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Pathogenicity, Epidemiology and Virulence Factors of *Salmonella* species: A Review

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Abstract

Salmonella infections are major public health problems worldwide. The hereby review aimed to establish an overview on the pathogenicity, epidemiology and virulence factors of *Salmonella* spp. in the world. A systematic search was conducted online using the keywords 'Salmonella', '*Salmonella* spp.', '*Salmonella s*pp. Epidemiology', 'virulence factors of *Salmonella spp.* in the world', 'bacteria responsible for the contamination of meat products', 'non-typhoid salmonella'. These keywords were entered into databases such as PubMed and Google Scholar using mainly French language. The obtained articles were included based on the reliability of their source, the study area (usually Benin and Africa) and the subject. The review revealed that *Salmonella* spp. is motile Gram-negative rod-shaped bacteria, of the family Enterobacteriaceae, currently counting more than 2,600 serovars. Human contamination occurs through the ingestion of contaminated water and food and can cause gastroenteritis or typhoid fever, which are two serious public health problems. A gene set constituting the pathogenicity islands determines the pathogenesis of *Salmonella* spp. The diagnosis is based on bacteriological, serological and molecular techniques. *Salmonella* infections are usually treated using antibiotics; however, emergence of antibiotic resistance in these microorganisms suggests that the anti-salmonella control should explore new sources such as medicinal plants.

Keywords: antibiotic, bacteriological and molecular diagnostics, *Salmonella* spp., serovar

Abbreviations: MLST: Multilocus Sequence Typing; MLVA: Multilocus Variable Tandem Analysis; NTS: Non Typhoidal Salmonella; PCR: Polymerase Chain Reaction; RAPD: Random Amplification of Polymorphic DNA; RFLP: Restriction Fragment Length Polymorphism; SPI: *Salmonella* Pathogenicity Island

Introduction

Salmonella infections are a major public health problem (OMS, 2013). In the United States, they cause disease in more than one million people every year, resulting in estimated medical costs of $ 365 million (CDC, 2014). In the European Union, they are the second most common cause of gastrointestinal infection, with a confirmed case rate of 20.4 cases per 100,000 individuals in 2011 (European Centre for Disease Prevention and Control, 2013). In China, it is estimated that 22.2% of foodborne diseases are due to *Salmonella* (Wang *et al.*, 2007). Worldwide, salmonella infections are the second leading cause of foodborne bacterial diseases (Dougnon *et al.*, 2017).

They are often the etiological agents of gastroenteritis associated with the consumption of crustaceans and contaminated meat products (Leriche and Carpentier, 2000; Chae and Schraft, 2001; Joseph *et al.*, 2001). *Salmonella* gastroenteritis is a major cause of food poisoning in developed countries, and infant mortality in developing countries.

Human contamination occurs primarily by oral route via ingestion of contaminated water or food. *Salmonella* spp. may indirectly affect humans through the transfer of animals and animal-derived foods, and cause life-threatening diseases. This aggravates its epidemiological burden.

The use of antibiotics is fundamental for controlling the epidemiology of salmonellae, but the emergence and widespread of multidrug-resistant strains to antibiotics responsible for gastroenteritis keeps worsening the situation (Hadjer, 2016). Global Monitoring data showed an overall increase of antibiotic resistance in *Salmonella* although there are a large geographical variability and serotype (Parry and Threlfall, 2008; Gong *et al.*, 2013; European Centre for Disease Prevention and Control, 2013; Lai *et al.*, 2014; Russel *et al.*, 2014; Andino and Hanning, 2015). It is urgent to develop a broader understanding of *Salmonella* infection in animals and humans (Kuang *et al.*, 2015) to guide research towards discovering alternatives to current antibiotics.

The current article aimed to identify, through a literature review, information on pathogenicity, epidemiology and virulence factors of *Salmonella* spp.

Materials and Methods

A systematic search was conducted online in French using the keywords 'Salmonella', 'Salmonella spp., 'salmonella spp epidemiolog', 'Virulence factors of *Salmonella spp* in the world', 'Bacteria responsible for the contamination of meat products' and 'non-typhoid salmonella'. These keywords were entered into PubMed and Google Scholar and the articles obtained were included based on the reliability of their source, the study area (mainly Benin and Africa) and the subject.

