

Original Research Article

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Screening of Multidrug Resistant *Salmonella* spp from Potable Water Collected from the Slum Areas of Bhopal, M.P. India

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ABSTRACT

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Salmonella is a most significant pathogenic bacteria causing typhoid and non-typhoidal Salmonellosis. Contaminated food and water consumption is one of the vital modes of *Salmonella* transmission cause illness in both human and animals, resulting about 93.8 million causes worldwide annually. Asian and African nations are viewed as the hugest gathering of patients contaminated with waterborne microbial infection. Antibiotic resistance in drinking water sources is of genuine grave concern. The present investigation was aimed at screening of antibiotic resistant *Salmonella* spp. incident in potable water collected from the some of the slum areas of Bhopal City, M.P. India. Water consumed by the slums when test was reported with higher number of microbial load even at the as low as 10^{-7} dilution of water samples. The *Salmonellas* spp. from water was selectively isolated on bismuth sulphide agar media followed by Gram's staining, microscopy & biochemical characterization. The 10 different isolates of *Salmonellas* spp. shows a difference in their diversity based on the resistance towards the particular type of antibiotics. The *Salmonella* isolated from different sample sources showed resistance towards at least 1 antibiotic and maximum 4 antibiotics. 80% of the isolates of *Salmonella* spp. screened from potable drinking water collected from slum areas were reported to show resistance towards the important antibiotics commonly prescribed by medical practitioners is the serious issue of concern.

Introduction

Salmonella is a most significant pathogenic bacteria causing typhoid and non-typhoidal Salmonellosis. *Salmonella* species are most frequently reported cause of foodborne illness in both humans and animals, resulting about 93.8 million causes worldwide annually (Majowicz, 2010 & Gillespie, 2003) whereas in developing countries like India, foodborne

illness are mostly under reported; however in the past 29 years (1980-2009) 3485 persons have been affected from 37 *Salmonella* related outbreaks (Sudershan, 2012).

Salmonellae are gram negative, non-lactose fermenting and non-sporing bacteria. With exception of *Salmonella pullorum* and *Salmonella gallinarum*, all *Salmonellae* are actively motile (Cheesbrough, 2000).

Contaminated food and water consumption is one of the vital modes of *Salmonella* transmission (D'Aoust, 1989; Quinn et al., 2002; Brooks et al., 2015).

As indicated by CDC, 1999 hosts for non-typhoidal salmonellosis including dairy cattle, swine, poultry, wild winged animals, and pets discharge this life form in dung, which can defile water, different nourishment sources and condition. *Salmonella* contaminations are grouped into clinical classifications are as following, Salmonellosis, gastroenteritis, bacteremia, osteomyelitis, reactive arthritis, enteric fever and food poisoning (Owens, 2009 & Klotchko, 2009 and Johnson, 2012).

The significant hotspots for transmitting *Salmonella* species to human with 40 % of the clinical cases ascribed to the utilization of egg and poultry items (Chashni, 2009 & Ruban, 2010). *Salmonella* disease in people is chiefly brought about by drinking water tainted with emissions and defecation of infected faunae (Zahraei, 2000; Quinn et al., 2002; Winn et al., 2006; Motlagh et al., 2013).

As indicated by Seas et al., (2000) kids under five years-age, primarily in Asian and African nations are viewed as the hugest gathering of patients contaminated with waterborne microbial infection.

As per writing, the setting of antibiotic resistance in drinking water sources is of genuine grave concern (Jiang et al., 2013; Flores et al., 2014; Guo et al., 2014; Machado and Bordalo, 2014; Mohanta and Goel, 2014).

The antibacterial estimation of medications is compromised intensely by the expanded pervasiveness of resistant microorganisms (Grabow et al., 1974 & Hinshaw et al., 1969). Considering the source water is legitimately related with anthropogenic activity and wellbeing, understanding the predominance of

antibiotic resistance in human drinking water sources is vital (Chen et al., 2017).

In view of the facts mentioned the present investigation was aimed at screening of antibiotic resistant *Salmonella* spp. incident in potable water collected from the some of the slum areas of Bhopal City, M.P. India.

Materials and Methods

Sample Collection Total 10 potable water samples were collected from different area of Bhopal. 50ml of each sample were collected from the sites in a clean, dry and sterile containers using aseptic condition. Details of the collected sample are given table 1.

CUF (Colony Forming Unit) Count

The collected water samples were subjected to total bacterial count by inoculating 0.1 ml of water from collected samples on nutrient agar media plates followed by incubation at 37°C for 24 hours. To have the idea of contamination on quantitative basis following formulae was used;

$$CFU \text{ per ml} = \text{No. of CFU} / \text{Total Vol. Plated} \times \text{Dilution factor}$$

Screening and isolation of *Salmonella* species

Referring to the aim of study *Salmonella* spp. were used to selectively isolate on Bismuth Sulphite Agar Media. 0.1 ml of water from each collected samples were aseptically transferred on to the separated media plates and spreaded with the help of plastic spreader thereafter incubated at 37°C. After 24 hours of incubation jet black, surrounded by metallic sheen, convex type colonies were picked for pure culture preparation by transferring them on to the fresh media.

