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RESEARCH ARTICLE

ANTIMICROBIAL RESISTANCE OF SALMONELLA SPECIES ISOLATED FROM DIFFERENT ENVIRONMENTS IN SAUDI ARABIA AT MAKKAH REGION

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Abstract

Salmonellais the most widespread type of bacteria, which causes many diseases around the world, as it spreads in developed and developing countries alike, and its resistance to antibiotics, which has recently increased, has caused panic and concern about global public health, as its spread in various countries of the world is almost at the same rate.The lack of control and quality of food and beverages, which leads to their contamination to a large extent, is one of the biggest reasons for the spread of Salmonella in different environments, in addition to infection with humans. So,Comparing ofSalmonella isolates from different environments with those isolated from humans, whether blood or feces, through their resistance to antibiotics was our target.In this study, 58 samples were isolated from different environments, which were divided into 18 samples of human feces - 2 samples of human blood, 24 samples of different types of vegetables. In addition to 2 samples from each of the sewage water and 2 of well water, and 10 samples were isolated from chicken internal organs.The isolates were identified by BPW, SSA and XLD media, as well as using of BECKMA COULTERcompact system for biomedical identification and test the antibiotics resistance of isolates.The results came out positive, 20 samples matching the phenomena, the morphology on the dishes of the different culture media, in addition to the physiological results of BECKMA COULTER. The sensitivity of the isolates to antibiotics was somewhat different from previous studies, where all the isolates were resistant to Ampicillin, and all of them were sensitive to many types of antibiotics such as Cefepime - Levofloxacine - Amoxcillin / clav and other antibiotics. Salmonella is found in different environments and spreads widely, which increases scientific health concern, as it increases its resistance to antibiotics known for their strength.

Introduction:-

Salmonella spreads in many different environments and causes constant disturbance to global health due to the various diseases it causes, despite the development and differences in control and quality systems for food and

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beverages. (Sánchez-Vargas et al;2011).Salmonella belongs to the family of Enterobacteracae and characterized with Gram negative, rod, facultative anaerobic bacteria (Holt et al; 1994 and Hataet al; 2016)with a diameter ranging between 0.7-1.5 µm, while its size ranges between 2-5 µm. motile(Ellermeier&Slauch;, 2006; Costa et al; 2012 and Patel et al; 2020). Due to its facultative pathway of respiratory it has mulible active enzymes such as it is positive to catalase,methylred, H_2S production, in addition to Simmons Citrate, and it is negative to oxidase, urease, Voges-Proskaurin addition to the indole test (Holt et al; 1994 and; Percivalet al; 2004 and Aktaret al; 2016).Salmonella was identified as the main cause of typhoid fever in 1873 by William Budd, and was confirmed to be the cause of the disease when it was isolated from a patient's bedside by George Jaffke in 1885(Ellermeier&Slauch, 2006 and Ashurstet al; 2022).There are 80 repeating forms for 2541 species falling intwo main subspecies of the two main genera of Salmonella(de Freitas et al; 2010).Because of this large number of Salmonella, genetic classification(Timbury; 2002) and classification based on the types of flagella were relied upon to identify Salmonella(Percival et al; 2004, Puiet al; 2011). Salmonella causes many diseases, the most famous of which is typhoid fever, which the World Health Organization indicates causes 1,222,000 deaths annually(Ochiaiet al ;2008 and Ni et al ;2017), with the symptoms that can be caused by salmonella ranging from headaches and high temperature to death(Amiciziaet al;2017).

In addition to the high ability of Salmonella to infect and cause disease, Salmonellaspis found in different environments of soil, water, food, etc. in almost everywhere around us (Abakpa et al; 2015).Contrary to what is widespread, the incidence of Salmonella is more in developing countries, where there is a lack of general hygiene, lack of control over the quality of food and beverages, and pollution occurring in almost everything. However, environmental changes, civilizational progress, and interference in the food chain in developed countries are a clear and fundamental reason for the spread of Salmonellasp in these countries. Therefore, we have to accept the idea of not eradicating Salmonellasp, but we must control the rate of its spread and the occurrence of infection, because it is an environmental organism whose elimination leads to interference in the food chain and the environment in general (Lindqvist; 2008).