Results and Discussion

Description of Salmonella species
Salmonella spp. is motile Gram-negative rod-shaped bacteria, facultative aerobes and non-spore-forming (Sabbag, 2013). The size of the rods varies between 2.0 and 5.0 μm in length and 0.7 to 1.5 μm width (Korsak *et al.*, 2004; Moreno *et al.*, 2009).

According to Bergey's Manual (2001), the Genus *Salmonella* belongs to the family Enterobacteriaceae, in the order of *Enterobacteriales*, class of gamma-proteobacteria and the phylum *Proteobacteria* (Scaria *et al.*, 2008). It is divided into two distinct species: *S. enterica* and *S. bongori*. *S. bongori* corresponds to the old V subspecies of *S. enterica* and represents only 1% of the genus (Barrow and Methner, 2013). As for *S. enterica* it is divided into six subspecies: *enterica (I), salamae (II), arizonae (III), diarizonae (IV), houteanae (V)* and *indica (VI)* (Popoff *et al.*, 2004; Tindall *et al.*, 2005).

Serotyping based on the characterization of somatic antigens and flagella proteins, classifies subspecies as serovars. Serotyping is an essential component of epidemiological surveillance and investigation of salmonellosis outbreaks (Bergeron, 2009). Currently, more than 2,600 serovars of *Salmonella* are listed in Kaufmann-White-Le Minor's scheme. The Reference Center of Research on Salmonella at the Pasteur Institute, WHO Collaborator, maintains this scheme. The subspecies *S. enterica* subsp. *enterica* serovar is divided into Dublin, Enteritidis, Infantis, Paratyphi, Typhi, Typhimurium, *Virchow* etc. (Aubry, 2013). These serovars are frequently involved in human and animal infections (Agasan *et al.*, 2002; Boko *et al.*, 2013).

ELISA is a serological method commonly used for the detection of antigens or products of *Salmonella* spp. In the ELISA test, a specific antigen *Salmonella* spp. is linked to the appropriate antibody linked to a Solid matrix. After formation of the antigen-antibody complex, the concentration of the antigen and the presence of *Salmonella* can be measured by the color change caused by the enzyme cleaving a chromogenic substrate (Tietjen and Fung, 1995; Blivet *et al.*, 1998).

The agglutination technique uses latex particles coated with antibodies that react with antigens on the surface of *Salmonella* cells to form visible aggregates for the identification of positive samples (Thorns *et al.*, 1994; Tietjen and Fung, 1995). These analyzes are used as much technical confirmatory analysis rather than for screening *Salmonella* strains (Love and Sobsey, 2007; Eijkelkamp *et al.*, 2009).

This is a *Salmonella* screening procedure based on the PATH-STIK test, an immuno-gauge test. Before the gauge, the sample is pre-enriched and then selectively enriched. PATH-STIK does not include washing and is a quick method requiring only 30 minutes for analysis (Brinkman *et al.*, 1995).

Epidemiology of Salmonella spp. worldwide
Among the diseases related to *Salmonella spp.*, two are of major importance and constitute public health problems in the world: gastroenteritis, usually caused by serotypes Typhimurium and Enteritidis (other serovars may cause this disease), and typhoid fever (enteric fever), the causative agent of which is serovar Typhi (Sabbagh, 2013).

In addition, *S.* Paratyphi A, B and C are responsible for paratyphoid fever: an enteric fever less common than typhoid in developing regions, but clinically similar (Jones and Falkow, 1998; House *et al.*, 2001). Several NTS represented by the Typhimurium, Enteritidis, Newport, and Heidelberg serovars have reservoirs in farmed animal species (Hohmann, 2001; Rabsch *et al.*, 2001). They are therefore the main causative agents of food poisoning in the world. The serovars Enteritidis and Typhimurium are respectively the first and second largest cause of salmonellosis caused by consumption of contaminated food in humans (Bishop *et al.*, 2011). With regard to typhoid fever, recent statistics estimated the incidence of the disease to more than 21 million cases and place it responsible for about 216,000 deaths 2000 (Crump *et al.*, 2004); whereas 93.8 million cases and 155,000 deaths are associated with gastroenteritis caused by NTS (Nontyphoidal Salmonella) every year on the planet. Inadequate food storage temperature, inadequate cooking and poor personal hygiene

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are among other factors, the contamination factors mentioned (Crump *et al.*, 2004; Majowicz *et al.*, 2010).

