

RO Water Poisoning By Salmonella Typhi among Children under Twelve Years in Fatima AL- Zahraa Hospital at Baghdad: An In Vitro Study

¹Nada Khazal Kadhimi Hindi, ²Shatha Khazal K. Hindi, ³Ammar Khazaal,
⁴Mohammed Malih Radhi, ⁵Rusull Hamza Kh. AL-Jubori, ⁶Hind Ghasan, ⁷Nawfel
Fadhil Hamza

ABSTRACT--In this paper we evaluated the of RO Water Poisoning by Salmonella Typhi among Children under 12 Years, Antimicrobial Activity of plant extracts and antibiotics against S. typhi in Fatima AL- Zahraa Hospital Analytical study has been conducted during January to July 2019. The study was carried out in Fatima AL-Zahraa Hospital in Baghdad province. A total of 966 of blood samples from children under 12 years (they were drank RO water) were collected from them who were attending to the Hospital with signs and symptoms of poisoning. The samples were cultured (3 times) on different media. Antimicrobial activity of extracts and antibiotics carried out by well diffusion method. The results depicts that (12%) were positive culture for microbes bacteria, among those positive culture, the Salmonella typhi were the most common bacteria transmit after RO water filtering. The maximum effects of garlic and lemon extracts rather than antibiotics were observed in S. typhi. It is conclude that children who attending the hospital and suffers from signs and symptoms of poisoning and who drinking RO water were have been risks for contaminated mostly with Salmonella Typhi bacteria. Based on our findings and their support, it is need to be replace water filters frequently or boil water to prevent those risks. Garlic and lemon extracts exhibit obvious antibacterial properties against bacteria. The effect of extract has ability to subside the growth of infectious pathogens which have antibiotics resistance.

Key words--RO Water, Poisoning, Salmonella Typhi, Children, plant extract and antibiotics.

I. INTRODUCTION

Many of the causes of intestinal diseases in poor living environments and nutrient can continue in the soil or sewage or lake water, food or drinking water before moving to a sensitive host^[1]. Once the host's mucous tissue is reached, these pathogens express virulence mechanisms that allow epithelial attachment and/ or penetration^[2]. Salmonella species affects 93.8 million cases of gastroenteritis worldwide each year by about 155,000 deaths

¹ A.Prof. , PhD in microbiology, Department of Basic and Medical Science, College of Nursing, Babylon University, Babylon Province, Iraq. nadakhzal@yahoo.com Mobil: 009647725708351

² MSc in microbiology, Fatima AL-Zahraa Hospital, Baghdad, Iraq. nadakhzal83@gmail.com, Tel no: 009647813456613.

³ PHD student at School of Biology, Universiti Sains Malaysia (USM). ammarkhazaal1991@gmail.com

⁴ M. Sc in Community Health Nursing, Kut Technical Institute/ Middle Technical University- Iraq). mohammed.amri92@gmail.com

⁵ M. Sc in Community Health Nursing/ Babylon Health Directorate- Iraq) nadakhzal83@vgmail.com

⁶ BSc in microbiology, Fatima AL-Zahraa Hospital, Baghdad, Iraq nadakhzal83@gmail.com, Tel no: 009647813456613

⁷ M. Sc student in Pediatric Nursing/ Babylon Health Directorate- Iraq).: nonoiq74@gmail.com

(percentage of 5 to 95, 39000-303000)^[3]. Types of *Salmonella* intestinal, intestinal serological types: Typhi and Paratyphi A and B and Paratyphi C infection causes severe systemic and invasive and deadly known as typhoid fever or enteric fever. From 12 to 22 million patients of typhoid fever in the year, the mortality rate ranges estimated between 129,000 and 217,000, and 80% of these cases and deaths occur in Asia alone. Because of improved sanitation, the most developed countries in Europe and North America free of intestinal fever, but intestinal fever disease is still worrying in Southeast Asia and the Indian sub-continent and Africa, and to a lesser extent in South America^{[4][5]}. Causing contamination water with salmonella outbreaks routinely self-inflammation of the stomach and intestine limit in the United States^[6]. Intestinal *Salmonella Typhi* and Paratyphi are serovars restricted by humans and cause systemic infection in the endothelial retina called typhoid fever^[7]. *Salmonella* contributed about 53.4% of all cases of outbreaks of water diseases from 2006 to 2017, and about 32.7% of cases of *salmonella* outbreaks of water-borne were associated with the consumption of drinking. It is a filter water system which produces and stores and spins the water under the background conditions "likely to create a bio-adhesive membranes or microorganisms" which can be a source of undesirable levels of micro-organisms or internal toxins in the water flowing. Recent studies have shown that almost all large water purification systems can cause the formation of biofilms in vitro^[8].