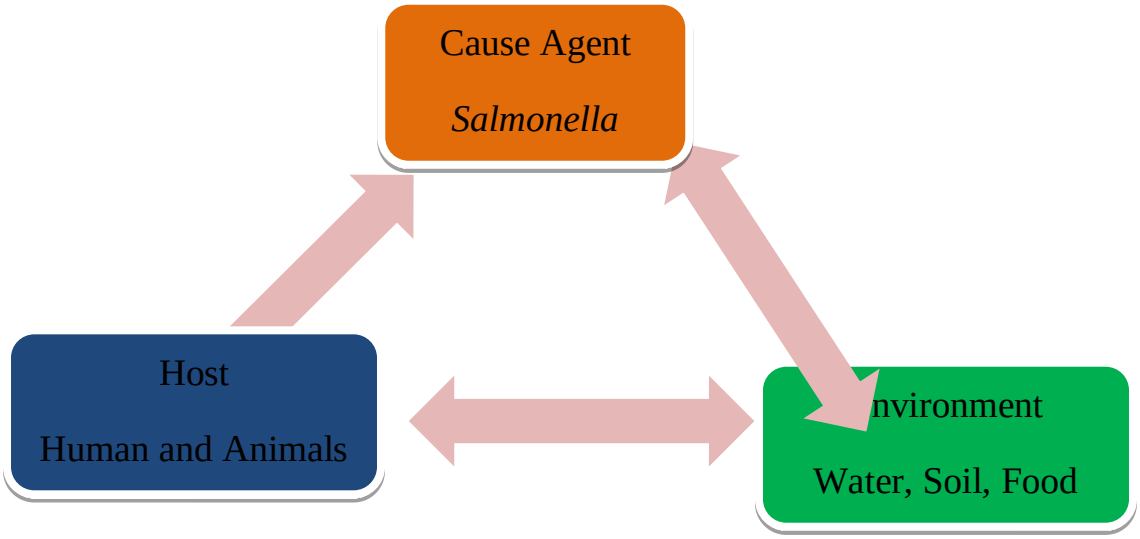


Fig.1:- Illustrates the relationship of the spread of Salmonellasp in various environments as a result of improper handling of the host (human and animal) with the environment, especially after human intervention in causing disruptions in the food chain and lack of control over the quality of food and beverages, which could lead to infection with Salmonella.

Salmonella infection rates came at a rate of 6.6% in North Africa and the Middle East, where Jordan and Oman came second after Morocco and Tunisia(Al-Rifaiet al;2019). Salmonella resistance to fluoroquinolone antibiotics had a greater prevalence in countries such as Kuwait and Qatar (Moghniehet al;2019). 0.9 % Of people with acute diarrhea in KSA were diagnosed with the presence of Salmonellasp, and this was attributed to the presence of Salmonella in the environment, meat and fish intended for human consumption (Elhadiet al;2013).The spread of food contamination with bacteria with what is known as food poisoning in the whole world and led to a clear threat to global food security, and among this threat was the outbreak of food accidents with Salmonellaspbacteria in many countries of the world such as the United Kingdom (UK), the United States (US) and Japan, beside the Arab

countries (World Health Organization;2002), In this part we will mention such food accidents in the Kingdom of Saudi Arabia, where it was seen in the recent period, recorded incidents of food poisoning with Salmonellasp bacteria, and the efficiency of the treatment of the authorities responsible for health and food security has been improved to deal with such incidents (Ministry of Health;2002). the World Health Organization has told that Salmonella is one of the most resistant bacteria to antibiotics, most of these antibiotics are; gentamicin, streptomycin ,amoxicillin , nalidixic acid ciprofoxacin , chloramphenicol , co-trimoxazole and tetracycline(WHO;2018).

Fig.2:- The percentage (%) of Salmonella serotypes is calculated from the positive samples of different environment in food chain of Arab world (Habibet al; 2021).

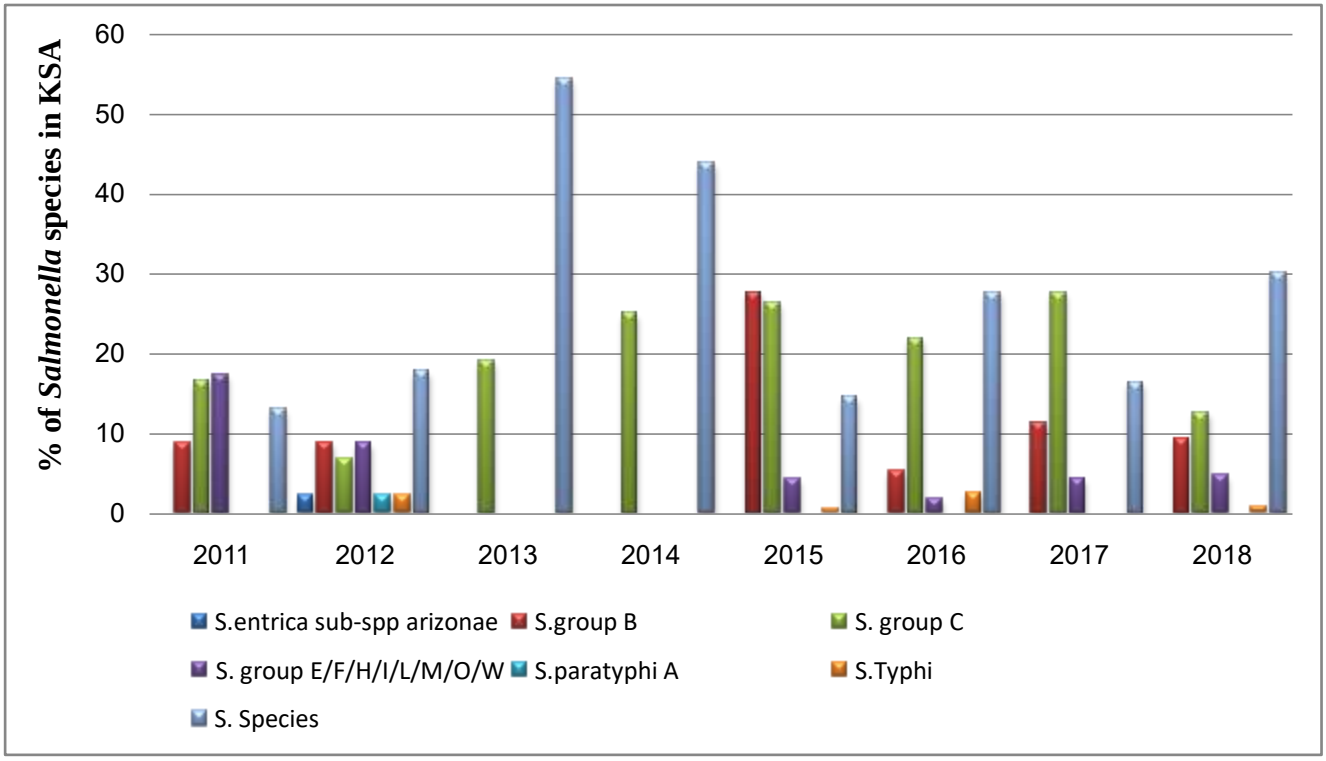


Fig 3:- Frequency of different Salmonella groups isolated from patients in KSA from (2011 to 2018) (Aljindan&Alkharsah;2020).

