Incidence Of *Salmonella* From Fresh Fruits And Vegetables And Study Of Its Resistance Towards Antibiotics

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ABSTRACT :

The present work was aimed to study the prevalence of *salmonella* from fresh fruits and vegetables and to determine the effect of some household cleaning methods in controlling *Salmonella* infection Collection of various fruits i.e. Apple, Mosambi, Pomegranate, Chiku, Guava and vegetables that is Fenugreek, Cabbage, Cauliflower, Bitter gourd, Spinach, Green Sorre from local market of Akola. Isolation an of *Salmonella sp.* from fresh fruits and vegetables were done. Different household cleaning methods were used to check the decrease of *Salmonella* load. Effective control measures should be implemented to improve the Microbiological quality of fresh produce .Therefore it is concluded that the tested basic decontaminating agents have the potential to control the no. Similarly household washing can assure the safety during consumption of fresh Fruits and vegetables

Keywords :- *Salmonella,* Fresh Fruits and Vegetables, Household Washing.

INTRODUCTION

In recent years, outbreak of Infections associated with the consumption of fresh or minimally proceed fruit and vegetables have increased, despite their nutritional and health benefit (Hedbergc. W., MacDonald. 1994, Beuchat, L. R. J. M. 2002). Human disease outbreak have been recognised as being caused by contaminated vegetables and fruit consumption several studies have been published describing the bacterial contamination of intact vegetable and fruit in open market (GARG, N, J. Churey, 1990). Raw vegetable host variety of pathogenic microorganism that may be spread over the plant or occur in the plant tissue as micro colonies (Beuchat, L. R. J. M. 2002). Some diverse factor may be affected the variation of microbial profile of vegetable including normal microflora of soil, animal manure derive flora ,irrigation or sewage water, transportation and unconscious handling by retailer.

Vegetables which have a large surface area that can be colonised by *Salmonella*, are particular serious risk (Phugamngoen C, Ritisak S. 2020). These vegetables are grown on large scale, both in the field and under roof, and are exported fresh as village minimally processed. (Azimirad *et al.,* 2021). Investigated the contamination of leafy vegetable, i.e watercress, leek, basil, Savory, parsley, radish. The detected *S.enterica*

Subsp in 15/274 (5.5%) of sample of leafy vegetable unwashed and unpackaged, Lettuce (*Lactum sativa* L.). One of the most consumed leafy vegetables in the world is obtain a career of *Salmonella spp*. and *Escherichia coli* O157 : H17 bacteria. Researchers from Brazil analysed 1,296 artical on *Salmonella* colonization of lettuce (de-Oliveira *et al*, 2019).

Salmonella sp. has the ability to growth in the temperature range of 2-45°C, with the optimum at 35-37 °C. The psychotropic attribute of *Salmonellae* and ability to growth slowly at cold temperature raises concerns on cold-induced *Bacteriostasis* as a food safety measure. *Salmonellae* can growth in the pH range with an optimum pH range of 6.5 - 75 for growth. The water activity for this genus is 0.93 or greater (*SILVA et al., 2010*).

The traditional technique for detecting *salmonella sp.* In food is a classic culture method for presence/absence, developed in order to ensure detection even under extremely unfavorable conditions. This is the case of food microbiology with a competitor microbiota much larger than the population of *Salmonella* and / or food in which the cells of *Salmonella sp* are very low in number and/or foods in which the cells are injured by the process of preservation (application of hesit, freezing, drying).

Antibiotics are the major drugs of choice of the physician's desk to treat the Pathogenic infections. It has been observed that some of the clinicians prescribe the medicine based on the Symptoms instead of performing diagnostic tests.

This prescribing pattern may be one of the reasons for the development of resistant for the antibiotics (Lalitha MK., 2004). Therefore, antibiotics susceptibility testing (AST) plays an important role to check the effectiveness of a Drug against a bacterium and select the best drug that Act against the bacterium.

Antibiogram was performed for each isolate by the Mueller Hinton agar diffusion method. According to the Recommendations of the European Committee on Antimicrobia Susceptibility Testing From a fresh bacterial culture on nutrient agar, a bacterial suspension was prepared in sterile normal saline to achieve a turbidity Equivalent to 0.5 McFarland standard, which corresponds to an inoculum of about 1 to 2x 108 CFU/mL Swabs over the entire surface of the Mueller Hinton agar were spread in three directions with the bacterial Suspension after which the antibiotic discs were deposited.

The disk diffusion sensitivity test Also known as Kirby Bauer disk method Is a simple and practical which uses antibiotic impregnated wafers (disk) to test whether particular Bacteria is susceptible to specific antibiotic or Otherwise (Jorgensen, JH, 2007, Wayne, 2009). The bacterial inoculums (approximately 1-2 X 108 CFU/mL) was uniformly spread using sterile cotton swab on a sterile Petri dish MH agar.