Many diseases occur because of bacteria that grow in the water, because the water, which is the center of a suitable solution for bacteria to grow and multiply in which 80% of the overall cellular spectrum, so the nomination process helps to reduce bacteria for as long as possible, growth where they cannot reproduce the bacteria away from moisture, because the water is where necessary for the growth of bacteria as well as the main source of human beings, and despite the use of modern equipment in the water filter, infection occurs despite the use of these devices and this indicates that the device used to filter water. In urgent need of change in a small period like (10-15) days lead to the absence of disease due to the growth of bacteria^{[9][10]}. In this paper we evaluated the of RO Water Poisoning by *Salmonella Typhi* among Children under 12 Years, Antimicrobial Activity of plant extracts and antibiotics against *S. typhi* in Fatima AL- Zahraa Hospital.

II. MATERIALS AND METHODS

Analytical study has been conducted during January to July 2019. The study was carried out in Fatima AL-Zahraa Hospital in Baghdad province, during January to July 2019. A total of 966 of blood samples from children under 12 years (they were drank RO water) were collected from them who were attending to the Hospital with signs and symptoms of poisoning. The samples were cultured (3 times) on nutrient agar, blood agar and MacConkey agar plates, using sterile standard loop (1ml) then incubated at 37°C for 24 hours.

III. METHODS

After positive results of growth were appear, only samples were identified with Gram stain and Biochemical test and vetch-system^[11].

For preparation of Aquatic Extract according to (17, 20). Antimicrobial activity test by Agar-well diffusion assay (In vitro): (18,19,20). Antibacterial activity assay: According to Forbes (11), the antimicrobial activity of ciprofloxacin was detected by agar-disc diffusion (the test were performed in triplicates).