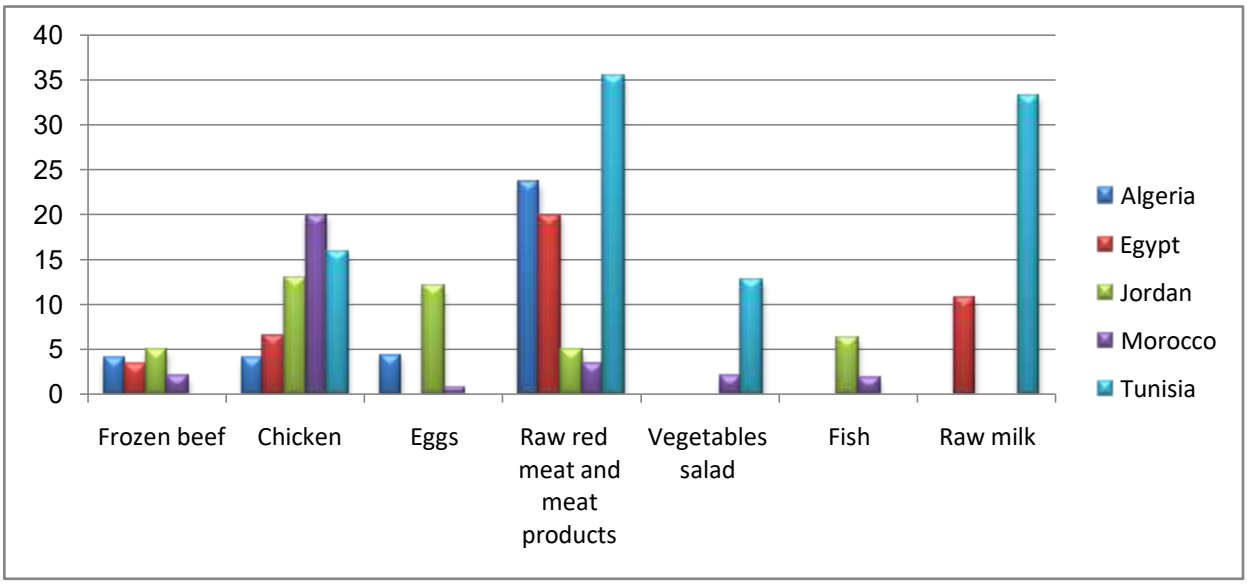
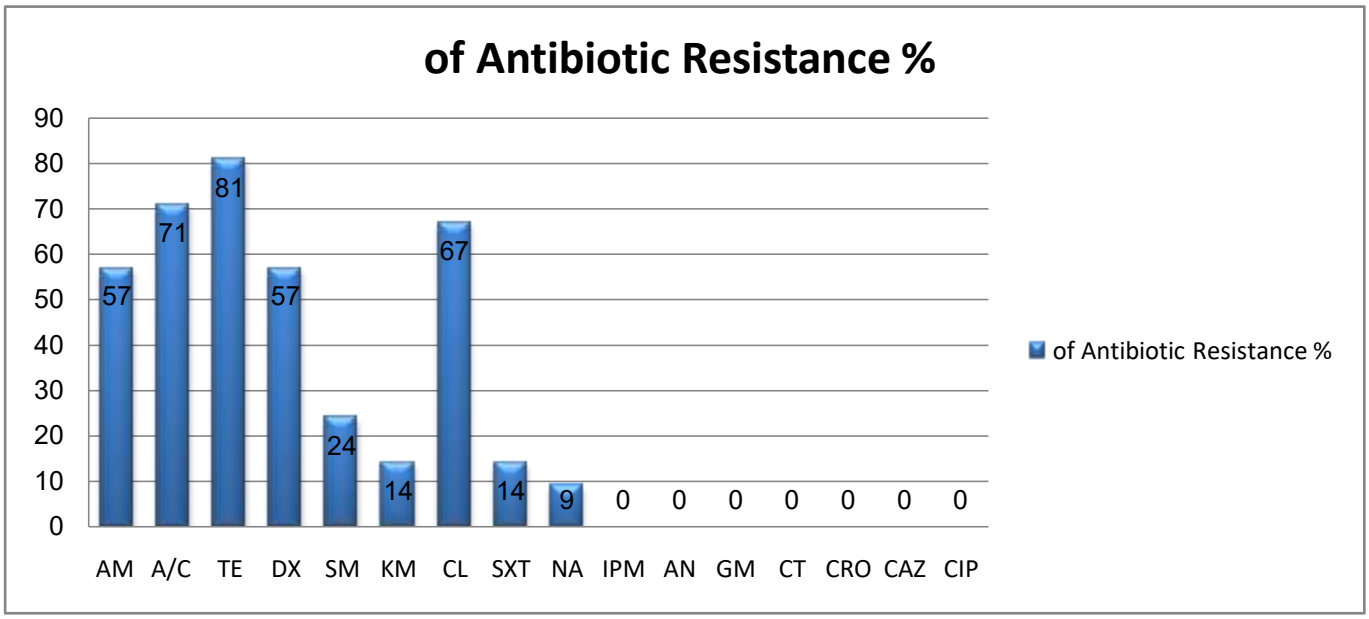


