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STUDY EFFECT OF ANTIBIOTICS ON SALMONELLA SPECIES

A research project Submitted To the council of the college of pharmacy/University Of Babylon As a partial fulfillment requirement of Bachelor's in pharmacy sciences.

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

(وَلَمَّا بَلَغَ أَشُدَّهُ آتَيْنَاهُ حُكْمًا وَعِلْمًا ۚ وَكَذَٰلِكَ نَجْزِي الْمُحْسِنِينَ)

صدق الله العظيم

"الاهـــــــــــــــــداء"

اهدي هذا العمل المتواضع الـــــى :

إلى من لا يضاهيهما أحد في الكون، إلى من أمرنا الله ببرّهما، إلى من بذلا الكثير، وقدّما ما لا يمكن أن يردّ، إليكما تلك الكلمات أمي وأبي.

إلى من تعلمت على ايديهم ولم يبخلوا حرفا عني اساتذتي في كل مراحل الدراسة وأخص بالذكر اساتذة كلية الصيدلة.

الى شهداء تشرين الذين سالت دمائهم من اجلنا وكل شهداء العراق .

CONTENT :

INTRODUCTION:

- The species Salmonella enterica comprises a group of gramnegative bacteria that are important pathogens for humans and livestock .(K. Prior et al microbiology physiology 2009)

In humans, ingestion of various Salmonella serovars gives rise to infection of the small intestine and to gastroenteritis. A small number of Salmonella serovars can lead to systemic infection and enteric fever. Typhoid fever in humans, caused by S. typhi, is the prototype of such disease. Without treatment, S. typhi represents a major health threat to humans, and in developing countries typhoid fever is still an important cause of morbidity and mortality, with more than 16 million cases and 600,000 deaths per year [Flaviano S.Martins et al Microbes and Infection 2013].

In contrast to the severe outcome of disease in humans, S. typhi is avirulent in most animals, including mice. However, in mice, infection withS. typhimurium gives rise to enteric fever, with symptoms similar to those observed in humans after infection with S. typhi.

S. typhimurium infection in mice is therefore widely accepted as an experimental model for typhoid fever in humans. Several attenuated strains of S. typhi and S. typhimurium have been generated that are being considered as live vaccines against disease caused by Salmonella. Furthermore, because of their high efficacy in inducing both cellular and humoral immune responses, recombinant Salmonella strains expressing heterologous antigens have been generated and are being analyzed for their potential as vaccines against a variety of pathogens (Lisa Maier et al AFFILIATION Institute of Microbiology)

Structure, Classification, and Antigenic Types

- Salmonellae are Gram-negative, flagellated, facultatively anaerobic bacilli possessing three major antigens: H or flagellar antigen; O or somatic antigen; and Vi antigen (possessed by only a few serovars). H antigen may occur in either or both of two forms, called phase 1 and phase 2. The organisms tend to change from one phase to the other. O antigens occur on the surface of the outer membrane and are determined by specific sugar sequences on the cell surface. Vi antigen is a superficial antigen overlying the O antigen; it is present in a few serovars, the most important being S typhi.(Baron S, editor et al Medical Microbiology 1996)
- Antigenic analysis of salmonellae by using specific antisera offers clinical and epidemiological advantages. Determination of antigenic structure permits one to identify the organisms clinically and assign them to one of nine serogroups (A-I), each containing many serovars .H antigen also provides a useful epidemiologic tool with which to determine the source of infection and its mode of spread.(John R. McQuiston et al Centers for Disease Control and Prevention 2020)
- As with other Gram-negative bacilli, the cell envelope of salmonellae contains a complex lipopolysaccharide (LPS) structure that is liberated on lysis of the cell and, to some extent, during culture. The lipopolysaccharide moiety may function as an endotoxin, and may be important in determining virulence of the organisms. This macromolecular endotoxin complex consists of three components, an outer O-polysaccharide coat, a middle portion (the R core), and an inner lipid A coat. Lipopolysaccharide structure is important for several reasons. First, the nature of the repeating sugar units in the