IV. RESULTS

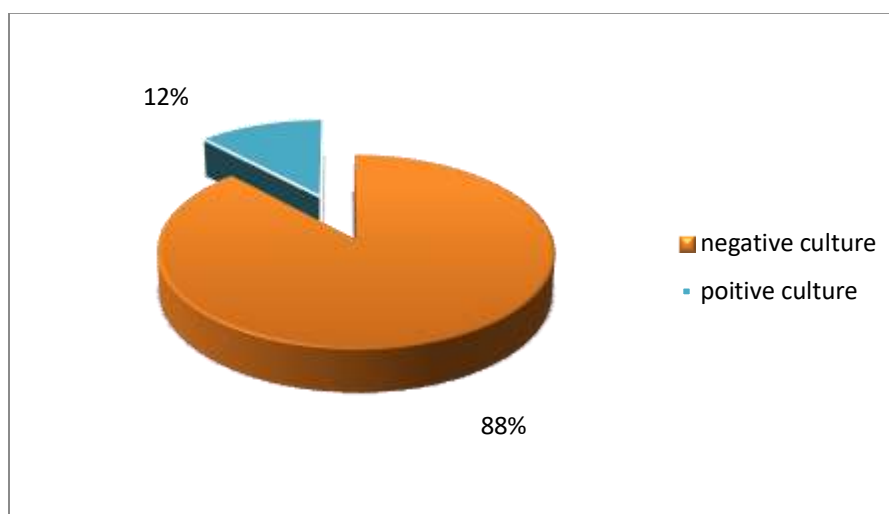


Figure 1: Positive and Negative Culture

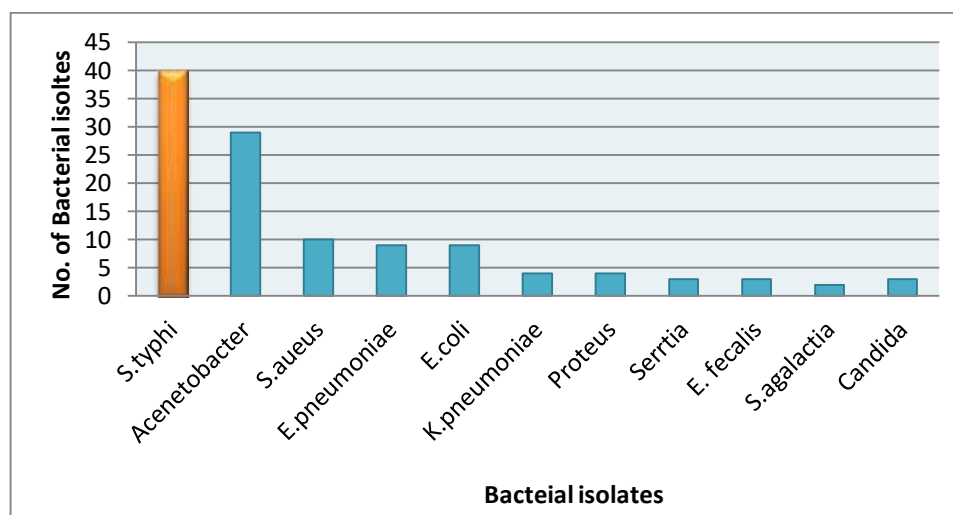


Figure 2: Number of Bacterial Isolates in Blood Culture

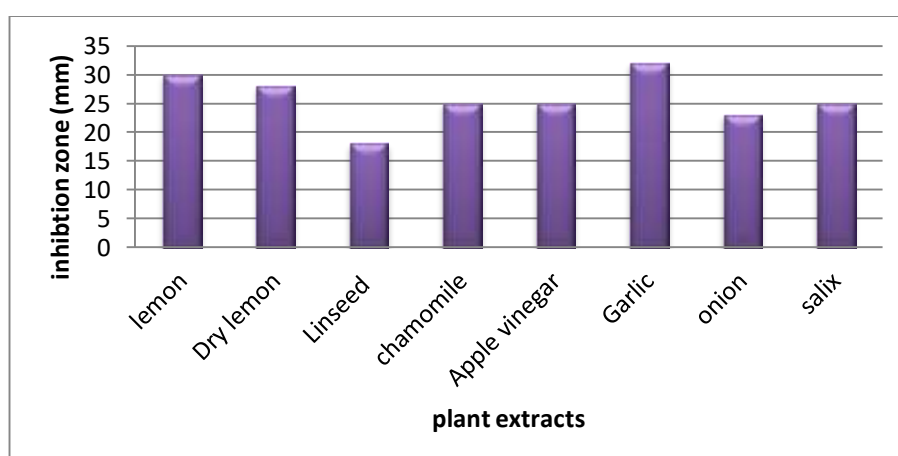


Figure 3: plant extracts activities against S.typhi

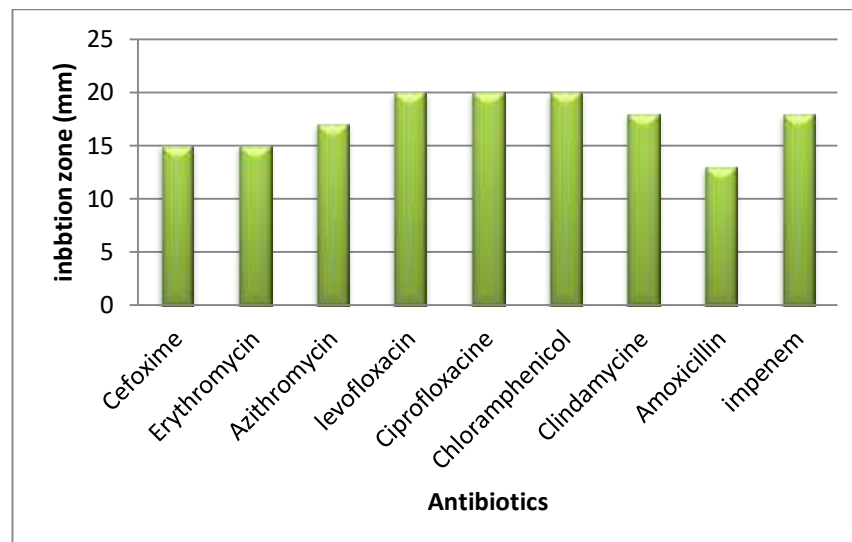


Figure 4: the effect of antibiotics against *S.typhi*

V. DISCUSSION

A total of 966 samples were transplanted at Al-Zahra Hospital for children suffering from signs and symptoms of poisoning and drinking RO water were cultured (3 times) on nutrient agar, blood agar and MacConkey agar plates. It was found that 88% of the cultivation is negative for bacteria and 12% is positive for bacteria. As being a source of undesirable levels of microorganisms or internal toxins during water filtration. Our findings come along with study has been conducted in Davao City in order to isolation of school water for bacteria. The results read that *Salmonella typhi* and *Pseudomonas aeruginosa* and staphylococci negative in Coagulase, positively isolated from drinking water in mostly^[12].

