

# Occurrence and Dissemination of Antimicrobial-Resistant Salmonella Isolated from Diarrheic Stool Samples of Human

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## Abstract

The current study was designed to isolate and identification of Salmonella from human stool using salmonella-shigella agar, the diagnosis of Salmonella relied on cultural properties where the Salmonella colonies on S.S. agar revealed pale with black center. Out of 72 samples were collected 59.7% were positive to Salmonella. The antibiotic susceptibility test of all positive isolates for Salmonella performed by using disk diffusion method against numerous antibiotics, The result of this test presented that the Salmonella isolates were highly resistant to Levofloxacin 43(100%), Amoxicillin/Clavulanate 40(93.0%), Streptomycin 22(51.2%), Tetracycline 19(44.2%), Imipenem 19(44.2%) and Amikacin 18(41.9%). While Salmonella isolates were moderately sensitive to Kanamycin 9(20.9%). The current study refers to Salmonella gastroenteritis treatment can be use Kanamycin as first treatment option.

**Keywords:** *Streptomycin, Salmonella spp., S.S. agar, Plasmid.*

## Introduction

The microorganisms resist antimicrobial agents by many ways such as; producing hydrolyzing enzymes capable of analyzing antibiotic molecule or modulating it to inert molecules or preventing antibiotics from reaching the target it affects. Among these enzymes are beta-lactamase, which breaks down penicillins and cephalosporins groups, which are main causes of the failure of many therapeutic cases. It was found most epidemics were caused by strains producing beta-lactamase enzymes by plasmids most of them are couplings with multiple resistance characteristics, some considered it much more dangerous than resistance from chromosomal enzymes that work against  $\beta$ -lactam antibiotic groups<sup>[1]</sup>. The antibiotic resistance has become one of the most common problems globally on health and economic level due to seriousness of plasmids and its multiple resistance to more than one antibiotic from different groups as well as its ability to move between different isolates carrying with it resistance character<sup>[2]</sup>. Because of importance the Enterobacteriaceae therapeutically, This research focused on isolation and identification of pathogenic Salmonella from human stool, in addition to determine antibiotic susceptibility profile of the Salmonella isolates .

## Materials and Method

A total of 72 diarrheic stool samples were collected for this study. The following substances were used to isolate and diagnoses of Salmonella, furthermore test their sensitivity to antibiotics:

- 1. Salmonella – shigella agar (S.S. agar):** Prepared according to manufacturer's instructions and used in diagnosis of bacteria. After collection of samples, Nutrient broth (NB) used for inoculation of each sample then incubated at 37°C for 24 hours, as well as, Salmonella-Shigella agar used for culture of a loop full from bacterial culture in incubated tubes, subsequently, carefully examination of plates for the existence of characteristic colonies of Salmonella<sup>[3]</sup>.
- 2. Nutrient Agar:** Prepared according to manufacturer's instructions and used in maintaining of bacteria.
- 3. Antibiogram:** The antibiotics used in this study comprise disks Himedia, India: Amicacine (AK), Streptomycin (S), Amoxicillin/Clavulanate (AMC), Tetracycline (TE), Imipenem (IMP), Kanamycin (KAN) and Levofloxacin (LEV). Kirby-Bauer disk diffusion method was used to evaluate the

susceptibility of *Salmonella* isolates on Mueller-Hinton agar according to CLSI<sup>[4]</sup>.

4. **Preservation and maintenance of bacterial isolates:** Bacterial strains are preserved on nutrient agar slants after inoculation in 37°C for 18hr . The isolates were subculture on another new culture media for monthly maintaining. All strains are kept in the refrigerator at 4 ° C. Nutrient broth added with 40 % glycerol was used For long maintenance then frozen for 6-8 months at -20 °C <sup>[5]</sup>.

## Results and Discussion

This study showed *Salmonella* was found in 59.7% of the collected total samples Fig:1, the presence of turbidity in NB indicates the growth of bacteria after 24 hr. incubation at 37°C . Also, the cultivation of bacteria on S.S. agar characterized by appearance of pale salmonella colonies with a black center, where the S.S. agar used to differentiate between *Salmonella* and *Shigella* from the rest of the species as this medium contains lactose sugar as a basis for differentiation between fermented and non-fermented species, the appearance of black center due to release of hydrogen sulfide and its deposition on the form Iron sulfide <sup>[6]</sup>.

