Abstract: Salmonella is a zoonotic disease widely spread in the environment, causing serious economic losses to the world. Objective: To improve people's understanding of Salmonella disease and reduce economic losses. Methods: Based on the recent research progress, the biochemical characteristics, clinical manifestations, laboratory detection methods and preventive measures of bacteria were discussed in this paper. Conclusion: Salmonella infection in China is becoming more and more serious, which has caused great harm to pig industry and even human beings.

Keywords: Salmonella, Swine, Identification, Diagnosis, Prevention, Treatment

1. Introduction

Salmonella belongs to Enterobacteriaceae, Gram-negative bacilli. It is a kind of zoonotic disease that can infect humans, livestock, poultry, rodents, etc., and brings huge economic losses to the world [1].

Pig industry has been facing the problem of Salmonella infection for many years [2] and public health protection becoming an increasingly important concern [3]. Pork is the second commonest source, for human salmonellosis after eggs [4]. *Salmonella Typhimurium* and *Salmonella Derby* are common serovars isolated from pigs worldwide, whereas the *Salmonella Choleraesuis* is frequently found in North America and Asia [5]. Recent systematic survey of ileo-caecal lymph nodes at slaughter in the European Union National, zero to 29% prevalence of salmonella was found in pigs [6].

Swine salmonellosis is an infectious disease caused by different Salmonella species including *Salmonella choleraesuis*, *Salmonella typhimurium*, *Salmonella delphicaria* and *Salmonella enteritidis* [7]. Salmonella Typhimurium is capable of causing paratyphoid in pigs [2]. It may infect piglets during pregnant period or early life up to 4 months of age, hence also called piglet paratyphoid [8].

1.1. Salmonella Classification and Biochemical Properties

Salmonella belongs to family Enterobacteriaceae. Other bacteria in the family include *Escherichia coli* (E.coli) and *Shigella* [7]. There are more than 2500 serotypes (also serovars) at present and their classification is defined on the basis of the somatic (O or (lipopolysaccharide) and flagellar (H) antigens (the Kauffman–White classification) [9]. Further differentiation of strains to assist clinical and epidemiological investigation may be achieved by antibiotic sensitivity testing and by other molecular biology techniques such as pulsed-field gel electrophoresis, multilocus sequence typing, and, increasingly, whole genome sequencing [10]. Historically, salmonellae have been clinically categorized as invasive (typhoidal) or noninvasive (nontyphoidal salmonellae) based on host preference and disease manifestations in humans [11, 12].

Salmonella are Gram negative, non-spore forming,
non-capsulated, flagellated and motile rod-shaped bacteria. Salmonella are aerobic or facultative anaerobic bacteria with an optimum growth temperature of 37°C and suitable pH of 6.7 - 7.7. This organism can be cultured on ordinary growth medium with limited nutritional requirements. On ordinary growth medium Salmonella colonies are round, 2-3 mm in diameter, smooth, colorless, translucent, moist, and have certain resistance to external factors such as light. Specimens for the diagnosis purpose should be cultured directly onto Brilliant Green (BG) and Xylose Lysine Deoxycholate agar (XLD) agar and also added to selenite F, Kappaport or tetraionate broth for enrichment and subsequent subculture. The plates and enrichment broth are incubated aerobically at 37°C for up to 48 hours. Subcultures are made from the enrichment broth at 24 and 48 hours. On BG agar, colonies and medium are red indicating alkalinity. On XLD agar, colonies are red (alkaline) with a black center, indicating H₂S production. Suspicious colonies, subcultured from the selective media into TSI agar and lysine decarboxylase broth, should be examined after incubation for 18 hours at 37°C to establish their biochemical identity as salmonellae. It can survive for weeks or months [13].

Salmonella is a Gram-negative organism. It shows a relatively higher tolerance and higher intrinsic resistance to disinfectants compared to Gram positive bacteria [14]. Salmonella has been shown to be less tolerance to hypochlorite and vinegar (low pH) than Staphylococcus aureus, and less tolerant to hydrogen peroxide than Listeria monocytogenes [14]. The oxidative disinfectants like hydrogen peroxide, peracetic acid and Chlorine dioxide that have been tested are effective at lower concentrations [16]. Salmonella remain alive for a long period of time on open and dry areas e.g. bench tops and cutting plasmids. Most of Salmonella have toxins, especially production. Suspicious colonies, subcultured from the different serotypes: 78kDa (S. Choleraesuis), 70kDa (S. Enteritis) and 56kDa (S. Typhi) and the amount of toxin produced in each serotype is also significantly different. In a report it has been shown that S. Enteritis produces a cytotoxin which is very much similar to a Shigella dysenteriae 1 like cytotoxin and could be neutralized by an anti-serum to S. dysenteriae 1 cytotoxin. Another exotoxin which appeared to be hemolytic, termed salmonolysin has been reported. A gene of Salmonella Typhimurium; slyA gene encodes that toxin. The best-studied exotoxin of Salmonella is the heat labile salmonella enterotoxin; Stn (29 kDa) is encoded by the stn gene. The expression of Stn causes an increase in AMP levels and prostaglandins. Most of the Salmonella toxins have been shown to be heat labile. Heating of contaminated food items at 75°C for 1 hour, the toxins remain effective and human consumption can cause poisoning [19].