outer O-polysaccharide chains is responsible for O antigen specificity; it may also help determine the virulence of the organism. Salmonellae lacking the complete sequence of O-sugar repeat units are called rough because of the rough appearance of the colonies; they are usually avirulent or less virulent than the smooth strains which possess a full complement of O-sugar repeat units. Second, antibodies directed against the R core (common enterobacterial antigen) may protect against infection by a wide variety of Gram-negative bacteria sharing a common core structure or may moderate their lethal effects. Third, the endotoxin component of the cell wall may play an important role in the pathogenesis of many clinical manifestations of Gram-negative infections. Endotoxins evoke fever, activate the serum complement, kinin, and clotting systems, depress myocardial function, and alter lymphocyte function. Circulating endotoxin may be responsible in part for many of the manifestations of septic shock that can occur in systemic infections. .(John R. McQuiston et al Centers for Disease Control and Prevention 2020).

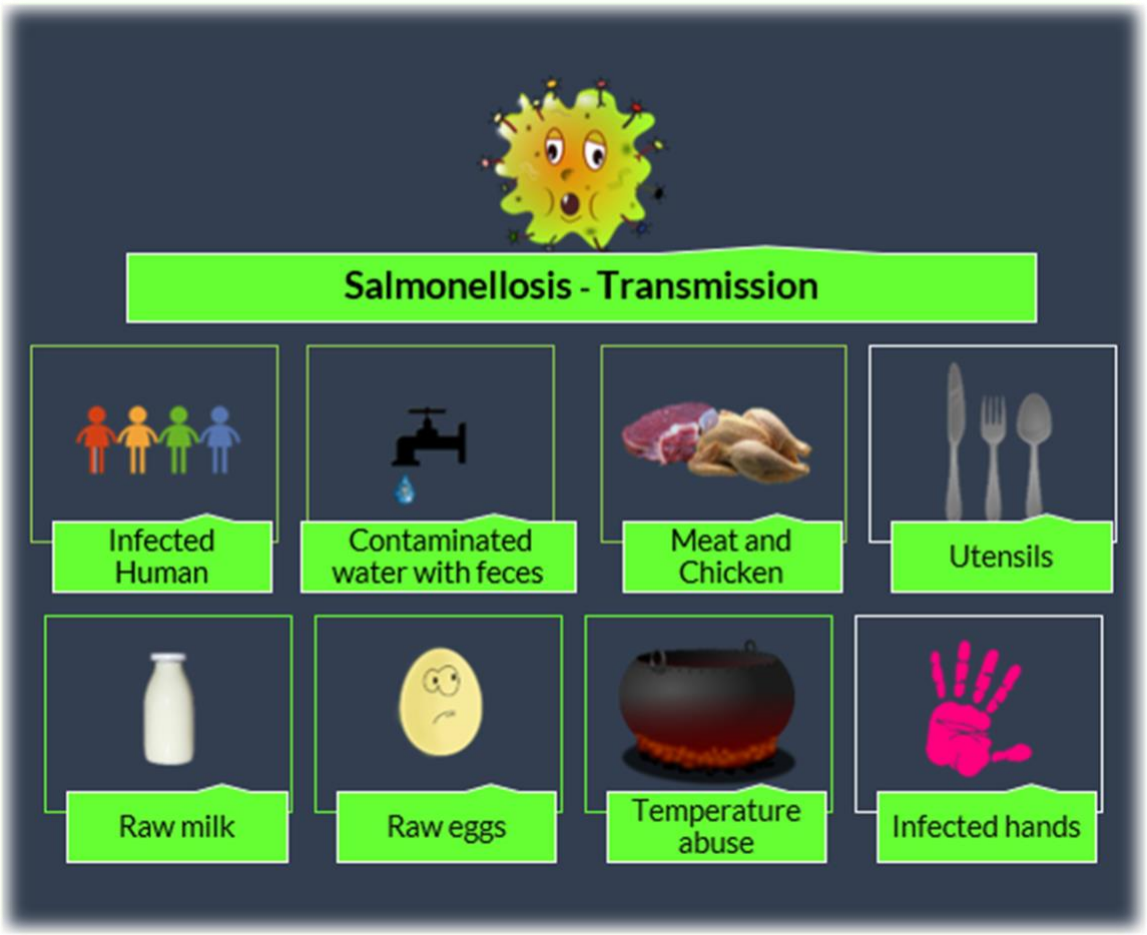
Epidemiology

- Typhoid fever is more common in children and young adults and is associated with low-income areas in which poor sanitation is prevalent.[Crump JA et al Clin Microbiol Rev. 2015 Oct]
- In 2000, typhoid fever was estimated to cause 21.7 million illnesses and 216,000 deaths globally, and the International Vaccine Institute estimated that there were 11.9 million cases of typhoid fever and 129,000 deaths in low to middle-income countries in 2010.However, these numbers are more than likely an under-representation of the true disease burden given a large proportion of patients are treated on an outpatient basis or receive

no treatment at all. In the United States, approximately 200 to 300 cases of Salmonella enterica serotype typhi are reported each year, and approximately 80% of these cases are from travelers returning from an endemic region.In the pre-antibiotic era, mortality rates were 15% or greater. However, mortality rates have fallen to less than 1% with the introduction of antibiotics.[Parry CM et al PubMed 2012][Luby SP et al Bull World Health Organ. 2004 May]

Transmission

- Contaminated foods are often of animal origin, such as poultry, pork, beef, poultry, milk, or eggs, but all foods, including vegetables may become contaminated.
- Many raw foods of animal origin are frequently contaminated, but fortunately, thorough cooking kills Salmonella.
