ml. Further, out of the 20 Ceftazidime resistant *Salmonella sp* (CzRS) isolates, 12 isolates have shown substantial increase in MIC values of 64-128µg/ml and further 08 isolates showed MIC of 16-32 µg/ml. The results were evaluated based on the observation of both agar dilution and broth dilution methods.

Discussion: The Foods derived from animal origin are causing majority of *Salmonella* infections in humans, and *Salmonella*e found in animals are often isolated from humans. Among the six subspecies of *S. enterica*, subsp. *diarizonae*, which is one of the predominant subspecies in salmonellosis, can be associated with food borne salmonellosis. However, among the 2,000 serotypes, serotype Typhimurium, is the cause of salmonellosis in higher

frequencies than other serotypes across the globe. Several animals, harbor serotype Typhimurium in a carrier state[20,21]. Non-typhoid *Salmonella* infection are due to consumption of contaminated food (poultry, meat, beaf other animal originated raw or unprocessed food or spoiled food contain salmonella), water, and carriers containing *salmonella*e[22].

In the current study, overall 19.57% of *salmonella sp* present in chicken and goat meat collected from retail outlets and sample wise, the incidence as follows chicken 22% whereas Goat meat 16.32% (n=24). It indicates the widespread occurrences of these pathogens meat and poultry products. Similar kind of studies by Van *et al*.[23] and Phan *et al*.[24] conducted in Vietnam, China, and Korea, stats that *Salmonella* incidence is higher in retail chicken and beef samples than the incidence in the current study. In a quality testing analysis in Bangkok indicated the presence of *Salmonella* sp is 28% in retail chicken meat samples[25]. *Salmonella* contamination is more in retail chicken and meats among the Asian regional countries. Contamination rates varies in different regions of European countries[26], like Ireland poultry samples (2.8 to 26.4%), 13.2% in Netherlands, meat samples from United kingdom (23 to 29%), animal samples from Spain (35.8%), 36.5% in Belgium with *Salmonella*, and other countries infection rates are as follows 43.3% in Australia, 20% in Argentina, 42% in Brazil[23,26]. Portugal with 60% having maximum contamination rates of *salmonella*. In general, climate and storage temperature influence the infection occurrence and spread[23]. Nevertheless, sampling processing and identification approaches could mainly affect the incidence of *Salmonella s*p. among tropical countries[27].

The most prevalent serotype identified was *Salmonella* serovar *Typhimurium*, and *Salmonella* serovar *enteridis*. The outcome of our research also supports the fact that serovar *Typhimurium* was the most frequently found in the raw meat samples types such as bovine, porcine, and poultry meats[28,8].

The present study, the incidence of *Salmonella* was more, 22% (n=32) in chicken meat, than goat meat samples with incidence rate of 16.32% (n=24) which is comparatively higher than the previously reported of 5.0% prevalence[29]. *S. Typhimurium* (n=49), and *Salmonella enteritidis* (n=07) isolates were detected in the present study (Table-3). Studies from Selvaraj *et al*.[30] also strengthen the present findings that, *S. Typhimurium* and *S. Enteritidis* are the prevalent serovars types in India from various meat and poultry food.

About 86% of the present *Salmonella* isolates were multidrug resistant (MDR) to more than three antibiotic; 60% (n=34) were MDR to four or more antimicrobial agents. only 7.1 % (n=04) were extended spectrum β-lactamase (ESBL) producers out of the 56 *Salmonella* strains, even though this enzyme is clinically significant and could be transferred among various species of *Enterobacteriaceae*. Occurrence of drug resistant *Salmonella* strains are raising the concern of invasive salmonella infections. Particularly the occurrence of ESBL in *Salmonella sp*. is a new threat worldwide[14].

Table-4: Percentage of antibiotic resistance among *Salmonella sp* isolates.

Antibiotic		No.of Resistant Strains	Percentage of Resistance	Percentage of intermediate	Percentage of susceptibility
ß-Lactams	Ampicillin	04	7.15%	7.15%	85.70%
	Azeotrenem	48	85.72%	10.71%	3.57%
Cephalosporin's	Cefexime	12	21.43%	32.14%	46.43%
	Ceftriaxone	04	7.15 %	10.71%	82.14%
	Ceftazidime	20	35.71%	25%	39.29%
	Cefetoxime	18	32.14%	21.43%	46.43%
Quinolones	Nalidixic acid	16	28.57%	53.54%	17.86%
	Ciprofloxacin	48	85.72%	00	14.29%
	Levofloxacin	00	00	00	100%
	Gatifloxacin	00	00	00	100%
Cotrimoxizole		00	00	7.15%	92.85%
Aminoglycosides	Streptomycin	04	7.15%	3.58%	89.29%
	Amikacin	02	3.58%	21.43%	77.78%
Macroloides	Azithromycin	38	67.86%	21.43%	10.71%
Tetracycline		08	14.29%	21.43%	66.67%
Chloramphenicol		04	7.15%	7.15%	85.70%

The other findings also revealed the existence of MDR *Salmonella* strains can have a possibly negative influence on human health. Little *et al*.[28] reported that 48.1% *Salmonella* strains showed reduced susceptibility to more than four antibiotics isolated from red meats in UK. There are also further reports of individual antibiotics; the most common resistance found in *Salmonella* isolates was to ampicillin (43.4%). In contrary our data shows less resistance to (06.06%) ampicillin, but it was relatively higher (85.72%) for Aztreonem. In an investigation conducted in Austria on beef, pork, and poultry meats, a total of 57.7% of the isolates are drug resistant strains, and most of the *salmonellae* showed reduced susceptibility to more than one antibiotics[31]. Indiscriminate usage of antibiotics in food industry, as growth-promoting substances in animal feeds and as prophylactics to yield good growth and prevention from infections may lead the emergence of MDR *Salmonella* in association with food[32].

Conclusion

In the present work, we have successfully isolated, identified and characterized *Salmonella* from retail meat. The consequence of one or more antimicrobial resistance among the strains isolated, could lead to treatment failures. The emergence and development of antimicrobial resistance and also the production of ESBL in *Salmonella*, as in other bacteria, may also contribute to the major public health problems worldwide.

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