JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

MULTIPLE DRUG RESISTANT *SALMONELLA* IN FRESH TOMATOES FROM VEGETABLE MARKET OF AURANGABAD CITY, MAHARASHTRA

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ABSTRACT

Contamination of fresh produce with *Salmonella* may occur during any point from fork to table *Salmonella* species (spp.) causes an estimated infection in around 20 million people worldwide each year and is responsible for several human deaths. Salmonella spp. have been isolated from all animal foods but also found in the vegetables such as tomatoes, spinach, alfalfa, etc. Globally several outbreaks of Salmonellosis have been found to be associated with the consumption of raw tomatoes. This is because of the fact that *Salmonella* attaches to the surface of tomatoes and also present in the interior due to geotropic transmission via contaminated soil and irrigation with contaminated water. In India, where most of the population is still using vendor based system to buy the vegetables, and no hygienic methods being employed and thus possibility of Salmonellosis cannot be ruled out as evident from large chunk of the population falls sick with Salmonellosis every year. Hence the present study was initiated with a total of 50 tomato samples randomly collected from different vegetable markets and food vendors. From these,6 samples were found to contain the *Salmonella* spp. All 6 isolates were then subjected to the antibiotic susceptibility test and were noticed to be resistant against several antibiotics.

Key words: *Salmonella*, tomatoes, soil, antibiotic susceptibility, resistant.

INTRODUCTION

The demand for fresh produce is intensifying, due to their nutritional value and consumer health awareness [1]. Studies have shown that consuming more fruits and vegetables can lead to a more productive and healthier lifestyle [2]. Despite the health benefits attained from fresh produce, microbial safety of fresh produce continues to be a major challenge as these foods are consumed raw, and are known for spreading infectious foodborne diseases [3]. Fresh produce has been recognized as a common source for *Salmonella* since the bacteria has ability to attach

and internalize in produce [5]. Fresh produce can become contaminated anywhere along the farm to plate continuum [6]. According to FDA [7], contamination with pathogenic bacteria may be directly or indirectly through contact with animals or insects, soil, water, dirty equipment, and human handling. Most *Salmonella* infections are caused by poultry products; however, it is estimated that fruit and vegetables are implicated in about 50% of *Salmonella* illnesses [8]. *Salmonella* outbreaks are frequently linked with animal products; however there have been outbreaks related to fresh produce, [9]. *Salmonella* has been isolated from produce such as mangoes [1], cantaloupe [10], cucumbers [11], alfalfa sprouts, lettuce [12, 13] and tomatoes[14].

Outbreak of infection associated with raw and minimally processed fruits and vegetable have occurred with increased frequency, in recent years. The last few decades have witnessed the emergence of highly virulent and antibiotic-resistant *Salmonella*, causing greater morbidity and mortality in humans. The emergence of several *Salmonella* serotypes resistant to multiple antibiotics in food animals underscores a significant food safety hazard. The presence of human pathogenic enteric bacteria on the surface and interior of the raw produce is a significant health concern; [16] studied fruits and vegetables grown on different farms, and found that bacteria mostly Gram negative motile rods are found in the interior of the vegetables. There are various sources of contamination of fruit and vegetables with pathogenic microorganism's viz. poor quality irrigation water, bio manure, wash water; improper handling by workers and contact with contaminated soil and sewage etc.[17]. Microorganisms may enter through various pathway available viz. via. water that can enter the plant stem, scar wounds, cuts and splits [18] up to maturation, harvesting and processing.

Tomatoes have been implicated in many *Salmonella* outbreaks [19].Several outbreaks of Salmonellosis have been associated with the consumption of raw tomatoes. Salmonella, the bacterium is Gram negative bacilli, either motile or non motile. They are non spore forming and grow on ordinary media, all ferment glucose with or without gas production. It causes enteric fever, gastroenteritis, septicemia, food poisoning etc. which is collectively known as Salmonellosis. The survival and growth characteristics of *Salmonella* as affected by the variety of tomatoes and stage of ripening, has received little research attention. Considering all above point it is very important to initiate the present study..

MATERIALS AND METHODS

Collection of samples

A total of 50 tomato samples randomly collected from different vegetable markets and food vendors of Aurangabad City during the month of February, 2023.The red- ripened tomatoes were selected and purchased early in the morning.. The tomatoes were collected in sterilized plastic bags and brought to the laboratory.