- Food may also become contaminated by the unwashed hands of an infected food handler, who did not wash his or her hands adequately after using the bathroom.
- Salmonella may also be found in the feces of some pets, and people can become infected if they do not wash their hands after contact with animals.
- Reptiles and baby chicks/ducklings are particularly likely to harbor Salmonella.
- People should always wash their hands immediately after handling these animals.
- Adults should also be careful that children wash their hands after handling a reptile**.[** Susan W.M. Hendriksen et al Utrecht University, Utrecht, the Netherlands]



Figure(1) transmission of salmonella

signs and symptoms of typhoid fever and paratyphoid fever?

- Typhoid fever and paratyphoid fever have similar symptoms. People usually have a sustained fever (one that doesn't come and go) that can be as high as 103–104°F (39–40°C).

- Sick with stomach pains

- Other symptoms of typhoid fever and paratyphoid fever include

- Weakness

- Stomach pain

- Headache

- Diarrhea or constipation

- Cough

- Loss of appetite

- Some people with typhoid fever or paratyphoid fever develop a rash of flat, rose-colored spots.[Chen PL, Chang CM, Wu CJ, Ko NY, Lee NY, et al. (2007)]

Diagnosing

- The diagnosis of salmonellosis requires bacteriologic isolation of the organisms from appropriate clinical specimens. Laboratory identification of the genus Salmonella is done by biochemical tests; the serologic type is confirmed by serologic testing. Feces, blood, or other specimens should be plated on several nonselective and selective agar media (blood, MacConkey, eosin-methylene blue, bismuth sulfite, Salmonella-Shigella, and brilliant green agars) as well as intoenrichment broth such as selenite or tetrathionate. Any growth in enrichment broth is subsequently subcultured onto the various agars. The biochemical reactions of suspicious colonies are then determined on triple sugar iron agar and lysine-iron agar, and

a presumptive identification is made. Biochemical identification of salmonellae has been simplified by systems that permit the rapid testing of 10–20 different biochemical parameters simultaneously. The presumptive biochemical identification of Salmonella then can be confirmed by antigenic analysis of O and H antigens using polyvalent and specific antisera. Fortunately, approximately 95% of all clinical isolates can be identified with the available group A-E typing antisera. Salmonella isolates then should be sent to a central or reference laboratory for more comprehensive serologic testing and confirmation.][Baron S, editor et al Medical Microbiology. 4th edition.]

