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Review article

Prevalent and Bacteriology of *Salmonella* serovars-Review

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In this review article the information derived from the literature has been duly acknowledged in the text and lists of references have been provided by the author.

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ABSTRACT

The review article summarizes the data related to prevalence and bacteriology of *Salmonella* serovars. Salmonellae are gram-negative, non-lactose fermenting, oxidase, catalase, urease, acetylmethyl-carbinol and KCN (i.e. KCN-sensitive) negative and non-sporing bacteria. With the exception of *Salmonella* Pullorum and *Salmonella* Gallinarum, all salmonellae are actively motile with peritrichous flagella. They are also non-capsulated with exception of *Salmonella* Typhi belonging to the family enterobacteriaceae. The *Salmonella* species inhabits the intestine of birds, reptiles, farm animals, humans and insects. There are three species of the genus *Salmonella* viz., *Salmonella* Enterica, *Salmonella* Bongori and *Salmonella* Subterranean. *Salmonella* Enterica is classified into six subspecies, viz., Enterica, Salamae, Arizonae, Diarizonae, Houtenae and Indica. *Salmonella* infection (also known as salmonellosis) is an infection caused by ingesting *Salmonella* directly or indirectly in food that is contaminated by faeces of animals or humans. Common sources of infection include meat and its products, eggs and its products. Some of the symptoms of salmonellosis are diarrhoea, vomiting, fever and abdominal pain. These occur 12-36 hours after eating infected food; in acute infection blood and mucous are present in faecal specimens. Salmonellae can be isolated from blood, stool, urine, bone marrow, duodenal aspirates and rose spots. *Salmonella* can be isolated and identified by culturing the organism onto a selective media and incubated at optimum temperature of 37^0C for 24 hours before biochemical and serological tests will be performed. To prevention and control Salmonellosis can be achieved by provision of potable water, food hygiene, breastfeeding, mass-campaign rehydration as well as good diagnosis and treatment of Salmonellosis.

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Key word: Salmonellae, Salmonellosis, Prevalent, Bacteriology, Food and Water borne.

INTRODUCTION:

The genus of *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist. Morphologically indistinguishable from *Escherichia coli*, they do not produce indole and do not hydrolyse urea. They are non-lactose fermenting but ferment glucose and various other sugars, most species producing acid and gas. Salmonellae exist in nature primarily as parasites of the intestinal tract of man and other animals [1, 2].

The nomenclature of the salmonellae has undergone several changes. Some of the species earlier described were named after the disease with which they were associated with and these names, such as *Salmonella* Choleraesuis, *Salmonella* Typhi, *Salmonella* Paratyphi and *Salmonella* Enteritidis, have for the most part been retained. A few species have been called after the persons who discovered or worked with them (e.g., *Salmonella* Schottmuelleri) or of the patient from whose body they were isolated (e.g., *Salmonella* Thompson). Many have been named after the locality in which they were first isolated, e.g., *Salmonella* Dublin, *Salmonella* London, *Salmonella* Poona, *Salmonella* Aberdeen, *Salmonella* Minnesota, etc. Since the number of species recognized is steadily increasing, over 1,000 have now been described, it has been suggested that the practice of giving a separate name to each new species should be abolished and new species designated by their antigenic formulae/composition [3].

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Socio-demographic characteristic like feeding habit, occupation, literacy, hygiene of individual have been reported to be the most predisposing factors that increase the risks of *Salmonella* infection in Nigeria and other developing countries [4]. Overcrowding, poor hygiene and sanitation facilitate the spread of resistant organisms especially, bacteria that cause salmonellosis, shigellosis, tuberculosis, and pneumonia. Control procedures of infections in hospitals are often inadequate, resulting in the spread of infectious diseases and resistant strains of organisms such as *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterococcus*, *Shigella* and *Salmonella* species [4, 5].

Although, in Nigeria *Salmonella* infection is endemic with the number of cases rising at certain periods of the year, the main source of typhoid is asymptomatic carriers; an individual can asymptomatically carry the *Salmonella* for days to years without showing any symptoms of *Salmonella* infection. In such carriers, the *Salmonella* bacillus continues to multiply in the gall bladder. It reaches the intestine through the bile duct. The silent carriers are the source of *Salmonella* germs for the continued episodes of infections. Women exceed men as carriers by a ratio of 3:1 [6].