MATERIALS AND METHODS

Salmonella is a significant foodborne pathogen responsible for numerous cases of gastroenteritis worldwide. Fresh fruits and vegetables, often consumed raw, can be a potential source of *Salmonella* contamination. The increasing consumption of fresh produce, coupled with the potential for contamination during cultivation, harvesting, processing, and distribution, underscores the importance of effective isolation and detection methods for *Salmonella* in these food items. This document outlines the materials and methods used for the isolation of *Salmonella* from fresh fruits and vegetables.

Collection of samples

Fresh fruit and vegetables sample from tomato (*Solanum, bicopersicum*). Chiku (*Manilkara, zapotai*). Apple (*Malus domestica*), Lemon (*Citrus limon*). Guava (*Psidium, gvajava* L.), pomegranate (*Punica granatum*) and vegetables spinach (*Spinaci aoleracea*), Fenugreek (*Irigonella foenum-graecum*), And Green Sorrel (Rumaxvestcartus) qwere collected from different vendors of Akola city

Preparation of sample:

Wash the fresh fruits and vegetables with sterile water to remove any dirt or debris. Dry the washed samples with sterile paper towels to remove excess moisture.

Isolation and identification of *Salmonella species*:

Collect your sample (e.g., food, water) and prepare it appropriately. If Necessary, dilute it in a sterile saline solution or buffered peptone water Using a sterile loop, streak a small portion of your sample onto the surface of Bismuth Sulfite Agar (BSA). This medium is selective for *Salmonella* due to its high content of Bismuth salts and sulfite. Incubate the inoculated BSA plates at 37°C for 18-48 hours.

Treatment of fruit and vegetable with different water samples

Tap water: Tap water treatment can be an effective method for cleaning fresh fruit and vegetables, but it is important to consider the potential negative effects, such as contamination and water spotting. Alternative methods, such as chlorine treatment,

Hot water: Hot water treatment involves submerging fruit and vegetables in hot water (typically between 45°C to 60°C) for a specified period (usually 1-10 minutes) to reduce microbial loads, kill pathogens, and extend shelf life.

NaCl treated water : The reduce to the risk of foodborne illness, various sanitizing agents and treatments have been explored for use on fresh produce. Sodium chloride (Nacl), commonly known as salt, has been shown to have antimicrobial properties, making it a potential for reducing microbial loads on fresh produce.
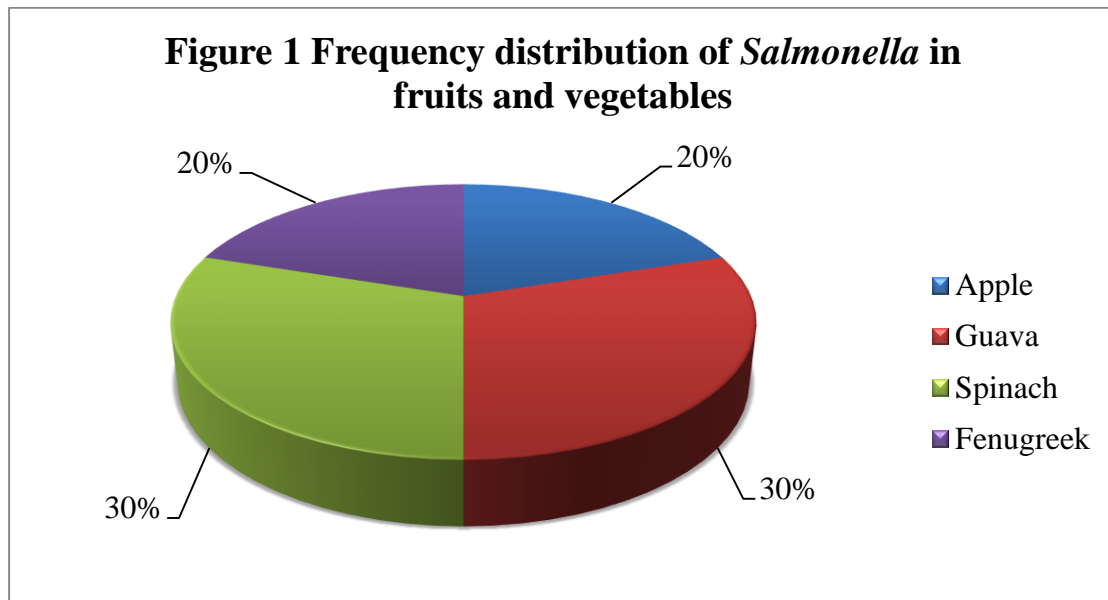
To check the Antibiotic Sensitivity Test by Disc Diffusion Method (Bauer, A. W. (1996) :

Mueller-Hinton agar plates commonly used for antibiotic sensitivity testing. Use sterile swabs to inoculate the culture onto the agar surface. Put Antibiotic disk a surface on agar media and press it to make contact with Agar medium Incubate the plate at 37°c for 24 hrs. After incubation the zone inhibition of is measured in terms of mm

RESULTS AND DISCUSSION

Worldwide demand for fresh fruits is ever increasing for its beneficial role. However, contamination of fresh fruits is of great public health concern and measures must be taken to reduce such microbial proliferation. Only washing with tap water and hot water are not sufficient to reduce the bacterial load completely. Some reliable and cleaning agents for the decontamination of fruits is needed.

