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Diversity of non-typhoidal *Salmonella* in Algeria

by

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List of publications

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Declaration

I declare that this Ph. D. thesis is my own work and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where states otherwise by reference or acknowledgment, the work presented is entirely my own.

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Abbreviations

AFLP	Amplified fragment length polymorphism
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
ET	Electromorph type
FDA	Food and Drug Administration
KCN	Potassium Cyanide
LPS	Lipopolysaccharide
MDR	Multi-drug resistant
MLEE	Multi-locus enzyme electrophoresis
MLST	Multi-locus sequence typing
NTS	Non-typhoidal *Salmonella*
ONPG	Ortho-Nitrophenyl-B-galactoside
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
SCV	*Salmonella* containing-vacuole
SGI	*Salmonella* genomic island
SPI	*Salmonella* pathogenicity island
ST	Sequence type
T3SS	Type three secretion system
WGS	Whole genome sequencing
WHO	World Health Organization

Salmonella nomenclature

For simplicity, in this dissertation *Salmonella* serovars are referred to by their serovar names, the preceding *Salmonella enterica* subspecies *enterica* is abbreviated by the letter "*S.*".

ABSTRACT

Non-typhoidal *Salmonella* (NTS) are globally recognized as important pathogens associated with gastroenteritis. In most cases, humans are infected through consumption of contaminated food products, especially food of animal origin. Poultry is reported to be the major source or reservoir of these pathogens. Most data on incidence and prevalence of NTS infection in both human and poultry are available from industrialized countries. In developing countries including Algeria, there is a lack of documentation, surveillance projects and initiatives. Thus, this has led to an underreporting of *Salmonella* serovars. This situation is making it harder for health authorities to implement and design preventive approaches for NTS infections. This concern has heightened after the emergence of multidrug-resistant *Salmonella* strains, as these pathogens are more virulent and responsible for adverse outcomes in infected patients. In Algeria, unregulated use of antibiotics is thought to have caused an increase in resistance by these organisms. Therefore, it is required more than before to initiate more projects documenting the background of NTS serovars in circulation, in order to build a strong and reliable data, useful in making policies for the establishment of routine surveillance systems. The overall aim of this thesis was to identify the different *Salmonella* serovars isolated from human and poultry in four cities in Algeria, including Guelma, Setif, Batna and Algiers, and to determine the prevalence of antimicrobial resistance in these isolates. Antimicrobial testing was conducted on the isolates using genotypic and phenotypic approaches.

Full genome sequences of the isolates were obtained using Whole Genome Sequencing (WGS) technology, and were analysed *in silico* for molecular characterization. Different serovars have been identified among the human isolates, naming *S*. Typhimurium as the most dominant, followed by *S*. Kentucky, *S*. Enteritidis, *S*. Heidelberg, *S*. Ohio, *S*. Lindenburg, *S*. Indiana, *S*. Virchow, and *S*. Bonn. Instead, *S*. Gallinarum was the only serovar found among the poultry isolates. The isolates displayed resistance to multiple antimicrobials. Genotyping showed that the resistance was mediated by various genes encoding for resistance to β-lactam antibiotics, carbapenems, quinolones, aminoglycosides and to co-trimoxazole (trimethoprim-sulfamethoxazole). Classical *Salmonella* genomic island 1 was identified in serovar Typhimurium, while new variant of SGI1 was identified in serovar Kentucky isolated from human, and it was given a name SGI1-K7. Fifty-four isolates (79%) carried various *Salmonella* pathogenicity islands (SPIs), including SPI-5, SPI-9, SPI-13, SPI-14 and C63PI. Fifty-four isolates (76%) carried at least one plasmid each. Plasmids belonging to incompatibility group FIB and FII were the most commonly identified among the isolates.

These findings are vital to public care system, and helpful for epidemiological control programmes. Furthermore, results presented above contribute in building strong and reliable databases, than can be effective to describe NTS infection and trace its public health consequences among the Algerian population.

BACKGROUND AND AIM OF THE STUDY

BACKGROUND

At the global level, diseases caused by non-typhoidal *Salmonella* (NTS) still have a massive impact on public health with a relevant impact in developing countries (Schlundt *et al.*, 2004; Djeghout *et al.*, 2017). It is estimated that NTS gastroenteritis are around 94 million cases, resulting 115,000 deaths every year (Majowicz *et al.*, 2010). On the other hand, NTS infections represent a socio-economic burden in both high- and low-income countries as it requires epidemiological surveillance systems and monitoring programs in order to effectively detect and control outbreaks (Crump *et al.*, 2011).

Different vehicles have been shown to be implicated in the transmission of these pathogens, and it is mainly associated with the consumption of contaminated raw meat, eggs and chicken, milk and other dairy products, fish and other sea foods, fruits and vegetables (FDA/NSTA, 2008).

Multi-drug-resistance (MDR) among *Salmonella* strains is another increasing concern for public health, as infections with MDR strains are causing more morbidity and mortality than those caused by susceptible strains (Fluit, 2005). Indeed, MDR *Salmonella* is giving rise to clinical worries for the antimicrobial therapy in both systemic gastroenteritis and bacteraemia caused by NTS serovars (Cooke *et al.*, 2007).

There is a paucity of data on *Salmonella* serovars in humans and from food sources in many developing countries. In Algeria, NTS represent one of the primary causes of

salmonellosis in both humans and food animal production, especially poultry (Ayachi *et al.*, 2010). The lack of data is probably linked to the inadequate setting of resources and shortage of epidemiological investigation.

To date, few studies have been reported information on NTS infection from various cities in Algeria, including Algiers, Boumerdes, Tizi-Ouzou, Bouira, Bejaia, Constantine, Batna, Annaba and El Taref (Fig. 1) (Aboun *et al.*, 2000; Elgroud *et al.*, 2009; Ayachi *et al.*, 2010; Bouzidi *et al.*, 2012; Mezali and Hamdi, 2012; Elgroud *et al.*, 2015; Djeffal *et al.*, 2017). Further research investigations are needed to effectively describe NTS infection and trace its public health consequences among the Algerian population.

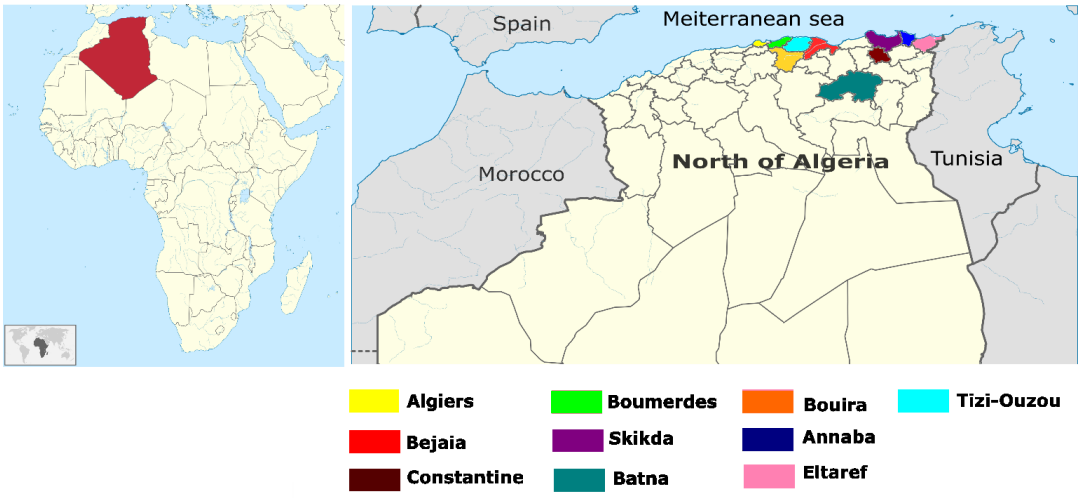

Fig. 1. **Geographical location and distribution of reported NTS infection with a focus on North Algeria**. Colors represent cities where NTS infection has been reported in the last 12 years (Djeghout *et al.*, 2017).

AIM OF THE STUDY

The purpose of the Ph. D. project was to identify the different *Salmonella* serovars isolated from human and poultry in four cities in Algeria, including Guelma, Setif, Batna and Algiers, and to determine the prevalence of antimicrobial resistance in these isolates using phenotypic and genotypic approaches.

The specific studies focused on the following objectives:

- To identify the different non-typhoidal *Salmonella* serovars isolated from human and poultry in four cities in Algeria
- To determine the prevalence of antimicrobial resistance in the isolates, using genotypic and phenotypic approaches.
- To characterise the multi-drug resistant invasive *S*. Kentucky ST198 from Algeria.

INTRODUCTION

INTRODUCTION

Serological and biochemical characteristics are the main methods used for *Salmonella* differentiation (Achtman *et al.*, 2012). Serotyping has been the core of public health monitoring of *Salmonella* infections for years. The Kauffmann White scheme is simplified and is commonly used in routine clinical laboratories, because it contains all serotypes and variants of existing serotypes confirmed and accepted by the WHO Collaborating Centre for Reference and Research on *Salmonella* (Bale, 2007). However, currently scientists commonly use DNA- based method such multi-locus sequence typing (MLST) to further divide every serotype into more subtypes, to detect outbreaks and effectively identify the pathogen in cause. Furthermore, Whole genome sequencing (WGS) technology have replaced traditional typing, and it is used now as routine typing tool in different reference laboratories including Public Health England (PHE) and Centers for Disease and Control Prevention (Ashton *et al.*, 2016).

Taxonomy of *Salmonellae*

Salmonella represents a leading cause of food-borne disease worldwide (Patrick A.D. Grimont, 1, 2001). *Salmonella,* a bacterial strain named after the American pathologist Daniel Elmer Salmon. It was first isolated in 1855 by Theobald Smith from the intestines of in infected pigs with swine fever (Eng *et al.*, 2015).

Genetic relatedness among *Salmonella* serovars put in evidence their clonal origin, and the degree of the sequence divergence permits to estimate that a common ancestor of the genus existed more than 25 years ago (Bäumler *et al.*, 1998). To present, nomenclature used for *Salmonella* is the one recommended by World Health Organization (WHO) and used by the Centers for Disease Control and Prevention (CDC) (Popoff *et al.*, 2003).

Species definition

In the 1880s, species were named according to the disease they cause and the host they infect (Ford Doolittle and Zhaxybayeva, 2009). Currently, the most adapted concept is the phylogenetic species concept (PSC). According to this concept, assignments to species is mostly made on the basis of overall genotypic similarity (Ford Doolittle and Zhaxybayeva, 2009). The assignment of two isolates to one species is made when the degree of the identity has a value of ≥70-80% in a standardized DNA–DNA hybridization experiment (Crosa *et al.*, 1973; Staley, 2006). This concept considers as well the similarity of 16S rRNA sequences, strains with <95-97% identity are placed in the same species (Staley, 2006). Multi-locus enzyme electrophoresis (MLEE) is also used to characterize the species of organisms, as every strain produces a different and unique profile known as eletromorph type (ET). This method detects mutations among amino acids responsible of the differences in genes loci that codes the enzymes (Stanley and Wilson, 2003). Based on the measurement of sequence divergence or what is known

as the nucleotide sequence variation, members in the genus *Salmonella* are assigned to two species *enterica* and *bongori* (Crosa *et al.*, 1973; Reeves *et al.*, 1989; Patrick A.D. Grimont, 1, 2001).

Subspecies typing

Subspecies typing is defined using biotyping, DNA hybridization (Crosa *et al.*, 1973), 16S rRNA analysis and MLEF (Reeves *et al.*, 1989). Biotyping or biochemical tests was widely used by taxonomists (Patrick A.D. Grimont, 2001). This method uses typical biochemical tests to distinguish species and sub-species such as the ability to ferment sugar, the presence of organic acids, and many other components (Bale, 2007). Currently, seven different sub-species have been classified based on the agreement between DNA hybridization and biotyping tests (Patrick A.D. Grimont, 1, 2001). The committee referee formed the sub-species *S. enterica* subsp. *enterica* (equivalent to subspecies I), including also other six subspecies naming; *S. enterica* subsp. *salamae* (subspecies II); *S. enterica* subsp. *arizonae* (subspecies IIIa); *S. enterica* subsp. *diarizonae* (subspecies IIIb); *S. enterica* subsp. *houtenae* (subspecies IV); *S. enterica* subsp. *indica* (subspecies VI) (Table 1) (Le Minor and Popoff, 1987).

Serotyping

Serological typing or serotyping is based on two antigenic determinants: the somatic (O) antigen, and the flagellar (H: phases 1 and 2) antigen (Brenner *et al.*, 2000; Porwollik, 2011). Each serotype of *Salmonella* has a unique antigenic formula that is a certain combination of the antigens O and H (Patrick A.D. Grimont, 1, 2001) and few of them produces the capsular antigen Vi (Kauffmann, 1961). The *rfb* locus encodes for the enzymes that synthesizes the antigen O, whilst the *fliC* and *fliB* genes encode the phase 1 and phase 2 flagellins, respectively (Li *et al.*, 1994). Sixty seven somatic O-antigens and 117 flagellar H-antigens have been identified to date (Grimont and Weill, 2008) in more than 2600 serotypes of *Salmonella enterica* (Gal-Mor *et al.*, 2014). The genetic variation for the Vi antigen locus is used to describe the serovar Typhi (Ferris *et al.*, 1990). The serotyping concept; each serovar carries a unique antigenic formula is untenable, as in some cases one serovar was found to be heterogeneous and in other cases, serovars shared several traits. This situation seems to pull-out the serotyping from the species rank (Patrick A.D. Grimont, 1, 2001).

Current identification of *Salmonella* strains is mostly based on serology that classifies more than 2600 serotypes (Patrick A.D. Grimont, 1, 2001; Cooke *et al.*, 2007). Thus, different typing methods have been designed to describe the ancestry of *Salmonella* isolates. These methods have been developed to be used in reason case-depending.

They are assorted as phenotypic, genotypic and sequence-based methods (Table 2) (Cooke *et al.*, 2007).

Table 1. Biochemical differentiation characters for *Salmonella* species and subspecies.

Salmonella enterica							
Species	***S. enterica***						***S. bongori***
Subspecies	*enterica* I	*salamae* II	*arizonae* IIIa	*diarizonae* IIIb	*houtenae* IV	*indica* VI	V
Characters							
Dulcitol	+	+	-	-	-	*	+
Lactose	-	-	- (75%)	+ (75%)	-	*	-
ONPG	-	-	+	+	-	*	+
Salicin	-	-	-	-	+	-	-
Sorbitol	+	+	+	+	+	-	+
Malonate	-	+	+	+	-	-	-
Mucate	+	+	+	*	-	+	+
Gelatine	-	+	+	+	+	+	-
Growth in KCN	-	-	-	-	+	-	+

Adapted from (Bale, 2007).

+: positive reaction with 90% average.
-: negative reaction with 90% average
*: different reactions given by different serotypes

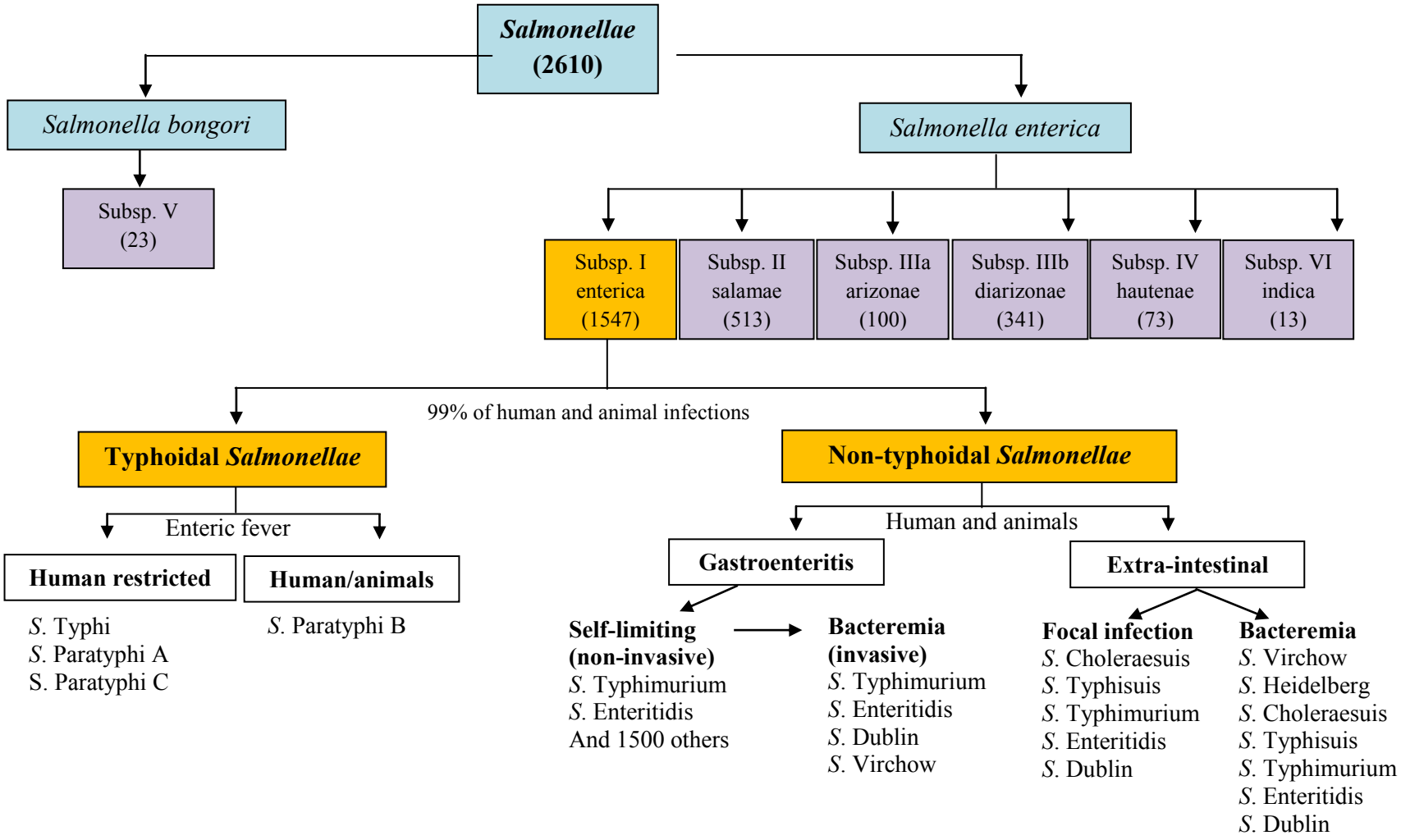

Fig. 2. General overview of *Salmonella enterica* classification. Two species have been defined using DNA-DNA hybridization and MLEE (Crosa *et al.*, 1973; Reeves *et al.*, 1989). Seven sub-species in *Salmonella* are defined by biotyping, DNA hybridization (Crosa *et al.*, 1973), 16sRNA analysis and MLEE (Reeves *et al.*, 1989). Serotypes within each subspecies are defined by serotyping (Guibourdenche *et al.*, 2010). Between brackets are numbers of serovars/serotypes identified in each sub-species.

Table 2. Common typing methods in *Salmonella*. Table adapted from (Cooke *et al.*, 2007).