Fig.4:- Rate (%) of antibiotic resistance in Salmonella typhimurium strains isolated from clinical samples from Tehran hospitals. AM: Ampicillin; A/C: Amoxicillin/clavulanic acid; TE: Tetracycline; DX: Doxycycline; SM: Streptomycin; KM: Kanamycin; CL: Chloramphenicol; SXT: Trimethoprim/sulfamethoxazole; NA: Nalidixic acid; IPM: Imipenem; AN: Amikacin; GM: Gentamicin; CT: Cefotaxime; CRO: Ceftriaxone; CAZ: Ceftazidime; CIP: Ciprofloxacin (Ranjbaret al; 2017).



Material and Methods:-

Chemicals and Instruments:

XLD (Xylose Lysine Desoxycholate Agar) (MOLEQULE, Auckland, New Zealand)
SSA (Salmonella Shiglla Agar) (MOLEQULE, Auckland, New Zealand)
BPW (Buffer Peptone Water) (OXOID)
RSB Rappapot Soy Broth (VASSILIADIS, MOLEQULE-NO)
BECKMA COULTER (MICROSCAN WALK AWAY 96 PLUS) 4911 Lab Compact system with Escherichia coli ATCC 25922, Pseudomonas aeruginosa NCTC 10662 and Salmonella enterica subspecies entericaserovarTyphimurium ATCC 14028 served as a control.

Bacterial collection and Isolation:

A total of 58 clinical and environmental samples were collected form Makkah, Jadahh and Taif cities and examined for the presence of Salmonellaesp. The 58 samples were consisted of human feces samples (18), human blood samples (2), well water (2), raw sewage (2), poultry intestinal swabs (6), poultry cecal contents (4); raw vegetables (24), all samples were collected between September 2022 and December 2022.

The instructions shown on each package of chemicals were followed for preparing culture media, and serial dilution was used for water samples, grinding and serial dilution was used for solid samples of vegetables and poultry.To isolate bacteria from vegetables, the method mentioned in (CAROLINA et al; 2009; Moussa et al; 2010; Nair et al; 2015; and Khan et al; 2022) was followed.For water samples, the method mentioned in (Abulreesh et al; 2004; Nair et al; 2015 and Ofred et al; 2017) was followed.Among poultry, the following method was followed (Moussa et al; 2010; Nair et al; 2015; Khan et al; 2018 and Khan et al; 2022) to isolate bacteria and to isolate bacteria from human samples, (Elhadi et al; 2013 and Reza et al; 2018) were followed.All samples were at 37 degrees for 24 to 48 hours

Identification of presumptive Salmonella isolates

All colonies appearing red with black centers on XLD agar (Maddockset al; 2002 and Khan et al; 2022) were considered as Salmonella. (Elhadiet al; 2013 and Khan et al; 2022) were also used to identify confirmed Salmonella isolates according to amnufacturer's instructions.

Antimicrobial susceptibility testing of confirmed Salmonellae:
All identified Salmonellasp were tested for their antimicrobial susceptibility profiles by using BECKMA COULTER (MICROSCAN WALK AWAY 96 PLUS) 4911 Lab Compact system with Escherichia coli ATCC 25922, Pseudomonas aeruginosa NCTC 10662 and Salmonella enterica subspecies entericaserovarTyphimurium ATCC 14028 served as a control.