Pathogenicity of Salmonella species

Virulence is the relative ability of a bacterium to induce infection (Berraho, 2009). The essential characteristics of the pathogenesis of salmonellae are their ability to invade host cells and remain as an intracellular parasite. When localization of the infection occurs, salmonellae often remain confined to the mesenteric lymph nodes. The first defense mechanisms used by the host are the acidity of the stomach and the bile salts of the small intestine, which have a bactericidal effect. Once in the small intestine, *Salmonella* should adhere as quickly as possible to the intestinal mucosa. They will pass through the lymphoid follicles of the ileum. At this point in the intestine, the epithelium is characterized by the presence, of M cells and the absence of mucus secretingcells. Apparently fimbriae (adhesins) allow recognition and binding of *Salmonella* to Peyer's plaques (Thorns and Woodward, 2000; Vimal *et al.*, 2000).

The entry into the Peyer plaques requires the presence of type III secretion systems. They are encoded by a set of genes forming the pathogenicity islands.

The pathogenesis of *Salmonella* spp. is defined by virulence factors encoded by genes organized into blocks on the chromosome, classified as "pathogenicity islands" or SPI (Salmonella Pathogenicity Island) and constituting an essential feature of the virulence of *Salmonella*. Five SPIs have been well characterized (Bonny *et al.*, 2011). SPI1 and SPI2 encode type III secretion systems, which provide the main virulence characteristics of *S. enterica* (Boko *et al.*, 2013). SPI1 is involved in invasion of host cells and inflammation in particular of phagocytic or non-phagocytic cells of the intestinal mucosa (Brisabois, 2001). It hosts the invasion *inv*A gene, which is found in most *Salmonella* strains. SPI2 is needed to encode the proteins involved in intracellular survival and replication within phagocytes. It also contributes to the systemic spread of salmonella. SPI2 contains the *spi*C gene that encodes the structural components and secretion and helps to initiate the production of mediators with considerable function in the virulence of *Salmonella*. *spi*C is also involved in the expression of flagella filament components and plays an important role in *Salmonella* infection (Brenner *et al.*, 2000). SPI3 is present in all lineages. However, distributions of SPI4 and SPI5 have not been established (Camart-Perie, 2006) even though their role is known. SPI4 intervenes in the initial interaction with the intestinal epithelium and contributes to long-term persistence (Bonny *et al.*, 2011). It contains the *orf*L gene, which is necessary for intra-macrophage survival and possibly carries a system involved in the secretion of toxins (Boko *et al.*, 2013). SPI5 is involved in the realization of several pathogenic processes during infection (Bonny *et al.*, 2011). Its first gene, *pip*D SPI5, has a target on the surface or inside the host cells (Carip *et al.*, 2008).

Salmonella has other virulence factors that are not located in the SPI. They can be observed on mobile genetic elements such as plasmids. The *Salmonella* virulence plasmid, composed of five genes (*spv*RABCD) potentiates the systemic spread of the pathogen and helps to replicate in extra-intestinal sites (Brisabois, 2001).

Bacteriological methods of Salmonella spp. diagnosis

Salmonella detection can be direct (bacteriological method). For the analysis of food samples (meat, eggs), various standards are used in food hygiene (Humbert *et al.*, 1998). The horizontal rules applicable to all types of products (ISO 6579 December 1993) and sectoral standards specific to a product type (NF V59 - 109 for food gelatin). The detection of *Salmonella* in food according to ISO 6579 has four basic steps: pre-enrichment, enrichment, isolation and biochemical identification.

Pre–enrichment: This is a non-selective step that uses a rich medium in which the sample is 10 fold diluted and incubated for 24 hrs at 35 °C or 37 °C. The pre-enrichment allows the sub-lethal bacteria to recover all their potentialities at the end of the incubation. The media used are liquid media, most often buffered peptone water or lactose broth (Humbert *et al.*, 1998).

Enrichment: Enrichment aims to minimize the growth of other bacteria that may be present in the sample, but also to enhance the selective growth of *Salmonella*. About 0.1 ml or 1 ml of the pre-enrichment solution is transferred into one or more enrichment media (10 ml of medium). The enrichment media are classified into three families: selenite broths, tetrathionate broths (Müller Kauffmann broth) and broths containing malachite green and magnesium chloride (Rappassort broth from Vassiliadis) (Humbert *et al.*, 1998).

