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Salmonellae in the Environment

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1. Introduction

Diarrhea due to *Salmonella* infections has been recognised since the late 19th century. Typhoid diseases were, in the early part of the 20th century, the commonest known waterborne diseases in both the United Kingdom and the United States of America (Hunter, 1997; Pui et al., 2011). In addition non-typhoid salmonellae have been recognised as a leading cause of bacterial enteritis in the UK and worldwide (Timbury et al., 2002; Pui et al., 2011). *Salmonella* infections in animals are common and have been well documented in the UK since 1958, with around 10,000 recorded incidences of bovine salmonellosis per year (Linton & Hinton, 1988).

Salmonella species are members of the family *Enterobacteriaceae*, being facultatively anaerobic, non-spore forming, Gram-negative rods (Group five of Bergey's Manual of Determinative Bacteriology) (Holt et al., 1994). Generally they are 2-5 μm long and 0.8-1.5 μm wide, straight rods, being motile by peritrichous flagella. As they are facultatively anaerobic, they have both respiratory and fermentative metabolism. Optimal growth temperature is 37 °C. D-Glucose and other carbohydrates are catabolised with the production of acid and usually gas. They are oxidase negative, catalase positive, indole and Voges-Proskauer negative, and methyl red and Simmons citrate positive. H_2S is produced; urea is not hydrolysed (Holt et al., 1994; Lightfoot, 2004; Percival et al., 2004). The genus *Salmonella* consists of two species: (1) *Salmonella enterica*, which is divided into six subspecies – *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), *S. enterica* subsp. *indica* (VI); and (2) *Salmonella bongori* (formerly subsp. V). There are around 2541 serovars/serotypes in the genus *Salmonella* (Table 1). This new nomenclature reflects recent advances in *Salmonella* taxonomy which are based on DNA-hybridization studies. For simplicity, serotypes can be abbreviated, for example *S. enterica* subsp. *enterica* serovar Enteritidis to *S. enteritidis* (Bopp et al., 1999; Timbury et al., 2002; Lightfoot, 2004; Percival et al., 2004; Lin-Hui & Cheng-Hsun, 2007; Pui et al., 2011).

Most of the serotypes pathogenic to humans and animals belong to *Salmonella enterica* subsp. *enterica* (i.e. subsp. I). Some serovars have a habitat limited to a particular host species, such as humans (serovars Typhi, Paratyphi A), sheep (serovars Abortusovis), or fowls (serovar Gallinarum). In general, subspecies I strains are usually isolated from humans and warm-blooded animals, whereas subspecies II, IIIa, IIIb, IV, VI and *S. bongori* are usually isolated from cold-blooded animals and the environment (rarely from humans) (Pui et al., 2011). Biochemical reactions of *S. enterica* serovars and differential characteristics of *Salmonella* species and subspecies are given in Tables 2 and 3.

Species / subspecies	Number of serovars
Salmonella enterica subsp.	
enterica	1504
salamae	502
arizonae	95
diarizonae	333
houtenae	72
indica	13
Salmonella bongori	22
Total	2541

Adapted from Lightfoot (2004); Lin-Hui and Cheng-Hsun (2007)

Table 1. Number of serovars in each species and subspecies of *Salmonella*

There are four clinically distinguishable forms of *Salmonella* infection in humans. These are gastroenteritis, enteric fever, bacteremia and other complications of non-typhoidal salmonellosis as well as chronic carrier state (Hunter, 1997; Percival et al., 2004; Pui et al., 2011). Gastroenteritis is caused by at least 150 *Salmonella* serotypes, *Salmonella enteritidis* being the most common serotype. Symptoms include watery, sometimes bloody diarrhea, fever and abdominal pain, and usually occur 18-48 hours after ingestion of the bacterium. The infection generally lasts 2-5 days. After recovery, faecal carriage may persist for up to 12 weeks. Less than 10 % of patients are reported as carriers for a longer period (Hunter, 1997; Percival et al., 2004; Pui et al., 2001).

	Typical *Salmonella*	*S. choleraesuis*	*S. pullorum*	*S. gallinarum*	*S. typhi*	*S. typhisuis*	*S. paratyphi* A
H_2S	+	-	±	±	+ (weak)	+	-
Citrate	+	+*	+	+	-	-	-
Gas from glucose	+	+	+	±	-	+	+
Dulcitol	+	-	-	+	-	+	+
Mucate	+	-	-	+	-	-	-
Maltose	+	+	-	+	+	+	+
Trehalose	+	-	+	+	+	+	+

* Simmon's citrate-negative, Christensen citrate-positive.
Adapted from Jones, et al. (2000)

Table 2. Biochemical reactions of *Salmonella enterica* serovars

Enteric fever is most often caused by *Salmonella typhi* (typhoid fever) and *S. paratyphi* A, B and C (paratyphoid fever). Enteric fever from *S. typhi* is more prolonged and has a higher mortality rate than paratyphoid fever. Symptoms for typhoid fever include sustained fever, diarrhea, abdominal pain and may involve fatal liver, spleen, respiratory and neurological damage. Paratyphoid fever has similar, but less severe symptoms. The incubation period for typhoid fever is 7-14 days and for paratyphoid fever 1-10 days. Between 1 and 3 % of patients become chronic carriers (Hunter, 1997; Percival et al., 2004; Pui et al., 2011).