PATHOGENISIS

- To be fully pathogenic, salmonellae must possess a variety of attributes called virulence factors. These include (1) the ability to invade cells, (2) a complete lipopolysaccharide coat, (3) the ability to replicate intracellularly, and (4) possibly the elaboration of toxin(s). After ingestion, the organisms colonize the ileum and colon, invade the intestinal epithelium, and proliferate within the epithelium and lymphoid follicles. The mechanism by which salmonellae invade the epithelium is partially understood and involves an initial binding to specific receptors on the epithelial cell surface followed by invasion. Invasion occurs by the organism inducing the enterocyte membrane to undergo “ruffling” and thereby to stimulate pinocytosis of the organisms .Invasion is dependent on rearrangement of the cell cytoskeleton and probably involves increases in cellular inositol phosphate and calcium. Attachment and invasion are under distinct genetic control and involve multiple genes in both chromosomes and plasmids.[

Galveston (TX): University of Texas Medical Branch at Galveston; 1996.]

- After invading the epithelium, the organisms multiply intracellularly and then spread to mesenteric lymph nodes and throughout the body via the systemic circulation; they are taken up by the reticuloendothelial cells. The reticuloendothelial system confines and controls spread of the organism. However, depending on the serotype and the effectiveness of the host defenses against that serotype, some organisms may infect the liver, spleen, gallbladder, bones, meninges, and other organs .[Giannella RA, Formal SB, Dammin GJ. et al. Pathogenesis of salmonellosis]
- Fortunately, most serovars are killed promptly in extraintestinal sites, and the most common human Salmonella infection, gastroenteritis, remains confined to the intestine.
- After invading the intestine, most salmonellae induce an acute inflammatory response, which can cause ulceration. They may elaborate cytotoxins that inhibit protein synthesis. Whether these cytotoxins contribute to the inflammatory response or to ulceration is not known. However, invasion of the mucosa causes the epithelial cells to synthesize and release various proinflammatory cytokines, including: IL-1, IL-6, IL-8, TNF-2, IFN-U, MCP-1, and GM-CSF. These evoke an acute inflammatory response and may also be responsible for damage to the intestine. Because of the intestinal inflammatory reaction, symptoms of inflammation such as fever, chills, abdominal pain, leukocytosis, and diarrhea are common. The stools may contain polymorphonuclear leukocytes, blood, and mucus.[. J Clin Invest.;et al pubmed,2013]
- Much is now known about the mechanisms of Salmonella gastroenteritis and diarrhea. And summarize the pathogenesis of

Salmonella enterocolitis and diarrhea. Only strains that penetrate the intestinal mucosa are associated with the appearance of an acute inflammatory reaction and diarrhea ; the diarrhea is due to secretion of fluid and electrolytes by the small and large intestines. The mechanisms of secretion are unclear, but the secretion is not merely a manifestation of tissue destruction and ulceration. Salmonella penetrate the intestinal epithelial cells but, unlike Shigella and invasive E. coli, do not escape the phagosome. Thus, the extent of intercellular spread and ulceration of the epithelium is minimal. Salmonella escape from the basal side of epithelial cells into the lamina propria. Systemic spread of the organisms can occur, giving rise to enteric fever. Invasion of the intestinal mucosa is followed by activation of mucosal adenylate cyclase; the resultant increase in cyclic AMP induces secretion. The mechanism by which adenylate cyclase is stimulated is not understood; it may involve local production of prostaglandins or other components of the inflammatory reaction. In addition, Salmonella strains elaborate one or more enterotoxin-like substances which may stimulate intestinal secretion. However, the precise role of these toxins in the pathogenesis of Salmonella enterocolitis and diarrhea has not been established.[Galveston (TX): University of Texas Medical Branch at Galveston; 1996.]