Technique	Principle	Characteristic	Disadvantage	Reference
Phenotypic				
Serotyping	Agglutination between the antigen somatic "O" and Flagellar "H" with a specific antibody.	Easily performed and instantly result.	Expensive; as many antiserum needed. Does not reflect true classification as it depends on markers of choice.	(Kauffmann, 1950; Popoff *et al.*, 2003)
Phage typing	Susceptibility of bacterial isolate to a panel of bacteriophage.	Useful for epidemiology. Widely used for *S*. Typhi, *S.* Typhimurium and *S*. Enteritidis	Limited use and is not applicable on all serotypes. Does not reflect true classification.	(Demczuk *et al.*, 2003)
Resistance typing	Susceptibility of bacterial isolate to a panel of antibiotics.	Inexpensive and easily performed. Useful data at population level.	It is not a classification, because phenotypic resistance pattern is not stable in the same serovar.	(Cooke *et al.*, 2007)
Genotypic				
Pulsed field gel electrophoresis gel (PFGE)	Genomic DNA restriction into fragments and migration in an electrical field of alternating polarity.	Highly discriminative molecular typing technique. Useful in epidemiological studies.	Expensive and limited to research laboratories.	(Cooke *et al.*, 2007)
Amplified fragment length polymorphism (AFLP)	PCR based modification of PFGE. Fluorescent markers used to better discriminate the fragment.	Robust and supplies higher level of discrimination.	Expensive equipment required.	(Nair *et al.*, 2000)
Multilocus enzyme electrophoresis (MLEE)	Analyses the relative mobilities under electrophoresis of a large number of intracellular enzymes.	Helpful in surveillance and epidemiological investigations.	Difficult technique. Manual use only.	(Ferris *et al.*, 1990)
PCR for specific genes or islands	PCR based method for resistance genes and virulence factors.	Useful for identification within serotypes.	Does not identify serotypes.	(Wain *et al.*, 2003)
Plasmid profiling	Identification and analysis of plasmids harbored by a bacterial isolate.	Useful for outbreaks investigation.	Not considered a classification. Bacterial isolates may lose or gain plasmids easily.	(Connerton *et al.*, 2000)
Sequence-based				
Multilocus sequence typing (MLST)	Analysis of 7 conserved genes to separate strains into sequence types.	Reproducible. Based on phylogenetic concept to define *salmonella* subtypes.	Expensive. Restricted to research/reference laboratories.	(Kidgell *et al.*, 2002)
Microarrays	DNA-DNA hybridization of the whole genome against a reference sequence.	Measure gene content. Excellent tool to describe genetic variation.	Cannot detect novel insertions.	(Thomson *et al.*, 2004)
Whole genome sequencing (WGS)	Entire genome sequencing and analysis. Provides raw nucleotide sequence of an individual's DNA.	Excellent research tool "*in silico* typing", is being introduced to clinical laboratories.	Requires a capacious computing power and skills to use *in silico* for genome analysis.	(Gilissen *et al.*, 2014)

Infection and clinical disease

Salmonella can be transmitted by the oral–fecal route via contaminated food and water (Figure 3) and are mostly associated with inadequate sanitation and hygiene (McElhaney, 1992). Clinically, *Salmonella enterica* is divided into two groups based upon the disease caused: typhoidal that is usually human-host restricted, causing systemic infection known as enteric fever. The other group includes all non-typhoidal *salmonella* (NTS) that causes self-limiting gastroenteritis (Figure 1) (Cooke *et al.*, 2007)**.**

Host restriction and adaptability in *Salmonella*

Host adaptation in *Salmonella* is mainly based on epidemiological evidence and surveillance investigations that can contribute to disclose characteristics of reservoirs of *Salmonella* serotypes. In *salmonella*, host adaptation can be defined as the ability of bacteria to circulate and cause disease in different populations of vertebrate hosts (Kingsley and Bäumler, 2000). For example, *S*. Typhimurium and *S*. Enteritidis are considered broad-host-range serovars, because they are frequently associated with *salmonella* infection in different animal species and also human (Hormaeche *et al.*, 1991; Cogan and Humphrey, 2003). Indeed, *S*. Dublin, which is mainly hosted in cattle, is predominantly responsible for the systemic form of salmonellosis in humans (Chen *et al.*, 2013). On the other hand, host restricted *Salmonella* is defined as the ability to cause disease only in a unique animal species it is adapted to. For instance, *S*. Typhi is linked

with only to disease incidents reported from human causing typhoid fever, and it is unable to infect other vertebrate species (Kingsley and Bäumler, 2000; McClelland *et al.*, 2004). *S.* Gallinarum and *S.* Abortusovis are highly adapted to animal hosts in particular in poultry and sheep respectively, and may only produce very mild symptoms in humans (Rubino *et al.*, 1993; Chen *et al.*, 2013).

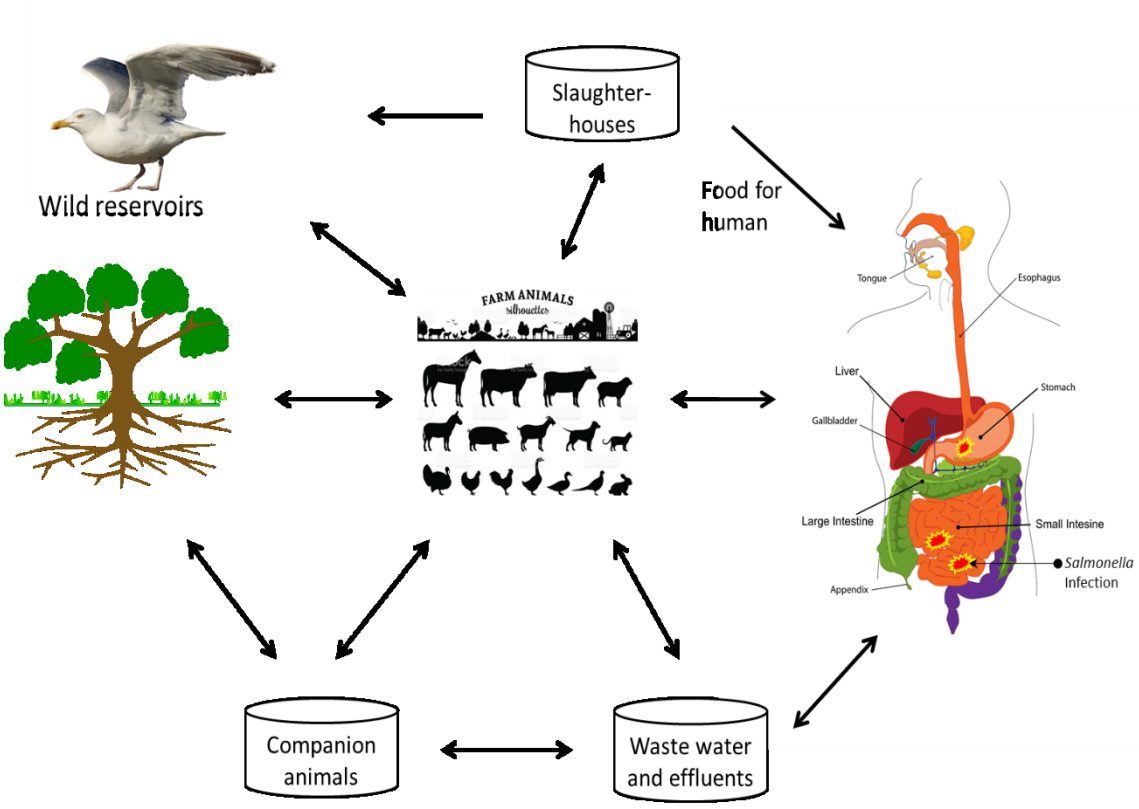

Fig. 3. Transmission of *Salmonella* in the human-animal-environment web. *Salmonella* is habitually spread after the consumption of contaminated raw meat, eggs and chicken, milk and other dairy products, fish and other sea foods, fruits and vegetables (FDA/NSTA, 2008). These pathogens can be transmitted between people and companion animals such as pets. Animals may become infected with *Salmonella* after roaming in contaminated environment. Wild animals such as birds may play a role in transmitting the bacteria to human (Patrick A.D. Grimont, 1, 2001).

Typhoidal *Salmonella*

Typhoid and paratyphoid fever are a systemic bacterial infection caused by *Salmonella enterica* subspecies *enterica* serovar Typhi and Paratyphi (hereafter *S.* Typhi) (McClelland *et al.*, 2004).

Clinically, paratyphoid fever is indistinguishable from typhoid fever. Therefore, *S.* Typhi, Paratyphi A, B and C are collectively referred to as typhoidal *Salmonella* serotypes (Sudeepa Kumar *et al.*, 2013). This disease is contagious, the bacteria can be transmitted from infected individuals via the fecal-oral route (Pang, 1998). At the global level and annually, typhoid infection comprises around 75% to 80% with more than 21 650 000 cases (with 216 500 deaths), and around 5 410 000 cases of paratyphoid fever (Ayele *et al.*, 2011).

Human-restricted typhoid

Human constitutes the only natural host and reservoir of infection caused by *S.* Typhi and *S.* Paratyphi A and C. These serovars are a highly host-adapted pathogens (McClelland *et al.*, 2004; Ayele *et al.*, 2011). *S.* Typhi was first isolated in 1880 by Karl J. Erberth and Robert Koch. It is known by the antigenic formula [9]Vi:d:-, and certain biochemical characters (Langridge, 2010). Substantially, during the late nineteenth and early twentieth centuries, typhoid fever caused by serovar *S.* Typhi was endemic in the majority of European countries and in both North and South America (Wolman and Gorman, 1931). Currently, the disease is uncommon in developed countries (Ackers *et*

al., 2000). Similarly to *S.* Typhi, *S.* Paratyphi identified with antigenic formula 1,2,12:a[1,5] is thought to cause milder disease, with mainly gastrointestinal symptoms (Grimont and Weill, 2008; Farrar *et al.*, 2013). Indeed, in some countries, particularly in Asia, enteric fever caused by *S.* Paratyphi A is increasing comparing with other enteric fevers (Woods *et al.*, 2006).

Although they are from different serogroup, genome analysis of *S.* Paratyphi A indicates that is genetically similar to *S.* Typhi, putting in suggestion that it contains more recent evolutionary origin because it has fewer pseudogenes. Both genomes carry around 4,400 protein coding sequences; over 173 pseudogenes in *S.* Paratyphi A and ~ 210 pseudogenes in *S.* Typhi (McClelland *et al.*, 2004). *S.* Paratyphi C, human restricted serovar (Patrick A.D. Grimont, 1, 2001), identified by the antigenic formula 6,7:c:1,5 and distinguished recently from other variants sharing the same antigenic formula by MLST analysis with the sequence types (STs) 146, 90 and 114 (Uzzau *et al.*, 2000; Grimont and Weill, 2008; Langridge, 2010). This serovar causes enteric fever with similar symptoms caused by *S.* Typhi and *S.* Paratyphi, but it is not commonly isolated.

Host-adapted typhoid

S. Paratyphi B, host-adapted and identified by the antigenic formula 2;25, appears to not have an important role in causing enteric fever but primarily causes gastroenteritis (Chart, 2003). This serotype is frequently isolated from terrapins and shares the same antigenic formula with *S.* Java. The subdivision of *S.* Paratyphi B and *S.* java is based on

the ability to produce a slime wall and the inability to utilize D-tartrate (Patrick A.D. Grimont, 1, 2001). Based on the fermentation of dextrorotatory (d-tartrate), *S*. Paratyphi B can be characterized into d-tartrate fermenting (dT+) and non-fermenting (dT-) (Barker, 1985). dT- variant *S*. Paratyphi B mainly causes paratyphoid fever while dT+ variant *S*. Paratyphi B (previously called *S*. Java) only provokes gastroenteritis in human (Chart, 2003; Ahmad *et al.*, 2012).

Non-typhoidal *Salmonella*

Salmonella strains other than *S*. Typhi and *S*. Paratyphi are referred to as non-typhoidal *Salmonella* (NTS) (Figure 2). Animals are the primary reservoir of NTS strains (Steve Yan *et al.*, 2003; Wales and Davies, 2013). Infection due to NTS is mainly associated with the consumption of contaminated raw meat, eggs and chicken, milk and other dairy products, fish and other sea foods, fruits and vegetables (FDA/NSTA, 2008). Despite close genetic relatedness, typhoidal and non-typhoidal *Salmonella* (NTS) provoke different illnesses and a divergent response from the human immune system (Gal-Mor *et al.*, 2014). Incubation period in NTS infection is shorter (6–12 hours) compared to typhoid infection, and symptoms are usually self-limiting (Crump *et al.*, 2008; Eng *et al.*, 2015).

Pathogenesis

Infection with NTS strains in human is clinically manifested with gastroenteritis, bacteraemia and other extra-intestinal complications (Darby and Sheorey, 2008). The

severity of infection varies depending on two factors; the serotype implicated and the health of the patient. Infants, the elderly, and immuno-compromised patients are more susceptible to NTS infections than healthy adults (Eng *et al.*, 2015).

Gastroenteritis

NTS commonly cause an acute gastroenteritis, an inflammatory condition of the gastrointestinal tract, which is accompanied by typical symptoms of salmonellosis such as diarrhea, nausea, vomiting, abdominal cramps and headache (Steve Yan *et al.*, 2003; Gharieb *et al.*, 2015). The symptoms appear 12 hours post-incubation, they last approximately for 10 days and they are usually self-limiting (Crump *et al.*, 2008; Eng *et al.*, 2015). *Salmonella* is excreted in faeces after infection, and remain detected for median of five weeks (Chen *et al.*, 2013). Patients with immunodeficiency may have prolonged or chronic *Salmonella* infection (Yuan *et al.*, 2011).

Bacteraemia

NTS actively invade intestinal epithelial cells Invasive NTS infection (bacteraemia) occurs when the bacteria enter the bloodstream, the meninges, bone, and joint spaces after invading the intestinal barrier (Acheson and Hohmann, 2001; Woods *et al.*, 2008; Eng *et al.*, 2015). Mostly, all NTS serotypes may cause bacteraemia. However, serovars *S*. Dublin and *S*. Choleraesuis are highly linked with invasive infections (Woods *et al.*, 2008). Bacteraemia is clinically manifested with high fever, but in the contrary of patients with enteric fever, no formation of rose spots is observed. These symptoms are

seen more in infections caused by NTS serovars than typhoidal serovars, and the infection can be developed to acute condition causing mortality when the immune system is trigged, resulting what is known as septic shock (Eng *et al.*, 2015).

Extra-intestinal infections

Five per cent of infected patients with NTS develop a bacteraemia, and in some of them extra-intestinal manifestations occur (Eng *et al.*, 2015). These manifestations are clinically important, in some cases the pathogen may even affect brain function (Arii *et al.*, 2002). For example, *S.* Typhimurium has the ability to adhere, invade, and penetrate human brain microvascular endothelial cells, the single-cell layer constituting the blood–brain barrier (BBB). It is believed to be a primary stage in the pathogenesis of meningitis (van Sorge *et al.*, 2011). NTS strains also are a leading cause of meningitis in Africa and Latin America, causing high level of mortality (GRAHAM *et al.*, 2000; Owusu-Ofori and Scheld, 2003). Other complications may be occurred in NTS infection, including cellulitis, pneumonia, urinary tract infections and endocarditis (Shimoni *et al.*, 1999; Arii *et al.*, 2002).

Treatment for non-typhoidal *Salmonella* infection

NTS infection is a self-limiting disease, mostly manifested with gastroenteritis and rarely requires antimicrobial therapy. Nevertheless, infections may become severe involving hospitalization and some patients may even become septic with bacteraemia (GRADEL *et al.*, 2007; Chen *et al.*, 2013). Antimicrobials are not recommended,

because these do not reduce the duration or acuteness of gastroenteritis, and instead may result in extended carriage and appearance of resistant strains (Steve Yan *et al.*, 2003; Su and Chiu, 2007). Indeed, fluid replacement is highly recommended in NTS infection (Chen *et al.*, 2013). However, antimicrobial therapy is considered for patients with severe diarrhea, and is commonly used for invasive salmonellosis or when individuals with immunodeficiency are affected (Steve Yan *et al.*, 2003).

In cases of extra-intestinal or focal infection, and similar to bacteraemia, antibiotics are chosen depending on the susceptibility pattern of the strains in cause, considering as well the clinical condition of the patients. Mostly, the therapy includes ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones or third generation cephalosporins, such as ceftriaxone (Wong *et al.*, 2000; Chen *et al.*, 2013). With potential risk of causing arthroplasty, fluoroquinolones remain last option in NTS infection for children, when there is no other alternative treatment available (Wong *et al.*, 2000; Chen *et al.*, 2013; Choi *et al.*, 2013).

Mechanisms of pathogenesis in non-typhoidal Salmonella infection

A range of virulence genes contribute to *Salmonella* pathogenicity (Marcus *et al.*, 2000). Virulence determinants can be harboured on the chromosome, usually encoded on pathogenicity islands, or on plasmids and bacteriophages (Fluit, 2005). Moreover, *salmonella* are surrounded by an outer membrane containing lipopolysaccharide (LPS) which is able to release lipid A endotoxin, potentially causing shock in the host

(Messina, 1993). Another component of virulence is the ability to synthesize enterobactin (Pollack and Neilands, 1970). Enterobactin is a siderophore secreted by *Salmonella* allowing it to sequester iron from the host and use it for growth (Nagy *et al.*, 2013).

Following the consumption of contaminated food with NTS strains, these pathogens colonize the intestines and may invade epithelium barrier (Hurley *et al.*, 2014). Serovars that succeed in passing this barrier have to face macrophages and may be subjected to phagocytosis, in their best state they actively invade these macrophages using T3SS-1 and fimbriae (Figure 4) (Rescigno *et al.*, 2001).

While *Salmonella* actively invade intestinal epithelial cells using a type III secretion system (TTSS) encoded in *Salmonella* pathogenicity island 1 (SPI-1) to inject effector proteins into host cells (Galán and Wolf-Watz, 2006), SPI-2 encodes genes involved in intracellular survival and replication in phagocytic and non-phagocytic cells, and has a crucial role in systemic infection (Abrahams and Hensel, 2006).