Salmonella bacteremia is characterised by chills, high remittent fever, anorexia and bacteraemia. The bacterium may localize in any organ in the body and produce focal lesions resulting in meningitis, endocarditis and pneumonia (Percival et al., 2004). Studies aimed at the determination of the infectious dose for salmonellosis suggests that infectious doses are certainly below10^3 and can be <10 organisms (Blaser & Newman, 1982; Hunter, 1997; Pui et al., 2011). Non-typhoidal serotypes may persist in the intestinal tract from 6 weeks to 3 months, with only 0.1 % of non-typhoidal *Salmonella* cases are shed in faeces for periods excceding 12 months. Up to 5 % of untreaed typhoid infections may result in chronic carrier state. Factors contributing to the chronic carrier state are not clearly understood, nonetheless, salmonellosis can be spread by chronic carriers who can infect other individual, particularly those who work in food industries (Pui et al., 2011).

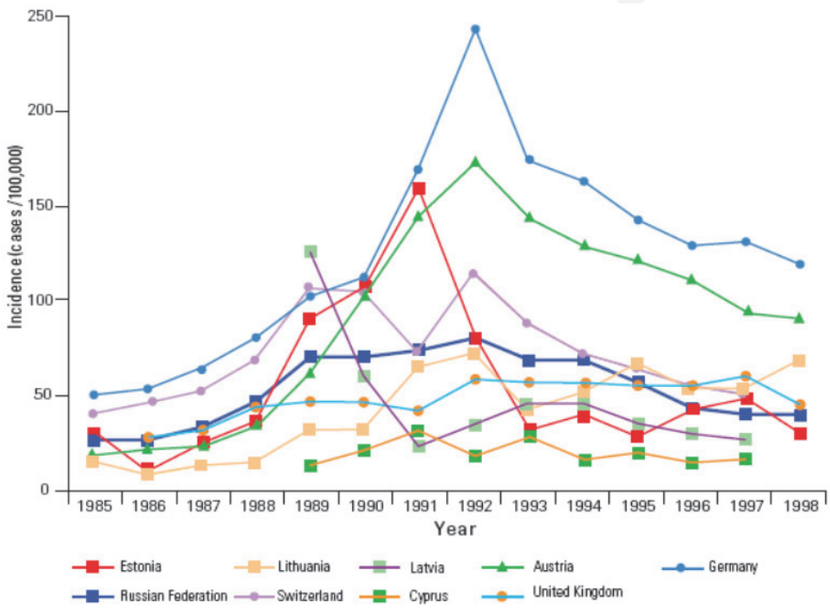

Fig. 1. The reported incidence of salmonellosis in nine European countries between 1985 and 1998 (from Schlundt et al. 2004)

Cases of typhoid (*Salmonella typhi*) and paratyphoid fevers (*S. paratyphi* A and B) have been reported since 1897. In England and Wales between 1911 and 1960 there were about 17 waterborne outbreaks of typhoid and paratyphoid fevers causing about 155 deaths (Galbraith, 1994). In the United States, more than 30 people out of every 100,000 died of typhoid in 1890 (Rusin et al., 2000). Although infections attributed to typhoid and paratyphoid salmonellae have declined in the UK and USA since 1960 (Galbraith, 1994; Leclerc et al., 2004), cases of waterborne typhoid and paratyphoid are still reported regularly from other parts of the world, mainly underdeveloped and poor countries in Asia and Africa, affecting 12.5 million people every year (Hunter, 1997). Waterborne and foodborne salmonellosis (non-typhoidal species) are now the second leading cause of gastroenteritis around the world (Fig. 1), and according to the US Centre for Disease Control and Prevention, 1.4 million cases of salmonellosis occur annually in the USA (Hunter, 1997; Lightfoot, 2004; Percival et al., 2004). Global surveillance data has suggested that increased salmonellosis is associated with the consumption of raw or undercooked eggs, poultry meat or dairy products, and salads prepared with mayonnaise

(Khakhria et al., 1997; Guard-Petter, 2001; Costalunga & Tondo, 2002). Contaminated drinking water is also an important vehicle of *Salmonella* infection (Hunter, 1997; Percival et al., 2004). Handling of pets, such as snakes and lizards, may also lead to infection (Schröter et al., 2004). By and large, salmonellosis is associated with poor hygiene and sanitation during food production (Lightfoot, 2004).