Isolation: Isolation is a selective step that uses solid media poured in Petri dishes. Isolation media contain a variety of selective factor associations. *Salmonella* appear as characteristic colonies by their shape, color and morphology. Solid media used for isolation are: Rambach media, Hektoen media, *Salmonella - Shigella* agar (SS agar), light green and phenol red agar (VB-RP), xylose-lysine-tergitol (XLT), *Salmonella* Compass media, mannitol lysine light green crystal violet, sucrose lactose citrate deoxycholate agar (DCLS) Xylose lysine desoxycholate agar (XLD) and Bismuth sulfite agar.

Biochemical and serological identification: Biochemical identification of *Salmonella* colonies is performed in two stages: the first step is the search for the family characteristics; very often, it is the Gram staining, the presence of catalase, the absence of a cytochrome oxidase, motility tests, respiratory type, culture on ordinary medium and fermentation of glucose. The second step is the search for differential traits, which requires pure cultures. Therefore, the reduced Leminor rack is used, which is a set of five media: Kliger-Hajna medium, Simmons citrate medium, iron lysine medium or Taylor medium, urea-tryptophan medium and mannitol mobility nitrate medium

Confirmation can also be made by serological identification. Serotyping identifies serogroups of cultures by antigenic analysis using the agglutination reaction subjected to structural antigens. However, the same serotype of *Salmonella* may vary with antigenicity, due to the change and loss of surface antigens, thus reducing the sensitivity of serological methods (Hoorfar *et al.*, 1999;

Sørensen *et al.*, 2004).

There are also other non-conventional bacteriological diagnostic techniques for *Salmonella*. These include sensitivity to phage 01 of Felix and Callow, standardized systems (API 20 E, RAPID 20 E, Enterotubes Rocks, MIS enterobacteria), lysis by phage 01 (Felix and Callow) which can be used as a confirmatory test for *Salmonella* spp.

Miniaturized biochemical assay

These systems include sterile, disposable microtiter plates, a multiple inoculation device, and substrates specifically designed to target organisms. Cells of *Salmonella* spp. are identified as a function of assimilation and use a special substrate, automated colony measurement and cell density. There are various kits for rapid miniaturized biochemical characterization of *Salmonella,* including commercially available API 20^E; Enterotube II; Enterobacteriaceae Set II; MICRO-ID; Quantum II; Sensititre; Tri Panel.

Molecular diagnostic methods for Salmonella spp.

Molecular methods are based on analysis of total DNA, chromosomal or plasmid DNA (Brisabois, 2001). The use of molecular tools in *Salmonella* Typing is widespread and based on several methods:

Random Amplification of Polymorphic DNA (RAPD)

The principle consists in using an oligonucleotide of about ten bases as a probe. The latter will bind to the bacterial genome at different non-specific locations, thus creating an important polymorphism in size and number of fragments generated afterwards. This typing method is widely used. The profiles of the obtained amplified products may be characteristic of the strain and allow good discrimination within a given serotype (Hilton *et al.*, 1996).

Ribotyping

Ribotyping consists of amplifying intergenic regions between the genes that encode the 16S and 23S ribosomal RNAs followed by digestion with restriction enzymes. This technique makes it possible to determine the phylogenetic relationships between the strains of the same species (Millemann, 1998; Grimont *et al.*, 2001).

Identification using PCR

PCR is used for early diagnosis while recommending the use of the Widal and Felix test when there is a diagnostic suspicion and eliminating it as a screening test. *Salmonella* Typhi, Paratyphi A, B, C are preferably isolated in blood and in feces of typhoid patients (Dumas, 1958). The first step is extracting chromosomal DNA from heat shock. A colony is suspended in 500 μl of sterile distilled water. Subsequently, this suspension is placed in a water bath at 95 °C for 10 min and then placed immediately on ice for 10 min. The operation is repeated 2 to 3 times in order to lyse the bacteria. After centrifugation for 10 min at 13,000 rpm, the supernatant is used for amplification by PCR or stored at -20 °C. The PCR technique amplifies a specific region of bacterial DNA "16S rRNA *Salmonella spp*" by using specific primers ITS1 -ITS2 (Gomez-Duarte *et al.*, 2009).