Table.1 Details of collected sample from different regions

S.N.	Sample	Sample Code	Regions
1	Portable water	S-1	Anna Nagar Bhopal
2	Portable water	S-2	BarkherhaPathani Bhopal
3	Portable water	S-3	Habibjang Slum Area Bhopal
4	Portable water	S-4	Narela Bhopal
5	Portable water	S-5	Bhim Nagar Bhopal
6	Portable water	S-6	NayaBasera Bhopal
7	Portable water	S-7	Kalpana Nagar Indrapuri Bhopal
8	Portable water	S-8	Neelbad Bhopal
9	Portable water	S-9	Meera Nagar Bhopal
10	Portable water	S-10	Jhatkhedi Bhopal

Characterization of isolates

The pure cultures of selectively isolated *Salmonella* spp. were subjected of morphological and microscopic characterization. Also, the biochemical tests like gram reaction, catalase test, gelatin hydrolysis, starch hydrolysis, IMViC test and TSI test were conducted for characterization (Cappuccino and Sherman, 1992).

Screening & Conformation Antibiotic Resistanc

The pure isolates of *Salmonella* spp. collected from different places were subjected to antimicrobial susceptibility assay using octadisc set of antibiotics (HiMedia, India Pvt Ltd). The isolates were first cultures in nutrient broth for 24 hours at 37°C followed by swabbing them on to the freshly prepared nutrient agar plates. After this, an antibiotic octadisc was carefully placed on the pre-inoculated media surface aseptically. The plates were observed for absence of inhibitory

zone around particular antibiotic discs as an indicator for drug resistance.

Results and Discussion

The presence of pathogenic microbes in water bodies is the issues of serious concern where total microbial load per ml and presence of antibiotic resistant bacteria shall be the matter of tests. The present study was intended to investigate the total microbial load in the potable water collected from the slum areas of various regions of Bhopal City.

Results of CUF Count in water samples

With reference to observed results of CFU count as depicted in table 2 of this article it is clear that the water consumed by the slums is loaded with higher number of microbial load even at the as low as 10^{-7} dilution of water samples. Sample S-6 was reported to have the lowest total CFU count at 10^{-7} dilution as 01 followed by S-5, S-8 and S-2 as 05, 06 and 08 CFU counts respectively. S-3, S-4 and S-9

were with higher CFU count as 45, 38 and 24 respectively.

Contaminated water plays an important role in diseases transmission and it performs a vital role in the public health and survival of organisms, especially in rural areas of developing countries (Makoni *et al.*, 2004; Pant, 2004; Katsi *et al.*, 2007).

People from developing countries encountered with higher risk of water-borne diseases as compared to the developed countries (Simpson *et al.*, 2002). Based on the report of World Health Organization (WHO), the mortality rate of diseases related to contaminated water is more than 5 million people per year.

Nowadays, efforts to attain higher quality of drinking water were emphasized, (WHO, 2008).

On global scale, drinking water pollution by pathogens is evaluated the most important risk for human health, and also it has been lead to numerous outbreaks of diseases and poisoning (WHO, 2008). Therefore this study

evaluates salmonella from drinking water source to determine to portability of the water.

Biochemical Characterization

The bacterial isolates of *Salmonella* spp. selectively isolated from the collected waters samples on bismuth sulphate agar media were subjected to number of biochemical tests the outcomes or observations of which are depicted in table 3.

Refereeing to the outcomes of the of biochemical tests the bacterial isolates were confirmed as *Salmonellas* spp. and becomes the matter of concerns in the sense that, the potable water used by the slums is flooded with the variety of *Salmonella* spp. and thus the population is prone to serious illness due to water born salmonellosis.

Identification of *Salmonella* spp. can be performed via both biochemical and molecular methods. In present work biochemical test parameters were considered for characterization of salmonella isolates according to Barrow, (2003).

Table.2 Total bacterial count of spread plate technique after incubation

Dilutions Used	CFU in each sample									
	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10
10^{-3}	148	78	214	311	56	39	142	68	307	181
10^{-5}	59	23	122	189	20	21	37	15	93	42
10^{-7}	11	08	45	38	05	01	13	06	24	13

Table.3 Results of biochemical test

S.N.	Tests Conducted	Samples Codes									
		S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10
1.	Gram's staining	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2.	Cell Morphology	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus
3.	Catalase Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
4.	Oxidase Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5.	Indole Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
6.	MR test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
7.	VP Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8.	Citrate Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
9.	TSI Test	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A
10.	H ₂ S Production	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
11.	Gas Production	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
12.	Sucrose Fermentation	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
13.	Fructose Fermentation	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
14.	Lactose Fermentation	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
15.	Mannitol Fermentation	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
16.	Glucose Fermentation	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Antimicrobial resistant activity

Antibiotic resistance is a global public health concern (Tule, 2017). Antibiotic-resistant bacteria as the etiology of infection have been escalating at a distressing rate (Fair, 2014). It is indicated that almost 1 crores people will die annually due to antimicrobial resistance (AMR) infections (Mckenna, 2014).