Character	*S. enterica*						
	Subsp. *enterica*	Subsp. *salamae*	Subsp. *arizonae*	Subsp. *diarizonae*	Subsp. *houtenae*	Subsp. *indica*	*S. bongori*
Dulcitol	+	+	-	-	-	d	+
OPNG (2h)	-	-	+	+	-	d	+
Malonate	-	+	+	+	-	-	-
Gelatinase	-	+	+	+	+	+	-
Sorbitol	+	+	+	+	+	-	+
Culture with KCN	-	-	-	-	+	-	+
$_{L}$(+)-tatrate	+	-	-	-	-	-	-
Galacturonate	-	+	-	+	+	+	+
γ-Glutamyltransferase	+	+	-	+	+	+	+
β-Glucuronidase	_	D	-	+	-	d	-
Mucate	+	+	+	- (70 %)	-	+	+
Salicin	-	-	-	-	+	-	-
Lactose	-	-	- (75 %)	+ (75 %)	-	d	-
Lysis by phage 01	+	+	-	+	-	+	d
Natural habitat	Warm blooded animals		Cold-blooded animals and the environment				

OPNG, *o*-nitropenyl-β-D-galactopyranoside; KCN, potassium cyanide, d, different reactions given by different serovars.
Adapted from: Bopp, et al. (1999); Jones, et al. (2000).

Table 3. Differential characteristics of *Salmonella* species and subspecies

2. Incidence and biodiversity of salmonellae in the environment

The transmission of *Salmonella* species takes the oral-faecal route, by means of contaminated food, primarily poultry and milk products and contaminated water, it is also believed that warm-blooded animals are an asymptomatic carriers of the organism in their gut (Guard-Petter, 2001; Costalunga & Tondo, 2002; Percival et al., 2004; Abulreesh et al., 2007). There is strong evidence to suggest that the organism is ubiquitous and widely distributed in the environment, where particular serovar may be associated with specific ecological niches (Murray, 2000).

2.1 Aquatic environments

Salmonellae are exogenous to aquatic habitats. Their presence in water, therefore, indicates faecal contamination. Sewage effluents, agricultural run-off and direct deposit of faecal

materials from wild animals and birds are the major sources of the bacteria in aquatic environments (Alcaide et al., 1984; Baudart et al., 2000; Johnson et al., 2003; Abulreesh et al., 2005). *Salmonella* species have been found in almost all types of aquatic environments that receive faecal contamination, that include drinking water (Bhatta et al., 2007), rivers (Pianetti et al., 1998; Polo et al., 1998; Polo et al., 1999; Dionisio et al., 2000; Lemarchand & Lebaron, 2003; Arvanitidou et al., 2005; Haley et al., 2009), lakes (Claudon et al., 1971; Arvanitidou et al., 1995; Sharma & Rajput, 1996), ponds (Shellenbarger et al., 2008), marine waters (Matinez-Urtaza et al., 2004a; Martinez-Urtaza et al., 2004b; Martinez-Urtaza & Liebana, 2005; Harakeh et al., 2006), run-off water (Claudon et al., 1971), treated and untreated wastewater (Ho & Tam, 2000; Melloul et al., 2002; Espigares et al., 2006, Mafu et al., 2009) worldwide. Abulreesh et al. (2004) were unable to detect salmonellae in water samples from a village pond that receives direct faecal contamination from waterfowl, nevertheless, they managed to isolate the bacterium from bottom sediments of the same pond. This might be attributed, in part, to concentration through sedimentation and also to greater survivability of *Salmonella* spp. in bottom sediments than in water (Burton et al., 1987; Fish & Pettibone, 1995; Winfield & Groisman, 2003). Higher salmonellae recovery rates from bottom sediment than from water in diverse aquatic environments were also observed by Hendricks (1971) and Van Donzel & Geldreich (1971).