Laboratory diagnosis of typhoid fever is dependent on either the isolation of *Salmonella* Typhi from clinical samples or detection of raised antibody titres by agglutination tests. Subsequently, antibody levels > 1:160 for *Salmonella* Typhi and *Salmonella* Paratyphi are regarded as diagnostic of enteric fevers [7]. Salmonellae can be isolated from blood, stool, urine, bone marrow, duodenal aspirates and rose spots [8]. The organisms can usually be detected in 75-90% of patients during the first ten days of infection and in about 30% of patients during the third week in the blood [8].

Increasing antibiotics resistance in *Salmonella* species has been a serious problem for public health worldwide. In Nigeria, the increasing treatment failure with the empirical therapy in recent times among patients with salmonellosis necessitates the need for frequent assessment and reporting of antibiotics resistance patterns of *Salmonella* serovar in our environment [1, 9]. The high rate of resistance is hampering the use of conventional antibiotics, and growing resistance to newer antibiotics is aggravating the situation. The circumstances of occurrence and spread of antibiotic resistance is complex; however, a major cause is the widespread use of antibiotics in food animals, particularly in animal feed. Genetic analysis has indicated that the source of resistance is frequently a transferable plasmid [1].

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DIVISION OF SALMONELLAE:

On the basis of their pathogenicity and clinical importance, the salmonellae may be divided into two groups:
Group 1: This group includes members of the genus that are involved as aetiologic agents of enteric fever i.e. *Salmonella* Typhi and *Salmonella* Paratyphi bacilli. These species are found only in the intestinal tract of man for whom they have a highest degree of pathogenicity and whom they frequently cause invasive disease [10].
Group II: This group includes members of the genus that are involved as aetiologic agent of food poisoning (non typhoidal salmonellosis). The food poisoning groups are essentially parasites of animals from which man is occasionally infected. Their pathogenicity for man is relatively lows, the usual result of infection being the production of gastroenteritis. In this condition they penetrate to the sub-epithelial tissues but rarely invade the blood stream, i.e. *Salmonella* Typhimurium and more recently serotype DT 104. Other members are *Salmonella* Enteritidis, *Salmonella* Heidelberg, *Salmonella* Agona, *Salmonella* Newport, *Salmonella* Hadar, and *Salmonella* Dublin [11, 12].

MORPHOLOGY AND STAINING REACTIONS OF SALMONELLAE:

Salmonellae are gram-negative bacilli, 2-4 x 0.6 µm, non-acid-fast, non-capsulate and non-sporing. Most strains of most serotypes form type 1 (Mannose-sensitive, haemagglutinating) fimbriae. *Salmonella* Pullorum and *Salmonella* Gallinarum and a few strains in other serotypes. Other form type 2 (non- haemagglutinating) fimbriae or are non-fimbriate, most strains of *Salmonella* Paratyphi A are non-fimbriate [13].
Cultural characteristics of salmonellae: Salmonellae are aerobic and facultatively anaerobic. Grow on artificial media in the temperature range 15-45°C, optimally at 37°C. Many strains are prototrophic, i.e. capable of growing on a glucose-ammonium minimal medium such as that of Davis and Mingioli, but some strains are auxotrophic and require enrichment of the minimal medium with one or more amino acids or vitamins, e.g., cysteine or nicotinamide; most Typhi strains require tryptophan [14].
Growth of salmonellae on artificial media: Salmonellae grow on artificial media such as; Nutrient agar, blood agar, peptone water and nutrient broth [15].
Differential and selective solid media: These media are valuable for the isolation of salmonellae from faeces and other materials contaminated with many bacteria of other kinds. From blood samples, the media are used for subculture of salmonellae from thioglycollate broth for the purposes of its identification. They include: MacConkey agar, Bile-salt lactose agar, Brilliant green macConkey agar, Deoxycholate citrate agar (DCA), Wilson and Blair's brilliant-green bismuth sulphite agar (BBSA), Taylor's xyline lysine deoxycholate (XLD) agar, Rambach's agar (PG) and SM-ID agar [14].
Enrichment media: These are liquid media used to assist the isolation of salmonellae from faeces, sewage, foodstuff and other materials containing a mixed bacterial flora. A larger amount of materials can be inoculated into enrichment media than onto an agar plate, for facilitating the isolation of salmonellae when these are presents only in small numbers [16]. The good enrichment media include: Tetrathionate broth, Kauffmann-Maler tetrathionate broth with Brilliant green, Selenite F broth, Rappaport's malachite green magnesium chloride broth [17,16].