The antibiotics towards which the resistance of indigenous Salmonella isolates was investigated at particular concentration by disc diffusion technique using octadisc are depicted in table 4. The 10 different isolates of Salmonellas spp. shows a difference in

their diversity based on the resistance towards the particular type of antibiotics. It was observed that sample S-7 showed resistance towards 5 antibiotics namely Ampicillin, Cotrimoxazole, Erythromycin, and Cephalexin while S-10 also was also resistant for Ampicillin, Tetracycline Erythromycin, and Cephalexin.

Samples S-1 and S-3 were reported to show resistance towards Ampicillin, Cotrimoxazole, Ciprofloxacin as set and Ciprofloxacin, Erythromycin, Cephalexin as set. Resistance for Ampicillin and Cephalexin was observed common in S-4 and S-9 Salmonella isolates while Samples S-5 & S-8

was inhibited by all the antibiotics present on the octa disc. The antibiotic observes ineffective against most of the isolates was Ampicillin & Cephalexin followed by Ciprofloxacin, Cotrimoxazole & Erythromycin then Tetracycline.

Salmonella is hard to wipe out from its reservoir hosts, and food animals frequently serve as reservoirs of the pathogen. Non-typhoidal *Salmonella* causes the most noteworthy number of sicknesses, hospitalizations, and passings related with foodborne ailment (Scallan, et al., 2011).

The circumstance exasperated, when antibiotic-resistant clones oftentimes inferred as the etiological operators in these flare-ups leads to treatment disappointments, higher risk of bloodstream infections, and amplified rate of hospitalizations (Nair, et al., 2018).

In prior examinations Taban, et al., (2013) while working with prepared to eat serving of mixed greens vegetable examples screened out the assorted variety of antibiotic-resistant *S. enterica* using RAPD-PCR

examinations.

White, (2001) announced salmonella pollution in 20% of the meet samples they investigated where 84% isolated were resistant to at least one antibiotic, and 53% were resistant to at least three antibiotics tried.

Similarly, Kebede, et al., (2016) led a cross-sectional investigation to evaluate the degree of *Salmonella* contamination in butchered bovines and ovines at Addis Ababa abattoir where out of 280 animals they examined, 4.64% (8 bovines and 5 ovines) were sure, with most examples (12/13, 92%) including *Salmonella* Dublin; 69% of all separates were resistant to multiple antibiotics especially human medicine at high level.

In present study also, 80% of the isolates of *Salmonella* spp screened from potable drinking water collected from slum areas of Bhopal City, India were reported to show resistance towards the important antibiotics commonly prescribed by medical practitioners is the serious issue of concern.

Table.4 Set of antibiotics used on octadisc with their concentrations on disc

S.N.	Antibiotic used	Concentration on Disc
1.	Ampicillin	10 µg
2.	Gentamycin	30 µg
3.	Cephalexin	25 µg
4.	Erythromycin	05 µg
5.	Tetracycline	10 µg
6.	Chloramphenicol	15 µg
7.	Cotrimoxazole	30 µg
8.	Ciprofloxacin	30 µg

Table.5 Resistance observed by indigenous *Salmonella* isolates towards the antibiotics use on present work

S.N.	Salmonella Isolates	Resistant towards Antibiotics
1.	S-1	Ampicillin, Cotrimoxazole, Ciprofloxacin,
2.	S-2	Ciprofloxacin
3.	S-3	Ciprofloxacin, Erythromycin, Cephalexin
4.	S-4	Ampicillin, Cephalexin
5.	S-5	No Resistance
6.	S-6	Cotrimoxazole, Ciprofloxacin
7.	S-7	Ampicillin, Cotrimoxazole, Erythromycin, Cephalexin
8.	S-8	No Resistance
9.	S-9	Ampicillin, Cephalexin
10.	S-10	Ampicillin, Tetracycline Erythromycin, Cephalexin

Ampicillin, Ciprofloxacin, Erythromycin, Chloramphenicol and Cotrimoxazole are the common antibiotics used in the treatment of typhoidal and non typhoidal diseases, hence the incidence of the isolated showing resistance towards these specific antibiotics possess serious threat of contracting Salmonellosis among the population using contaminated water.

With due concern to the public health, it is advisable that water supplies in the slum areas must be maintained with hygiene and also the families must take measures to make water microbial contamination free like use of alum and chlorine tablets or at least boiling & filtration before consumption.

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