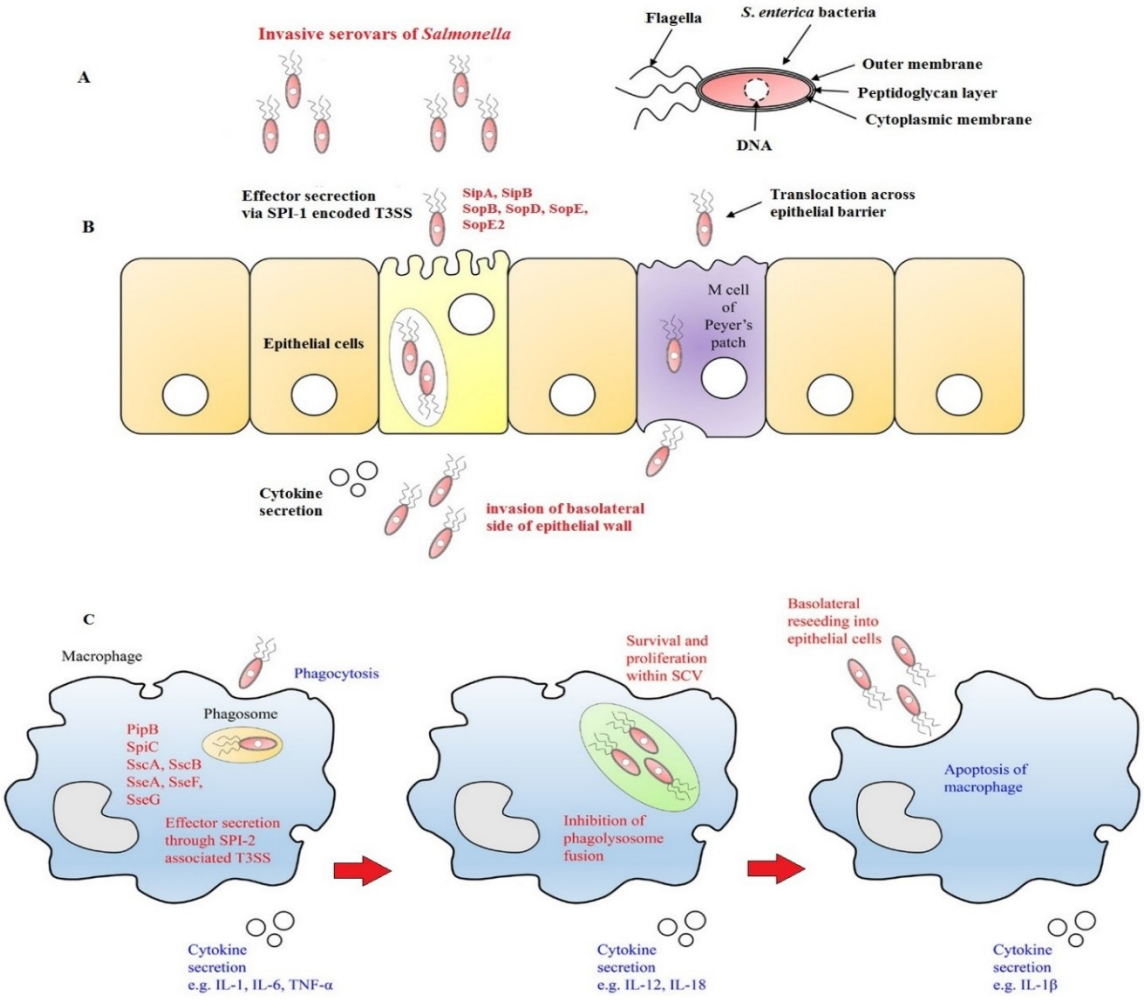

Fig. 4. Schematic illustration of invasive non-typhoidal *salmonella* infecting lower intestine. adapted from (Hurley *et al.*, 2014). (A) After reaching the lower intestine of the host, the pathogen adheres to the mucosal membranes and actively invades epithelial cells (Rescigno *et al.*, 2001). (B) Then, *Salmonella* follow two different paths; translocation across M cells of Peyer's patches or using SPI-1 encoded T3SS-1 to inject effector proteins into host cells (Galán and Wolf-Watz, 2006). (C) *Salmonella* is then surrounded by macrophages. SPI-2 encoded T3SS-2 releases effector proteins into the cytosol of the host cell (SigD/SopB, SipA, SipC, SodC-1, SopE2, and SptP) preventing fusion of the phagosome with the lysosome. *Salmonella* propagate within the *Salmonella*-containing vacuole (SCV) inducing secretion of cytokines by macrophage. As result, macrophage undergoes in apoptosis, *Salmonella* escape and will invade basolateral side of epithelial wall and even other phagocytic cells of innate immune system of the host (Hurley *et al.*, 2014).

Antimicrobial resistance and virulence in non-typhoidal *Salmonella*

Antimicrobial resistance in NTS is mostly promoted by the use of those antimicrobials in food animals (Angulo *et al.*, 2000; Gupta *et al.*, 2003; Dutil *et al.*, 2010; Crump *et al.*, 2011). It can be due to genetic mutations or through the acquisition of resistance encoding genes on mobile elements (Fluit, 2005). Plasmids are typical carriers of determinants that confer resistance against conventional antibiotics such as ampicillin, chloramphenicol and tetracycline (Guerra *et al.*, 2001). However, the chromosome can also harbour these determinants, *e.g*. on the multidrug resistance region of *Salmonella* Genomic Island 1 (SGI1) (Fluit, 2005).

In *Salmonella*, virulence determinants have been identified in different coding genes, mostly linked with a combination of chromosomal and plasmid factors (Oliveira *et al.*, 2002). For instance, The *Salmonella*-encoded fimbria *lpf operon* provides bacteria affinity for Peyer's patches and adhesion to intestinal M cells. While *agf operon* promotes interaction between the bacteria and intestine by stimulating bacterial self-aggregation, improving its survival rates within the intestine. On the other hand, *sef operon* enhances interactions between the bacteria and macrophages (Collinson *et al.*, 1996; Bäumler *et al.*, 1998; Edwards and Puente, 1998; Borges *et al.*, 2013).

Transmission of virulence and antibiotic resistance in non-typhoidal *Salmonella*

Likewise, other gram-negative organisms, genes in NTS are transmitted via genetic mobile elements. For instance, many plasmids and integrons are associated in the

dissemination of resistance to antibiotics via horizontal gene transfer. These genes play an important role in infection, host adaptation and disease development (Porwollik and McClelland, 2003).

IS elements

Insertion-sequence (IS) elements, firstly identified in *E. coli* in the *gal operon*, are genetic mobile elements originally from bacterial DNA. They are able to move from a position to another on the same or different genome. When IS elements are inserted in the middle of a gene sequence, they may interrupt the coding sequence, inhibiting the expression of that gene (Harper, 2012). Generally, genome of *salmonella* carries several copies of IS element naming *IS200*, *IS*3 and *IS*1617. Presence of an IS element may be an indication of variability in genetic content of a specific genetic region (Haack and Roth, 1995).

Transposons

Transposons known also as jumping genes, are DNA sequences that are able to change their location within a genome. They were first identified by Barbara McClintock more than fifty years ago. They are found in both eukaryotes and prokaryotes (Pray, 2008). Originally, they were distinguished from *IS* elements because transposons contain detectable genes, often conferring resistance to antibiotics (Calos and Miller, 1980). Generally, transposons are flanked between two copies of the same *IS* element.

Transposons and *IS* elements are now grouped together under the single term transposable elements (Griffiths, 2005).

Integrons

Integrons are mobile genetic units containing determinants of the components of site-specific recombination system that has the ability to capture and translocate genes contained in mobile elements known as gene cassettes that can be inserted into or deleted from their receptor elements (Hall and Collis, 1995a; Hall, 1997). Gene cassettes are known to encode for antibiotic resistance in gram negative pathogens, for example genes conferring resistance to β-lactams, aminoglycosides, trimethoprim, chloramphenicol, streptothricin (Hall, 1997). Three classes of integrons involved in antibiotic resistance have been characterized in detail (Ploy *et al.*, 2003). However, Class 1 integron is the most common class found in *Salmonella enterica* serovars (Hall and Stokes, 1993). It has been detected as well in many *Salmonella* Genomic Islands 1 SGI1 (Boyd *et al.*, 2002; Meunier *et al.*, 2002; Ebner *et al.*, 2004).

Salmonella genomic island 1 (SGI1)

Salmonella genomic island 1 (SGI1), a 43 kb integrative chromosomal island clustering antibiotic resistance genes in a 13 kb region known as Multi-drug resistant (MDR) region. It confers the bacteria resistance to antimicrobials. It was firstly identified in the epidemic *S.* Typhimurium DT104 that emerged during the 1980s, and had caused a

global health concern in both human and animals. Later this genomic island was identified in several *Salmonella enterica* serovars (Boyd *et al.*, 2002; Doublet *et al.*, 2005).

Antibiotic resistance genes cluster is located near the 3' end of SGI1, forming a complex class 1 integron belonging to the In4 group (Boyd *et al.*, 2002). The gene cluster of SGI1 is bounded by inverted repeats of 25 bp IRi and IRt and have a 3'-CS (conserved sequence) that includes a copy of *IS6100* (Brown *et al.*, 1996). Further, it is surrounded by 5 bp direct repeats, which suggests that it was integrated in SGI1 by a transposition event. SGI1 contains also a duplication of a part of the 5'-CS, that leads to a second attI1 site followed by a gene cassette (Partridge, Brown, *et al.*, 2001; Boyd *et al.*, 2002; Doublet *et al.*, 2005). SGI1 contains also a duplication of a part of the 5-CS, that leads to a second attI1 site followed by a gene cassette (Doublet *et al.*, 2005).

Variants in SGI1

Variants of SGI1 are identified based on the variation of the genetic organization in their MDR region. Different variants of SGI1 containing different sets of resistance genes have been identified (Boyd *et al.*, 2002; Carattoli *et al.*, 2002; Benoît Doublet *et al.*, 2004; Levings *et al.*, 2005; Cloeckaert *et al.*, 2006). The original SGI1 was isolated from *S.* Typhimurium phage type DT104. It contains five genes *aadA2*, *sul1*, *floR*, *tetA(G)*, and *blaP1* (variant form *blaPSE-1* or *blaCARB-2*). These genes respectively confer resistance to streptomycin and spectinomycin, sulfonamides, chloramphenicol and

florfenicol, tetracyclines, and β-lactam antibiotics (Briggs and Fratamico, 1999; Boyd *et al.*, 2001), and all of them are located within the boundaries of a complex class 1 integron named In104 (Levings *et al.*, 2005) (Fig. 5). Sequence of the MDR region in SGI1 has revealed that the integron contains a backbone similar to that of In4 (Briggs and Fratamico, 1999; Partridge, Brown, *et al.*, 2001; Partridge, Recchia, *et al.*, 2001; Boyd *et al.*, 2001) , but it has in addition duplications of parts of the integron conserved segments (CS) naming 5′-CS and part of the 3′-CS (qacEΔ1 and partial *sul1* genes), where integrated gene cassettes are usually flanked (Hall and Collis, 1995b; Hall and Collis, 1998). A second integrated element named retron phage, is found inserted between end of SGI1 and the *yidY* gene, but it is detected only in the serovar Typhimurium (Boyd *et al.*, 2000; Boyd *et al.*, 2001).

Variants from SGI1-A to SGI1-J, seem to have gained, lost, or exchanged resistance genes by gaining and/or losing various segments of DNA, mostly occurred by homologous recombination. Some of them have lost parts of In104, and others have different gene cassettes from those found in SGI1, naming the *dfrA1-orfC* or the *aacCA5-aadA7* pairs (Doublet *et al.*, 2003; Benoit Doublet *et al.*, 2004; Levings *et al.*, 2005). Further variants with different gain/loss have been identified (Boyd *et al.*, 2002; Levings *et al.*, 2005).

The genetic variation in the MDR region seems to be a useful tool to track the evolution of the structure of SGI1, especially that it is occurring in a defined context and location, the backbone of the SGI1, which is integrated within the end of the *thdF* gene in the *Salmonella enterica* chromosome (Levings *et al.*, 2007).

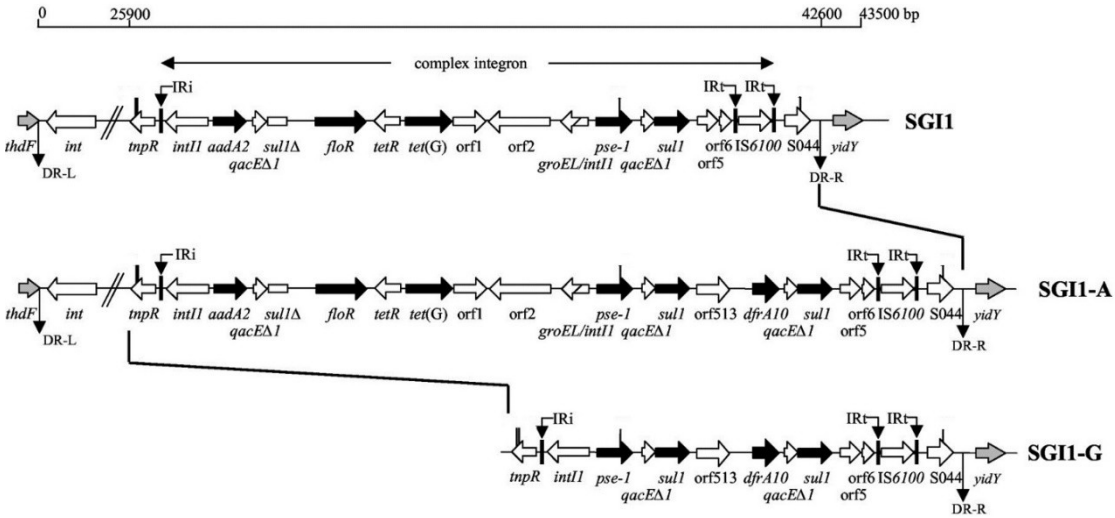

Fig. 5. Schematic view of the genetic organization of the MDR gene clusters of SGI1. Adapted from (Benoît Doublet *et al.*, 2004). The variants SGI1-A and SGI1-G are represented below to illustrate the variation. Left and right junctions (*thdf* and *yidY*), bracketing SGI1 are represented in grey arrows. Black arrows correspond to antibiotic resistance genes. Vertical thick black bars indicate homology regions between SGI1 variants.

Salmonella pathogenicity islands (SPIs)

SPIs are genomic island that contain important genes conferring virulence to *Salmonella enterica.* They are located on the chromosome and can be transferred from an organism to another through horizontal gene transfer. Usually, pathogenicity islands accommodate vast clusters of genes that contribute to a specific virulence phenotype. In general, this

phenotype is manifested at a specific moment during the infection, and may convert normally benign microorganism into a pathogen (Marcus *et al.*, 2000). Some SPIs are conserved in the *genus Salmonella* and others are serovar-restricted (Siriken, 2013). Different SPIs have been identified, but the most important ones namely SPI1 and SPI2 that code for genes involved in the intestinal phase of the infection. The remaining SPIs are required for fimbrial expression, magnesium and iron uptake, antibiotic resistance and other functions required for survival inside the host (SIRIKEN, 2013).

Plasmids

Many *Salmonella* serovars harbour virulence plasmids that are important for systemic infection (Gulig, 1990; Wallis *et al.*, 1995). Plasmids in *Salmonella* are heterogeneous in size, depending on the serovar, ranging from 50 to 110 kb (Chu *et al.*, 1999). Moreover, all of them share a 7.8 kb region named *spv*, required for bacterial multiplication in the reticulo-endothelial system. Plasmids carrying virulence determinants can be transferred from a bacterium to another by horizontal gene transfer, this mechanism is known by transformation (Johnston *et al.*, 2014). However, current evidence suggests that the contribution of virulence plasmids to pathogenesis in *Salmonella* is less important (Casadesüs, 1999), as these last do not affect the ability of *Salmonella* of causing a gastroenteritis (Gulig *et al.*, 1993). Plasmids found in *Salmonella enterica* can contain a variety of genes encoding antimicrobial and heavy metal resistance, toxins or virulence genes that are beneficial to adapt to different environments. These plasmids have been

found in different *Salmonella serovars* namely *S*. Enteritidis, *S*. Typhimurium, *S*. Dublin, *S*. Choleraesuis, *S*. Gallinarum, *S*. Pullorum and *S*. Abortusovis. These plasmids ranging in size from few to several hundred kbp. They contribute in the spread of genes in bacterial populations. (Rychlik *et al.*, 2006). They are mostly classified into incompatibility groups (Inc) based on their mode of replication and maintenance inside the bacterial cell. Consequently, plasmids exploiting the same replication machinery are mutually incompatible and unable to persist in the same cell for extended period (Ou, 1993). Knowledge on plasmids is limited. However, their presence or absence is frequently used for strain differentiation in epidemiological studies.

Bacteriophages

Bacteriophages (phages) are the most abundant organism in the biosphere (Clokie *et al.*, 2011). They can transfer antibiotic resistance from a bacterium to another using a mechanism known by transduction. This process is a significant contributing factor to dissemination of antibiotic resistance genes in food-borne pathogens of the *Enterobacteriaceae* family, naming non-typhoidal *Salmonella* (Colavecchio *et al.*, 2017).

Epidemiology of non-typhoidal *Salmonella* infection

Food-borne diseases have become a global economic burden on health care systems. More than 550 million individuals fall sick every year, including 220 million children under the age of 5 years. *Salmonella* is one of the most reported causes of this disease

globally (WHO, 2016). In fact, the prevalence of *Salmonella* infection is higher than is reported, because most infected people do not undergo copro-culture diagnosis (kay M.Tomashek, Tyler M.Sharp, 2015).

Epidemiology related to NTS varies depending upon serovar, and its ability to cause gastrointestinal or invasive infection. With certain subtypes, *S*. Typhimurium is recognized as capable of causing bacteraemia, whereas *S*. Heidelberg, *S*. Dublin, and *S*. Choleraesuis represent a significant potential to cause hospitalization, and even death. In comparison, serovar *S*. Newport was observed to cause fewer fatalities than *S*. Typhimurium (Crump *et al.*, 2011).

Several outbreaks of food-borne infections of antibiotic resistant NTS have been reported globally while individual epidemiology studies in more-developed African countries provide some basic insight until more data are available (Enwere *et al.*, 2006). Such information is crucial to understand the spread of multidrug-resistant *Salmonella* strains (Harrois *et al.*, 2014). In Algeria, limited data is available on epidemiology of NTS infection. *S*. Typhimurium, *S*. Heidelberg and *S*. Enteritidis are commonly reported in both human and animal food sources (Ayachi *et al.*, 2009; Elgroud *et al.*, 2009; Bounar-Kechih *et al.*, 2012; Bouzidi *et al.*, 2012; Mezali and Hamdi, 2012). In other parts of Africa, particular serovars are prevalent in specific regions, namely *S*. Concord in Ethiopia (Beyene *et al.*, 2011), *S*. Bovismorbificans in Malawi (Bronowski *et al.*, 2013), *S*. Stanleyville and *S*. Dublin in Mali (Tennant *et al.*, 2010), and S. Isangi in South Africa (Wadula *et al.*, 2006). In Kenya, an average of 166 per 100,000 children

under five acquire an NTS infection every year (Oundo *et al.*, 2002; Berkley *et al.*, 2005). In sub-Saharan Africa, a serious invasive form of NTS linked to *S.* Typhimurium sequence type ST313 has emerged, and become a leading public health issue in this region. This invasive *S.* Typhimurium had an estimated mortality rate of 20-25% in children and up to 50% in adults (Kingsley *et al.*, 2009).

In the US, food-borne *Salmonella* is the largest health burden of all bacterial pathogens (Scallan *et al.*, 2011). During 2014, more than 19,000 laboratory-confirmed cases of food-borne infections were identified. *Salmonella* was linked to 7439 of these cases, in which 2144 persons were hospitalized, and among 32 patients died (CDC, 2014). The most commonly detected serovars of *Salmonella* in humans include *S.* Enteritidis and *S.* Typhimurium, followed by *S.* Newport, *S.* Javiana, and *Salmonella* with the antigenic formula *4, [5],12:i:-* (CDC, 2014).

Although developed countries have a better awareness among food handlers and optimized surveillance programs, NTS infection has been present and responsible for patient morbidity and mortality for decades. For developing countries, the situation is worsened with the limited sources currently available. In addition, massive human migration from African countries and the increase in food trade between developed and developing countries may play a significant role in spreading such pathogens all over the world.

STUDY DESIGN AND METHODS

STUDY DESIGN AND METHODS

Bacterial isolation and identification

Sixty-nine *Salmonella* were isolated according to a standard ISO method (ISO, 2007). Human clinical isolations was obtained from stools of diarrheagenic patients admitted at different hospitals in three cities in Algeria: Guelma, Setif and Algiers during 2014-2015. Animal origin strains were isolated from poultry houses in Batna city. The isolates were serotyped by the slide agglutination method using Omni-O *Salmonella* antisera (Bio-Rad Laboratories, USA).

Antimicrobial resistance phenotyping

The isolates were tested for susceptibility to antimicrobials on Muller-Hinton agar following the Kirby-Bauer disk diffusion method, using a panel of 12 antibiotics, and interpreted according to the recommendation of the Clinical and Laboratory Standards Institute 2016. Antimicrobial disks (Bio-Rad Laboratories, USA) with the following drug contents were used: ampicillin (10 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), streptomycin (10 μg), tetracycline (30 μg), meropenem (10 μg), imipenem (10 μg), cefotaxime (30 μg) and gentamicin (10 μg). Isolates resistant to three or more classes of antimicrobials were specified as MDR.