BIOCHEMICAL REACTIONS OF SALMONELLAE

Although most strains are identified by the pattern of reactions of salmonellae, the decision that a bacterium is not *Salmonella* should not be based on the result of only a single test. Some strains show exceptional reaction in particular tests and it is necessary to consider the general pattern of the reactions in a group of tests. [18].
Fermentation tests of salmonellae: Carbohydrates are generally fermented with the production of acid and gas. Typhi, Gallinarum and rare anaerogenic variants in other serotypes, e.g., Typhimurium, form only acid, typically glucose, mannitol, arabinose, maltose, dulcitol and sorbitol are fermented, but not lactose, sucrose, salicin or adonitol; the ONPG test for β-galactosidase is negative. Among exceptional strains, Choleraesuis and some strains of Typhi do not ferment arabinose, whereas Choleraesuis, the Pullorum biotype of Gallinarum, most strains of Typhi and some strains of Paratyphi A and Paratyphi B do not ferment dulcitol [16].

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Decarboxylase tests of salmonellae: Salmonellae decarboxylate the amino acids; lysine, ornithine and arginine, but not glutamic acid. Typhi is exceptional in lacking ornithine decarboxylase and Paratyphi in lacking lysine decarbaxylase [13].
Other biochemical tests of salmonellae: Most salmonellae have the following reactions; indole not produced, methyl-red positive, acetyl methyl carbinol not produced (i.e. voges-proskauer negative), citrate utilized, except by Typhi and Paratyphi A, malonate not utilized, gluconate not utilized, urease not produced, phenylalanine deaminase not produced, hydrogen sulphide produced in ferrous chloride-gelatin medium, except by Paratyphi A, Choleraesuis, Typhisuis and Sendai, no growth in KCN medium, gelatin not liquefied [19].

KAUFFMANN-WHITE CLASSIFICATION OF SALMONELLAE:

This scheme, first developed in 1931, classifies the salmonellae into different O groups, or O serogroups, each of which contains a number of serotypes possessing a common O antigen not found in other O groups. The O group's first defined were designated by capital letters A to Z and those discovered later by the number (51-67) of the characteristics O factors, i.e. to abandon the letters A-Z used to designate early O groups. Hence, O groups become: O2(A), O4(B), O7(C_1), O8 (C_2-C_3), O9, 12 (D_1), O9, 46 (D_2), O3, 10 (E_1) *etc* Groups O2 to O3, 10 (A-E_1) contains nearly all the salmonellae that are important pathogens in man and animals [20, 21]. The Kauffman and White classification scheme is a system that classifies the genus *Salmonella* into serotypes, based on surface antigens. It is named after Philip Bruce White and Fritz Kauffmann [22].

ANTIGENIC STRUCTURE OF SALMONELLAE:

Many different serotypes have one or more of their O or H antigens in common and their distinctive antigens have to be demonstrated in tests with 'single-factor' antisera which have been absorbed with heterogonous bacteria to free them from antibodies to the shared antigens [23]. In other variations of antigens organisms may lose H antigens and become non-motile. Loss of O antigen is associated with a change from smooth to rough colony form. Vi antigen may be lost partially or completely. Antigens may be acquired (or lost) in the process of transduction [15].