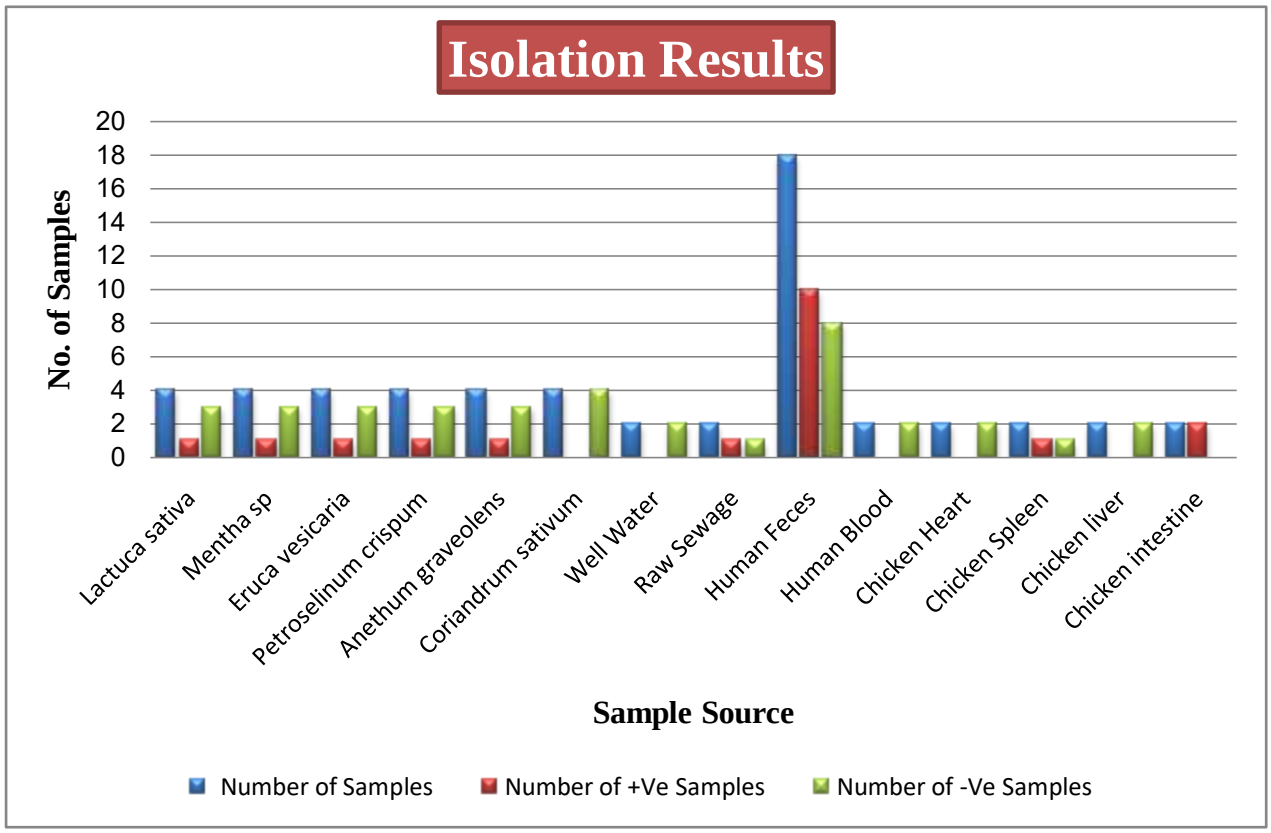
Results:-

The results of isolation and identification came for 58 samples that were isolated from different environments in addition to human feces and human blood as shown in (Table1), where the results indicated positivity of 10 samples from human feces, 1 sample from human blood, and 2 samples for each of vegetables and sewage water in addition to 5 samples from intestinal content. The results also showed that all samples taken from well water were negative.

Table 1:- Numbers of positive and negative isolates for samples taken from different environments.

Sample type	N	P	Presumptively confirmed isolates†
Human Feces	18	10	10
Human Blood	2	0	0
Raw Vegetables	24	5	5
Sewage Water	2	1	1
Well Water	2	0	0
Intestinal cotent	10	4	4
Total	58	20	20
P††	<0.001		
P‡	0.001<		

Fig 5:- Shows the numbers of isolates used and their sources where they were extracted, in addition to number of positive samples of them.



The 20 positive samples previously described were tested to measure their sensitivity to different antibiotics, using the BECKMA COULTER system, and the results were as shown in Table No (5).The results of the sensitivity test for different antibiotics showed that all samples were resistant to Ampicillin, in addition to that all samples were sensitive to 12 types of antibiotics, namely (Amox/Clav- Aztreonam- Cefepime- Cefotaxime- Ceftazidime- Ertapenem- Imipenem- Levofloxacin- Meropenem- Pip/ Tazo - Tigecycline- Trimeth/Sulfa) in addition to ciprofloxacin, which showed an effective effect on all isolates except (**VD5 - VE6 - SW5 - C1I - C2I -C2S** and **MX9**). The results also showed that the antibiotics had an intermediate effect for each of (Amp/Sulbatam), except for the sample **ST5**, which was resistant to it, and the effect of (Moxfloxacin) was moderate on all samples except **ST2 - ST4** and **ST9**, and its effect on them was very effective. In addition, all samples showed a sub-MIC response to each of (Amikacin- Cefazolin- Cefotaxime/kc- Cefoxitin- Ceftazidime/k- Cefuroxime- Colistin- Gentamicin- Nitrofurantoin- Norfloxacin- Tobramycin).