2. Clinical Symptoms of Salmonella Infection in Pigs

2.1. Clinical Manifestations of Different Types of Salmonella Infection in Pigs

2.1.1. Salmonella Paratyphi

Acute form of S. paratyphi is also known as septic type, mostly occurs in piglets before and after weaning, often cause piglets suddenly died. Pigs with a slightly longer course of disease have shown elevated body temperature (41-42°C), abdominal pain, diarrhea, difficult breathing, and purple spots on the skin of the ear, chest and abdomen, mostly ending in death [13]. The course of disease is 1 to 4 days. In case of chronic form of the disease common symptoms include elevated body temperature, inflammation of the conjunctiva, purulent discharge; diarrhea after constipation, gray or yellowish green stench. The sick pig is thin and has eczema on the skin. The course of the disease lasts for several weeks and ends up being dead or becoming paralyzed.

2.1.2. Salmonella Choleraesuis

Pigs are well thought-out to be the host of Salmonella choleraesuis, it is mean that infection and disease due to this organism is restricted almost solely to pigs and inapparent persistent infections can occur only in pigs. The important thing is that that signs of disease may not be shown. Salmonella choleraesuis is the main pathogen causing paratyphoid fever in piglets. During the infection the temperature of the sick animal is elevated, animal looks depressed with loss of appetite. Death may be result due to infection of Septicemic S. choleraesuis, without appearance of signs. Almost all affected pigs are off feed, have cough, fever and may give signs of pneumonia. pregnancy of sows may be
abortion. Some parts of the body, like ears, tail, nose, feet, and abdomen, turn light red to dark purple. Pigs that survive 3 or 4 days develop yellow diarrhea with flakes of fibrin or, less commonly, blood. Animals that recover usually have decreased weight gain and stunting [5, 20].

2.1.3. Salmonella Enteritidis

*S. enteritidis* is the main pathogen causing acute gastroenteritis, and typical symptoms after infection include fever, diarrhea and vomiting [21].

2.1.4. Salmonella Typhimurium

*Salmonella typhimurium* (*S. typhimurium*) causes invasive nontyphoidal infection in pigs [22]. The clinical manifestations can be divided into 4 forms: gastroenteritis, septicemia, focal infection and chronic carriage. Flies and fleas act as vectors and can cause transmission of bacteria from one animal to other. Infection can also be transmitted through contaminated food. *Salmonella typhimurium* is an invasive bacterium, mainly invading the ileum and colon and cause enterocolitis [23, 24].

2.2. Changes in Clinical Anatomy

The body temperature of the sick pig is as high as 40.2-41.4°C, with nervous signs, depression, chills, loss of appetite and gray yellow diarrhea. There is ocular discharge and difficult breathing in some pigs. Some pigs also develop cyanosis and bluish-purple spots on their ears, chest and abdomen. Most of the infected pigs died in 1-3 days. The subcutaneous edema of the corpse can be observed on examination. Postmortem examinations reveal enlarged kidneys with rupture capsule and small haemorrhages [25]. Gastrointestinal mucosa and serosa have scattered punctate haemorrhages; pleural effusion and pericardial effusion. The spleen is enlarged, dark blue, elastic, with a numerous haemorrhages and necrotic foci. The mesenteric lymph nodes are swollen and hemorrhagic. Liver is enlarged, congested and show grey yellow necrotic spots.

A large amount of fluid accumulates in the intestine. The other lesions in the intestine include: necrotic enteritis, thickening of the colon and cecum wall and covering the mucosa with a layer of diffuse bran-like necrotic. On peeling off the pseudomembrane, visible red, irregular edge ulcer surface can be seen [26]. The most typical lesion for the diagnosis of salmonella infection is presence of gray yellow necrotic foci on the liver, as shown in Figure 1.