VIABILITY OF SALMONELLAE:

Salmonellae are readily killed by moist heat, e.g., in l hour at 55^{o}C or 15 minutes at 60^{o}C and most strong disinfectants. Also, it can be killed by temperature of 130^{o}F or higher for 2 hours or at 165^{o}F for a few seconds. Cultures on slopes of Dorset's egg, kept tightly capped to prevent drying and stored in the dark at room temperature usually remain viable for at least 10-20 years [24]. Typhi and other salmonellae gradually die incontaminated moist natural environment outside the body, but some bacilli may survive for over 4 weeks in for example, sewage-polluted water or moist soil. They die more quickly when dried, Typhi often within a few hours, so that spread is less likely to take place by dust or dry fumets than by water or moist foodstuffs [24].

SOURCES AND TRANSMISSION OF SALMONELLA INFECTIONS:

Salmonella Typhi, *Salmonella* Choleraesuis, and perhaps *Salmonella* Paratyphi A, *Salmonella* Paratyphi B and *Salmonella* Paratyphi C are primarily infective for humans, and *Salmonella* Paratyphi C mainly causes septicaemia. Paratyphoid caused by *Salmonella* Paratyphi C is usually more serious than those of *Salmonella* Paratyphi A and *Salmonella* Paratyphi B [3]. The organisms almost always enter via the oral route, usually with contaminated food or drink, among the host factors that contribute to resistance to *Salmonella* infection are gastric acidity, normal intestinal microbial flora and local intestinal immunity [25].
Other sources of *Salmonella* infections includes; Recreational drugs, e.g., marijuana and other drugs, animal dyes, e.g., dyes like carmine used in drugs, foods and cosmetics. House pets, e.g., turtles, dogs, cats can be contaminated with salmonellae and serve as sources of *Salmonella* infections [26].

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PATHOGENESIS AND SYMPTOMS OF SALMONELLAE:

The pathogenesis of bacterial infection includes initiation of the infectious process and the mechanisms that lead to the development of signs and symptoms of disease [27, 28]. Three clinical types of salmonellosis have been described in humans;

Enteric fevers or systemic salmonellosis (typhoid fever and paratyphoid fever): These are caused by *Salmonella* Typhi (typhoid fever) and *Salmonella* Paratyphi A, B and C (paratyphoid fever). Are clinical entities distinct from other salmonellosis, with characteristic clinical development and followed by lasting immunity. Contamination is made orally, by eating infected food or by drinking infected water [27].

Enteric salmonellosis is food poisoning (acute gastroenteritis): These represent the common form, widespread in all countries. They are caused most frequently by *Salmonella* Enteritidis and *Salmonella* Typhimurium. Symptoms appear in 10-24 hours after eating contaminated food or drinking contaminated water. Characteristic symptoms are diarrhea, abdominal pain, vomiting, fever, which disappear within 2-4 days [28].

Bacteraemia: With or without the existence of enteritis outbreaks, is caused by *Salmonella* Typhimurium, *Salmonella* Paratyphi A and B and by *Salmonella* Choleraesuis. Mainly affect two age groups. In young children is manifested by fever and gastroenteritis, and in adults is manifested by transient bacteraemia during episodes of gastroenteritis, or signs of sepsis without gastroenteritis (in the immunosuppressed) [29].

Chronic carrier: Asymptomatic are represented by a rate of 1-5% of patients with typhoid fever or paratyphoid fever. The germs are located in the gallbladder and are excreted continuously or intermittently through feaces [29].

PATHOGENICITY OF SALMONELLAE:

Salmonella species are facultative intracellular pathogenic bacteria. They can invade macrophages, dendrites and epithelial cells. The responsible virulence genes for invasion, survival, and extra-intestinal spread are located in *Salmonella* **pathogenicity islands (SPIs).** SPIs are thought to be acquired by horizontal gene transfer. Some of the SPIs are conserved throughout the *Salmonella* genus, and some of them are specific for certain serovars. There are differences between *Salmonella* serotypes in terms of adaptation to host cell, virulence factors and the resulting infection according to SPA presence and characteristics. The most important *Salmonella* virulence gene clusters are located in 12 pathogenicity islands [30]. Virulence genes that are involved in the intestinal phase of infection are located in SPI-1 and SPI-2 and the remaining SPIs are required for intracellular survival, fimbrial expression, magnesium and iron uptake, multiple antibiotic resistances and the development of systemic infections. In addition SPIs, Sigma (RpoS) factors and adaptive acid tolerance response (ATR) are the other two important virulence factors. RpoS and ATR found in virulent *Salmonella* strains help the bacteria to survive under inappropriate conditions such as gastric acidity, bile salts, inadequate oxygen concentration, and lack of nutrients, antimicrobial peptides, mucus and natural microbiota and also to live in phagosomes or phagolysosomes. This review article summarizes the data related to pathogenicity islands in *Salmonella* serotypes and some factors which play role in the regulation of virulence genes [30].