Whole Genome Sequencing (WGS) and *in silico* analysis

Genomic DNA of the isolates was extracted using Wizard Genomic DNA Purification Kit. DNA extracts were converted into a Nextera XT library for sequencing on an Illumina NextSeq 500 platform according to the manufacturer's instructions. The *Salmonella* library was diluted to 4nM (as determined by analysis on an Agilent Technologies 2200 Tapestation and using the Qubit HS dsDNA assay) and pooled in equimolar amounts with other barcoded libraries. The entire library pool was then diluted to 1.8 pM and sequenced using the NextSeq 500 v2 2x150 bp paired-end protocol.

Genomes were assembled using Velvet de novo genomic assembler (Afgan *et al.*, 2016). Web-based tool SeqSero 1.0 was used to determine the serotype and the antigenic profile of the isolates (Zhang *et al.*, 2015). The genome assemblies were then subjected to sequence type (ST) analysis using *Salmonella* in *Silico* Typing Resource platform (SISTR) [https://lfz.corefacility.ca/sistr-app/] (Yoshida *et al.*, 2016). Further investigation was conducted on the genome assemblies for acquired resistance genes, *Salmonella* Pathogenicity Islands (SPI), plasmids and incompatibility group using ResFinder (Zankari *et al.*, 2012), SPIFinder-1.0 (CGE online platform: http://www.genomicepidemiology.org/) and PlasmidFinder 1.3 platforms respectively (Carattoli *et al.*, 2014). Presence of the *Salmonella* Genomic Island 1 (SGI1) was

investigated by PCR primers DR-S004 targeting SGI1 left junction, *int, xis and rep*, and with S044-DR targeting SGI1 right junction with retronphage (Carattoli *et al.*, 2002). SGI1 mapping was conducted *in silico* using Geneious R10 software.

RESULTS

Genomic characterization of non-typhoidal *Salmonella* isolated from human and poultry in four cities in Algeria

Genomic characterization of non-typhoidal *Salmonella*

Information and surveillance on NTS serotypes are limited in Africa, and also in Algeria. Reliable source of data is not always easy to be found, whilst few individual studies supply some basic information. One of the aims of this Ph. D. study is to identify and characterize the different non-typhoidal *Salmonella* serovars isolated from humans and poultry in four cities in Algeria. Serotypes of the isolates were determined utilizing whole genome sequencing data.

Human isolates were collected from hospitalized patients suffering from diarrhea in three cities, including Guelma, Setif and Algiers. Poultry isolates were collected from slaughterhouses in Batna city. Altogether, 10 different serotypes were recovered from poultry and humans, being *S*. Typhimurium the dominant serotype among human isolates, followed by *S*. Kentucky, *S*. Enteritidis, *S*. Heidelberg, *S*. Ohio, *S*. Lindenburg, *S*. Indiana, *S*. Virchow, and *S*. Bonn, while *S*. Gallinarum was the only serovar found among the poultry isolates (Table 5).

***Salmonella* Gallinarum isolated from poultry**

Twenty-nine (42%) *Salmonella enterica* isolated from poultry were of serovar Gallinarum. They belonged to serogroup D1, holding antigenic formula 9:g,m:-, and sequence type ST78. Five strains harbored two plasmid each, belonging to incompatibility group N and FII. Eighteen strains harbored one plasmid each of IncFII

group. Six strains did not contain any plasmid. *In silico* analysis of genome sequences identified different *Salmonella* pathogenicity islands (SPIs), including SPI-5 carrying *sopB*, *sigD*, *pipB* genes encoding for effector proteins required in function of SPI-1 and SPI-2. This SPI was acquired from *S.* Typhimurium strains LT2 and has inserted in the *serT* allele on the chromosome. SPI-13 have been found inserted in the allele *pheV* on the chromosome, carrying *gtrB* and *gtrA* genes encoding for *LysR* transcriptional regulator, those genes are implicated in the bacterial stringent response and in the virulence of *Salmonella* (Maddocks and Oyston, 2008). This SPI was originally found in *S.* Gallinarum strain SGA-10. SPI-14 was detected in the genome carrying two genes, naming *gpiA* encoding for electron transfer favoprotein beta subunit, and *gpiB* implicated in the regulation of transcription. The origin of this SPI is *S.* Gallinarum strain SGA-8. Furthermore, another SPI was detected, named C63PI, inserted in *fhlA* allele. This SPI is acquired from *S.* Typhimurium strain SL1344. It is implicated in transcription of the *sit* operon under iron-lack growth conditions, forming Putative Iron Transport System (PITS) within the Centisome of this pathogenicity island (Zhou *et al.*, 1999).

Salmonella **Typhimurium isolated from humans**

Nineteen strains (27%) were identified as *S.* Typhimurium among the human clinical isolates. They all belonged to serogroup B, with antigenic formula 4:i:1,2, and sequence

type (ST19). 84% (16 out of 19) Typhimurium carried plasmids belonging to incompatibility group FII, and FIB. Different SPIs were found in the chromosome of these strains. Sixteen strains had similar SPIs previously found in *S*. Gallinarum in this study, including SPI-5 inserted in the chromosomic allele *serT* and carrying *SopB*, *SigD*, *PipB* genes. Three different functional SPI-13 were found inserted in the allele *pheV* on the chromosome, one carrying *gacD* originally acquired from *S*. Gallinarum strain SGD-3, encoding for Acetyl-coA dehydrolase, and other two harbouring *gtrB* and *gtrA* genes, originally from *S*. Gallinarum SGG-1 and *S*. Gallinarum SGA-10 respectively. SPI-14 was detected in the genome carrying two genes, naming *gpiA* and *gpiB*. The origin of this SPI is *S*. Gallinarum strain SGA-8. The pathogenicity island C63PI were found inserted in *fhlA* allele of the chromosome, with the similar functions as in strains of *S*. Gallinarum isolated from poultry. Two strains of *S*. Typhimurium found to be carried only two SPIs on their chromosomes, including SPI-13 and SPI-14.

Salmonella Kentucky isolated from humans

Seven strains (10%) were identified as *S*. Kentucky. They belonged to serogroup C2-C3 with antigenic formula 8:i:z6, and sequence type ST198. Three strains carried one plasmid each belonging to incompatibility group I2. A plasmid of IncHI2 was found in one strain, while three strains did not harbor any plasmid. Only pathogenicity island C63PI was detected on the chromosome of two isolates. It was inserted in the allele *fhlA*.

Salmonella Enteritidis isolated from humans

Five *S.* Enteritidis have been identified from humans. They belonged to serogroup D1, with antigenic formula 9:g,m:-, and sequence type ST11. Two plasmids IncFII and IncFIB were found in each of forth *S.* Enteritidis isolates, the fifth strains did not harbor any plasmid. Two strains were found to harbor SPI-5, SPI-13, SPI-14 and the pathogenicity island C63PI inserted in the chromosomal allele *fhlA*, encoding for iron uptake system.

On the other hand, three strains were identified as *S.* Heidelberg. They belong to serogroup B, with antigenic formula 4:r:1,2, with sequence type ST15. Two of the three strains carried two plasmids each, belonging to incompatibility group X1 and I1. One of the three strains carried one only plasmid belonging to incompatibility group X1. They carried three different SPIs, including SPI-9, SPI-13 and SPI-14. Two strains were identified as *S.* Indiana. They belong to serogroup B, with antigenic formula 4:z:1,7, and do not harbor any plasmids or SPIs.

Two other isolates were identified as *S.* Ohio with sequence type ST329 and antigenic formula 7:b:l,w, and as *S.* Bonn sequence type ST2522 with antigenic formula 7:l,v:e,n,x, Both isolates carried one plasmid each belonging to IncA/C2 group. Four different SPIs were found inserted on the chromosome of *S.* Bonn including SPI-5, SPI-

13, SPI-14 and C63PI, while *S*. Ohio harboured only C63PI inserted in *fhlA* allele on the chromosome.

Finally, one *S*. Lindenburg with antigenic formula 8:i:1,2 and one *S*. Virchow with antigenic formula 7:r:1,2 carrying one plasmid of IncA/C2 group were identified among the *Salmonella* isolates. *S*. Lindenburg carried four different SPIs inserted on the chromosome, including SPI-5, SPI-13, SPI-14 and C63PI. In *S*. Virchow only SPI-13 was found inserted in *pheV* allele on the chromosome. Genotypic characters are summarised in Table 3.

Table 3. *In silico* typing of *Salmonella enterica* isolated from humans and poultry in four cities in Algeria.

N° of isolates	Serotype	Antigenic formula	Sequence Type (ST)	Plasmids, Inc. Group	SPIs	Source
15	*S.* Typhimurium	4:i:1,2	ST-19	FIB + FII	SPI-5, SPI-13, SPI-14	Human
1	*S.* Typhimurium	4:i:1,2	ST-19	ND	SPI-13, SPI-14	Human
2	*S.* Typhimurium	4:i:1,2	ST-19	FIB + FII	SPI-13, SPI-14	Human
2	*S.* Enteritidis	9:g,m:-	ST-11	FIB + FII	SPI-5, SPI-13, SPI-14	Human
2	*S.* Enteritidis	9:g,m:-	ST-11	FIB + FII	ND	Human
1	*S.* Enteritidis	9:g,m:-	ST-11	ND	SPI-13, SPI-14	Human
2	*S.* Heidelberg	4:r:1,2	ST-15	X1 + I1	SPI-9, SPI-13, SPI-14	Human
1	*S.* Heidelberg	4:r:1,2	ST-15	X1	SPI-9, SPI-13, SPI-14	Human
3	*S.* Kentucky	8:i:z6	ST-198	I2	ND	Human
2	*S.* Kentucky	8:i:z6	ST-198	ND	ND	Human
1	*S.* Kentucky	8:i:z6	ST-198	ND	C63PI	Human
1	*S.* Kentucky	8:i:z6	ST-198	HI2	C63PI	Human
1	*S.* Ohio	7:b:l,w	ST-329	A/C2	C63PI, SPI-5, SPI-13, SPI-14	Human
2	*S.* Bonn	7:l,v:e,n,x	ST-2522	A/C2	C63PI, SPI-5, SPI-13, SPI-14	Human
1	*S.* Lindenburg	8:i:1,2	NI	A/C2	C63PI, SPI-5, SPI-13, SPI-14	Human
1	*S.* Virchow	7:r:1,2	NI	A/C2	SPI-13	Human
2	*S.* Indiana	4:z:1,7	NI	ND	ND	Human
4	*S.* Gallinarum	9:g,m:-	ST-78	N + FII	SPI-5, C63PI, SPI-13, SPI-14	Poultry
1	*S.* Gallinarum	9:g,m:-	ST-78	N + FII	C63PI, SPI-13, SPI-14	Poultry
18	*S.* Gallinarum	9:g,m:-	ST-78	FII	C63PI, SPI-5, SPI-13, SPI-14	Poultry
6	*S.* Gallinarum	9:g,m:-	ST-78	ND	ND	Poultry

NI: Not identified, **ND**: Not detected.

Antimicrobial resistance testing by phenotype and genotype of non-typhoidal *Salmonella*

Antimicrobial resistance testing by phenotype and genotype of non-typhoidal *Salmonella*

Antimicrobial-resistant non-typhoidal *Salmonella* (NTS) are leading cause of infection in many african countries (Murgia *et al.*, 2015), but there is still a paucity of knowledge about their genetic background and molecular characteristics of genes responsible for resistance to antibiotics, also in Algeria. Part of this Ph. D. study, contributes to fill this lack through investigation of the molecular basis of antimicrobial resistance in these NTS strains isolated from humans and poultry in four cities in Algeria. Phenotypic resistance tests were conducted only on fifty-three isolates using disk method, while genotypic analysis for resistance genes was conducted on full genome sequences of all the sixty-nine isolates.

Resistance phenotyping

As a whole, all tested *Salmonella* isolates were resistant to one or more antimicrobials. Among the antimicrobials tested, the frequency of resistance was more common to nalidixic acid (85%), ciprofloxacin and streptomycin (66%), ampicillin (63%), tetracycline (44%) and chloramphenicol (20%). Frequency of resistance to ampicillin, chloramphenicol and tetracycline in *Salmonella* isolates from poultry was relatively low

compared to that seen in humans. For instance, resistance to tetracycline was 84% in isolates obtained from humans while only 34% of poultry isolates were resistant to tetracycline. Tetracyclines are broad-spectrum antibiotics, displaying activity against a wide range of gram-positive and gram-negative bacteria, and are widely used for treatment in human infections (Pezzella *et al.*, 2004). On the other hand, tetracyclines are inexpensive antibiotics, which have been used extensively in animal feed as growth promoters. It is strongly believed that this unregulated use may have resulted resistance to tetracycline in different bacterial pathogens including *Salmonella*.

Resistance to nalidixic acid, was detected in 99% of isolates obtained from poultry, all *S.* Gallinarum. This high resistance is probably linked to the extensive use of fluoroquinolones in animal husbandry in the Batna region, east of Algeria.

Decreased susceptibility to ceftriaxone was detected among the isolates, only 10% in humans in *S.* Typhimurium, *S.* Ohio, and *S.* Heidelberg, and 7% in poultry in *S.* Gallinarum. Resistance to ceftriaxone is a matter of concern, because this antimicrobial agent is important in treatment of salmonellosis, especially in children (Chen *et al.*, 2004).

On the other hand, all isolates were sensitive to meropenem, imipenem, cefotaxime and gentamycin. Majority of the isolates (85%) displayed a multiple resistance to the tested antimicrobials. Resistance pattern of each isolate is presented in Table 4.

Table 4. Antimicrobial resistance patterns of NTS isolated from humans and poultry in four cities in Algeria.

City of isolation	N° of isolates	Serotype	Resistance phenotype	Source of isolation
Guelma	6	*S*. Typhimurium	A,C,NA,S,TE	Human
	1	*S*. Typhimurium	A,C,S	Human
	1	*S*. Typhimurium	A,C,S,TE	Human
	1	*S*. Typhimurium	A,CRO,NA,SXT,S*,TE	Human
	1	*S*. Typhimurium	A*,NA,S*,	Human
	1	*S.* Enteritidis	CIP	Human
	1	*S.* Enteritidis	NA	Human
	2	*S*. Heidelberg	A,CRO,NA,S*	Human
	3	*S.* Kentucky	A,NA,CIP,S,TE	Human
Setif	1	*S.* Ohio	A,CRO*,S*,TE*	Human
	2	*S.* Enteritidis	A,NA,CIP*	Human
Algiers	1	*S*. Typhimurium	A. C	Human
	1	S. Kentucky	A	Human
	1	*S.* Kentucky	CIP	Human
	1	*S.* Lindenburg	A,CIP	Human
Batna	1	*S.* Gallinarum	A*,C,NA,CIP,SXT,S,TE	Poultry
	2	*S.* Gallinarum	A*,CAZ*,NA,CIP*,S*	Poultry
	3	*S.* Gallinarum	A,NA,CIP*,S,GMN	Poultry
	1	*S.* Gallinarum	C,CAZ*,CIP*,S	Poultry
	3	*S.* Gallinarum	AMC,NA,CIP*,S,TE	Poultry
	4	*S.* Gallinarum	A,AMC,NA,CIP,SXT,S,TE	Poultry
	1	*S.* Gallinarum	CRO,NA,CIP*,S	Poultry
	1	*S.* Gallinarum	CRO,NA,CIP,SXT,S*,TE	Poultry
	11	*S.* Gallinarum	NA,CIP*,S	Poultry
	1	*S.* Gallinarum	NA,CIP,SXT,S*,TE	Poultry
	1	*S.* Gallinarum	CAZ*,NA,S	Poultry

A: Ampicillin, **AMC**: Amoxicillin, **CAZ**: Ceftazidime, **C**: Chloramphenicol, **CRO**: Ceftriaxone, **CIP**: ciprofloxacin, **NAL**: Nalidixic Acid, **S**: Streptomycin, **SXT**: Trimethoprim-Sulfamethoxazole, **T**: Tetracycline, * : intermediate

Resistance genotyping

There was good correlation between the presence of resistance genes and corresponding resistance phenotypes. For instance, most of chloramphenicol-resistant strains contained *cat1* or *floR* genes and 11 of 40 streptomycin-resistant strains contained *aadA2*, *aadA3, aadA7, aac(3)-Id*, *strA* or *strB* genes. However, 8 of 22 tetracycline-resistant strains did not contain any tet(A), tet(B), tet(C) or tet(G) genes. On the other hand it was interesting to note that some strains harboured two different resistance genes for the same antibiotic, such as *aadA7* and *aac(3)-Id* in *S*. Kentucky and *strA* and *strB* in *S*. Gallinarum. Additionally, the *aadA2* and *sul1* genes were often found in strains of *S*. Typhimurium rather than the *aadA3* and *sul2* genes.

Molecular mechanisms of resistance to beta-lactamases

The full genome sequences of the isolates were analysed *in silico* for β-lactam genes (*bla*). Out of 69 *Salmonella* isolates 21 (30%) contained β-lactam genes, and it was detected only in humans (Table 5). The dominant *bla* gene responsible for resistance to beta-lactam antimicrobials in majority of *Salmonella* isolates was found to be variants of *blaCARB-1* and *blaCARB-2* genes, it was detected in nine *S*. Typhimurium.

In five of the human isolates *S*. Kentucky, and one *S*. Bonn three *bla* genes (*blaTEM-1B*, *blaTEM-63* and *blaTEM-110*) were detected. These genes encode for the enzymes

implicated in extended-spectrum β-lactamases (ESBLs) activity. In one *S*. Kentucky isolate *bla*SHV-12 gene was detected. The TEM- and SHV-ESBLs were widespread in the ESBL landscape during the 1980s till the 1990s, mostly associated with outbreaks in hospitals (Cantón *et al.*, 2012). Among beta-lactams, variant corresponds to the *CTX-M* family, *CTX-M-1* was detected in one *S*. Heidelberg isolate. This family is largely disseminating around the world, and referred as the "*CTX-M* pandemic"(Cantón and Coque, 2006).

Molecular mechanisms of other antibiotic resistance

Analysis for antimicrobial resistance genes using Res.Finder, revealed that aminoglycosides resistance such as streptomycin was mainly mediated by *aadA2* and *aadA3* in *S.* Typhimurium, *aad7, aac(3)-Id* and *aph(3')-Ia* in *S.* Kentucky and *S*. Indiana, and was mediated by *strA* and *strB* genes in both *S*. Bonn and *S*. Gallinarum. The genes *strA* and *strB* are distributed worldwide and confer streptomycin resistance in at least 17 genera of gram-negative bacteria (Sundin and Bender, 1996). While tetracycline resistance in *S*. Typhimurium was mainly found mediated by *tet(G)* gene. However, in *S*. Heidelberg and *S*. Gallinarum was found mediated by *tet(A)* gene. On the other hand it was mediated by *tet(C)* gene in *S*. Ohio b*,* and by by *tet(J)* in both *S.* Kentucky and *S*. Indiana.

Salmonella isolates that displayed resistance to florfenicol/chloramphenicol including some strains of *S*. Typhimurium and *S*. Bonn, they contained *floR* as resistance gene. However, the same resistance was found to be mediated by *cat* gene in a number of *S*. Kentucky, *S*. Indiana, *S*. Lindenburg and other strains of *S*. Typhimurium.