3. Detection Methods for Salmonella

The incidence of *Salmonella* infection is very high in food poisoning cases all over the world. According to statistics, 70% to 80% of bacterial food poisoning in China is caused by *Salmonella*, and almost 90% of the food which cause salmonella poisoning is meat, eggs, milk and other animal products. At present, the detection of *Salmonella* has become one of the indispensable hygienic indicators in the measurement and certification of food quality and safety monitoring in China. Conventional detection and diagnostic methods use a process consisting of non-selective pre-enrichment of the given sample, followed by selective enrichment of bacteria, bacterial culture on selective agar, biochemical reaction and serological identification (Figure 2). This whole process is time consuming and very complicated. The whole processes of conventional microbiological methods are easy to use, reliable, sensitive, specific, and less expensive as compared to molecular biology technologies [24]. However, preparations are made for these procedures because multiple subcultures are required for several identification steps, which take more than 5 days for completion of isolation and confirmation process. Biochemical reactions for the diagnosis of Enterobacteriaceae bacteria often take 4 to 7 days to complete. Other than these false, positive results may happen due to repeated handling of sample. To process excess of samples, such a requirement may not be addressed due to laborious and time-consuming culture-based techniques. PCR and other molecular technique have the advantage that these are rapid and highly sensitive. These techniques have been widely used in food, clinical samples and environmental detection of *Salmonella*.

![Figure 2. Scheme of Salmonella culture, plating, screening and confirmation [27].](image-url)

RV, Rappaport-Vassiliadis medium; BS, bismuth sulfite agar; HE, Hektoen enteric agar; XLD, xylose lysine desoxycholate agar; TT, tetrahionate broth, TSI, Triple sugar iron agar, and LIA, Lysine iron agar.
3.1. Traditional Isolation and Identification

Firstly, for the pathological samples, pig’s liver, feces, ileum, cecum or colon contents are collected to carry out the isolation and identification of Salmonella. It usually includes following steps (Figure 2):

1. Pre-enrichment: putting the specific amount of sample in the nutrient medium to ensure the survival of the normal and pathogenic Salmonella

2. Selective enrichment: to ensure the growth of Salmonella and inhibit the growth of other microorganisms. Mostly two kinds of selective media are used for parallel experiments;

3. Isolation and purification: Plating of culture of Salmonella on selective media and single colony was selected for pure culture

4. Identification: For identification microscopic examination, biochemical identification and serological tests (like serum agglutination test) are used. Gram’s staining is performed on the purified Salmonella culture. Further the biochemical tests are carried out to confirm whether it is Salmonella or not. Serum can also be used for slide agglutination test of pure cultured strains, with sterile saline as control. Within 1-minute obvious agglutination can be observed which indication of positive result is whereas no agglutination is negative. Figure 3 shows the isolation and purification of Salmonella (DHL plate) and Gram staining (bilateral symmetrical short rods) [27].

At present, the current national standard for the detection of Salmonella in animal and animal products is NY / T 550 - 2002. The traditional culture and detection methods are still widely used. Although the method has high accuracy, it takes a long time. It takes 4 days to get the preliminary results. It needs 4 to 7 days to get the definite diagnosis results. It is not convenient and fast enough [28].

3.2. Immunological Techniques

Salmonella spp. have characteristic somatic or flagella antigens. Immunological assays use specific antibodies; monoclonal or polyclonal which bind with these antigens. Immunological assays are used for rapid detection of specific pathogens. Using the specificity of antibody antigen binding and recognition of antigen antibody complex, a very sensitive detection method can be established. At present, the immunological methods which are widely used in the detection of Salmonella, include enzyme-linked immunosorbent assay (ELISA), dot-linked immunosorbent assay (Dot-ELISA), immunofluorescence labeling (FIA), immunomagnetic separation (IMS), latex agglutination tests, immunodiffusion [29]. The assays which are immunology based are sensitive, specific and also reliable. There are some drawbacks of these methods which include a longer supplementation time to get the proper number of cells; closely related antigens show cross-reactions and variations in antigen, some sample may have restrictions for sensitivity relative to stressed cells matrices and expensive automation [30].