CLINICAL MANIFESTATION OF SALMONELLA INFECTION:

The clinical manifestation of *Salmonella* infection includes the following;
The enteric fever: Enteric fever is also known as typhoid and paratyphoid fever caused by a gram negative *Salmonella* Enterica, either *Serover* Typhi (*Salmonella* Typhi) which is the most serious form or *Serover* Paratyphi (*Salmonella* Paratyphi) [30].
Diarrhoea diseases (Enterocolitis): *Salmonella* species cause a disease called enterocolitis, a type of diarrhoea in which the stools sometime contain blood and mucus. For many years, *Salmonella* species were thought to be the only bacteria that caused diarrhoea, but it is now clear that some strains of *Shigella* species and *Escherichia coli*

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cause the same symptoms. Diarrhoea is also caused by an amoeba, *Entamoeba histolytica* (amoebic dysentery). In diarrhoea caused by *Salmonella* and *Shigella*, the specimen has an alkaline pH unlike those caused by amoeba which has an acid pH [31].

Bacteraemia and septicaemia: Non-typhi salmonellae (NTS), particularly *Salmonella* Typhimurium and less frequently *Salmonella* Enteritidis are common causes of bacteraemia and septicaemia in young children in developing countries [32, 33].

Bacteraemia is rare (2-4%) except in immune-deficient person. Blood cultures are usually negative, but stool cultures are positive for salmonellae and remain positive for several weeks after clinical recovery. Food-poisoning *Salmonella* strains can also cause bacteraemia, inflammation of the gall bladder, osteitis especially in children with sickle cell disease, and occasionally abscesses [34, 35].

Septicaemia is a common cause of paediatric morbidity and mortality. Deaths from paediatric septicaemia are likely to be higher in low-income settings. Children with septicaemia present with fever, difficult breathing, tachycardia, malaise, inability to feed or lethargy, but those with asymptomatic bacteraemia tend to show no obvious sign of illness [27]. In Nigeria, septicaemia is a major cause of death in neonates and children. The outcome of treatment of neonates with septicaemia has remained poor in Nigeria as shown by reports of mortality rate of 33% to 41% from two tertiary hospitals in the country [32].

Reiter's syndrome (An autoimmune response): Another complication of *Salmonella* infections is an arthritis that appears after the intestinal infection has passed. This condition, called Reiter's syndrome, is also a complication of other invasive bacterial infections caused by *Yersinia enterocolitica*, *Shigella* species and *Klebsiella pneumoniae*. This condition is suspected to be caused by an autoimmune response triggered by bacterial antigens that affects joint tissues, leading to inflammation of the joints, but it could also be due to an infection of the joints in some cases [35].

Lipopolysaccharide (LPS): Lipopolysaccharide (LPS) appears not to play a role in invasion, intracellular replication, or cell-to-cell spread of *Salmonella* species, but may contribute to tissue damage. Rough strains of *Salmonella* (strains with little or no O antigen) invade and replicate normally in tissue culture cells. However, they do not produce keratoconjunctivitis when inoculated in the eye of a guinea pig (Sereny test), an experimental test of the ability of the bacteria to cause an inflammatory response [36].

Nephrotyphoid: This is an immune disorder of the kidney usually associated with typhoid in patients suffering from schistosomiasis. Its clinical features include fever, oedema, raised albuminnuria and haematuria [37].

Osteomyelitis: Infection with *Salmonella* Typhi can also cause osteomyelitis and typhoid arthritis particularly in those with sickle cell disease and thallassaemia. Patient suffers from osteomyelitis (inflammation of the bone); typhoid nodules may be found in the bone marrow. Disease caused by *Salmonella* Paratyphi A and B is generally milder than typhoid [16].