Collection of samples:

Figure 1 Frequency distribution of *Salmonella* in fruits and vegetables

The present work is carried out with the fresh fruits and vegetables materials. During the study from August to November total 20 samples of Guava (*Psidium guajava L.*), Pomegranate (*Punicagranatum*), Apple (*Malusdomestica*), vegetables, palak(*Spinacia oleracea*), maithi (*Trigonella foenum-graecum*) were collected from different places in Akola market and some fruits and vegetables were washed by household cleaning method. The fruits and vegetables material is treated with different water samples such as tap water, hot water, and NaCl treated water to check the effect of different water samples on growth of bacteria

Abadias M, *et al.,* (2008) tap water treatment can be an effective method for cleaning fresh fruit and vegetables, but it is important to consider the potential negative effects, such as contamination and water spotting. The most common household processes such as washing with tap water are not proven to be enough for the complete removal of microorganisms. Washing fresh produces with water removes sand, soil, and other debris but unable to remove microorganisms completely.

Sapers *et al.* (2006) reported that Hot water treatment involves submerging fruit and vegetables in hot water (typically between 45°C to 60°C) for a specified period (usually 1-10 minutes) to reduce microbial loads. Kill pathogens, and extend shelf life. Hot water can kill bacteria, viruses, and other microorganisms that may be present on the surface of fresh fruit and vegetables. Reduces risk of foodborne illness:

Isolation and identification:

Isolation of *Salmonella* was done by using selective media. Liang *et al.*, (2017) reported that for the detection of *Salmonella* a pre enrichment in buffered peptone water for 24 h at 37°C was carried out, then two broths, Rappaport-Vassiliadis (41.5°C) and Muller-Kauffmann with Tetra Thionate-novobiocin (37°C) were used for enrichment. For isolation, Xylose Lysine Desoxycholate (XLD) and Hektoenagar media were used. The colonies presenting the typical appearance of *Salmonella* were subjected to biochemical tests namely Kligler-Hajna agar which consisted of looking for a slope (red), a pellet (yellow with formation of gas and hydrogen sulphide (blackened agar).

According to Gorski *et al.*, (2006) specific biochemical test were used for the detection of *Salmonella spp.* was Indol negative, Methyl red positive, VP negative, Citrate positive, Urease negative, Catalase positive, TSI positive and other sugar fermentation viz., Lactose negative, Arbabinase positive, Maltose positive, Sorbitol positive and dulcitol positive.

TableNo.1: House hold methods use for washing the fruits and vegetables

Sr. No	Tap water	*Salmonella*
1	R.O. water	Reduced no.of colonies
2	Hot water	Very less colony
3	NaCl treated water	Moderate colany

- Tap Water – This is regular water from the tap. Since it is not purified or treated, it is likely to have a high number of *Salmonella* bacteria. This means that tap water can be a potential source of infection if it is contaminated.

- RO (Reverse Osmosis) Water – This is purified water that goes through a filtration process to remove bacteria and impurities. As a result, the number of *Salmonella* colonies is greatly reduced. This suggests that RO water is much safer to drink because it removes most harmful microorganisms.

- Hot Water – When water is heated, many bacteria are killed, but some might still survive depending on the temperature and duration of heating. In the table, hot water still shows a moderate number of colonies, meaning it helps reduce bacteria but may not completely eliminate them. This explains why boiling water is recommended to make it safer for drinking.