3.3. Molecular Biology Methods

Molecular biology method uses in vitro molecular probes/primers to detect specific sequence fragments of bacterial nucleic acids (RNA/DNA). Labelling the nucleic acid molecules with enzymes, isotopes and other markers is a common practice to achieve specific DNA or RNA detection. Specific nucleic acid target sequence utilizes these assays within the organism. The assays have been most seriously reconnoitered and established among Salmonella detection methods for being sensitive, specific, and it is inclusive, because without obtaining pure cultures it is rapidly identifying Salmonella [10]. At present, the main molecular methods to detect salmonella nucleic acids include: PCR, multiplex PCR, real-time fluorescence quantitative PCR, in situ fluorescence loop-mediated isothermal amplification (LAMP), gene chip technology, nucleic acid probe technology. In a study a DNA primer pair was used to amplify a 284 bp fragment of Salmonella DNA [31]. The primer sequence is shown in Table 1, and the bands of 284 bp of positive PCR product are shown in Figure 4.
Figure 4. Agarose gel electrophoresis.

M: DNA Maker DL 2000, The remaining lanes were purified Salmonella DNA samples. PCR product are detected by real time Polymerase chain reaction by monitoring the increased fluorescence signal within a system which is equipped with integrated real-time and fluorescence detector. This solved the issue of false Positive results caused by amplicon contamination.

Table 1. PCR/RT-PCR primer sequences.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Primer sequence (5’→3’)</th>
<th>annealing temperature/°C</th>
<th>Amplification size /bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>5'-TCATCGCACCGTCAAAGGAACC-3’</td>
<td>55</td>
<td>284</td>
</tr>
<tr>
<td></td>
<td>5'-GTGAAATTATCGCCACGTTCGGGCAA-3’</td>
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Salmonella has a complex antigenic structure, generally divided into somatic O antigen and flagella H antigen, very few Salmonella contain capsular Vi antigen. At present, according to Kauffman-White (K-W) serotyping method, based on the difference of Salmonella antigen and flagella antigen, there 2600 different kinds of Salmonella serotypes [32]. O antigen is a specific polysaccharide contained in LPS and it is also a significant element of gram-negative bacteria. O antigen encoding Genes are usually placed in a cluster of genes called RFB on chromosomes. The RFB gene is involved in the biosynthetic pathway of nucleoprotein and the transfer of repetitive units. The Wzx gene (rfb X gene) encodes a transmembrane protein that is composed of 12 transmembrane fragments and is present in clusters of all Salmonella O antigen gene. In different O antigen clusters, (Wzx) protein has structural homology [33]. These proteins are almost similar if level of amino acid sequence is considered. Studies have pointed out that the function of the Wzx protein is accountable for the O-unit from cytoplasm transported to the cell membrane side which is known as periplasmic side [34]. In contrast, H1 and H2 flagellar antigens encodes by fli-C and flj-B genes, correspondingly. The ends of both genes are conserved, while the central region of the flagellar antigen is highly variable [35,36].

Molecular detection methods have been studied to replace or supplement traditional serotyping methods, such as ribose typing, RAPD molecular marker technology [37], IS200 typing technology [38] analysis. With the continuous deepening of research, PCR and other nucleic acid-based molecular diagnostic techniques have been widely used in biological classification and identification, which greatly shortens the laboratory testing time, is easy to standardize, and does not require high staffing, to a certain extent, to make up for the shortcomings of traditional identification and typing methods [39]. In a study of Shi et al., they identified 21 common serotypes of Salmonella by MLST prediction and PCR direct determination [40]. The PCR method was based on genes encoding different antigens O antigen, H1 antigen and H2 antigen. Compared with MLST prediction method, the PCR method could identify Salmonella serotypes more accurately and directly. It has the advantages of less time-consuming and low cost, and can be combined with other classification methods, so it has a broader application space [10].

4. Prevention and Treatment of Salmonellosis

4.1. Salmonella Prevention

Good management and husbandry practices make sure preventions of clinical disease. The most desirable thing is the all-in-all out management systems and suitable sanitation between different groups [41]. The important thing is that pigs having different range of ages or sources should not be assorted. Before adding new pigs to empty pens or buildings there should be proper cleaning and disinfection and these practices are very important. The prevention for salmonellosis also depends on vaccination. Preventive medication is not recommended. Regular monitoring of the pathogenic Salmonella (including S. Enteritidis and Salmonella typhimurium) in the house during the pig raising process will help to prevent the outbreak of the disease. Water and feed for should also be preserved to avoid contamination by Salmonella [42].