GENETICS OF SALMONELLAE VIRULENCE:

The *Salmonella* virulence is the ability of salmonellae to invade epithelial cells, multiply intracellularly, and spread from cell to cell to expand the focus of infection. This cycle of invasion, multiplication, and spread leads to the destruction of the epithelial layer of intestine that is characteristic of salmonellosis [38]. The ability of *Salmonella* species to produce the gene products required for virulence is regulated by growth temperature. *Salmonella* species grown at 37^{o}C are fully virulent and capable of invasion, multiplication and spread. However, when grown at temperatures lower than 34^{o}C, the organisms become phenotypically avirulent and noninvasive. Gene expression in response to temperature is a characteristic that *Salmonella* species share with other bacterial pathogens of humans, including; *Shigella* species *Bordetella pertussis*, uropathogenic *Escherichia coli* and *Yersinia* species [23, 39].

EPIDEMIOLOGY OF SALMONELLAE:

The majority of new cases in the UK are believed to be related to foreign travel, there were around 230 cases of each *Salmonella* Paratyphi and *Salmonella* Typhi reported in England and Wales in 2005. Increased incidence of typhoid during the rainy season has been noted before in West Africa, though the seasonal incidence elsewhere in Africa is different. The explanation usually offered is that surface drinking water supplies are probably polluted during rainy season floods [40]. However, the increased incidence of malaria in the rainy season is well known, and it would be interesting to postulate a relation between malaria in children and susceptibility to enteric fever. Such a relation

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could also explain the severe anaemia already commented upon, for which no satisfactory cause can be found [41, 42].

LABORATORY DIAGNOSIS OF SALMONELLAE:

Collections of commonly used clinical specimens for isolation of salmonellae
Laboratory diagnosis of *Salmonella* infections depends mainly on the isolation and identification of the causal *Salmonella* from specimens of the patients' blood, faeces, urine, bone marrow and vomit [43];
Blood specimens: Blood for culture must be taken repeatedly in enteric fevers and septicaemias, blood cultures, are often positive in the first week of the disease [44].
Stool specimens: Stool specimens also must be taken repeatedly. In enteric fevers, the stools yield positive results from the second or third week. In enterocolitis during the first week, a positive culture of duodenal drainage establishes the presence of salmonellae in the biliary tract of carries [44].
Rectal swabs: Rectal swabs are convenient to collect, but compare un-favourably with faeces in their yield of isolations [43].
Faecal swabs: Which are swabs dipped into and heavily charged with faeces after it has been passed, are good specimens, If delay in transit of swabs to the laboratory is inevitable, they should be placed and submitted in Stuart's transport medium [43].
Pus swabs: Pus swabs should be treated as rectal or faecal swabs [45].
Urine and Vomit or Bile specimens: Specimens of urine and vomit or bile should be centrifuged and the deposit is cultured. Urine cultures may be positive for salmonellae after second week of infection [45].
Duodenal aspirate and bone marrow: Specimens should be collected. Cultures may be positive for salmonellae after culturing the specimens [45].

SEROLOGICAL IDENTIFICATION OF SALMONELLAE:

Serological identification of salmonellae is based on agglutination test. The principle of bacterial agglutination test is based on the reaction between the antigen (bacteria) and its corresponding antibodies in which known antigen can be used to detect the presence of corresponding antibody and vice versa [46];

Rapid preliminary identification of salmonellae cultures/isolates using Commercial kits: Agglutination tests used for salmonellae identifications is achieved by the use of known sera and unknown culture are mixed on a slide. Clumping, when it occurs, can be observed within a few minutes. This test is particularly useful for rapid preliminary identification of cultures. There are commercial kits available to agglutinate and serogroup salmonellae by their O antigens: A, B, C_1, C_5, D, and E. Diagnostic antisera can be prepared in the laboratory by immunizing rabbits with the appropriate antigens, but agglutinating antibodies to cross-reacting antigens must be removed by absorption and the method is not only tedious but also requires considerable expertise. It is usual, therefore, to employ commercially prepared antisera (e.g., from Wellcome). The choice of sera to be held in the laboratory will vary with the type of specimens received, but the following sera would be suitable for a large hospital or public health laboratory [47].