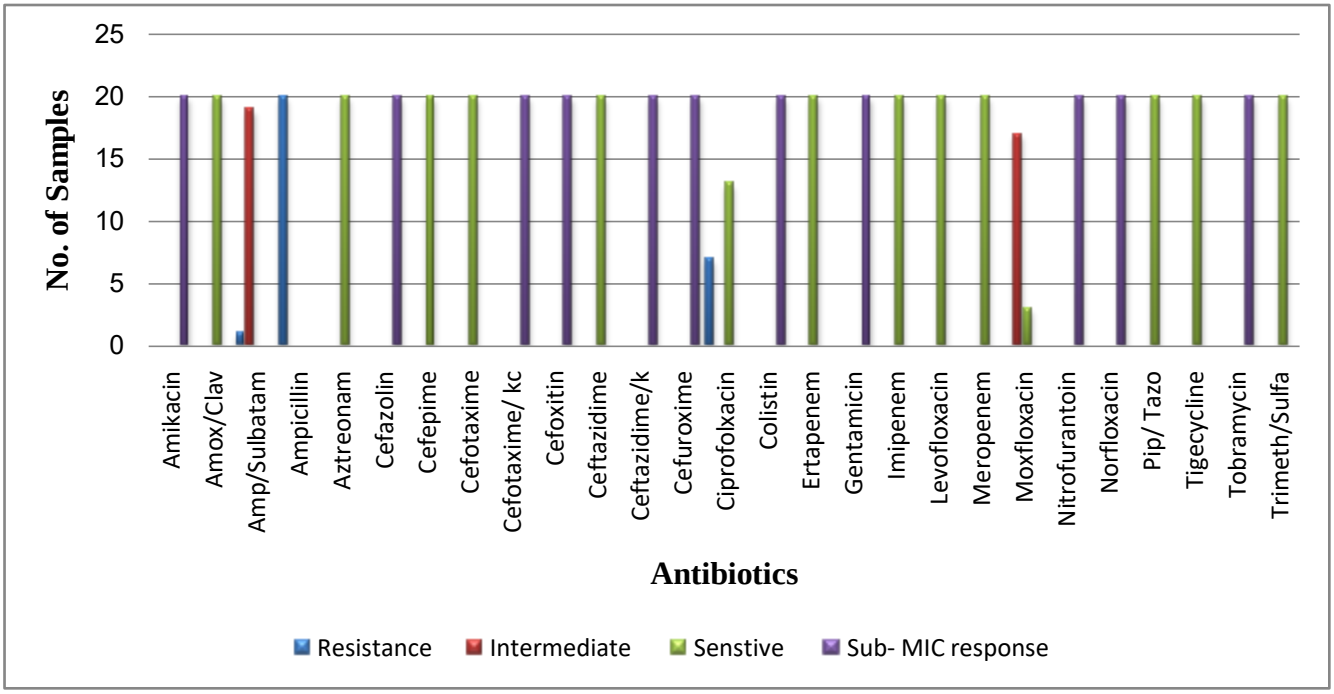
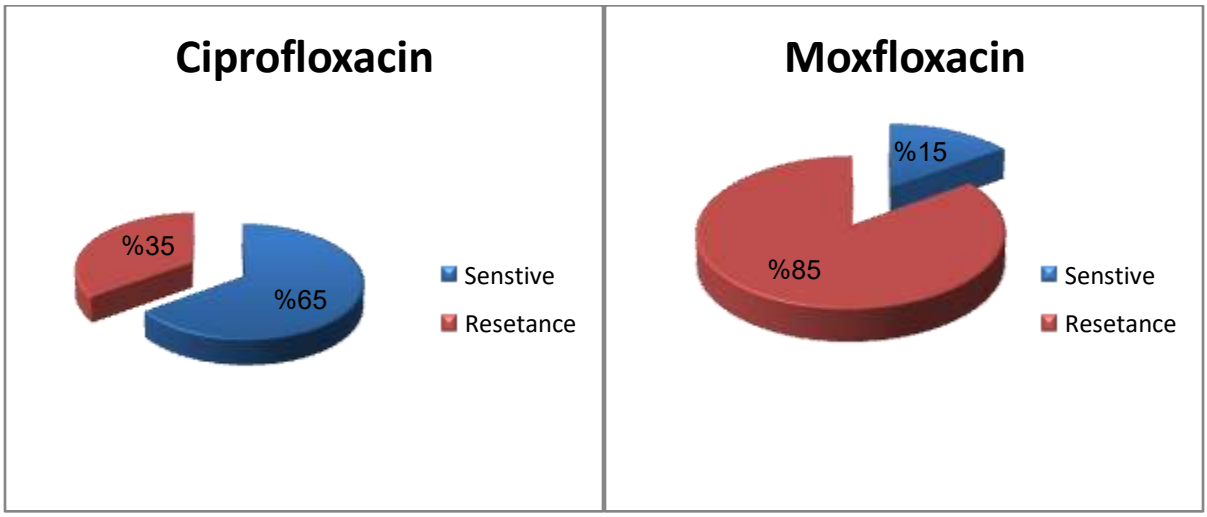


Fig 6:- Antibiotic Resistances to Salmonellasp Isolates.



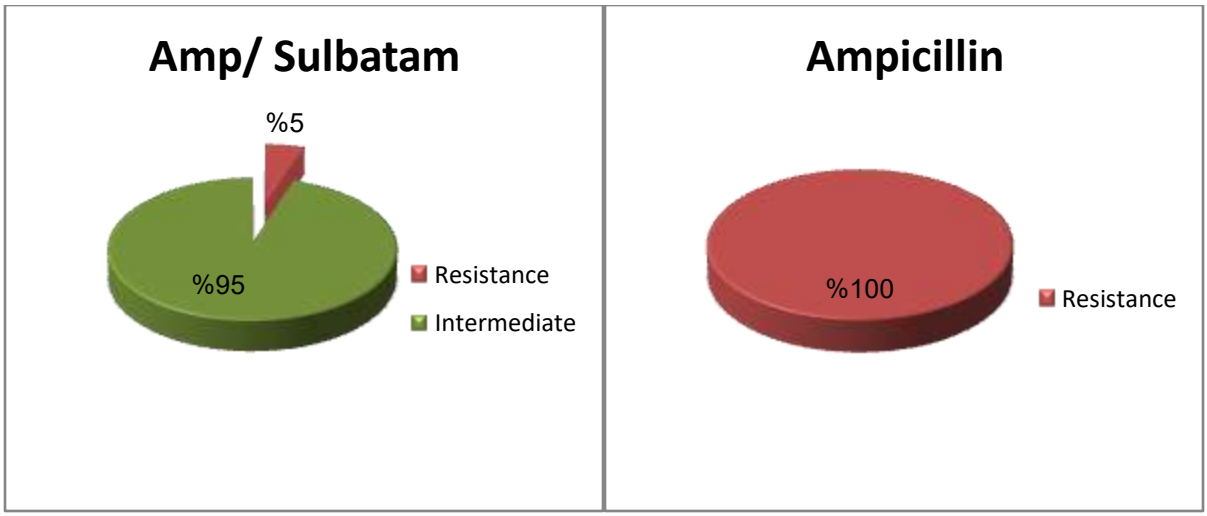


Fig 7:- Shows the distinct types of antibiotics that were used in testing the sensitivity of isolates to antibiotics.