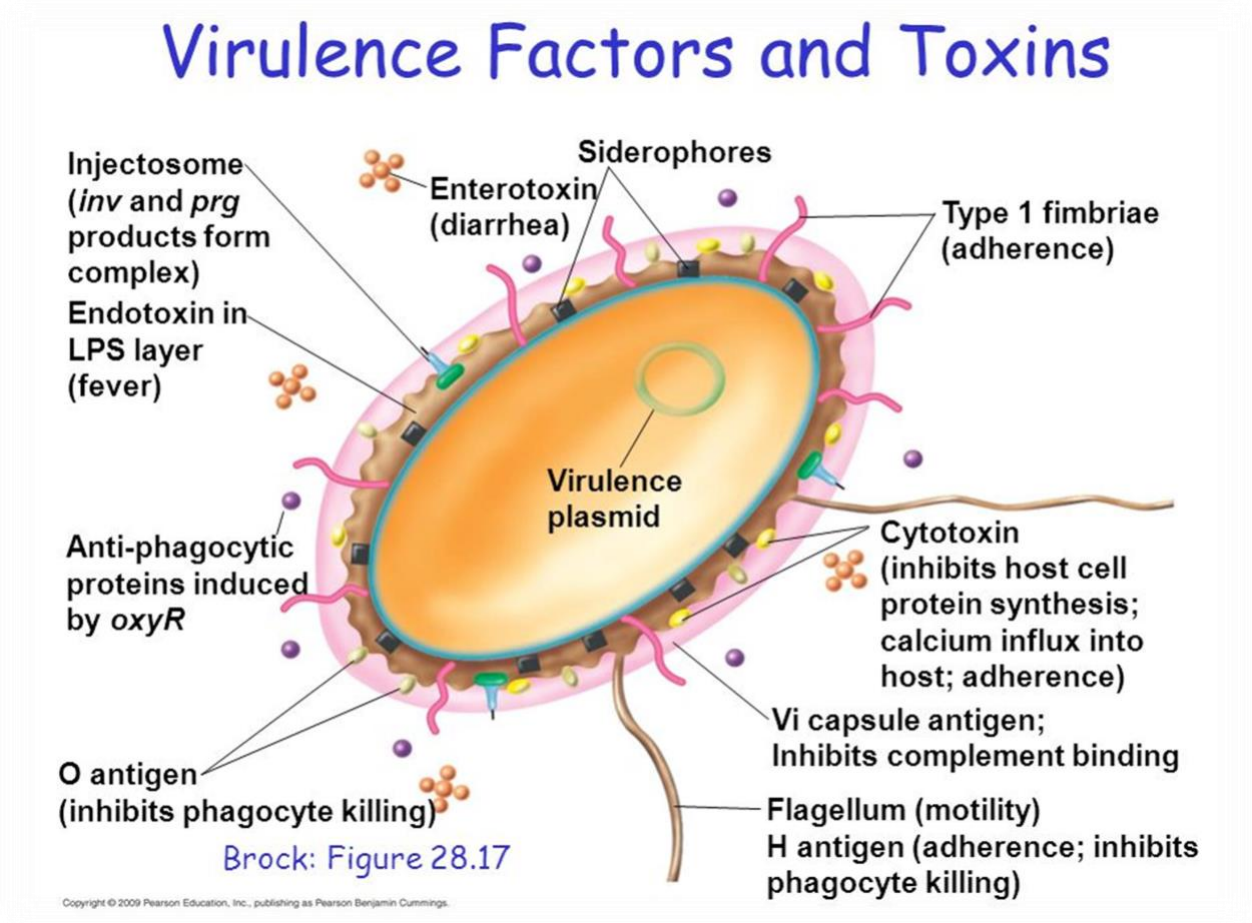


Figure (2) virulence factors of salmonella

Treatment:

Treatment of salmonellosis depend on severity and health problem and area of living

- **Non-typhoidal salmonellosis**

a-chlormephenicol ,trimethoprim/sulfamethaxazole ,ampicillin :these drugs used from 1980 but MDR SALMONELLA PRSENT 30-40 % OF INFECTED PERSON

B-FLOUROQUINOLONES : NALDIXIC ACID has been used but emerg resistant , ciprofloxacin 500 mg twice daily for 7to 10 days and

levofloxacin have been used and effective 500mg once daily for 7 to 10 days

C resistance to fluoroquinlone use azithromycin as alternative

d- third generation cephalosporines show good efficacy in resistance to fluoroquinolone

also carbapenem effective but resistance have been reported

2-gastroenteritis caused by salmonella it self limited and need only fluid and electrolyte