Resistance to sulfonamides was found mostly mediated by *dfrA14* gene in *S*. Typhimurium and in some strains of *S*. Gallinarum. *dfrA14* is a group A drug-insensitive dihydrofolate reductase, which can not be inhibited by trimethoprim (Young *et al.*, 1994). On the other hand, the resistance was mediated by *sul1* gene in *S*. Typhimurium, S. Kentucky, in some strains of *S.* Gallinarum and by *sul2* in *S*. Heidelberg, *S*. Lindenburg, *S*. Indiana and in *S*. Gallinarum strains. The *sul1* gene is normally found linked to other resistance genes in class 1 integrons, while *sul2* is usually located on plasmids (Antunes *et al.*, 2005). Therefore, among the Algerian *Salmonella* isolates in this study, *sul1* gene was found inserted in In4-type integron as part of SGI1 and SGI1-K7 integrated in the chromosomes of *S*. Typhimurium and *S*. Kentucky respectively (Fig. 5).

Finally*, QnrS1* gene responsible of plasmid-mediated quinolone resistance was detected in only five *S*. Gallinarum isolated from poultry.

Table 5. Prevalence of antibiotic resistant genes detected in *S. enterica* serovars.

Resistance genes	*Salmonella enterica* serovars								Antimicrobial agent
	Typhimurium	Kentucky	Indiana	Ohio	Heidelberg	Lindenburg	Bonn	Gallinarum	
blaCARB-1,2	+								Beta-lactams
blaTEM-1B		+					+		Beta-lactams
blaTEM-63		+					+		Beta-lactams
blaTEM-110		+					+		Beta-lactams
blaCTX-M-1					+				Beta-lactams
blaSHV-12		+							Beta-lactams
aadA2	+								Streptomycin
aadA3	+								Streptomycin
aadA7		+	+						Streptomycin
aac(3)-Id		+	+						Streptomycin
aph(3')-Ia		+	+						Streptomycin
strA							+	+	Streptomycin
strB							+	+	Streptomycin
tet(G)	+								Tetracycline
tet(A)					+			+	Tetracycline
tet(C)				+					Tetracycline
tet(J)		+	+						Tetracycline
floR	+						+		Chloramphenicol
cat	+	+	+			+			Chloramphenicol
dfrA14	+							+	Sulfonamides
sul1	+	+						+	Sulfonamides
sul2			+		+	+		+	Sulfonamides
QnrS1								+	Quinolones

+: serovars positive for resistance genes.

Salmonella Genomic Island 1

Sixty-nine *Salmonella* isolated from humans and food of animal origin in Algeria were investigated for the presence of *Salmonella* genomic Island 1 (SGI1). Nineteen isolates (27%) were identified to harbour classical SGI1 conferring resistance to ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfametoxazole and tetracycline). This genomic island was detected only in serovar *S.* Typhimurium. It was similar to previously described in *S.* Typhimurium DT104 (Levings et al., 2005, Boucherif et al., 2009). The resistance genes are located within the boundaries of the class 1 integron In104. In104 integron has a similar backbone structure to In4 integron (Partridge, Brown, *et al.*, 2001). It has duplications of parts of the integron conserved segments (CS), precisely, part of the *intI1* gene from the 5′-CS and part of the 3′-CS (*qacEΔ1* and partial *sul1* genes) where gene cassettes are flanked. Because of this complex structure, In104 includes two *attI1* sites, into which the *aadA2* gene cassette is incorporated in one and the *blaP1* cassette is incorporated in the other. On the other hand, three of *S.* Kentucky were found to carry new variants of SGI1 belonging to Ks family. Genetic organization of their MDR region is described further (Fig 6).

Genomic analysis of multi-drug resistant *S.* Kentucky ST198 from Algeria

Genomic analysis of multi-drug resistant *S*. Kentucky ST198 from Algeria.

S. Kentucky has been previously reported as travel related pathogen, and highly resistant to antibiotics, especially the strains isolated from those travellers returning from Africa (Le Hello, Harrois, *et al.*, 2013). NTS infections are usually treated by fluoroquinolones, such as ciprofloxacin (CIP) and extended-spectrum cephalosporins (ESCs). Recently *S.* Kentucky resistant to CIP and ESCs has emerged in the Mediterranean region (Le Hello, Harrois, *et al.*, 2013). This resistance was reported in France from a French tourist who had an inflammation of the gastrointestinal tract during his stay in Egypt in 2002 (Weill *et al.*, 2006). Moreover, a propagation of *S.* Kentucky CIP resistant (hereafter CIP^R) was reported in two hospitals during 2003 and 2004 in The Slovak Republic where the origin of these isolates were linked to a travel in Egypt (Majtán *et al.*, 2006). One year after, a high level of CIP^R *S.* Kentucky was detected for the first time in Belgium from a traveller that was infected in Libya with MDR *S.* Kentucky (Collard *et al.*, 2007). This strain continued to spread across the globe in particular in Northeast of Africa naming Kenya, Sudan and Tanzania (Weill *et al.*, 2006). Studies suggest that infections with CIP^R *S.* Kentucky are mostly travel related, and though to be caused by preventative use of quinolones (Weill *et al.*, 2006; Collard *et al.*, 2007).

Beside antimicrobial resistance testing, genotypic analysis was conducted on the full genome sequences of three clinical isolates of *S*. Kentucky isolated in a Hospital of Guelma city, which displayed multidrug resistance phenotypes. Furthermore, the isolates were investigated for the presence of *Salmonella* genomic island 1 (SGI1) and the genetic organization of its antibiotic resistance genes cluster. *S*. Kentucky strains were given unique names: HSK31, HSK61 and HSK71.

Antimicrobial resistance phenotype of *S*. Kentucky isolated from human in Algeria

The isolates were sensitive to ceftriaxone, chloramphenicol, trimethoprim, meropenem, imipenem, cefotaxime and gentamicin. They displayed MDR phenotypes with similar pattern of resistance to ampicillin, nalidixic acid, ciprofloxacin, streptomycin and tetracycline. Ciprofloxacin-resistance in *S*. Kentucky has recently emerged (Mulvey *et al.*, 2013), raising concerns for the selection of antibiotic therapy. Only few cases have been notified, and the source of contamination is not properly investigated; only hypothesis about the country of infection are discussed (Kariuki *et al.*, 2005; Collard *et al.*, 2007; Le Hello *et al.*, 2011). Studies suggest that contamination may have been acquired abroad, in particular people returning from Africa (Weill *et al.*, 2006). Resistance to ciprofloxacin is commonly found in certain serovars of *Salmonella* including *S*. Typhimurium, *S*. Choleraesuis, and *S*. Schwarzengrund (Olsen *et al.*, 2001; Baucheron *et al.*, 2005), and in recent years, it is repeatedly seen in the serovar Kentucky. This situation may lead to a therapy failure for NTS infections, and may

increase costs, which can put healthcare systems under more tension, in particular in low and middle-income countries, including Algeria.

In silico analysis of the chromosome

The isolates were identified as *S*. Kentucky of Sequence Type ST198. Infection with this sequence type strongly involves poultry as the principle vehicle (Le Hello *et al.*, 2011; Le Hello, Harrois, *et al.*, 2013). In the US, it represents the most common serovar isolated from chickens and the second most common reported among retail chicken (Rickert-Hartman and Folster, 2014)

Analysis for antimicrobial resistance genes revealed that aminoglycoside resistance genes were present and that clinically important beta-lactam resistance was mediated by *blaTEM-1* and *aac(3)-Id* genes, streptomycin resistance was mediated by *aad7* and *strAB* genes, sulfonamides resistance by *sul1* genes and tetracycline by *tetA*. On the contrary, none of them presented the *qnrA*, *B* or *S* genes responsible of plasmid-mediated quinolone resistance. The three isolates did not harbour any plasmid.

An intact SGI1 was detected in the isolates showing high similarity to SGI1 variant K [Accession number AY463797], but different genetic rearrangement of their MDR regions following mapping analysis of the full genome of each isolate. Therefore, the three chromosomes were investigated for the genetic organization of these three SGI1 that they harbour.

Genetic characterization of the MDR region in SGI1 variant K7 (SGI1-K7) identified in *S*. Kentucky strains HSK31, HSK61 and HSK71

In silico analysis of the chromosomes of the three isolates revealed novel rearrangement of the antibiotic resistance genes cluster in their SGI1s. This new organisation is resulted by insertional events and transposon-mediated genetic rearrangements. SGI1-K1 was used as reference in this analysis, because this variant was previously identified in the serovar Kentucky, and it is commonly used in investigating new genetic rearrangement among SGI1-Ks group. The variant found in this study was given a unique name SGI1-K7 (Fig 6). This variant belongs to Ks group, and was found to be integrated in the 3' end of the chromosomal *thdF* gene in three MDR serovars Kentucky strains HSK31, HSK61 and HSK71. This genomic island harbours the complex class 1 integron In104 that belongs to the In4 group, conferring the penta-resistance previously described (Cloeckaert and Schwarz, 2001; Mulvey *et al.*, 2006). The integron contains two gene cassettes: *aacCA5-aadA7* and *sul1*, adjacent to a mercury resistance module. However, in SGI1 commonly found in *S.* Typhimurium, the integron does not contain *sul1* gene cassette (Levings *et al.*, 2007). Similar to SGI1-K1, downstream of the *mer* module part of the transposon *Tn*1721 was found containing the tetracycline resistance genes *tetR* and *tet(A)*, followed by a *Tn*3-like region containing the *tnpR* gene and the β-lactamase *blaTEM-1* gene flanked by two *IS26* elements in opposite orientations. At the leftmost of the integron, an *IS26* element was found to be inserted into the SGI1 backbone. This

rearrangement on the MDR region confers the antibiotic resistance phenotype for these three strains of *S*. Kentucky

The full genome sequences of the three strains are available for comparison with other ciprofloxacin-resistant *S*. Kentucky at the ENA [project PRJEB66229].

Story of the MDR *S*. Kentucky ST198 is emerging globally (Rickert-Hartman and Folster, 2014), putting in evidence the need for international initiatives of surveillance networks for antimicrobial drug resistance, in order to facilitate tracking of genetic evolution of these strains in different geographical locations.

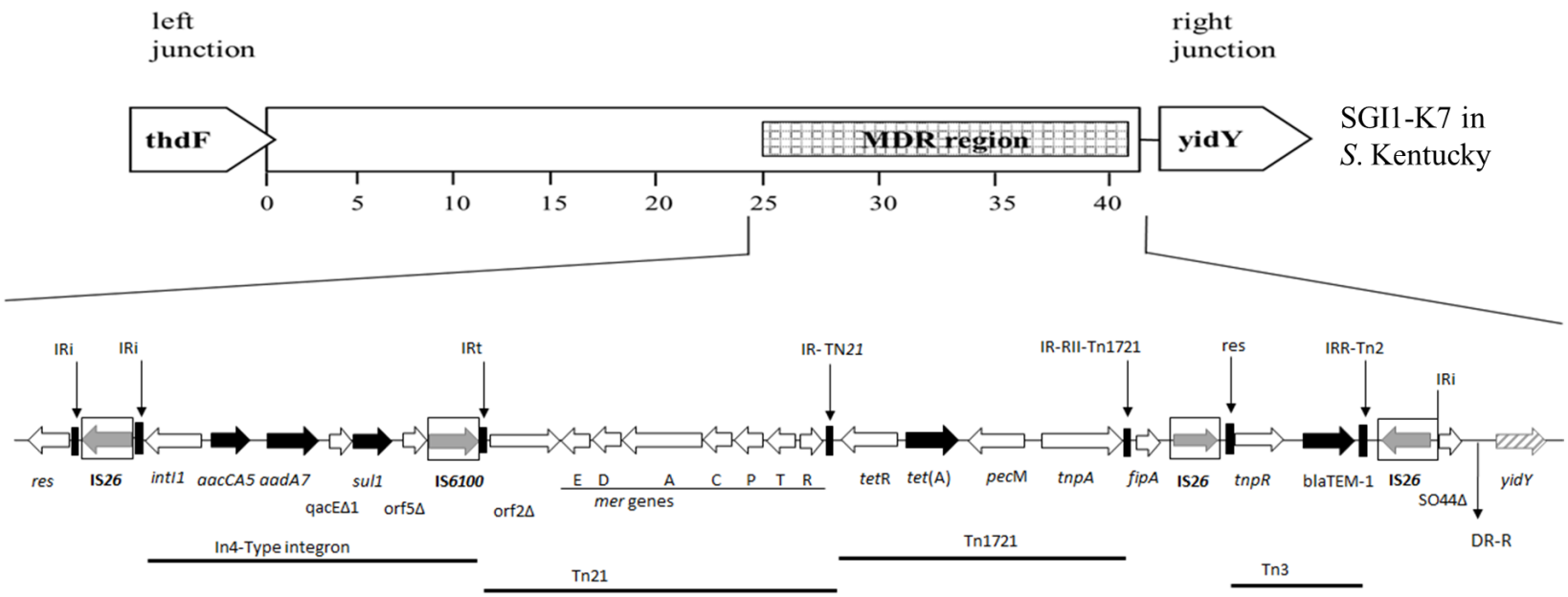

Fig 6. Schematic view of the MDR region in SGI1-K7 identified in *S.* Kentucky. Map of MDR region in SGI1-K7, the integron contains two gene cassettes: *aacCA5-aadA7* and *sul1*, adjacent to a mercury resistance module. The complete sequence of the SGI1-K7 of the three S. Kentucky strains HSK31, HSK61 and HSK71 are available at the ENA [project PRJEB66229]. Black arrows, and grey arrows reprent antibiotic resistance genes, IS elements flanking SGI1, respectively. DR-R corresponds to the right 18 bp direct repeat at the end of SGI1. IRi and IRt are 25 bp imperfect-inverted repeats defining the left and right ends of In4-type complex class 1 integrons. Vertical black bars corresponds to inverted repeats, and resolution site of transposon or integron sequences. Base pair coordinates are from the complete SGI1 sequence [GenBank accession number AF261825]. A distance scale in kbp is indicated.

OVERALL DISCUSSION

OVERALL DISCUSSION

Data on NTS infection in developing countries including Algeria is still limited. This is possibly linked to poor diagnostic ability, paucity of resources and lack of surveillance initiatives. Furthermore, individuals in Algeria do not usually undergo copro-culture diagnosis for diarrheal diseases, unless infection becomes acute with worsened symptoms. These factors have importantly contributed in underreporting of NTS serovars, and their propagation among the human, animal and environment in the country.

Prevalence of non-typhoidal *Salmonella* in poultry and humans in four cities in Algeria

Salmonella serovars isolated from poultry and humans in this study represent those usually reported worldwide, namely USA, and Europe (Authority EFS, 2015).

Ten different *Salmonella* serovars were recovered among poultry and humans in four cities in Algeria, being *S*. Typhimurium, *S*. Kentucky, *S*. Enteritidis, *S*. Heidelberg, *S*. Ohio, *S*. Lindenburg, *S*. Indiana, *S*. Virchow, and *S*. Bonn, while *S*. Gallinarum was the only serovar found among the poultry isolates.

However, *S*. Typhimurium ST19 was the dominant serovar isolated (27%). This serotype has a wide animal reservoirs, and it is one of the most frequently isolated serovar from food-borne outbreaks globally (Guerra *et al.*, 2002; El-Aziz, 2013).

It is of special interest, in particular strains belonging to phage type DT104 that emerged in several countries including UK, Germany and the USA (Baggesen and Aarestrup, 1998).

S. Typhimurium strains isolated in this study were considerably pathogenic, holding different virulence determinants, including pathogenic islands (SPIs and SGI1), and antimicrobial resistance determinants namely the clinically important beta–lactams. All of them, carried from two to four SPIs on their chromosomes each. For instance, SPI-5 carrying *sopB*, *sigD*, *pipB* genes encoding for effector proteins required in function of SPI-1 and SPI-2, those two last are implicated in invading intestinal epithelial cells using a type III secretion system (TTSS) to inject effector proteins into host cell, in intracellular survival, and has a crucial role in systemic infection (Abrahams GL *et al*., 2006). Furthermore, 84% of *S.* Typhimurium from this study carried two plasmids each belonging to incompatibility group FII, and FIB. IncFIB plasmids (also commonly known as ColV plasmids) can encode both virulence factors and antimicrobial resistance genes (Han *et al.,* 2012).

In a previous study, DNA sequence based phylogenetic diversity analyses showed that the IncFIB plasmid-encoded *Sit* (regulated iron transporter) and aerobactin iron acquisition systems are conserved among bacterial species including *S. enterica*.

IncFIB plasmids (also commonly known as ColV plasmids) can encode both virulence factors and antimicrobial resistance genes, and have been shown to be implicated in the virulence of extra-intestinal pathogenic *Escherichia coli* (Gao *et al.*, 2012). On the other hand, IncFII plasmid found in these *S.* Typhimurium is commonly found in this serovar (Guerra *et al.*, 2002). However, seventy-six percent of all the isolated *Salmonella* in this study carried at least one plasmid. Plasmids belonging to incompatibility group FIB and FII was the most commonly identified among the isolates.

In Algeria, there are few studies on the spread and distribution of serovars, or antimicrobial resistance in NTS. In Constantine region (east of Algeria), it was reported that 37% of broiler farms and 53% of slaughterhouses were contaminated with NTS and *S.* Typhimurium was among the most frequent serovar detected (Elgroud *et al.*, 2009) (Elgroud *et al.*, 2015). While *S.* Enteritidis and *S.* Typhimurium were previously reported to be the most frequent isolated serovars from human clinical cases in the same region (Elgroud *et al.*, 2015). Some of Algerian strains of *S.* Enteritidis isolated from humans shared similar antimicrobial resistance phenotype with strains of *S.* Enteritidis reported in Constantine (Elgroud *et al.*, 2015), which is a neighbour city of the source of isolation. However, data on their genotype is very limited, making it difficult for genetic comparison.

Similar situation has been reported in other African countries namely Egypt, Morocco and Senegal, where *S.* Enteritidis and *S.* Typhimurium have been the serotypes most frequently associated with human and avian salmonellosis even at low incidences of NTS infection (Rouahi *et al.*, 2000; Sow *et al.*, 2007; El-Sharkawy *et al.*, 2017; Tarabees *et al.*, 2017).

Resistance rates of *S.* Enteritidis isolated from humans are generally low. However, resistance to nalidixic acid was commonly observed among our isolates. Nalidixic acid is the prototype quinolone, widely used in veterinary medicine. It is strongly suggested that the intensive use of this antimicrobial agent has resulted dissemination of nalidixic acid resistance in *Salmonella enterica* across food animals, which may be transmitted to humans (Angulo *et al.*, 2004; Stevenson *et al.*, 2007).

The other *Salmonella* serovars identified in this study as Virchow, Heidelberg, Lindenburg, Indiana and Ohio are commonly reported worldwide. Therefore, virulence determinants including SPIs and SGIs, and antibiotic resistance genes, altogether they put in evidence the prevalence of pathogenic strains of NTS among humans and food animals circulating in Algeria.

Salmonella Gallinarum in the poultry industry in Algeria

From poultry, twenty-nine *S.* Gallinarum were recovered. In general, most of the strains displayed multiple resistance to antibiotics, and nalidixic acid resistance commonly

occurred. Furthermore, various virulent determinants were found on the chromosomes of these strains, including SPIs. This avian-adapted serovar was endemic in poultry flocks in Europe and the Americas during the early 20th century (Bullis, 1977). *S*. Gallinarum causes fowl typhoid (FT), a severe systemic disease responsible for significant substantial economic losses to the poultry industry worldwide (Shah *et al.*, 2005). FT caused by this serovar has been controlled and eradicated from most of industrialized countries like Australia, North America and most of the European countries. However, it still remains endemic in many countries of Africa, the Middle East, Central and South America and Asia (Shah *et al.*, 2005).