- NaCl (Salt-Treated) Water – This is water with added salt (sodium chloride). Salt is known to have antibacterial properties, which slow down the growth of bacteria. In the table, NaCl-treated water shows a moderate number of colonies, meaning it reduces bacterial growth but does not completely stop it. This is why salt is sometimes used for food preservation, but it may not be enough to fully kill harmful bacteria in drinking water.Tap water has the highest number of bacteria, making it the least safe.RO water has theleast bacteria, meaning it is the safest to drink. Hot water and NaCl-treated water reduce bacteria but do not remove them completely
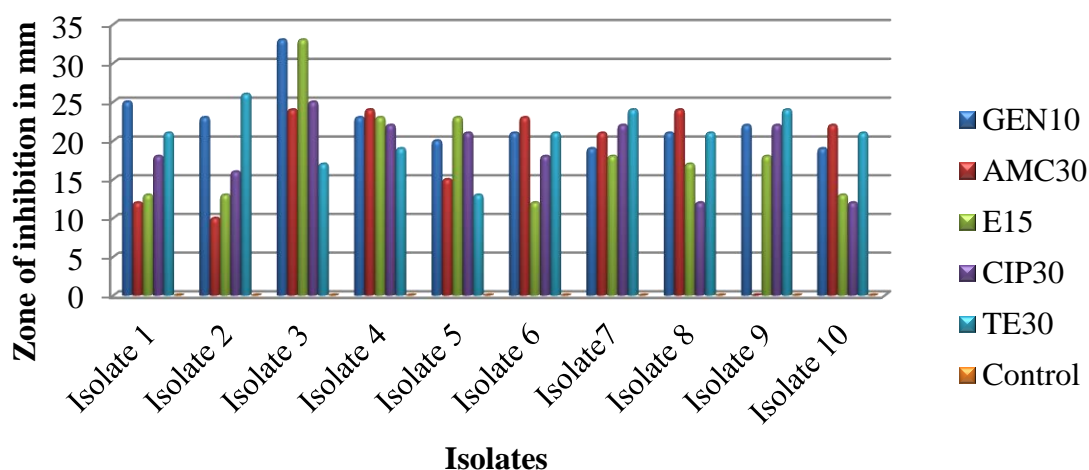
This table helps us understand that purified (RO) water is the safest for drinking, while tap water has the highest risk of contamination. Heating and adding salt can help reduce bacteria, but they are not foolproof methods for making water completely safe.

Table No.2 :To check the Antibiotic sensitivity Test by Disk Diffusion method

Sr.No	Isolates	GEN10	AMC30	E15	CIP30	TE30	Control
1	Isolate1	25 mm	12 mm	13 mm	18 mm	21 mm	-
2	Isolate2	23 mm	10 mm	13 mm	16 mm	26 mm	-
3	Isolate3	33 mm	24 mm	33 mm	25 mm	17 mm	-
4	Isolate4	23 mm	24 mm	23mm	22mm	19mm	-
5	Isolate 5	20 mm	15 mm	23mm	21mm	13mm	-
6	Isolate 6	21 mm	23 mm	12mm	18mm	21mm	-
7	Isolate7	19 mm	21 mm	18mm	22mm	24mm	-
8	Isolate 8	21mm	24 mm	17mm	12mm	21mm	-
9	Isolate 9	22mm	13m m	18mm	22mm	24mm	-
10	Isolate 10	19mm	22mm	13mm	12mm	21mm	-

Fig. 2 : Antibiotic sensitivity Test by Disk Diffusion method

Antibiotic sensitivity of all the *Salmonella* isolation was checked. Isolate 1 shows highest sensitivity for Gen. 10, having zone size 25 mm whereas least activity weas shown for AMC 30 12 mm zone. Isolate 3 shows highest zone of inhibition of 33 mm for Gentamycin followed by isolate 2 and 4 (23 mm) and Isolate 6 and 8 (21 mm) .

Moderate activity was shown by Erythromycin for all isolates ranging between 12 – 18 mm only effective against isolate 3 measuring 33 mm zone size. Overall studies shows that Antibiotics shows moderate activity in controlling all the *Salmonella spp.* indicating multidrug resistance by *Salmonella*.

Several studies have documented high resistance of *Salmonella* to the tetracyclines (Yoke-Kqueen *et al.,* 2008; Learn-Han *et al.,* 2009), which is in agreement with the result obtained in this study. The high

resistance phenotypes rate of tetracycline observed in the study could be as a result of the use of tetracycline in food animal production which has led to worldwide spread of tetracycline resistance observed in *Salmonella* isolates (White *et al.,* 2001; Logue *et al.,* 2003; Parveen *et al.,* 2007). Thereby indicating longitudinal transfer of resistance genes between *Salmonella* isolates from animal-related sources to vegetable sources (Learn-Han *et al.,* 2009). *Salmonella* isolates from cabbage vegetable were highly sensitive to the quinolones and fluoroquinolones antibiotics compared to isolates from spinach vegetable.

Photoplate 1 Treatment of Fruits and Vegetables

Fig 1 : **Fruits and Vegetables soak in Tap Water., RO Water and NaCl 10 min,**

Fig 2 : **Slight growth of *Salmonella spp.* observed after treatment**

Photoplate 2 :- Antibiotics Susceptibility Test

CONCLUSION

- *Salmonella spp.* was detected in the fresh fruits and vegetables analysed in this study

- Effective control measures should be implemented to improve the Microbiological quality of fresh produce .

- Therefore it is concluded that the tested basic decontaminating agents have the potential to be used, an household washing can assure the safety during consumption of fresh Fruits and vegetables

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