3- Patient who are at risk for extraintestinal salmonella infection: oral fluoroquinolone(levofloxacin 500mg once daily for 7to 10 days), azithromycin 500mg once daily for 7days,ceftriaxone 1g to 2g daily for 7 to 10 days
4- Bacteremia induced by salmonella :fluoroquinolone alone (levofloxacin 750mg once daily or ciprofloxacin 500 mg to 750mg for 10 days) or combination of fluoroquinolone with third generation cephalosporine (ceftriaxone)

- **Typhoidal salmonellosis**:

  Aim of treatment is decrease mortality , shortening the resolution of fever ,eradication of fecal shedding salmonella ,prevent s typhi relapse of infection

  a- Fluoroquinolone (effective more than tmp/smx, ampicillin,chlormephenicol)

     It cosist of ciprofloxacin , levofloxacin . gatifloxacin 95% effective in children

  b- Azithromycin can be use as alternative in 20 mg /kg /day

c- Cephalosporines (cefixime ,ceftriaxone)

Chronic typhoidal carriers

Amoxicillin 2g 3times for 28 days

Ampicillin 1g 4times for 6weeks

Ciprofloxacine 500 mg to750 mg twice daily for 3-4 weeks

Norfloxacin 400 mg twice daily for 4 weeks. [TREAMENT OF SALMONELOSIS ,APPLIED THERAPIEUTICS 11TH EDITHION ,BY CAROLIN S. ZEINED .]

Salmonella resistance :

-In Salmonella the main mechanisms of antibiotic resistance are mutations in target genes (such as DNA gyrase and topoisomerase IV) and the over-expression of efflux pumps.

- The horizontal transmission of resistance genes plays a vital role in the dissemination of antibiotic resistance in Salmonella enterica species. These resistance genes can be found in the resistant plasmids or within the chromosome of bacteria

- However, other mechanisms such as changes in the cell envelope; down regulation of membrane porins; increased lipopolysaccharide (LPS) component of the outer cell membrane; quorum sensing and biofilm formation can also contribute to the resistance seen in this microorganism.

- Overcome MDR Salmonella

To overcome this problem new therapeutic approaches are urgently needed.

In the case of efflux-mediated multidrug resistant isolates, one of the treatment options could be the use of efflux pump

inhibitors (EPIs) in combination with the antibiotics to which the bacteria is resistant. By blocking the efflux pumps resistance is partly or wholly reversed, allowing antibiotics showing no activity against the MDR strains to be used to treat these infections.

Compounds that show potential as an EPI are therefore of interest, as well as new strategies to target the efflux systems.

Quorum sensing (QS) and biofilm formation are systems also known to be involved in antibiotic resistance. Consequently, compounds that can disrupt or inhibit these bacterial "communication systems" will be of use in the treatment of these infections. (Griggs D.J., Gensberg K., Piddock L.J .et. pubmed.2000)