In Algeria, Salmonellosis caused by *S*. Gallinarum represents a real scourge for poultry farmers and the poultry industry. To face this major problem, control measures have been taken by the authorities in 20th of January 2003, defining measures for the prevention and specific control of avian salmonellosis with *S*. Gallinarum. The twenty-nine strains isolated from poultry during this study has revealed that those measures are probably not sufficient. It might be associated with the lack of education and awareness among farmers in the country, or more effective regulations are needed to be undertaken in the poultry sector in Algeria.

Salmonella Kentucky in travellers

Salmonella enterica serotype Kentucky represents one of the non-typhoidal types of *Salmonella* that is encountered in gastroenteritis infections. For decades, *S*. Kentucky was not a matter of concern for public health systems, but this pathogen has managed to develop resistance to a number of antimicrobial agents, making it difficult to treat. During the last 10 years, it was widely reported from different developing countries in both humans and domestic poultry, in particular the African ones. MDR *S*. Kentucky ST198 was firstly isolated in returning tourists from Egypt, and later from Libya, Morocco, Tunisia, Kenya, Sudan and Tanzania (Figure 6) (Majtán *et al.*, 2006; Weill *et al.*, 2006; Collard *et al.*, 2007; Bouchrif *et al.*, 2008). Between 2002 and 2015, fifteen sporadic MDR resistant *S*. Kentucky ST198 were reported. These isolates were found to be producing carbapenemases (*blaVIM-2*, *blaOXA-48*), ESBLs (*blaCTX-M-1*, *blaCTX-M-15*, *blaCTX-M-25*), cephamycinase (*blaCMY-2*) or even combinations of carbapenemases and ESBLs (*blaOXA-48* and *blaVEB-8*). All these isolates were originally from the Mediterranean (Collard *et al.*, 2007; Le Hello, Harrois, *et al.*, 2013; Le Hello, Bekhit, *et al.*, 2013; Harrois *et al.*, 2014; Seiffert *et al.*, 2014). In Algeria, research investigations on genetic background of such growing resistant-serovar is needed. Therefore, results found here, put in evidence prevalence of *S*. Kentucky ST198 in two different regions namely Guelma (east of Algeria) and Algiers (centre of Algeria). It is strongly believed that the origin of these strains is Egypt, because during the period

2002-2005, *S.* Kentucky resistant to ciprofloxacin caused a major health problem among the Egyptian population, and was spread throughout Africa and Middle East (Le Hello, Harrois, *et al.*, 2013). However further genetic studies are needed to demonstrate the origin of *S.* Kentucky ST198.

Fig. 7. Geographical spread of epidemic population of MDR *S.* Kentucky ST198, with a focus on the Mediterranean region and Middle East. Colours represent countries where the infection has been reported in the last 14 years.

Antimicrobial resistance among non-typhoidal *Salmonella* isolated in four cities in Algeria

The dissemination of MDR *Salmonella* from animals to humans is a major adverse consequence for public health, which is leading to a treatment failure in human infectious diseases (Jordan *et al.,* 2009). In Algeria, an increase in NTS has been observed among bacterial isolates in laboratories both clinically and in animals (Elgroud

et al., 2015). At present, the available data on NTS infection are not sufficient to understand the propagation of NTS through the food chain and into humans. One of the major consequences of such propagation could be the resistance to antibiotics.

As a whole, all tested *Salmonella* isolates in this study were resistant to one or more antimicrobials, with high proportion of multi drug resistance. Frequency of resistance was more common to nalidixic acid (85%), ciprofloxacin and streptomycin (66%), ampicillin (63%), tetracycline (44%) and chloramphenicol (20%). Resistance to nalidixic acid prevalent in isolates obtained from poultry, all S. Gallinarum. This can be explained as a consequence of the extensively use of this antibiotic in animal husbandry in the Batna region, in the east part of Algeria, Similar situation was reported in *S*. Gallinarum outbreak in Egypt (Sannat *et al.*, 2017). Beta lactamase producer *S*. Gallinarum was confirmed as cause of increased mortality in layer birds farms (Sannat *et al.*, 2017). Frequency of resistance to ampicillin, chloramphenicol and tetracycline in *Salmonella* isolates from poultry was relatively low compared to that seen in humans. For instance, resistance to tetracycline was 84% in isolates obtained from humans while only 34% of poultry isolates were resistant to tetracycline. In Morocco, located in the western frontiers of Algeria, tetracycline resistance in *Salmonella,* namely *S*. Typhimurium and *S*. Enteritidis, was reported to be the most commonly observed among the tested antibiotics. This resistance was predictable as a consequence of the high use of veterinary and human medicine in the country (Bouchrif *el al.,* 2009).

Decreased resistance to ceftriaxone was observed among *Salmonella* isolates in this study, only 10% in humans in *S*. Typhimurium, *S*. Ohio, and *S*. Heidelberg, and 7% in poultry in *S*. Gallinarum. Resistance to ceftriaxone is a matter of concern for public care systems, because this antimicrobial agent is important in treatment of salmonellosis, especially in children (Chen *et al.*, 2004). Cases of ceftriaxone-resistant *Salmonella* is not frequently notified. For instance, in UK and Denmark, ceftriaxone-resistant S*almonella* have been reported very rarely by national surveillance systems (Fey *et al.*, 2000). However, in USA ceftriaxone-resistant *Salmonella* infections have been notified. It is thought that the use of ceftiofur in food-producing animals is probably contributing to ceftriaxone resistance in *Salmonella*, particularly resistance caused by an *ampC* resistance gene (Dunne *et al.*, 2000). The same resistance was reported in Kenya, from patients infected with *S*. Typhimurium ST313. Resistance was associated with the *blaCTX-M-15* gene integrated in an IncHI2 plasmid (Kariuki *et al.*, 2015).

On the other hand, investigation for resistance mediation genes revealed that thirty percent of *Salmonella* isolates in this study contained β-lactam genes, and it was detected only in humans. The dominant *bla* gene responsible for resistance to beta-lactam antimicrobials in majority of *Salmonella* isolates was found to be variants of *blaCARB-1* and *blaCARB-2* genes, it was detected in nine *S*. Typhimurium. In five of the human isolates *S*. Kentucky, and one *S*. Bonn three *bla* genes (*blaTEM-1B*, *blaTEM-63* and *blaTEM-110*) were detected. *QnrS1* gene responsible of plasmid-mediated

quinolone resistance was detected in only five *S*. Gallinarum isolated from poultry. Furthermore, Twenty-seven percent of *Salmonella* were identified to harbor classical SGI1 conferring resistance to ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfametoxazole and tetracycline). This genomic island was detected only in serovar *S*. Typhimurium. On the other hand, three of *S*. Kentucky were found to carry new variants of SGI1 belonging to Ks family named SGI1-K7.

On a global scale, genes exchange within bacterial communities is recognized as a major contributor in evolution of antibiotic resistance. In Algeria, the majority of antibiotic resistance determination is based upon phenotypic characterization, and there is limited use of molecular biology to understand the spread of antibiotic resistance among food-borne bacteria. Therefore, to be able to understand molecular mechanisms by which antimicrobial resistance emerges and spreads, it is required to design intervention strategies to reduce its progression in the future.

Burden of NTS infection

Worldwide, infection caused by NTS is one of the largest health burden among bacterial pathogens. In the US, among 19 057 laboratory-confirmed cases of bacterial infections, salmonellosis was linked to 7439 of these cases, in which 2144 persons were hospitalized, and among them 32 patients died (CDC, 2014). In survey reports, *S*. Typhimurium and *S*. Enteritidis are the most frequent reported serovars. They account

for nearly half of all cases of salmonellosis. (Tauxe, 1999). However, other serovars are widespread in particular countries, causing more acute infections and consequences.

In many developing countries, especially African countries, the situation is worsened by their socio-economic conditions, which is aggravating the rate and seriousness of infections. Specific serovars are prevalent in certain regions, namely *S.* Concord in Ethiopia (Beyene *et al.*, 2011), *S.* Bovismorbificans in Malawi (Bronowski *et al.*, 2013), *S.* Stanleyville and *S.* Dublin in Mali (Tennant *et al.*, 2010), and *S.* Isangi in South Africa and Zimbabwe (Wadula *et al.*, 2006; Paglietti *et al.*, 2013). In Kenya, an average of 166 per 100,000 children under five acquire an NTS infection every year (Oundo *et al.*, 2002; Berkley *et al.*, 2005). In sub-Saharan Africa, a serious invasive form of NTS linked to *S.* Typhimurium ST313 has emerged, and has become a leading public health issue in this region. This invasive *S.* Typhimurium had an estimated mortality rate of 20-25% in children and up to 50% in adults (Kingsley *et al.*, 2009). However, none of our Algerian *S.* Typhimurium belonged to this sequence type, and they were all ST19.

According to Eurostat, Directorate-General of the European Commission, EU trade in food and live animals with the northern Africa and the Middle East countries has risen over the last decade, particularly from 2008 onwards. Furthermore, the EU imported mostly from Morocco, and among these countries, Algeria is the main destination of EU food and live animals exports. Thus, the raise in inter-trade has broken the boundaries

between developed and developing countries and such pathogenic strains of bacteria are being transmitted globally across animal food origin. Consequently, this reinforce the need for harmonised antimicrobial resistance surveillance in *Salmonella*.

Conclusion

Based on the findings presented above, information on the prevalence of non-typhoidal *Salmonella* serovars present in humans and poultry in four cities in Algeria, as well as their resistance to antimicrobials have been provided. Data on their genetics has also been provided. A novel variant of genomic island (SGI1-K7) integrated in the chromosome of three MDR strains of *S*. Kentucky ST198, where clusters of antimicrobial resistance genes are located has been described.

The findings presented above support the fact that continual surveillance of antibiotic resistance in *Salmonella* is of primary importance. Consequently, maintenance of different resistance genes in different serovars reported in this study constitute a serious public health concern among the Algerian population. Furthermore, NTS infection is responsible for heavy economic losses to the commercial poultry industry through morbidity, mortality and reduced egg production. Now more than before, it is crucial for monitoring programmes for the surveillance of *Salmonella* in both humans and food animal origin in order to improve public health system in Algeria. On the other hand, these findings contribute in providing information that can be useful for further studies.

Limitations of the study

Unfortunately, detailed information on the health condition of patients from which *Salmonella* was isolated were not available. The number of *Salmonella* isolates collected from hospitals was higher than presented in this thesis, this because of the problems encountered for the transport of all isolates from University of Batna in Algeria to the University of Sassari in Italy. Moreover, since the isolation of the strains was conducted in Algeria, storing conditions of the isolates were not always appropriate, which resulted in the loss of many isolates. Furthermore, lack of whole genome sequences of *S.* Kentucky on databases online did not permit further genetic comparisons with the *S.* Kentucky strains recovered in this study.

REFERENCES

Aboun, A., Benelmouffok, A., Bougueddour, R., Taril, A., Rezkallah, M., and Selatnia, L. (2000) Avian *Salmonella* diagnosed at the Pasteur Institute of Algeria from 1988 to 2002: Serotypes encountered, their antimicrobial resistance and regulatory aspects. *Arch l'institut Pasteur d'Algérie* **2003**: 93–114.

Abrahams, G.L., and Hensel, M. (2006) Manipulating cellular transport and immune responses: Dynamic interactions between intracellular *Salmonella enterica* and its host cells. *Cell Microbiol* **8**: 728–737.

Acheson, D., and Hohmann, E.L. (2001) Nontyphoidal Salmonellosis. *Clin Infect Dis* **32**: 263–269.

Achtman, M., Wain, J., Weill, F.X., Nair, S., Zhou, Z., Sangal, V., *et al.* (2012) Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathog* **8**.

Ackers, M.L., Puhr, N.D., Tauxe, R. V, and Mintz, E.D. (2000) Laboratory-based surveillance of *Salmonella* serotype Typhi infections in the United States: antimicrobial resistance on the rise. *JAMA* **283**: 2668–2673.

Afgan, E., Baker, D., Beek, M. van den, Blankenberg, D., Bouvier, D., Čech, M., *et al.* (2016) The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. *Nucleic Acids Res* **44**: W3–W10.

Ahmad, N., Gee Hoon, S.T., Abd Ghani, M.K., and Tee, K.Y. (2012) The discrimination of d-tartrate positive and d-tartrate negative *S. enterica* subsp. *enterica* serovar Paratyphi B isolated in Malaysia by phenotypic and genotypic methods. *Malays J Pathol* **34**: 35–39.

ANGULO, F.J., JOHNSON, K.R., TAUXE, R. V., and COHEN, M.L. (2000) Origins and Consequences of Antimicrobial-Resistant Nontyphoidal *Salmonella:* Implications for the Use of Fluoroquinolones in Food Animals. *Microb Drug Resist* **6**: 77–83.

Angulo, F.J., Nargund, V.N., and Chiller, T.C. (2004) Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. *J Vet Med B Infect Dis Vet Public Health* **51**: 374–9.

Antunes, P., Machado, J., Sousa, J.C., and Peixe, L. (2005) Dissemination of sulfonamide resistance genes (sul1, sul2, and sul3) in Portuguese *Salmonella enterica* strains and relation with integrons. *Antimicrob Agents Chemother* **49**: 836–839.

Arii, J., Tanabe, Y., Miyake, M., Mukai, T., Matsuzaki, M., Niinomi, N., *et al.* (2002) Clinical and pathologic characteristics of nontyphoidal *salmonella* encephalopathy. *Neurology* **58**: 1641–5.

Ashton, P.M., Nair, S., Peters, T.M., Bale, J.A., Powell, D.G., Painset, A., *et al.* (2016) Identification of *Salmonella* for public health surveillance using whole genome sequencing. *PeerJ* **4**: e1752.

Authority, E.F.S. (2015) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSa J* **13**.

Ayachi, A., Alloui, N., Bennoune, O., and Kassah-Laouar, A. (2010) Survey of *Salmonella* serovars in broilers and laying breeding reproducers in Eastern Algeria. *J Infect Dev Ctries* **4**: 103–106.

Ayachi, a, Alloui, N., Bennoune, O., Yakhlef, G., Amiour, S.D., Bouzid, W., *et al.* (2009) Antibacterial activity of some fruits; berries and medicinal herb extracts against poultry strains of *Salmonella*. *Am J Agric Environ Sci* **6**: 12–15.

Ayele, M., DeLong, S.M., Wong, D.M.A.L.F., Wagenaar, J.A., Karlsmose, S., Maxwell, N., *et al.* (2011) WHO Global Foodborne Infections Network (GFN): Over 10 years of strengthening national capacities to detect and control foodborne and other enteric infections globally. .

Baggesen, D.L., and Aarestrup, F.M. (1998) Characterisation of recently emerged multiple antibiotic-resistant *Salmonella enterica* serovar Typhimurium DT104 and other multiresistant phage types from Danish pig herds. *Vet Rec* **143**: 95–97.

Bale, J. (2007) *Kauffmann-White scheme - 2007 : Salmonella identification serotypes and antigen formulae*. Centre for Infections, Health Protection Agency, .

Barker, R.M. (1985) Utilization of d-tartaric acid by Salmonella paratyphi B and *Salmonella* java: comparison of anaerobic plate test, lead acetate test and turbidity test. *Epidemiol Infect* **95**: 107–114.

Baucheron, S., Chaslus-Dancla, E., Cloeckaert, A., Chiu, C.H., and Butaye, P. (2005) High-level resistance to fluoroquinolones linked to mutations in gyrA, parC, and parE in *Salmonella enterica* serovar Schwarzengrund isolates from humans in Taiwan [2]. *Antimicrob Agents Chemother* **49**: 862–863.

Bäumler, A.J., Tsolis, R.M., Ficht, T.A., and Adams, L.G. (1998) Evolution of Host Adaptation in *Salmonella enterica*. *Infect Immun* **66**: 4579–4587.

Berkley, J.A., Lowe, B.S., Mwangi, I., Williams, T., Bauni, E., Mwarumba, S., *et al.* (2005) Bacteremia among Children Admitted to a Rural Hospital in Kenya. *N Engl J Med* **352**: 39–47.

Beyene, G., Nair, S., Asrat, D., Mengistu, Y., Engers, H., and Wain, J. (2011) Multidrug resistant *Salmonella* Concord is a major cause of salmonellosis in children in Ethiopia. *J Infect Dev Ctries* **5**: 23–33.

Borges, K.A., Furian, T.Q., Borsoi, A., Moraes, H.L.S., Salle, C.T.P., and Nascimento, V.P. (2013) Detection of virulence-associated genes in *Salmonella* Enteritidis isolates from chicken in South of Brazil. *Pesqui Vet Bras* **33**: 1416–1422.

Bouchrif, B., Karraouan, B., Ennaji, M.M., and Timinouni, M. (2008) Quinolones-resistant *Salmonella* spp. in Casablanca-Morocco. *Med Mal Infect* **38**: 615–616.

Bouchrif, B., Paglietti, B., Murgia, M., Piana, A., Cohen, N., Ennaji, M., Rubino, S., and Timinouni, M. (2009) Prevalence and antibiotic-resistance of *Salmonella* isolated from food in Morocco. J Inf Dev Ctries **1**: 35-40.

Bounar-Kechih, S., Hamdi, T.M., Mezali, L., Assaous, F., and Rahal, K. (2012) Antimicrobial resistance of 100 *Salmonella* strains isolated from Gallus gallus in 4 wilayas of Algeria. *Poult Sci* **91**: 1179–1185.

Bouzidi, N., Aoun, L., Zeghdoudi, M., Bensouilah, M., Elgroud, R., Oucief, I., *et al.* (2012) *Salmonella* contamination of laying-hen flocks in two regions of Algeria. *Food Res Int* **45**: 897–904.

Boyd, D.A., Peters, G.A., Ng, L.K., and Mulvey, M.R. (2000) Partial characterization of a genomic island associated with the multidrug resistance region of *Salmonella enterica* Typhymurium DT104. *FEMS Microbiol Lett* **189**: 285–291.

Boyd, D., Cloeckaert, A., Chaslus-Dancla, E., and Mulvey, M.R. (2002) Characterization of variant *Salmonella* genomic island 1 multidrug resistance regions from serovars Typhimurium DT104 and agona. *Antimicrob Agents Chemother* **46**: 1714–1722.

Boyd, D., Peters, G.A., Cloeckaert, A., Boumedine, K.S., Chaslus-Dancla, E., Imberechts, H., and Mulvey, M.R. (2001) Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. *J Bacteriol* **183**: 5725–5732.

Brenner, F.W., Villar, R.G., Angulo, F.J., Tauxe, R., and Swaminathan, B. (2000) *Salmonella* nomenclature. *J Clin Microbiol* **38**: 2465–2467.

Briggs, C.E., and Fratamico, P.M. (1999) Molecular characterization of an antibiotic resistance gene cluster of *Salmonella* Typhimurium DT104. *Antimicrob Agents Chemother* **43**: 846–9.

Bronowski, C., Fookes, M.C., Gilderthorp, R., Ashelford, K.E., Harris, S.R., Phiri, A., *et al.* (2013) Genomic Characterisation of Invasive Non-Typhoidal *Salmonella* enterica Subspecies enterica Serovar Bovismorbificans Isolates from Malawi. *PLoS Negl Trop Dis* **7**.

Brown, H.J., Stokes, H.W., and Hall, R.M. (1996) The integrons In0, In2, and In5 are defective transposon derivatives. *J Bacteriol* **178**: 4429–4437.

Bullis, K.L. (1977) The History of Avian Medicine in the U.S- Pullorum Disease and Fowl Typhoid. *Avian Dis* **21**: 422–429.

Calos, M.P., and Miller, J.H. (1980) Transposable elements. *Cell* **20**: 579–595.