A study conducted in Vellore District deals with isolation and identification of bacteria from water. Their results confirmed that water is commonly contaminated with microbes and this contamination may be playing a role in the transmission of potentially harmful organisms^[13].

Also, the study has been conducted in rural Venda in order to investigated quality microbial sources of river water. It has been confiromed that *Vibrio Cholerae* and *Salmonella* are the main pathogens found in water^[14].

Using sterile standard loop (1ml) then incubated at 37°C for 24 hours. After positive results of growth were appear, only samples were identified with Gram stain and Biochemical test and vetch-system. Our findings reveals that *Salmonella Typhi* records the highest level among other types of bacteria. *Salmonella* can be found in water sources such as private wells that are contaminated with feces of infected humans or animals. You can enter waste water in different ways, including the flooding of sewage and sewage systems that do not work properly, and the flow of contaminated rainwater, runoff for agriculture. Wells may be more susceptible to such contamination after the flood, especially if the shallow wells, or drilled or Mellha, or inundated by flood waters for long periods of time. Moreover, it can be transmitted to the intestines by drinking RO water by not treating the water litter properly, which can accumulate in the filter filters^[15].

In another manual, To kill or disable salmonella, bring the water to a boiling point for one minute. Then allow water to cool and store in a clean, sterile container with a tight lid, then cool. This is a virtue of the process of water filtration especially in children (considered a preventive aspect of typhoid disease)^[14].

In Nepal study deals with Household drinking water filter for arsenic and pathogen treatment. It is confirmed that water filter system contains fibers called Brubylene. These fibers after two or three week will be attract to bacteria ^[16].

As well as, it can contaminate drinking water transports a number of infectious diseases. It can result in the construction and proper maintenance and regular testing to reduce the risk of infection. However, bacterial contaminants can be natural, such as *Salmonella* are well intervention through natural processes and can make you sick. The good news is that when you discovered; it is possible to treat the piece of water filters by replacing frequently to get rid of the danger^[15].

Furthermore, Antibacterial Activity of extracts Estimation against *S.typhi* During the course of this work, the eight designated antimicrobial procedures were compared. The watery extract of Lemon, Dry lemon, Linseed, chamomile, Apple vinegar, Garlic, and onion were used. Results revealed that the highest activity of the lemon and garlic against *S.typhi* (figure 3). The highest inhibition zone was detected (32mm). On the other hand, the results showed a lowest activity of Linseed against microorganism. The used aqueous extracts were applied at 30% concentration by agar diffusion methods. Statistical analysis of data, exhibited no significant differences between the inhibitory effects of plant extracts on bacterial isolates at level ($P \leq 0.05$).

In contrast, antibacterial activity of plant extract was compared to antibiotics (Cefoxime, Erythromycin, Azithromycin, levofloxacin, Ciprofloxacin, Chloramphenicol, Clindamycin, Amoxicillin, impenem), The highest inhibition zone was detected (20mm) (Figure 4) summarized response to this drug, which showed less effect than the plant extract.

Drugs derived from active components of plants are effective, less expensive, easily available, and without secondary effects. The specialists of indigenous and traditional medicine principally depend on medicinal plants to prepare therapeutic materials. The crude extracts of plants may be used directly against antibacterial activities. These were match with (Qin et al., 2009(21) who found that the antimicrobial activity of vinegar is strong on bacterial. Vinegar and extract of stored garlic both of them had heavy antimicrobial activity against both fungi and bacteria. Zasshi and his group (1997) (22) pointed out, as the microbiocidal and microbiostatic actions of vinegar yields on growth of *E. coli*, as bactericidal influence of vinegar was independent on the size of bacterial inoculums, but it depends on the phase of growth. In Thailand, vinegar was utilized in treat dandruff and other skin infection, due to the vinegar has antimicrobial effect against gastrointestinal tract disorder and dermatitis bacteria, also it has an antioxidant activity. As, the antioxidant agents may play a vital role as antiinflammatory caused by skin diseases and wounds. Vinegars inhibited growth of *P. acnes*, *S. epidermidis*, *S. aureus*, *S. faecalis*, and *E. coli* (23). The phenolic compounds as cresols or phenol are well recognized to have antimicrobial features and contribute to the antimicrobial effect (24). The phenolic compounds antioxidant ability depends on the presence of methoxyl, alkyl and hydroxyl groups (Lopez, et al., 2003) (25). Eja et al. (2007) (26) revealed that the microbiocidal effect of extract garlic against *Streptococcus*, *Helicobacter pylori*, *Staphylococcus*, *Proteus*, *Escherichia*, *Clostridium*, *Klebsiella*, *Bacillus*, *Salmonella* and *S. enterica* Enteritidis. Also, the extracts of garlic can stop the thermo nuclease and *Staphylococcal* enterotoxins A, B, and C1 formation (27). Cavallito and Bailey (1994) (28) who demonstrate