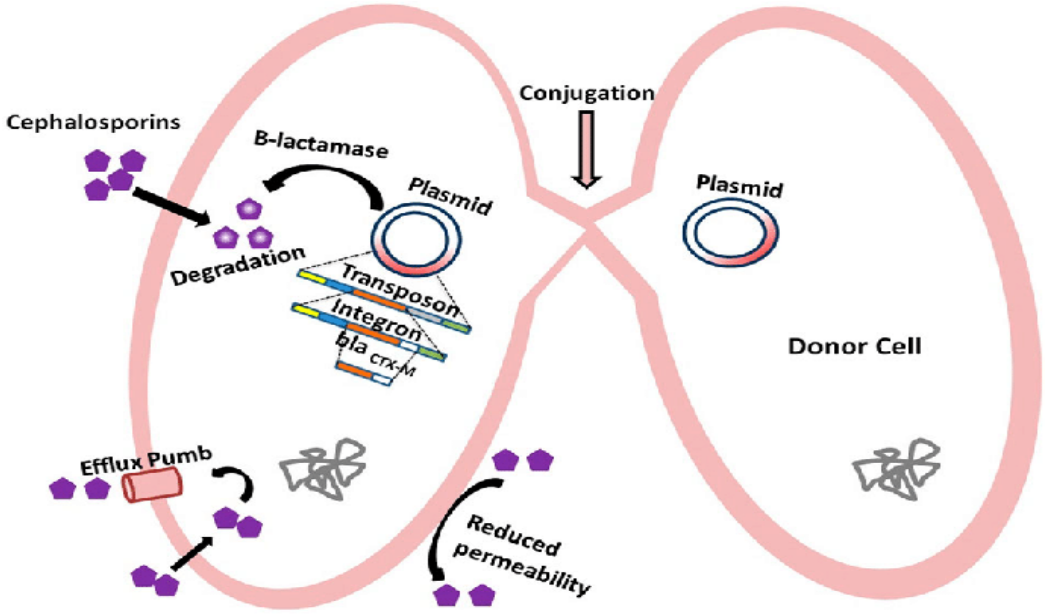


Figure (3) resistance mechanism of salmonella

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DATA COLLECTION

- the data collected from margan general hospital from 1/11/2021 to 15/3/2022

There is 370 patient suspected and 41 only confirmed

-the data were collected from babil hospital for gyne and ped from 15/1 to 15/3/2022

There is 3358 admission with fever and 115 suspected as salmonellosis and only 32 confirmed as salmonellosis

- the data collected from alkafel general hospital from 15/1 to 15/4/2022

There are 633 suspected and only 59 confirmed salmonellosis

RESULT:

- TABLE (1) Distribution of salmonella according to age :

AGE GROUPS	NO. OF PATIENT
1-10 YRS	28
11-20YRS	5
21-30YRS	19
31-40 YRS	11
41-50 YRS	22
51-60 YRS	29
61-70YRS	18

- Table (2) Distribution of salmonella according to

Area of living	No of patient
rural	86
urban	46

- Table (3) explain the antibiotics for treatment of salmonellosis

antibiotics	No of patients	Clinical cure rate
ceftriaxone	53	87%
ciprofloxacin	24	84%
Azithromycin	17	75%
levofloxacin	10	90%
Chloromaphenicol	2	resistance
Ceftriaxone+amikacin	5	100%
Ceftriaxone +meropenem	5	100%
Ceftriaxone +ciprofloxacin	6	100%
Ceftriaxone +vancomycin	10	100%

DISCUSSION :

As results :-

- **Children** under 5 years old are the most likely to get a Salmonella infection. **Infants** (children younger than 12 months) who are not breast fed are more likely to get a Salmonella infection. **adults** aged 55 and older, and people with a weakened immune system are the most likely to have severe infections.
  - Spread more in rural area because these diseases are spread through sewage contamination of food or water with animal infected with salmonella.
  - -**Non-typhoidal** salmonellosis typically resolves in a few days without treatment, but will be treated with rest and rehydration. Patients with severe diarrhea or dehydration, however, will be hospitalized and put on intravenous fluids and electrolytes، Innate and acquired immune response for non typhoidal Salmonella is enough to eradication organis , however in typhoid Salmonella immune response need support drug to complete eradication of Salmonella
  - -**Typhoid fever** is always treated with antibiotics, if possible. The mortality rate for untreated typhoid fever is 10-30%; antibiotic treatment lowers the mortality rate to 1%. Also, typhoid fever can cause intestinal tearing (perforation) that will require surgery.
  - Common first-line oral antibiotics for susceptible Salmonella infections are **fluoroquinolones (for adults) and azithromycin (for children)**. Ceftriaxone is an alternative first-line treatment agent ,Fluoroquinolones act against Salmonella by inhibiting their DNA replication. However, several zoonotic serotypes of Salmonella have developed resistance or are less susceptible to fluoroquinolones. Salmonella presents its resistance by substituting amino acids within the topoisomerase subunits, overexpression of multidrug efflux pumps, or decreasing the expression of outer

membrane porins. The resistance level is further increased with the plasmid-mediated quinolone resistance genes which could horizontally transfer the resistance from strain to strain. The development of resistance in Salmonella shows that it is a multifactorial process and the acquisition of fluoroquinolone resistance might have significant influences on the bacterial fitness and virulence.