Cantón, R., and Coque, T.M. (2006) The CTX-M b-lactamase pandemic. *Curr Opin Microbiol* **9**: Webb, H. E., Granier, S. A., Mrult, M., Millemann,.

Cantón, R., González-Alba, J.M., and Galán, J.C. (2012) CTX-M enzymes: Origin and diffusion. *Front Microbiol* **3**.

Carattoli, A., Filetici, E., Villa, L., Dionisi, A.M., Ricci, A., and Luzzi, I. (2002) Antibiotic resistance genes and *Salmonella* genomic island 1 in *Salmonella enterica* serovar Typhimurium isolated in Italy. *Antimicrob Agents Chemother* **46**: 2821–2828.

Carattoli, A., Zankari, E., Garci-Fernndez, A., Larsen, M.V., Lund, O., Villa, L., *et al.* (2014) *In Silico* detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* **58**: 3895–3903.

Casadesüs, J. (1999) The virulence plasmids of *Salmonella*. *Int Microbiol* **2**: 177–184.

CDC (2014) Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet Surveillance Report for 2012 (Final Report). *US Dep Heal Hum Serv CDC* .

Centers for Disease Control and Prevention (2014) Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet Surveillance Report for 2014 (Final Report). *US Dep Heal Hum Serv CDC* .

Centers for Disease Control and Prevention (CDC) (2016) National *Salmonella* Surveillance Annual Report, 2013. 1–89.

Chart, H. (2003) The pathogenicity of strains of *Salmonella* Paratyphi B and *Salmonella* Java. *J Appl Microbiol* **94**: 340–348.

Chen, H.M., Wang, Y., Su, L.H., and Chiu, C.H. (2013) Nontyphoid *Salmonella* infection: Microbiology, clinical features, and antimicrobial therapy. *Pediatr Neonatol* **54**: 147–152.

Chen, S., Zhao, S., White, D.G., Schroeder, C.M., Lu, R., Yang, H., *et al.* (2004) Characterization of Multiple-Antimicrobial-Resistant *Salmonella* Serovars Isolated from Retail Meats. *Appl Environ Microbiol* **70**: 1–7.

Choi, S.H., Kim, E.Y., and Kim, Y.J. (2013) Systemic use of fluoroquinolone in children. *Korean J Pediatr* **56**: 196–201.

Chu, C., Hong, S.F., Tsai, C., Lin, W.S., Liu, T.P., and Ou, J.T. (1999) Comparative physical and genetic maps of the virulence plasmids of *Salmonella* enterica serovars Typhimurium, Enteritidis, Choleraesuis, and Dublin. *Infect Immun* **67**: 2611–2614.

Cloeckaert, A., Praud, K., Doublet, B., Demartin, M., and Weill, F.X. (2006) Variant *Salmonella* genomic island 1-L antibiotic resistance gene cluster in *Salmonella enterica* serovar Newport. *Antimicrob Agents Chemother* **50**: 3944–3946.

Cloeckaert, A., and Schwarz, S. (2001) Molecular characterization, spread and evolution of multidrug resistance in *Salmonella enterica* Typhimurium DT104. In *Veterinary Research*. pp. 301–310.

Clokie, M.R.J., Millard, A.D., Letarov, A. V., and Heaphy, S. (2011) Phages in nature. *Bacteriophage* **1**: 31–45.

Cogan, T. a, and Humphrey, T.J. (2003) The rise and fall of *Salmonella* Enteritidis in the UK. *J Appl Microbiol* **94 Suppl**: 114S–119S.

Colavecchio, A., Cadieux, B., Lo, A., and Goodridge, L.D. (2017) Bacteriophages contribute to the spread of antibiotic resistance genes among foodborne pathogens of the *Enterobacteriaceae* family - A review. *Front Microbiol* **8**.

Collard, J.-M.M., Place, S., Denis, O., Rodriguez-Villalobos, H., Vrints, M., Weill, F.-X.F.-X., *et al.* (2007) Travel-acquired salmonellosis due to *Salmonella* Kentucky resistant to ciprofloxacin, ceftriaxone, and co-trimoxazone and associated with treatment failure. *J Antimicrob Chemother* **60**: 190–2.

Collinson, S.K., Liu, S., Clouthier, S.C., Banser, P.A., Doran, J.L., Sanderson, K.E., and William, W. (1996) the *Salmonella* Enteritidis and / or *S* . Typhimurium XbaI-BlnI genomic restriction maps. *Gene* **169**: 75–80.

Connerton, P., Wain, J., Hien, T.T., Ali, T., Parry, C., Chinh, N.T., *et al.* (2000) Epidemic typhoid in Vietnam: Molecular typing of multiple-antibiotic- resistant *Salmonella enterica* serotype Typhi from four outbreaks. *J Clin Microbiol* **38**: 895–897.

Cooke, F., Threlfall, E., Wain, J., Rhen, M., Maskell, D., Mastroeni, P., and Threlfall, J. (2007) Current trends in the spread and occurrences of human salmonellosis: Molecular typing and emerging antibiotic resistance. *Salmonella Mol Biol Pathog* 1–34.

Crosa, J.H., Brenner, D.J., Ewing, W.H., and Falkow, S. (1973) Molecular relationship among the *Salmonelleae*. *J Bacteriol* **115**: 307–315.

Crump, J.A., Kretsinger, K., Gay, K., Hoekstra, R.M., Vugia, D.J., Hurd, S., *et al.* (2008) Clinical response and outcome of infection with *Salmonella enterica* serotype Typhi with decreased susceptibility to fluoroquinolones: A United States FoodNet multicenter retrospective cohort study. *Antimicrob Agents Chemother* **52**: 1278–1284.

Crump, J.A., Medalla, F.M., Joyce, K.W., Krueger, A.L., Hoekstra, R.M., Whichard, J.M., and Barzilay, E.J. (2011) Antimicrobial resistance among invasive nontyphoidal *Salmonella enterica* in the United States, National Antimicrobial Resistance Monitoring System, 1996-

2007. *Antimicrob Agents Chemother* **55**: 1148–1154.

Darby, J., and Sheorey, H. (2008) Searching for *Salmonella*. *Aust Fam Physician* **37**: 806–810.

Demczuk, W., Soule, G., Clark, C., Ackermann, H.W., Easy, R., Khakhria, R., *et al.* (2003) Phage-based typing scheme for *Salmonella enterica* serovar Heidelberg, a causative agent of food poisonings in Canada. *J Clin Microbiol* **41**: 4279–4284.

Djeffal, S., Bakour, S., Mamache, B., Elgroud, R., Agabou, A., Chabou, S., *et al.* (2017) Prevalence and clonal relationship of ESBL-producing *Salmonella* strains from humans and poultry in northeastern Algeria. *BMC Vet Res* **13**: 132.

Djeghout, B., Ayachi, A., Paglietti, B., Langridge, G.C., and Rubino, S. (2017) An Algerian perspective on non-typhoidal *Salmonella* infection. *J Infect Dev Ctries* **11**: 583.

Doublet, B., Boyd, D., Mulvey, M.R., and Cloeckaert, A. (2005) The *Salmonella* genomic island 1 is an integrative mobilizable element. *Mol Microbiol* **55**: 1911–1924.

Doublet, B., Butaye, P., Imberechts, H., Boyd, D., Mulvey, M.R., Chaslus-Dancla, E., and Cloeckaert, A. (2004) *Salmonella* genomic island 1 multidrug resistance gene clusters in *Salmonella enterica* serovar agona isolated in Belgium in 1992 to 2002. *Antimicrob Agents Chemother* **48**: 2510–2517.

Doublet, B., Lailler, R., Meunier, D., Brisabois, A., Boyd, D., Mulvey, M.R., *et al.* (2003) Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster in *Salmonella enterica* serovar Albany. *Emerg Infect Dis* **9**: 585–591.

Doublet, B., Weill, F.-X., Fabre, L., Chaslus-Dancla, E., and Cloeckaert, A. (2004) Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster containing a novel 3'-N-aminoglycoside acetyltransferase gene cassette, aac(3)-Id, in *Salmonella enterica* serovar newport. *Antimicrob Agents Chemother* **48**: 3806–3812.

Dunne, E.F., Fey, P.D., Kludt, P., Reporter, R., Mostashari, F., Shillam, P., *et al.* (2000) Emergence of domestically acquired ceftriaxone-resistant *Salmonella* infections associated with AmpC beta-lactamase. *JAMA* **284**: 3151–3156.

Dutil, L., Irwin, R., Finley, R., Ng, L.K., Avery, B., Boerlin, P., *et al.* (2010) Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. *Emerg Infect Dis* **16**: 48–54.

Ebner, P., Garner, K., and Mathew, A. (2004) Class 1 integrons in various *Salmonella enterica* serovars isolated from animals and identification of genomic island SGI1 in *Salmonella enterica* var. Meleagridis. *J Antimicrob Chemother* **53**: 1004–1009.

Edwards, R.A., and Puente, J.L. (1998) Fimbrial expression in enteric bacteria: A critical step in intestinal pathogenesis. *Trends Microbiol* **6**: 282–287.

El-Aziz, D.M.A. (2013) Detection of *Salmonella* Typhimurium in retail chicken meat and chicken giblets. *Asian Pac J Trop Biomed* **3**: 678–681.

El-Sharkawy, H., Tahoun, A., El-Gohary, A.E.-G.A., El-Abasy, M., El-Khayat, F., Gillespie, T., *et al.* (2017) Epidemiological, molecular characterization and antibiotic resistance of *Salmonella enterica* serovars isolated from chicken farms in Egypt. *Gut Pathog* **9**: 8.

Elgroud, R., Granier, S.A., Marault, M., Kerouanton, A., Lezzar, A., Bouzitouna-Bentchouala, C., *et al.* (2015) Contribution of Avian *Salmonella enterica* Isolates to Human Salmonellosis Cases in Constantine (Algeria). *Biomed Res Int* **2015**: 352029.

Elgroud, R., Zerdoumi, F., Benazzouz, M., Bouzitouna-Bentchouala, C., Granier, S.A., Frémy, S., *et al.* (2009) Characteristics of *Salmonella* contamination of broilers and slaughterhouses in the region of Constantine (Algeria). *Zoonoses Public Health* **56**: 84–93.

Eng, S.-K., Pusparajah, P., Ab Mutalib, N.-S., Ser, H.-L., Chan, K.-G., and Lee, L.-H. (2015) *Salmonella* : A review on pathogenesis, epidemiology and antibiotic resistance. *Front*

Life Sci **8**: 284–293.

Enwere, G., Biney, E., Cheung, Y., Zaman, S.M.A., Okoko, B., Oluwalana, C., *et al.* (2006) Epidemiologic and Clinical Characteristics of Community-Acquired Invasive Bacterial Infections in Children Aged 29 Months in The Gambia. *Pediatr Infect Dis J* **25**: 700–705.

Farrar, J., Hotez, P.J., Junghanss, T., Kang, G., Lalloo, D., and White, N.J. (2013) *Manson's Tropical Diseases: Twenty-Third Edition*. .

FDA/NSTA (2008) Science and our food supply: Food safety A to Z reference guide. 8–9 www.worldbank.org.

Ferris, K., Tall, B.D., Cravioto, A., Musser, J.M., Selander, R.K., Beltran, P., *et al.* (1990) Evolutionary genetic relationships of clones of *Salmonella* serovars that cause human typhoid and other enteric fevers . Evolutionary Genetic Relationships of Clones of *Salmonella* Serovars That Cause Human Typhoid and Other Enteric Fevers. *Infect Immun* **58**: 2262–2275.

Fey, P.D., Safranek, T.J., Rupp, M.E., Dunne, E.F., Ribot, E., Iwen, P.C., *et al.* (2000) Ceftriaxone-Resistant *Salmonella* Infection Acquired by a Child from Cattle. *N Engl J Med* **342**: 1242–1249.

Fluit, A.C. (2005) Towards more virulent and antibiotic-resistant *Salmonella FEMS Immunol Med Microbiol* **43**: 1–11.

Ford Doolittle, W., and Zhaxybayeva, O. (2009) On the origin of prokaryotic species. *Genome Res* **19**: 744–756.

Gao, Q., Wang, X., Xu, H., Xu, Y., Ling, J., Zhang, D., *et al.* (2012) Roles of iron acquisition systems in virulence of extraintestinal pathogenic *Escherichia coli*: salmochelin and aerobactin contribute more to virulence than heme in a chicken infection model. *BMC Microbiol* **12**: 143.

Gal-Mor, O., Boyle, E., and Grassl, G. (2014) Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ. *Front Microbiol* **5**: 391.

Galán, J.E., and Wolf-Watz, H. (2006) Protein delivery into eukaryotic cells by type III secretion machines. *Nature* **444**: 567–573.

Gharieb, R.M., Tartor, Y.H., and Khedr, M.H.E. (2015) Non-Typhoidal *Salmonella* in poultry meat and diarrhoeic patients: prevalence, antibiogram, virulotyping, molecular detection and sequencing of class I integrons in multidrug resistant strains. *Gut Pathog* **7**: 34.

Gilissen, C., Hehir-Kwa, J.Y., Thung, D.T., Vorst, M. van de, Bon, B.W.M. van, Willemsen, M.H., *et al.* (2014) Genome sequencing identifies major causes of severe intellectual disability. *Nature* **511**: 344–347.

Gradel, K.O., Dethlefsen, C., C. Schonheyder, H., Ejlertsen, T., T. Sorensen, H., Thomsen, R.W., and Nielsen, H. (2007) Severity of infection and seasonal variation of non-typhoid *Salmonella* occurrence in humans. *Epidemiol Infect* **135**: 93.

Graham, S.M., Molyneux, E.M., Walsh, A.L., Cheesbrough, J.S., Molyneux, M.E., and Hart, C.A. (2000) Nontyphoidal *Salmonella* infections of children in tropical Africa. *Pediatr Infect Dis J* **19**: 1189–1196.

Griffiths, A.J.F. (2005) *An Introduction to Genetic Analysis*. 7th editio., W. H. Freeman, New York.

Grimont, P., and Weill, F.-X. (2008) Antigenic formulae of the *Salmonella* servovars. *WHO Collab Cent Ref Res Salmonella* **9**: 1–167.

Guerra, B., Soto, S., Helmuth, R., and Mendoza, M.C. (2002) Characterization of a self-transferable plasmid from *Salmonella enterica* serotype Typhimurium clinical isolates carrying two integron-borne gene cassettes together with virulence and drug resistance

genes. *Antimicrob Agents Chemother* **46**: 2977–2981.

Guerra, B., Soto, S.M., and Argüelles, J.M. (2001) Multidrug Resistance Is Mediated by Large Plasmids Carrying a Class 1 Integron in the Emergent *Salmonella enterica* Serotype Multidrug Resistance Is Mediated by Large Plasmids Carrying a Class 1 Integron in the Emergent *Salmonella enterica*. *Antimicrob Agents Chemother* **45**: 1305–1308.

Guibourdenche, M., Roggentin, P., Mikoleit, M., Fields, P.I., Bockemühl, J., Grimont, P.A.D., and Weill, F.X. (2010) Supplement 2003-2007 (No. 47) to the White-Kauffmann-Le Minor scheme. *Res Microbiol* **161**: 26–29.

Gulig, P.A. (1990) Virulence plasmids of *Salmonella* Typhimurium and other *Salmonellae*. *Microb Pathog* **8**: 3–11.

Gulig, P.A., Danbara, H., Guiney, D.G., Lax, A.J., Norel, F., and Rhen, M. (1993) Molecular analysis of spv virulence genes of the *Salmonella* virulence plasmids. *Mol Microbiol* **7**: 825–830.

Gupta, A., Fontana, J., Crowe, C., Bolstorff, B., Stout, A., Duyne, S. Van, *et al.* (2003) Emergence of Multidrug-Resistant *Salmonella enterica* Serotype Newport Infections Resistant to Expanded-Spectrum Cephalosporins in the United States. *J Infect Dis* **188**: 1707–1716.

Haack, K.R., and Roth, J.R. (1995) Recombination between chromosomal IS200 elements supports frequent duplication formation in *Salmonella* Typhimurium. *Genetics* **141**: 1245–1252.

Hall, R.M. (1997) Mobile gene cassettes and integrons: moving antibiotic resistance genes in gram-negative bacteria. *Ciba Found Symp* **207**: 192-202-205.

Hall, R.M., and Collis, C.M. (1995a) Mobile gene cassettes and integrons: capture and spread of genes by site- specific recombination. *Mol Microbiol* **15**: 593–600.

Hall, R.M., and Collis, C.M. (1995b) Mobile gene cassettes and integrons: capture and spread of genes by site- specific recombination. *Mol Microbiol* **15**: 593–600.

Hall, R.M., and Collis, C.M. (1998) Antibiotic resistance in gram-negative bacteria: the role of gene cassettes and integrons. *Drug Resist Updat* **1**: 109–19.

Hall, R.M., and Stokes, H.W. (1993) Integrons: Novel DNA elements which capture genes by site-specific recombination. *Genetica* **90**: 115–132.

Han, J., Lynne, A.M., David, D.E., Tang, H., Xu, J., Nayak, R., *et al.* (2012) DNA Sequence Analysis of Plasmids from Multidrug Resistant *Salmonella enterica* Serotype Heidelberg Isolates. *PLoS One* **7**.

Harper, P.S. (2012) *Practical Genetic Counselling 7th Edition*. CRC Press, .

Harrois, D., Breurec, S., Seck, A., Delauné, A., Hello, S. Le, Pardos de la Gándara, M., *et al.* (2014) Prevalence and characterization of extended-spectrum β-lactamase-producing clinical *Salmonella enterica* isolates in Dakar, Senegal, from 1999 to 2009. *Clin Microbiol Infect* **20**: O109-16.

Hello, S. Le, Bekhit, A., Granier, S.A., Barua, H., Beutlich, J., Zając, M., *et al.* (2013) The global establishment of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain. *Front Microbiol* **4**.

Hello, S. Le, Harrois, D., Bouchrif, B., Sontag, L., Elhani, D., Guibert, V., *et al.* (2013) Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: A microbiological study. *Lancet Infect Dis* **13**: 672–679.

Hello, S. Le, Hendriksen, R.S., Doublet, B., Fisher, I., Nielsen, E.M., Whichard, J.M., *et al.* (2011) International spread of an epidemic population of *Salmonella enterica* serotype Kentucky ST198 resistant to ciprofloxacin. *J Infect Dis* **204**: 675–684.

Hormaeche, C.E., Joysey, H.S., Desilva, L., Izhar, M., and Stocker, B.A.D. (1991)

Immunity conferred by Aro- *Salmonella* live vaccines. *Microb Pathog* **10**: 149–158.

Hurley, D., McCusker, M.P., Fanning, S., and Martins, M. (2014) Salmonella-host interactions - modulation of the host innate immune system. *Front Immunol* **5**.

ISO, (International Organization for Standardization) (2007) 6579:2002/AMENDMENT 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. *Br Stand Inst* Webb, H. E., Granier, S. A., Mrult, M., Millemann,.

Johnston, C., Martin, B., Fichant, G., Polard, P., and Claverys, J.-P. (2014) Bacterial transformation: distribution, shared mechanisms and divergent control. *Nat Rev Microbiol* **12**: 181–196.

Kariuki, S., Revathi, G., Kariuki, N., Muyodi, J., Mwituria, J., Munyalo, A., *et al.* (2005) Increasing prevalence of multidrug-resistant non-typhoidal *Salmonellae*, Kenya, 1994-2003. *Int J Antimicrob Agents* **25**: 38–43.

Kariuki, S., Okoro, C., Kiiru, J., Njoroge, S., Omuse, G., Langridge, G., *et al.* (2015) Ceftriaxone-resistant *Salmonella* enterica serotype Typhimurium sequence type 313 from Kenyan patients is associated with the blaCTX-M-15 gene on a novel IncHI2 plasmid. *Antimicrob Agents Chemother* **59**: 3133–3139.