The vaccines currently used to prevent Salmonella in livestock and poultry are divided into two categories: 1. Inactivated vaccines: mainly including Salmonella enteritidis inactivated vaccine and Salmonella typhimurium inactivated vaccine [43,44]. 2. Attenuated live vaccines: mostly live Salmonella typhimurium vaccine and live Salmonella enteritidis vaccines is used, etc. Different studies have shown that live vaccine strains against Salmonella infections give better protection if we compare it to the inactivated vaccines, perhaps due to the more obvious cellular immune response and the stimulation of mucosal immunity [45]. Attenuated Salmonella refers to Salmonella strains obtained by physicochemical, genetic engineering and other methods to cause irreversible mutations in certain virulence-related genes to achieve the purpose of reducing virulence. At present, the
attenuated strain of Salmonella is also used as a vaccine carrier to carry a foreign gene, and the corresponding immunogenic protein is continuously expressed along with the proliferation of the attenuated strain, thereby achieving the purpose of immunization [44]. Various virulence genes have been identified by different research studies, and it plays a crucial role in different phases of the Salmonella Typhimurium pathogenesis causing infections in pigs. These conclusions may donate to the development of more resourceful and safer type of vaccines.

The prevention of salmonellosis in livestock and poultry breeding mainly uses antibacterial drugs, while the infection of Salmonella is often accompanied by mixed infection. Most of the farmers use broad-spectrum antibiotics, such as amoxicillin and other antibiotics for bacterial disease prevention.

4.2. Salmonella Treatment

The goal to treat a Salmonella outbreak is to cure ill and diseased pigs which stop the spread of the pathogenic organism to transmit or spread in other animals. Salmonella spp. often shows resistance to many common antibiotics which makes this goal difficult to achieve. Drugs against Salmonella can be used to treat septicemic salmonellosis however these are not effective for enterocolitis. In case of enterocolitis the severity of illness does not decrease but may increase the course of shedding of Salmonella in faeces.

*S. choleraesuis* usually causes septicemic salmonellosis in pigs and antibacterial therapy is proved effective in most outbreaks. The use of systemic antibacterial is widely practiced and has been shown to decrease the severity of the disease and to increase animal’s survival rate. Mortality due to salmonellosis in pigs is high, but early treatment will increase survival rate. Antibiotics should initially be selected on the basis of susceptibility of the majority of *S. choleraesuis* isolates in the geographic area. Isolation, identification, biochemical testing and antimicrobial sensitivity are required for a confirmatory diagnosis in a particular disease outbreak as well as for the selection of suitable antibiotics for the treatment. Anti-inflammatory drugs are also effective in severely affected pigs.

Medication in feed/water for the oral administration should be started in the beginning of an outbreak not only effectively reduces the morbidity rate but also decrease in number of new cases by decreasing shedding of organism. Sick pigs often go off feed because of loss of appetite. In such cases oral medication is not helpful and other routes (Intra/venous or Intra/muscular) should be adopted.

The current common drugs for the treatment of Salmonella include:

- Neomycin: It can be used to treat gastrointestinal infections caused by Salmonella. Different preparations of neomycin are present; premixed neomycin can be given at a the dose rate of 77 ~ 154 g / 1000 kg of feed where as whereas soluble powder / solution can be administered at a dose rate of 50~75 mg/L water for 3~5 days;
- Oxytetracycline: Oral administration of single dose in pig at a dose rate of 10~25 mg/kg body weight, 2~3 times a day for 3~5 days.
- Methotrexate: Oral administration of a single dose, at a dose rate of 5-10 mg / kg body weight, 2 times a day, for 2-3 days.
- Colistin: It is usually available as mixed drink. In pigs the dosage is 40~200 mg/L in water. Lactating pigs are given 2~40 g/1 000 kg feed, piglet 2~20 g/1 000 kg feed.
- Sulfadiazine: mixed feeding, single daily dose, pig 15 ~ 30 mg / kg body weight, used for 5 d [46].

On this basis, it is recommended that farmers use that antibiotic which shows susceptibility in *in vitro* antibiotic susceptibility testing of pathogenic bacteria in the treatment of Salmonella infection, which can effectively avoid the problems of poor efficacy, high treatment cost and drug resistance caused by the abuse of antibiotics.

Antibacterial drugs are also used in feed as a prophylactic purpose. It may decrease the incidence and the severity of clinical infection but does not prevent infection or eliminate Salmonella. This practice is costly, encourages antibiotic resistance, less effective and less desirable for prevention and control of Salmonella. Prophylactically anti-bacterial should be used as feed additives in feed instead of using at growth promoting levels, for short periods of time [47]. To decrease the risk of drug resistance, the same anti-bacterial drug should not be given for a longer period of time.

5. Conclusion

To sum up, Salmonella has caused great harm to pig industry and even human beings. This article summarizes the biochemical characteristics, clinical manifestations, laboratory detection methods, treatment and prevention methods of Salmonella, and forms a review article on Salmonella. It is expected to provide help for further understanding of Salmonella.

References