- **Azithromycin** has been used as an alternative drug for treating typhoid fever. It achieves low intravascular levels, has high intracellular tissue penetration, and a long elimination half life of 72 hours mechanism of resistance to azithromycin a non-synonymous mutation (R717Q) in the acrB gene, which encodes an efflux pump.
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- The third-generation cephalosporin, ceftriaxone, **has been extensively used for the treatment of invasive Salmonella infection** , to ceftriaxone, was associated with carriage of a combination of bla ctx-m-15, bla oxa-1, and bla tem-1 genes. The genes encoding resistance to heavy-metal ions were borne on the novel IncHI2 plasmid pKST313, which also carried a pair of class 1 integron
- The combination of antibiotics have been used for treatment of s.typhi because of emerging resistance of these species .

CONCLUSION :

1- Children under 5 years old are the most likely to get a Salmonella infection.
2- Spread more in rural area because these diseases are spread through sewage contamination of food or water with animal infected with salmonella.
3- Selection of suitable antibiotics for treatment of salmonellosis most important challenge because of emerged resistance of salmonella
4- The main causative agent for resistance is gene mutation by plasmid transmission to non plasmiod bacteria.
5- The combination of antibiotics have been used for treatment of S.typhi because of emerging resistance of these species.

References:

-Advances in Microbial Physiology Volume 56, 2009, Pages 1-28

-Microbes and Infection Volume 15, Issue 4, April 2013, Pages 270-279

-Balamurugan Periaswamy AFFILIATION Institute of Microbiology, D-BIOL, ETH Zürich, Zürich, Switzerland

-Lisa Maier , Vikalp Vishwakarma , Emma Slack AFFILIATION Institute of Microbiology, D-BIOL, ETH Zürich, Zürich, Switzerland

-Medical Microbiology. 4th edition. Baron S, editor.

-Galveston (TX): University of Texas Medical Branch at Galveston;1996. CHAPTER 21

-Enteric Diseases Laboratory Branch, Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

- National Salmonella Reference Laboratory, Division of Foodborne, Waterborne, and Environmental Diseases, MS-C03, Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA

- Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, Clinical Presentation, Laboratory Diagnosis, - Antimicrobial Resistance, and Antimicrobial Management of Invasive Salmonella Infections. Clin Microbiol Rev. 2015 Oct;28(4):901-37. [PMC free article[

- Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med. 2002 Nov 28;347(22):1770-82. [PubMed]

-Luby SP, Crump JA, Mintz ED. The global burden of typhoid fever. Bull World Health Organ. 2004 May;82(5):346-53. [PMC free article] [PubMed[

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-Chen PL, Chang CM, Wu CJ, Ko NY, Lee NY, et al. (2007) ------ Extraintestinal focal infections in adults with nontyphoid Salmonella bacteraemia: predisposing factors and clinical outcome. J Intern Med 261: 91–100-

-Medical Microbiology. 4th edition. Ralph A. Giannella

-Galveston (TX): University of Texas Medical Branch at Galveston; 1996. CHAPTER 21

- Giannella RA, Formal SB, Dammin GJ. et al. Pathogenesis of salmonellosis. Studies of fluid secretion, mucosal invasion, and morphologic reaction in the rabbit ileum. J Clin Invest. 1973;52:441. [PMC free article] [PubMed[

- TREAMENT OF SALMONELOSIS ,APPLIED THERAPIEUTICS 11TH EDITHION ,BY CAROLIN S. ZEINED.

-Griggs D.J., Gensberg K., Piddock L.J. Mutations in gyrA gene of quinolone-resistant Salmonella serotypes isolated from humans and animals. Antimicrob. Agents Chemother. 1996;40:1009–1013. doi: 10.1128/AAC.40.4.1009. [] [PubMed]