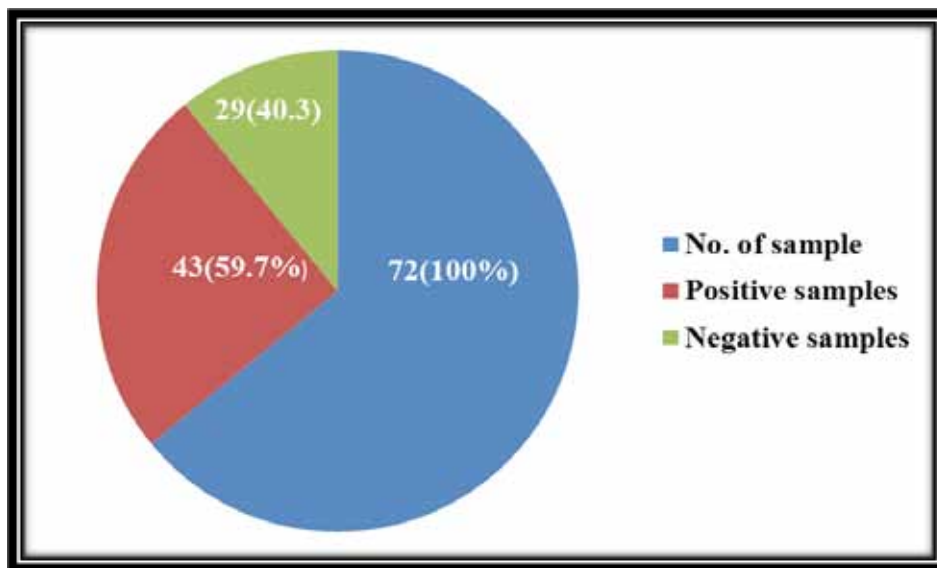


Fig (1): Frequency of positive and negative infection

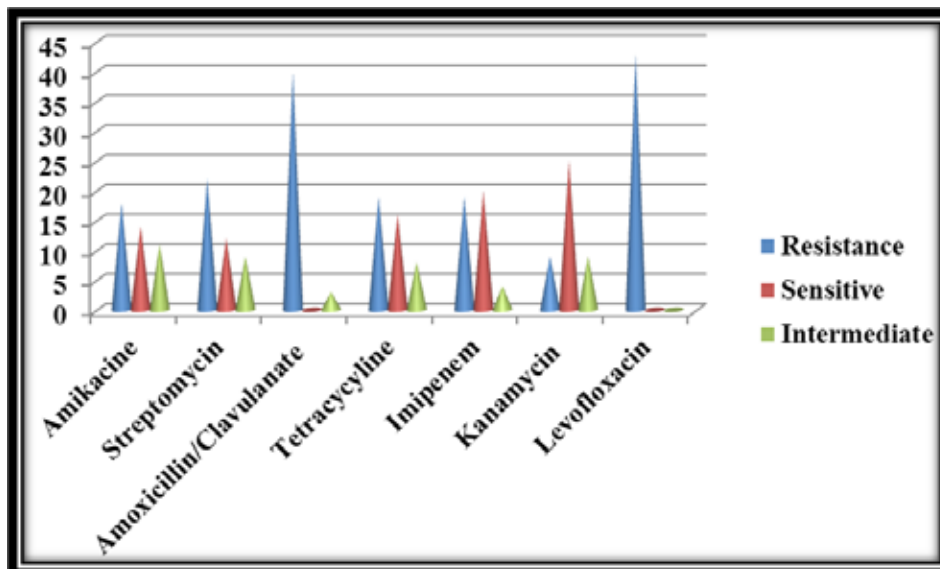


Fig. (2) : Antibiotics susceptibility profile for salmonella isolates (n=43).

All *Salmonella* isolates were examined for a number of the above mentioned antibiotics, it was noted that the bacteria were resistant to antibiotics as shown in Fig: 2, Amikacin 18(41.9%), Streptomycin 22(51.2%), Amoxicillin/Clavulanate 40(93.0%), Tetracycline 19(44.2%), Imipenem 19(44.2%), Kanamycin 9(20.9%) and Levofloxacin 43(100%).

The *Salmonella* resistance to antibiotics has been steadily increasing, numerous research related to surveillance reports revealed that *Salmonella* resistance phenotypes increases a two-fold from 20%-30% to about 70% during the period 1990 to 2000<sup>[7]</sup>. In this current study, we observed all *Salmonella* isolates were resistant to levofloxacin Fig: 2. The causes of *Salmonella* resistance to Levofloxacin may be due to point mutations to protect DNA gyrases or determinants of plasmid mediated resistance or efflux pumps that ejects antibiotic molecules outside the bacterial cell<sup>[8]</sup>. This type of resistance is important in public health due to latent spread among species of bacteria, in addition to fluoroquinolones resistance development among *Salmonella spp.* is usually slow<sup>[9]</sup>. This result poses a real risk because fluoroquinolones is one of the central drugs in the treatment of human salmonellosis. Certainly, Misuse of antibiotics, as also the use of these antibiotics frequently in food animals, pharmaceutical industries, and prophylactic. The cause of bacterial sensitivity to the mentioned antibiotics is due to several reasons, including: absence of plasmids with a definite or antibiotics-altered character, these antibiotics have not been used previously for studied bacteria, so bacteria do not have genetic genes inherited from their ancestors to resist these antibiotics and some of these antibiotics neutralize plasmid and therefore lose resistance in bacteria<sup>[10]</sup>. From the current study, we conclude that it is necessary to increase health education about the use of antibiotics properly to prevent the emergence of antibiotic resistance also to decrease the prevalence of Salmonellosis among the people to evade food borne illness. Strict health measures with proper treatment must be seriously done.

**Ethical Clearance:** This research underwent to the terms of ethical considerations by higher education and scientific research ministries in Iraq.

**Conflict of Interest:** The authors announce that they have no conflict of interest.

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