Widal screening test (Tile agglutination test for enteric fever): The patient's serum is tested by tube agglutination for its titres of antibodies against H, O and Vi suspensions of the enteric fever bacteria likely to be encountered. Although suspensions may be prepared from suitable stock laboratory cultures, there is little need for that nowadays because commercially prepared suspensions are widely available (e.g., Stained *Salmonella* Suspensions, Wellcome) [47]. Customary practice in areas of low prevalence is to perform a rapid screening test in tests with commercial suspensions and neat serum and, if the patient's serum is positive, to prepare a series of dilutions of serum to estimate O, H and Vi titres [47].

Procedure for widal screening test (tile agglutination test for enteric fever):

i) The antisera of (*Salmonella* Typhi, Paratyphi A.B.C.) are dropped separately on clean, dried tile.
ii) A loopful to the test serum is then added each of the antigens

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iii) And rocked whichever shows agglutination is responsible for the cause.

Widal confirmatory test (tube dilution agglutination test for enteric fever):
After screening test tube test should be carried out to:
(1) Rule out the non-specific antibodies that flight have caused false positive result in the slide test;

(2) To determine the titre (extent) of the antibody in the serum.

However, in this method of agglutination tests for salmonellae identifications, equal volume of known sera and unknown culture/sample are mixed in a tube. Clumping, when it occurs, can be observed within a few minutes. Sometime serial dilution of equal volume of known sera and unknown culture/sample are mixed in a tube and a significant titre is obtained or recorded [48].

The reciprocal of the highest dilution giving a positive agglutination is regarded as the titre. Following an infection, the somatic (O) antibody appears first followed later by the antibody to the flagella antigen (H) that usually persists longer. The antibody to the Vi antigen though not often measured indicates that the *Salmonella* organism is present either in acute or chronic state if the level is more than 1:5 [48].

IDENTIFICATION OF SALMONELLAE BY NUCLEIC ACID AMPLIFICATION IN VITRO (THE POLYMERASE CHAIN REACTION):

In-vitro nucleic acid amplification techniques are relatively new, and methods in addition to PCR are likely to make a significant impact in diagnostic microbiology in the near future. Techniques include ligard chain reaction (LCR), nucleic acid sequence based amplification (NASBA), strand displacement amplification (SDA) and Q-beta replicate-dependent amplification (QBRDA) [49].
Some of the methods used for diagnosis of salmonellae using polymerase chain reaction tests are as follows:-
Identification of drug resistance genes in clinical isolates of Salmonellae for Development of diagnostic multiplex polymerase chain reactions:

It was investigated that multiplex PCR targeting specific genes related to diagnosis and antibiotic resistance could be very useful. In this direction a multiplex PCR that can be used for diagnosis of typhoid and determination of antibiotic resistance pattern of the particular strain was developed [50]. After identifying the *Salmonella* Typhi isolates that antibiotic resistance to antibiotics used, PCR conditions were optimized for simultaneous amplification of specific *Salmonella* Typhi *fliC* gene and all relevant drug resistance genes, so that the result could be obtained in a single experiment. It would provide information on diagnosis and antibiotic resistance pattern in a single day [50].

Identification of Salmonellae by real-time polymerase chain reactions (Taqman assay):
A basic PCR technique combined with a DNA probe specific to the Vi antigen of *Salmonella* Typhi could be used to reinforce the clinical diagnosis of typhoid fever with a negative blood culture. However, the detection of *Salmonella* Typhi using these techniques is not sensitive enough to detect fewer than 500 bacteria in a 1 ml blood sample [51]. In the real time PCR study, there is development of a *TaqMan*-based real-time PCR assay (*TaqMan* assay) for quantifying *Salmonella* Typhi directly from blood specimens of patients suspected of having typhoid fever, especially in cases with a negative blood culture test result. The genes copies from *Salmonella* Enterica serovar Typhi were quantified (*Salmonella* Typhi) in the blood of patients suspected of having typhoid fever by using *TaqMan*-based real-time PCR to target the *Salmonella* Typhi flagellin gene in genomic DNAs isolated from blood samples [51].