Discussion:-

Salmonella is a bacteria that spreads in different environments (Linscott;2011) and causes many diseases, especially in developing countries (Majowiczet al; 2010 and de Freitaset al; 2010), where there is no adherence to protection systems and prevention of diseases related to eating and drinking, or even personal hygiene to an adequate extent, due to the absence of health control or quality control over food and beverages (Nairet al; 2015) in some of these countries due to their poverty The basic capabilities and resistances to this process, and in some others, the matter is due to old cultures or unhealthy habits (CDC;1999).Salmonella infection spreads in different environments, as previously mentioned, and most of its serums show different intestinal and infectious diseases, and we find this consistent with what previous studies reported regarding the spread of Salmonellasp through foods, vegetables, and fruits, in addition to its direct transmission through water or dealing with pets, (Waldner et al; 2012)confirming that in his study of Salmonella in North America.

Fifty-eight samples were isolated in this study from different environments, such as human blood and feces, and some of the leafy vegetables spread in our Arab world, in addition to samples from drinking water and sewage, as well as samples from the internal guts of poultry as an example of the food environment.

The results came as a surprise to us, as the percentage of positive isolates was 34.48%, with 20 positive samples, which is not a small percentage. Perhaps the reason for this is the presence of isolates of human feces, which are known to contain Salmonellasp since they are intestinal bacteria.

It can be clarified that the positive percentage in the isolation samples is large, but compared to the previous literature, in which the number of isolates increased, we find that the presence of Salmonella in most of the literature is a small percentage.This is due to the practice of sound healthy habits and follow-up on the cleanliness of food and drink, in addition to the great attention paid by the sectors specialized in quality control of food and drink, in addition to the great awareness of personal hygiene, especially after the Corona virus crisis.Despite previous reports that proved that the infection with Salmonellasp is increasing and reached 30% in 2018, according to (Al-Jandan and Al-Kharsa, 2020). Despite the handling of livestock, sheep and other animals, which contributes to the spread of Salmonella infection, as mentioned (CDC; 1999; Foddaiet al 2015 and Nair et al; 2015).

On the other hand, the transmission of infection with Salmonellasp between adults may interfere with other factors related to the synthesis of Salmonella, as previous studies that have been relied on in their hypothesis that the formation of biofilm of Salmonella can play an important role in the transmission of infection (Jacqueset al; 2010). This is through the law of preserving life, which is a law preserved by pathogenic microorganisms such as bacteria, which is the formation of such thin biofilms to gather and invade the host in groups and ensure their survival, while from the point of view of infection, the formation of thin biofilms may be an anti-virulence feature (MacKenzieet al; 2017).Where it was found by comparing some Salmonellasp that make thin biofilms and others that do not have the

ability to secrete biofilm and left them to infect mice in a previous study. The result was in favor of Salmonella that do not secrete biofilm (MacKenzieet al; 2015 and Adcoxet al; 2016).

In view of the above analysis of the presence of Salmonella in simple isolates in human feces, contrary to what was expected from previous studies, whether in the world or in the KSA, we can say that the Kingdom of Saudi Arabia has a critical quality control system, as food or drinking water contaminated with Salmonellasp is low in most of the time. of the year, despite the increase in the number of infections in the season of Umrah and Hajj, due to the presence of many tourists to the holy areas, who can penetrate the health and safety system for food and water, and this is what we can call that Salmonella in humans in the Kingdom is somewhat seasonal.

In addition, some previous studies mentioned that infection with Salmonellatyphi in humans again is less severe in symptoms in 5-10% of people (Gunnet al; 2014), and it can be latent in immature white blood cells in the bone marrow in 2-4% of people (Dougan& Baker; 2014), and this means that there is a percentage even if a few people suffer from Salmonella infection and it is not detected, or it can be diagnosed in another way because of the lack of severity of the symptoms, so we did not find them, and this explains the importance of such a study to expand the consideration of the danger of Salmonella and raise awareness of infection with it.

We note in this study a slight contradiction with that literature, as out of 20 positive samples, 50% of them were for human feces, and this means that there is infection among members of the Saudi people even in non-seasons. Despite that, our study comes in complete agreement with the previously mentioned literature. Human blood samples had a positive rate of 0%.

The results of our study indicated that the isolates of poultry containing intestines had a positive percentage among the isolates of their peers, where 10 samples were isolated, of which 2 were positive, of which 20% were positive, in addition to 10% of the samples of the spleen content of poultry. . The results were similar to those of (Fanelliet al; 1971), in which he confirmed in his study that 5-45% of the gut contents of isolated chickens were samples of Salmonella-positive bacteria, in addition to that 100% of the cecal samples were positive for Salmonella (Apajalahtiet al; 2004 and Stanley et al; 2014). Looking at the results announced from 2010 to 2020 in the continent of Africa, which is considered one of the countries that use the most poultry proteins because it is unique in most countries of the continent, we find that these studies are consistent with the results of our current study, as the percentage of Salmonellasp in the isolates of those studies ranged between 12% The Zimbabwean isolates increased to 100% in samples isolated from countries such as Nigeria, Senegal, Egypt, Ethiopia and South Africa (Ramtahalet al; 2022). Returning to the continent of Asia and its poor countries, such as Bangladesh, where poultry is also a cheap and reliable source of protein, the proportion of Salmonellasp was found in intestinal swabs, which was 42% of intestinal swabs. The isolates were positive, and 60% of the isolates of the intestinal question were also positive for Salmonella (Haqueet al, 2021), which means that the intestinal tract of poultry is a fertile environment for the growth of Salmonella (Hoqueet al; 2019).