Kauffmann, F. (1950) The diagnosis of *Salmonella* types. *The Diagnosis of Salmonella Types* .

Kauffmann, F. (1961) The Species-Definition in the Family *Enterobacteriaceae*. *Int Bull Bacteriol Nomencl Taxon* **11**: 5–6.

kay M.Tomashek, Tyler M.Sharp, H.S.M. (2015) Infectious Diseases Related To Travel. *Cent Dis Control Prev* Chapter 3.

Kidgell, C., Reichard, U., Wain, J., Linz, B., Torpdahl, M., Dougan, G., and Achtman, M. (2002) *Salmonella* typhi, the causative agent of typhoid fever, is approximately 50,000 years old. *Infect Genet Evol* **2**: 39–45.

Kingsley, R.A., and Bäumler, A.J. (2000) Host adaptation and the emergence of infectious disease: The *Salmonella* paradigm. *Mol Microbiol* **36**: 1006–1014.

Kingsley, R.A., Msefula, C.L., Thomson, N.R., Kariuki, S., Holt, K.E., Gordon, M.A., *et al.* (2009) Epidemic multiple drug resistant *Salmonella* Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. *Genome Res* **19**: 2279–2287.

Langridge, G.C. (2010) Metabolic capability in host-restricted serovars of *Salmonella enterica*. .

Levings, R.S., Lightfoot, D., Partridge, S.R., Hall, R.M., and Djordjevic, S.P. (2005) The genomic island SGI1, containing the multiple antibiotic resistance region of *Salmonella enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other *S. enterica* serovars. *J Bacteriol* **187**: 4401–4409.

Levings, R.S., Partridge, S.R., Djordjevic, S.P., and Hall, R.M. (2007) SGI1-K, a variant of the SGI1 genomic island carrying a mercury resistance region, in *Salmonella enterica* serovar Kentucky. *Antimicrob Agents Chemother* **51**: 317–323.

Li, J., Nelson, K., McWhorter, a C., Whittam, T.S., and Selander, R.K. (1994) Recombinational basis of serovar diversity in *Salmonella enterica*. *Proc Natl Acad Sci USA* **91**: 2552–2556.

Maddocks, S.E., and Oyston, P.C.F. (2008) Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. *Microbiology* **154**: 3609–3623.

Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., *et al.* (2010) The Global Burden of Nontyphoidal *Salmonella* Gastroenteritis. *Clin Infect Dis* **50**: 882–889.

Majtán, V., Majtán, T., Majtán, J., Szabóová, M., and Majtánová, L. (2006) *Salmonella enterica* serovar Kentucky: antimicrobial resistance and molecular analysis of clinical isolates from the Slovak Republic. *Jpn J Infect Dis* **59**: 358–62.

Marcus, S.L., Brumell, J.H., Pfeifer, C.G., and Finlay, B.B. (2000) *Salmonella* pathogenicity islands: Big virulence in small packages. *Microbes Infect* **2**: 145–156.

McClelland, M., Sanderson, K.E., Clifton, S.W., Latreille, P., Porwollik, S., Sabo, A., *et al.* (2004) Comparison of genome degradation in Paratyphi A and Typhi, human-restricted serovars of *Salmonella enterica* that cause typhoid. *Nat Genet* **36**: 1268–1274.

McElhaney, R.N. (1992) *Mycoplasmas: Molecular Biology and Pathogenesis*. Horizon Scientific Press, .

Messina, F.J. (1993) Heritability and "evolvability" of fitness components in Callosobruchus maculatus. *Heredity (Edinb)* **71**: 623–629.

Meunier, D., Boyd, D., Mulvey, M.R., Baucheron, S., Mammina, C., Nastasi, A., *et al.* (2002) *Salmonella enterica* serotype Typhimurium DT 104 antibiotic resistance genomic island I in serotype Paratyphi B. *Emerg Infect Dis* **8**: 430–433.

Mezali, L., and Hamdi, T.M. (2012) Prevalence and Antimicrobial Resistance of *Salmonella* Isolated from Meat and Meat Products in Algiers (Algeria). *Foodborne Pathog Dis* **9**: 522–529.

Minor, L. Le, and Popoff, M.Y. (1987) Designation of *Salmonella enterica* sp. nov., nom. rev., as the Type and Only Species of the Genus *Salmonella*. *Int J Syst Bacteriol* **37**: 465–468.

Mulvey, M.R., Boyd, D.A., Finley, R., Fakharuddin, K., Langner, S., Allen, V., *et al.* (2013) Ciprofloxacin-resistant *Salmonella enterica* serovar Kentucky in Canada. *Emerg Infect Dis* **19**: 999–1001.

Mulvey, M.R., Boyd, D.A., Olson, A.B., Doublet, B., and Cloeckaert, A. (2006) The genetics of *Salmonella* genomic island 1. *Microbes Infect* **8**: 1915–1922.

Murgia, M., Bouchrif, B., Timinouni, M., Al-Qahtani, A., Al-Ahdal, M.N., Cappuccinelli, P., *et al.* (2015) Antibiotic resistance determinants and genetic analysis of *Salmonella enterica* isolated from food in Morocco. *Int J Food Microbiol* **215**: 31–39.

Nagy, T.A., Moreland, S.M., Andrews-Polymenis, H., and Detweiler, C.S. (2013) The ferric enterobactin transporter fep is required for persistent *Salmonella enterica* serovar Typhimurium infection. *Infect Immun* **81**: 4063–4070.

Nair, S., Schreiber, E., Thong, K.L., Pang, T., and Altwegg, M. (2000) Genotypic characterization of *Salmonella* Typhi by amplified fragment length polymorphism fingerprinting provides increased discrimination as compared to pulsed-field gel electrophoresis and ribotyping. *J Microbiol Methods* **41**: 35–43.

Olsen, S.J., DeBess, E.E., McGivern, T.E., Marano, N., Eby, T., Mauvais, S., *et al.* (2001) A nosocomial outbreak of fluoroquinolone-resistant *Salmonella* infection. *N Engl J Med* **344**: 1572–9.

Ou, J.T. (1993) The 90 kilobase pair virulence plasmid of *Salmonella* serovar Typhimurium coexists in strains with a plasmid of the 23 incompatibility groups. *Microb Pathog* **15**: 237–242.

Oundo, J.O., Muli, F., Kariuki, S., Waiyaki, P.G., Iijima, Y., Berkley, J., *et al.* (2002) Non-typhi *Salmonella* in children with severe malaria. *East Afr Med J* **79**: 633–639.

Owusu-Ofori, A., and Scheld, W.M. (2003) Treatment of Salmonella meningitis: Two case reports and a review of the literature. *Int J Infect Dis* **7**: 53–60.

Paglietti, B., Falchi, G., Mason, P., Chitsatso, O., Nair, S., Gwanzura, L., *et al.* (2013)

Diversity among human non-typhoidal *salmonellae* isolates from Zimbabwe. *Trans R Soc Trop Med Hyg* **107**: 487–492.

Pang, T. (1998) Vaccination against intracellular bacterial pathogens. *Trends Microbiol* **6**: 433.

Partridge, S.R., Brown, H.J., Stokes, H.W., and Hall, R.M. (2001) Transposons Tn1696 and Tn21 and their integrons In4 and In2 have independent origins. *Antimicrob Agents Chemother* **45**: 1263–1270.

Partridge, S.R., Recchia, G.D., Stokes, H.W., and Hall, R.M. (2001) Family of class 1 integrons related to In4 from Tn1696. *Antimicrob Agents Chemother* **45**: 3014–3020.

Patrick A.D. Grimont, Francine Grimont and Philippe Bouvet (2001) *Salmonella in Domestic Animals*. CABI, .

Pezzella, C., Ricci, A., DiGiannatale, E., Luzzi, I., and Carattoli, A. (2004) Tetracycline and Streptomycin Resistance Genes, Transposons, and Plasmids in *Salmonella enterica* Isolates from Animals in Italy. *Antimicrob Agents Chemother* **48**: 903–908.

Ploy, M.-C., Chainier, D., Hoa, N., Thi, T., Poilane, I., Cruaud, P., *et al.* (2003) Integron-Associated Antibiotic Resistance in *Salmonella enterica* Serovar Typhi from Asia. *Antimicrob Agents Chemother* **47**: 1427–1429.

Pollack, J.R., and Neilands, J.B. (1970) Enterobactin, an iron transport compound from *Salmonella* Typhimurium. *Biochem Biophys Res Commun* **38**: 989–992.

Popoff, M.Y., Bockemühl, J., and Gheesling, L.L. (2003) Supplement 2001 (no. 45) to the Kauffmann-White scheme. *Res Microbiol* **154**: 173–174.

Porwollik, S. (2011) *Salmonella: From Genome to Function*. Horizon Scientific Press, .

Porwollik, S., and McClelland, M. (2003) Lateral gene transfer in *Salmonella*. *Microbes Infect* **5**: 977–989.

Pray, L. (2008) Transposons: The jumping genes. *Nat Educ* **1**: 204.

Reeves, M.W., Evins, G.M., Heiba, A.A., Plikaytis, B.D., and Farmer, J.J. (1989) Clonal nature of *Salmonella* Typhi and its genetic relatedness to other *Salmonellae* as shown by multilocus enzyme electrophoresis and proposal of Salmonella bongori comb. nov. *J Clin Microbiol* **27**: 313–320.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., *et al.* (2001) Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* **2**: 361–367.

Rickert-Hartman, R., and Folster, J.P. (2014) Ciprofloxacin-resistant Salmonella enterica serotype kentucky sequence type 198. *Emerg Infect Dis* **20**: 910–911.

Rouahi, N., Zouhdi, M., Zidouh, A., Elyachioui, M., and Mahjour, J. (2000) Antibiotic resistance of Moroccan strains of *Salmonella* Enteritidis isolated between 1996 and 1997. *East Mediterr Heal J* **6**: 1107–1113.

Rubino, S., Rizzu, P., Erre, G., Colombo, M.M., Leori, G., Gulig, P.A., *et al.* (1993) Mechanism of pathogenicity in *Salmonella* Abortusovis. In *Biology of Salmonella*. Springer, pp. 141–147.

Rychlik, I., Gregorova, D., and Hradecka, H. (2006) Distribution and function of plasmids in *Salmonella enterica*. *Vet Microbiol* **112**: 1–10.

Sannat, C., Patyal, A., Rawat, N., Ghosh, R.C., Jolhe, D.K., Shende, R.K., *et al.* (2017) Characterization of *Salmonella* Gallinarum from an outbreak in Raigarh, Chhattisgarh. *Vet World* **10**: 144–148.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R. V., Widdowson, M.A., Roy, S.L., *et al.* (2011) Foodborne illness acquired in the United States-Major pathogens. *Emerg Infect Dis* **17**: 7–15.

Schlundt, J., Toyofuku, H., Jansen, J., and Herbst, S. a (2004) Emerging food-borne zoonoses. *Rev Sci Tech* **23**: 513–33.

Seiffert, S.N., Perreten, V., Johannes, S., Droz, S., Bodmer, T., and Endimiani, A. (2014) OXA-48 carbapenemase-producing *Salmonella enterica* serovar Kentucky isolate of sequence type 198 in a patient transferred from Libya to Switzerland. *Antimicrob Agents Chemother* **58**: 2446–2449.

Shah, D.H., Lee, M.J., Park, J.H., Lee, J.H., Eo, S.K., Kwon, J.T., and Chae, J.S. (2005) Identification of *Salmonella* Gallinarum virulence genes in a chicken infection model using PCR-based signature-tagged mutagenesis. *Microbiology* **151**: 3957–3968.

Shimoni, Z., Pitlik, S., Leibovici, L., Samra, Z., Konigsberger, H., Drucker, M., *et al.* (1999) Nontyphoid *Salmonella* Bacteremia: Age- Related Differences in Clinical Presentation, Bacteriology, and Outcome. *Clin Infect Dis* **28**: 822–827.

SIRIKEN, B. (2013) *Salmonella* Pathogenicity Islands. *Mikrobiyol Bul* **47**: 181–188.

Sorge, N.M. van, Zialcita, P. a, Browne, S.H., Quach, D., Guiney, D.G., and Doran, K.S. (2011) Penetration and activation of brain endothelium by *Salmonella enterica* serovar Typhimurium. *J Infect Dis* **203**: 401–405.

Sow, A.G., Wane, A.A., Diallo, M.H., Boye, C.S.B., and Aïdara-Kane, A. (2007) Genotypic characterization of antibiotic-resistant *Salmonella* Enteritidis isolates in Dakar, Senegal. *J Infect Dev Ctries* **1**: 284–288.

Staley, J.T. (2006) The bacterial species dilemma and the genomic-phylogenetic species concept. *Philos Trans R Soc B Biol Sci* **361**: 1899–1909.

Stanley, T.G., and Wilson, I. (2003) Multilocus enzyme electrophoresis: a practical guide. *Mol Biotechnol* **24**: 203–220.

Steve Yan, S., Pendrak, M.L., Abela-Ridder, B., Punderson, J.W., Fedorko, D.P., and Foley, S.L. (2003) An overview of *Salmonella* typing: Public health perspectives. *Clin Appl Immunol Rev* **4**: 189–204.

Stevenson, J.E., Gay, K., Barrett, T.J., Medalla, F., Chiller, T.M., and Angulo, F.J. (2007) Increase in nalidixic acid resistance among non-typhi *Salmonella enterica* isolates in the United States from 1996 to 2003. *Antimicrob Agents Chemother* **51**: 195–197.

Su, L.-H., and Chiu, C.-H. (2007) *Salmonella*: Clinical Importance and Evolution of Nomenclature. *Chang Gung Med J* **30**: 210–219.

Sudeepa Kumar, M., Vijaykumar, G.S., Prakash, R., Prashanth, H. V., Raveesh, P.M., and Nagaraj, E.R. (2013) Comparison of *Salmonella* Typhi and Paratyphi A occurrence in a Tertiary care hospital. *J Clin Diagnostic Res* **7**: 2724–2726.

Sundin, G.W., and Bender, C.L. (1996) Dissemination of the strA-strB streptomycin-resistance genes among commensal and pathogenic bacteria from humans, animals, and plants. *Mol Ecol* **5**: 133–143.

Tarabees, R., Elsayed, M.S.A., Shawish, R., Basiouni, S., and Shehata, A.A. (2017) Isolation and characterization of *Salmonella* Enteritidis and Salmonella Typhimurium from chicken meat in Egypt. *J Infect Dev Ctries* **11**: 314–319.

Tauxe, R. V (1999) Salmonella Enteritidis and *Salmonella* Typhimurium DT104: successful subtypes in the modern world. In *Emerging infections 3*. American Society of Microbiology, pp. 37–52.

Tennant, S.M., Diallo, S., Levy, H., Livio, S., Sow, S.O., Tapia, M., *et al.* (2010) Identification by PCR of non-typhoidal *Salmonella enterica* serovars associated with invasive infections among febrile patients in Mali. *PLoS Negl Trop Dis* **4**: e621.

Thomson, N., Baker, S., Pickard, D., Fookes, M., Anjum, M., Hamlin, N., *et al.* (2004) The role of prophage-like elements in the diversity of *Salmonella enterica* serovars. *J Mol Biol*

339: 279–300.

Uzzau, S., Brown, D.J., Wallis, T., Rubino, S., Leori, G., Bernard, S., *et al.* (2000) Host adapted serotypes of *Salmonella enterica*. *Epidemiol Infect* **125**: 229–255.

Wadula, J., Gottberg, A. von, Kilner, D., Jong, G. de, Cohen, C., Khoosal, M., *et al.* (2006) nosocomial outbreak of extended-spectrum-lactamase-producing *Salmonella* isangi in pediatric wards. *Pediatr Infect Dis J* **25**: 843–844.

Wain, J., Nga, L.T.D., Kidgell, C., James, K., Fortune, S., Diep, T.S., *et al.* (2003) Molecular analysis of incHI1 antimicrobial resistance plasmids from *Salmonella* serovar Typhi strains associated with typhoid fever. *Antimicrob Agents Chemother* **47**: 2732–2739.

Wales, A., and Davies, R.H. (2013) Environmental Aspects of *Salmonella*. *Salmonella Domest Anim* Webb, H. E., Granier, S. A., Mrult, M., Millemann,.

Wallis, T.S., Paulin, S.M., Plested, J.S., Watson, P.R., and Jones, P.W. (1995) The *Salmonella* Dublin virulence plasmid mediates systemic but not enteric phases of Salmonellosis in cattle. *Infect Immun* **63**: 2755–2761.

Weill, F.X., Bertrand, S., Guesnier, F., Baucheron, S., Grimont, P.A.D., and Cloeckaert, A. (2006) Ciprofloxacin-resistant *Salmonella* Kentucky in travelers . *Emerg Infect Dis* **12**: 1611–1612.

Westrell, T., Monnet, D.L., Gossner, C., Heuer, O., and Takkinen, J. (2014) Drug-resistant *Salmonella enterica* serotype Kentucky in Europe. *Lancet Infect Dis* **14**: 270–271.

Wolman, A., and Gorman, A.E. (1931) The significance of waterborne typhoid fever outbreaks, 1920-1930. *J Am Water Works Assoc* **23**: ix, 82.

Wong, C.S., Jelacic, S., Habeeb, R.L., Watkins, S.L., and Tarr, P.I. (2000) The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med* **342**: 1930–6.

Woods, C.W., Murdoch, D.R., Zimmerman, M.D., Glover, W.A., Basnyat, B., Wolf, L., *et al.* (2006) Emergence of *Salmonella enterica* serotype Paratyphi A as a major cause of enteric fever in Kathmandu, Nepal. *Trans R Soc Trop Med Hyg* **100**: 1063–1067.

Woods, D.F., Reen, F.J., Gilroy, D., Buckley, J., Frye, J.G., and Boyd, E.F. (2008) Rapid multiplex PCR and real-time TaqMan PCR assays for detection of *Salmonella enterica* and the highly virulent serovars Choleraesuis and Paratyphi C. *J Clin Microbiol* **46**: 4018–4022.

World Health Organization (2016) updates fact sheet on non-typhoidal *Salmonella*.

Yoshida, C.E., Kruczkiewicz, P., Laing, C.R., Lingohr, E.J., Gannon, V.P.J., Nash, J.H.E., and Taboada, E.N. (2016) The *Salmonella* in silico typing resource (SISTR): An open web-accessible tool for rapidly typing and subtyping draft *Salmonella* genome assemblies. *PLoS One* **11**: e0147101.

Young, H.-K., Qumsieh, M.J., and Mclntosh, M.L. (1994) Nucleotide sequence and genetic analysis of the type Ib trimethoprim-resistant, Tn 4132-encoded dihydrofolate reductase. *J Antimicrob Chemother* **34**: 715–725.

Yuan, T., Liu, Z.Y., Hu, L.Z., and Xu, G.B. (2011) Electrochemical and electrochemiluminescent aptasensors. *Fenxi Huaxue/ Chinese J Anal Chem* **39**: 972–977.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., *et al.* (2012) Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* **67**: 2640–2644.

Zhang, S., Yin, Y., Jones, M.B., Zhang, Z., Kaiser, B.L.D., Dinsmore, B.A., *et al.* (2015) *Salmonella* serotype determination utilizing high-throughput genome sequencing data. *J Clin Microbiol* **53**: 1685–1692.

Zhou, D., Hardt, W.D., and Galán, J.E. (1999) *Salmonella* Typhimurium encodes a putative iron transport system within the centisome 63 pathogenicity island. *Infect Immun* **67**: 1974–1981.