Isolation: Following steps were involved in isolation of *Salmonella* from tomatoes.

i. **Enrichment of culture**

Tetrathionate broth and Selenite F broth (Hi-Media) were used for the enrichment of the culture. Surface swabs were taken for the isolation of Salmonella spp. from the surface of the tomatoes and inoculated on Tetrathionate broth. Tomatoes were disinfected using 1% HgCl2 solution and later cut into small pieces aseptically and transferred to the tetrathionate broth for estimating the presence of *Salmonella* in the interior of tomatoes. All the inoculated tubes were incubated for 24 hours at 37°C.

ii. **Plating of enriched culture**

A loopful of enriched culture was inoculated on selective media plate i.e. Bismuth sulphite agar (BSA), Deoxycholate citrate agar (DCA), Xylulose lysine dextrose (SLD) agar, Brilliant green agar (BGA). To rule out any false positive results, tests were run in triplicate by using three plates per enriched sample of each media. The Plates were then incubated at 37°C for 24 hours.

iii. **Screening of test organisms**

Typical colonies on the selective media were screened for colony characteristics, Gram staining and motility test was performed by using a hanging drop method. A single colony then was inoculated on fresh Bismuth sulphate agar plates and further incubated at 37°C for 24 hours. After incubation the biochemical tests were carried out.

iv. **Antibiotic susceptibility testing**

Salmonella spp. isolated were then analyzed for in-vitro antimicrobial testing using sensitivity discs for selected antibiotics mentioned down under (Hi-Media) on Mueller hinton using 12 hour old nutrient broth culture by Kirby and Bauer method.The zone of inhibition around the discs was measured and interpreted as sensitive, moderately sensitive and resistant using the interpretation chart supplied by the antibiotic disc manufacturers (HI-Media, Mumbai).

RESULT AND DISCUSSION

A total of 6 isolates of *Salmonella* spp were isolated from the tomatoes selected from various vegetable markets in Aurangabad city. Characteristics of screened Salmonella spp. were studied. Among all the isolated *Salmonella* spp using Brilliant green agar, colourless and opaque colonies on XLD agar, black selective medium typical jet black colonies on BSA agar, red colour colonies on Hektoen enteric agar were observed. Isolates exhibiting production of acid and gas during carbohydrate fermentation along with the ability to consume citrate as sole source of carbon were confirmed and identified as belonging to Salmonella species. On triple sugar iron reddish purple slope and yellowish butt were observed indicating the dextrose fermentation. Upside lift of the butt was observed which a typical indicator of H,S production. On comparing the cultural characteristics and biochemical tests with the available standard key literature [15] the Salmonella species was confirmed.

Out of 50 samples of tomatoes analyzed, 6 samples were found positive for Salmonella spp. Amongst these 6 isolates 2 were isolated from the interior of the tomatoes and remaining 4 from the surface of the tomatoes by swabbing.

The reason for the prevalence of the Salmonella in the interior of the tomatoes may be due to the cultivation of vegetables in the commonly used agronomic practices and also due to the use of polluted water for the irrigation. *Salmonella* isolates were screened for the drug sensitivity resistant pattern against 11 different antibiotics which are routinely observed in therapeutic use (Table.1). From the result, it was concluded that, all the isolates found resistant to Ampicillin, Tetracycline, Methicillin, Vancomycin, and Bacitracin. The resistance to these antibiotics may be due to the presence of R-plasmid, however, the confirmation of which can be done after plasmid analysis.It was further shown that, all the isolates were sensitive to Ciprofloxacin, Norfloxacin, Ofloxacin, and Chloramphenicol. Ciprofloxacin and Norfloxacin can be used as a favourable option for treating the multi drug resistant cases of Salmonellosis. Ciprofloxacin and Norfloxacin clinical use constituted a significant advancement in therapy not only for multiple drug resistant *Salmonella* but many Gram negative and Gram positive pathogens as well.

CONCLUSION

Multidrug resistant *Salmonella* spp. isolated from tomato samples shows the possibility of disease outbreak due to contaminated tomatoes and hence it is recommended that a great care should be taken while purchasing such tomatoes and raw eating should be avoided in terms of getting prevented by Salmonellosis. The utmost threat to the consumer is when vegetables and fruits are consumed without being washed. Practicing good agricultural practices (GAPs) on farms and good handling practices on farms and homes is often recommended to elude *Salmonella* in fresh produce.

Table.1. Antimicrobial activity of isolated Salmonella spp. Against different antibiotics (Zone size in millimeter)

Isolate No.	Antibiotics										
Potency(mcg/disc)	A	AM	TR	M	C	CF	NF	K	V	OF	B
	10	30	10	10	30	30	30	30	30	30	30
T1	R	20	R	R	26	33	28	20	R	25	R
T2	R	20	R	R	25	32	28	21	R	26	R
T3	R	21	R	R	26	33	27	20	R	25	R
T4	R	20	R	R	24	31	28	20	R	24	R
T5	R	19	R	R	26	32	29	18	R	25	R
T6	R	20	R	R	26	33	28	20	R	24	R

Legends:

T1 to T6= Bacterial isolates;

R= Resistant; A= Ampicillin; K= Kanamycin; AM= Amoxicillin; V = Vancomycin;

TR = Tetracycline; B = Bacitracin; M = Methicillin; OF = Ofloxacin; C = Cloramphenicol;

NF = Norfloxacin; CF = Ciprofloxacin.

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