The digestive system of chickens is a fertile environment for the growth of microbes and bacteria, as more than 900 types of bacteria can grow in it;this is due to the acidic environment that the digestive system of chickens enjoys, in addition to providing means for fermentation and hydrolysis of starch into sugars, and these are the requirements for bacteria to grow(Weiet al; 2013 and Miccicheet al; 2018). The reason for this is due to several factors, mainly the fodder that poultry feeds on, as some farms use some high-protein feed such as cheap fish feed, which causes Salmonellasp contamination (Okoliet al;2006 and da Costaet al; 2007).In addition to the lack of cleanliness of slaughter tools in many cases, especially in small poultry shops, contamination with Salmonella also results (Rahmanet al; 2011).

The isolates from sewage were positive, as the percentage of positive isolates reached 50%. This was fully confirmed by previous studies on contaminated water and the analysis of more than 288 samples of drinking water in Canada between 1971-2001 (Schuster et al; 2005), in addition to What was confirmed by studies in North America in 2008. The contamination of drinking water with the runoff of animal waste led to the isolation of Salmonella (Kozlickaet al; 2010). Salmonellasp isolates also appeared in the USA in irrigation and drinking water in a study conducted between 2002-2005 by (Greene et al; 2008)

Perhaps the reason for this is the fertile environment of carbon and nitrogenous substances that bacteria feed on and are widely available in addition to the fermentative nature of Salmonella that allows it to survive in contaminated

sewage or drinking water. This is consistent with (Santiago et al; 2018) in his study of Salmonella isolates from wastewater.

The prevalence of intestinal bacterial infection in drinking water has caused panic in many countries, especially developing countries, but contrary to what appears in some studies, our results indicated that there are no Salmonella bacteria in drinking water or even ground water, and we can. Deferring this to previous studies that relied on isolating Salmonella from drinking water, as this water was treated from wastewater, rain, or the like, as Chauhan mentioned in his study in 2015, and this is completely consistent with our results, where Salmonella was isolated from wastewater health. 50% of them are positive. Despite the negativity of all samples of drinking water and well water, this indicates that the pure water is not contaminated with Salmonellasp or any member of the internal bacteria, but when mixed with a vital source or any source that supplies it with the materials. Necessary for the growth of bacteria, we find that the isolates are positive (Chauhan ;2015).

Fresh vegetable samples were 20.8% contaminated with Salmonellasp, so 5 out of 24 samples were positive. The bacterial content of fresh vegetables, especially table salad vegetables, is significant and this has been reported in previous studies in Zimbabwe, Taiwan and Ijib (Nyenjeet al; 2012), as well as studies isolated from restaurants and coffee shops in Riyadh, Saudi Arabia as well as the Tabuk region, Saudi Arabia (Darwish; 2018 and Al-Harbiet al; 2019). This was in agreement with what was found in the current study, perhaps the reason for this was the ability of bacteria to attach themselves to plant tissues and form a biofilm (Sheffield & Crippen; 2012), or it could be that vegetables were watered with untreated or improperly treated water. This may be exactly what previous studies have reported (Quiroz-Santiago et al; 2009).

Through the results of the isolates, we found a great sensitivity of the isolates towards antibiotics, in contrast to what was stated in the previous literature, which attributed the effective resistance of Salmonella against many antibiotics to methods of Salmonella resistance to antibiotics, including plasmids that contain antibiotic resistance genes, especially beta-lactams (Carattoli (2003), et al. 2012). This is indeed consistent with the current results in our study, as the only antibiotic that all isolates were resistant to was ampicillin.

In contrast to previous literature, which explained Salmonella resistance by the presence of genes carrying resistance enzymes such as chloramphenicol via the floR gene (Alamet al; 2020), tetracycline via the tetA gene (Mridhaet al; 2020), ampicillin and streptomycin are due to the presence of many plasmids that contain systems Genetic resistance to these antibiotics, and the low percentage of Salmonella resistance to ciprofloxacin may be a chromosomal mutation that occurs in gyrA, and this was confirmed by the study, as the isolates in our current study, including 7 isolates, were resistant to ciprofloxacin, and the opposite of what came from the explanation of the resistance of Salmonella to some antibiotics from families, which increases the cephalosporins, and cefamycin, resistance to ceftriaxone and cefixime was greater than 98% (Dotelet al; 2010) and this is also due to the evolving spectrum of genetic mutations (Enget al; 2015).

The isolates of the current study were sensitive to these antigens, and the resistance to ampicillin, amoxicillin, oxacillin, and cloxacillin was in great disagreement with what was reported (Akondet al; 2012), and the results were largely inconsistent with (Rumi et al; 2019).

Conclusion:-

Salmonella is present in many environments spread around us. Salmonella causes many diseases, the most famous of which is typhoid fever. Studies have shown that it is resistant to a number of antibiotics. Doctors must be consulted before taking any antibiotic, in addition to following personal hygiene and food hygiene instructions to prevent the spread of Salmonella.

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