TREATMENT, PREVENTION AND CONTROL OF SALMONELLA INFECTIONS:

The term “Chemotherapy”, is used for the “Antibiotic treatment of parasitic infections”, in which the parasites (bacteria, viruses, protozoa, fungi, worms, *etc*), are destroyed or removed without injuring the host [52].
Management of enteric fever can be achieved by the following:

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Management of mild carrier state of *Salmonella* infections: Supportive adequate rest, rehydration and correction of electrolyte disturbances, Antipyretic therapy is required, hygiene; carriers must be meticulous with hand washing and the disposal of faeces and urine, early diagnosis and rapid commencement of treatment is important. Antibiotics shorten the course, reduce the rate of complications if begun early and reduce mortality [53].
Management of chronic carrier state of *Salmonella* infections: Ciprofloxacin 750 mg b.d. and norfloxacin 400 mg b.d. have both been effective in the past. Norfloxacin has a cure rate of 78%. Bacteriological surveillance after recovery should continue until 6 consecutive negative results are obtained on faecal and urine cultures. Long-term urinary carriers should be assessed for urinary tract abnormalities, including schistosomiasis. In long term faecal carriage, cholecystectomy is not very effective as the liver is a reservoir [54].
Prognosis of *Salmonella* Infections: In the days before antibiotics mortality was 20%. Between 10% and 20% of patients treated with antibiotics have a relapse, usually a week after stopping the antibiotic but it can be much later, and the relapse rate is lower with quinolones as they penetrate the cell? Patients with invasive salmonellosis require antibiotic treatment. Increasing antibiotic resistance may add to the difficulty or delay in administration of microbiologically effective therapy, leading to increased morbidity and mortality [29].

ANTIBIOTIC RESISTANCE OF SALMONELLAE:

Most of the antibiotic resistance which is now making it difficult to treat some infectious diseases is due to the extensive use and misuse of antibiotics which have favoured the emergence and survival of resistant strains of micro-organisms. Antibiotic-resistant strains are common among staphylococci, gonococci, meningococci, pneumococci, enterococci, gram- negative bacteria (e.g., *Salmonella, Shigella, Klebsiella, Pseudomona* species and *Mycobacterium tuberculosis*) [55].

PREVENTION AND CONTROL OF SALMONELLA INFECTIONS:

The control of disease should be made on limited knowledge on the epidemiological information. The epidemiological knowledge should deduce information on a given infection and overall diseases of given infections of the population. Also before establishing control program of diseases it is necessary to know the mechanism for recognizing the infection and confirming the diagnosis, finding the sources of infection and also should be able to determine the extent of outbreak [56]. Based on Koch's' postulates there are three methods of controlling diseases;
Elimination of *Salmonella* infections' predisposal condition by vector: Elimination of *Salmonella* infections' predisposal condition by Vector is very important method of preventing and controlling of *Salmonella* infections' in our community. Jatau and Yakubu [57] reported that, salmonellosis is transmitted by vector's mechanical transmission in which parasites development does not occur in such vector for transmission of such parasitic diseases. Cockroaches (*Periplaneta americana*) and flies have been incriminated in the transmission of *Mycobacterium leprae, Salmonella* species, *Shigella* species, *Vibrio cholerae* and other pathogenic microbes [56].
Interrupting of the pathway for transmission of *Salmonella* infections: *Salmonella* infections usually occur in sporadic epidemic and pandemic forms. As in the case with other intestinal infection, the most effective method for controlling *Salmonella* infection is provision of safe and abundant water and effective faeces disposal [58].

Protection of susceptible host through immunization: Vaccines are microbial preparation of killed or modified microorganisms which can stimulate immune response in the body in order to prevent future infection with similar microorganisms [59]. In 2002, at least two effective vaccines available for typhoid both of which were recently licensed for use in the United States. The oral live attenuated vaccine (for use in children aged 6 years and older) and the parenteral (i.e., injectable capsular polysaccharide vaccine (for use in children aged 2 years and older) each have efficacy of 50%-80%, and fewer adverse events associated with their use than earlier typhoid vaccines [59].

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