DNA probe hybridization assay

The technique is based on a hybridization assay of the DNA molecule by a probe comprising a sequence complementary to the target sequence of a DNA or RNA molecule in the target organism (de Boer and Beumer, 1999; Fung, 2002; Mozola, 2006). In a first stage, the target cells are lysed, and then the nucleic acids are purified. The stable hybrid with the labeled DNA is detected by radiographic and enzymatic techniques. However, a sufficient concentration of the target organism (here *Salmonella*) after pre-enrichment is necessary for detection (Jones *et al.*, 1993).

The mass spectrometer: MALDI-TOF

In the MALDI-TOF process, the sample to be analyzed is mixed with a substance called 'matrix'. The mixture is disposedon a slide, which is placed in the instrument and illuminated by a laser. The matrix absorbs light from the laser, evaporates with the sample, and gains an electrical charge (ionization). The electric fields guide the ions in the mass spectrometer which will separate them according to their mass and give results in the form of a series of peaks (spectrum) corresponding to the different fragments originating from the original molecule. By analyzing the characteristics of these fragments, it is then possible to deduce the structure of the molecule. VITEK MS compares the spectrum of the sample with a spectrum library including a large number of recorded bacteria to allow accurate identification of the species present.

WGS: Whole Genome Sequencing (WGS)

Whole genome sequencing (WGS) refers to highly automated and parallelized genome sequencers used to sequence entire genomes of bacterial pathogens within hours. When coupled with bioinformatics analytical pipelines such as the one established at CFSAN-FDA, precise and stable genetic changes can be identified that can distinguish *Salmonella* foodborne epidemic strains at the source level, including specific farms, food types and geographical regions.

Metagenomics

Metagenomics is the analysis of the genomic DNA of an entire community. This distinguishes it from genomics, which is the analysis of the genomic DNA of an individual organism or cell (Chen *et al.*, 2010; Gilbert and Dupont, 2011). According to Marchesi and Ravel (2015), a metagenome is the collection of genomes and genes from members of a microbiota obtained by sequencing DNA rifle extracted from a sample.

Targeted bacterial metagenomics studies have focused on the use of 16S rRNA gene sequences that utilize the ability to use the conserved regions of the 16S rRNA gene to identify bacterial taxa.

Antibiotic resistance of Salmonella spp.

Antimicrobial resistance is one of the major health problems in human and animal medicine (Guillot, 1989). The WHO also recognizes it as an emerging public health problem. The phenomenon is more important as it concerns pathogens that can be transmitted to humans (Duffy *et al.*, 1999; Madec, 2012). In recent years, *Salmonella* Typhimurium DT 104 of stem isolated in various human and animal environments often have a multidrug resistance phenotype with respect to the following antibiotics: ampicillin, chloramphenicol,

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streptomycin, sulfonamides and tetracycline (Leriche and Carpentier, 2000; Tindall *et al.*, 2005). Resistance to sulfamethoxazole-trimethoprim, nalidixicacid, doxycycline, tetracycline, cephalosporins, fluoroquinolones and penicillin has also been established (Carip *et al.*, 2008). Mutations (3 or 4) at the level of genes associated with gyrase (subunit encoded by *gyrA)* and topoisomerase IV (subunits A and B encoded by *parC* and *parE,* respectively) were connected to the resistance mechanisms to ciprofloxacin (Sabbagh, 2013). A recent study reported that medicinal plants such as *Persea americana, V. amygdalina, Corchorus olitorius, Moringa oleifera* (leaves), *Carica papaya* (leaves and seeds) and *Vernonia amygdalina* (leaves) are used for salmonellosis treatment (Dougnon *et al.*, 2017).

Conclusions

Salmonella infections are among the first known causes of collective foodborne illnesses. At the economic and social levels, they are of crucial importance, given the loss of lives that they generate. They have several pathogenicity factors defined by the pathogenicity islands, which give them a certain virulence enabling them to bypass the defense mechanisms of their host. Official surveillance systems for zoonotic diseases need to be strengthened to enable the collection of essential epidemiological data in order to better ensure prevention and information. A better knowledge of the ecology of *Salmonella* is therefore an important step in the fight against these species. Moreover, it is necessary to control the cases of antimicrobial resistance by resorting to new sources of antibiotics such as medicinal plants.

Acknowledgements

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