that the antimicrobial effect of garlic extract due to active component of garlic is allicin, some strains of bacteria resistant to methicillin but its sensitive to allicin . The Allicin antimicrobial activity is by inhibition of synthesis of RNA, DNA and protein syntheses. Allicin interferes with lipid synthesis and RNA production (primary target of allicin action is the RNA). If RNA produced in less amount, or it cannot be produced; thus severely disturbance of protein synthesis will be occur. It may be paused at any stage contributed to the deficiency of tRNA, mRNA, and rRNA. When amino acids, subsequently proteins failed to be made, thus development and growth of the this organism will not happen as they are necessary for all structures of cell and growth. Concerning, the lipid synthesis is also affected, with its effect chiefly, inhibiting the formation of cell wall phospholipid biolayer in both Gram-stained bacterial types, and because of its reaction chemically with various enzymes through thiol groups, such as thioredoxin reductase, alcohol dehydrogenase and RNA polymerase. The action of diallyl disulphide or diallyl thiosulphinic acid or allicin, the antifungal and antibacterial activities of extracted garlic juice are because of the inhibition of succinic dehydrogenase via the thiol group inactivation. Anionic components of garlic is chlorides, sulfates and nitrates and other water soluble components which could be responsible for its antimicrobial action (29). All of these elements lead to the inability of the bacteria to mature in the existence of garlic or allicin. Thus the diameter size of the zone of inhibition obtained with garlic extract showed a higher zone, as it is compared to the activity of the marketable antibiotics (30). Natural plant products may provide new various sources of antibacterial agents; from all these results, that the medical importance of plant extracts is equivalent to the daily used antibiotics

In addition, Abdullah (2009) (31) found that the juice of *C. limon* has significant inhibition against both *S. aureus* and *K. pneumoniae* with inhibition zones 17.4 and 13.3 mm respectively, These results were agreed with our results as the juice of *C. limon* was more effective. This could be due to the acidic pH of this juice that will affect the charges of the amino acids that constitute the peptidoglycan, and it may affect the active sites of enzymes leading to defect in their activity (31). The resistance of Gram-negative bacteria to plant extracts was not sudden. In general, this category of bacteria is more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (32). Infections caused by *P. aeruginosa*, especially those with multidrug resistance, are among the most difficult to treat with conventional antibiotics. In this study, the growth of *P. aeruginosa* was slightly inhibited by lemon extracts. Such results are very fascinating and with expectation, increasing the concentration of the extracts may produce more inhibition to this bacterium. They may inhibit the bacterium by a particular mechanism than that of presently used antibiotics and should have therapeutic price as an antibacterial agent against multi-drug resistant bacterial strains. There are clinical studies found that daily usage of pure hand gel that contain *C. limon* in its ingredients is associated with reduction in the microbial load, which is important to prevent risk of transmitting nosocomial infections by healthcare workers (33). This result united with our outcome as it showed that *S. Typhi* is affected with these extracts of *C. limon*. Dhanavade et al., 2011 (34) suggest that different alcoholic extracts of lemon peel give antimicrobial activity against different bacterial isolates especially *P. aeruginosa* and *S. Typhi* murium better than the aqueous extract that we used in this study as it gave no effect against most of the study isolates including the two mentioned above. Generally, Rahman et al (2011) (35) documented that, throughout the detection of microbial susceptibility to totally different plant extracts, the scale of inhibition zone to purpose relative

antibacterial activity is not adequate. The zone must be affected by the solubility and rate of diffusion in agar medium or its volatilization; and so the results may well be affected.

Durairaj (2009) (36) reported that the aqueous garlic extract has ability to inhibited the growth of *E. coli*, *S. typhi* and *S. aureus*. Combined effects can avert the development of resistance to antibiotic.

VI. CONCLUSION

Children who attending the hospital and suffers from signs and symptoms of poisoning and who drinking RO water were have been risks for contaminated mostly with *Salmonella Typhi* bacteria. Based on our findings and their support, it is need to be replace water filters frequently or boil water to prevent those risks. Garlic and lemon extracts exhibit obvious antibacterial properties against bacteria. The effect of extract has ability to subside the growth of infectious pathogens which have antibiotics resistance.

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