It is expected that the diversity of salmonellae population in aquatic environments may depend on sources of contamination. However, salmonellae serotypes that prevail in aquatic environments do not often coinside with the common zoonotic or human serotypes identified in the areas surrounding these aquatic environments (Polo et al., 1999; Dionisio et al., 2000; Matinez-Urtaza et al., 2004; Setti et al., 2009). For instance, Setti et al. (2009) isolated about 57 strains along 122 Km coastline in Morocco, where only three serotypes were identified. Interestingly, these serotypes were Kentucky, Blockey and Senftenberg, were not included among those frequently reported serotypes from human infections or animal origin in Morocco. Likewise, the results obtained by Haley et al. (2009) showed that serotypes Enteritidis, Typhimurium and Heidelberg were not among the serotypes isolated from freshwater environments in U.S.A, even though common sources of these serotypes were present in the watersheds that were examined. The explanation of this discrepancy may be attributed, in part, to different survival rates of different salmonellae serotypes. Other environmental factors such as rainfall and temperature may also play a major role in the diversity and dynamics of salmonellae serotypes in aquatic environments (Martinez-Urtaza et al., 2004a; Simental & Martinez-Urtaza, 2008; Haley et al., 2009; Setti et al., 2009).

The use of faecal indicators (faecal coliforms, *Escherichia coli*, faecal streptococci and *Clostridium perfringens*) aims to evaluate water sources intended for water supply or recreation, by predicting the presence of waterborne pathogens. Significant correlations have been found between total coliforms, faecal coliforms and faecal streptococci and *Salmonella* in marine bathing sites in Portugal (Polo et al., 1998). Similarly, Arvanitidou et al. (2005) noted a close relationship between the presence of *Salmonella* serovars and total coliforms in Greek rivers. Lake Jabalpure in India was found to receive sufficient pollution of organic matter, where high significant correlation was found between the abundance of *Salmonella* and the abundance of total coliforms, faecal coliforms and faecal streptococci (Sharma & Rajput, 1996). Morinigo et al. (1993) found significant correlation between densities of faecal indicators and the presence of *Salmonella* spp. in Spanish fresh and marine natural waters that received faecal discharge.

Together, these and other studies suggest that faecal indicators are potentially a useful warning of the potential presence of salmonellae in aquatic environments (Geldreich, 1996). However, relationships are not always found between faecal indicators and salmonellae in aquatic environments; an observation that may be related to various reasons such as different survival rates between salmonellae and faecal indicators, also the possibility that salmonellae being in a viable but nonculturable state. Lemarchand and Lebaron (2003) found no correlations between salmonellae and any given faecal indicator in French rivers. Detection of *Salmonella* spp. was achieved in water samples from coastal areas in Portugal in the presence of low counts of faecal indicators (Dionisio et al., 2000). No close relationships between the presence of salmonellae and counts of faecal indicators were also noted in fresh and marine waters that receive industrial and domestic effluents in Spain (Morinigo et al., 1993). Furthermore, *Salmonella* spp. were successfully detected in Spanish fresh and marine water that received faecal pollution in the absence of faecal indicators, as well as in aquatic environments with low degree of pollution (Pianetti et al., 1998; Morinigo et al., 1990; Baudart et al., 2000; Dionisio et al., 2000). Thus, the ability of faecal indicators to predict the presence of salmonellae in polluted environmental waters remains questionable, and the absence of faecal indicators is not always a reliable indication of the absence of *Salmonella* spp.

2.2 Domestic and agricultural waste

Sewage effluents serve as frequent source of environmental contamination with *Salmonella* serovars. Obviously, infected individuals are the source of salmonellae in sewage effluents (Sahlström et al., 2004, 2006). In Spain, the most frequently identified serovars in clinical samples from human origin were Enteritidis, Hadar and Typhimurium, these serovars were also noted to be the most frequently encountered in sewage effluents, particularly Hadar (38.1%), followed by Enteritidis (23.8%) (Espigares et al., 2006). Discharge from agricultural waste may, in part, play a role in the presence of different serovars in sewage effluents (Berge et al., 2006), however some salmonellae serovars may present in sewage effluents but could not be traced to a human or animal source (Danielsson, 1977).

It is well-established that waste treatment aims to stabilize sewage sludge, accordingly pathogens may be activated rather than removed (Godfree, 2003). This has been clearly noticed with different *Salmonella* serovars. In Poland a study showed that serovar Virchow was detected in raw and treated sewage. The same serovar was also detected in primary and excess sludge (Olańczuk-Neyman et al., 2003). Similar observation was noted in Sweden, were *Salmonella* spp. were detected in 55% of treated sludge samples, with serovar Hadar being the most frequently isolated from treated and raw sludge (Sahlström et al., 2004). Salmonellae can grow in sewage sludge and effluents after treatment, particularly at low temperatures (Danielsson, 1977), consequently, the application of treated sludge on agricultural land and/or irrigation with treated wastewater, and the discharge of treated effluents in aquatic environments may constitute potential public health hazard (Hutchinson et al., 2008). *Salmonella* was detected in 68.75% of vegetable samples in agricultural land irrigated with wastewater in Morocco (Melloul et al., 2001), moreover, high infection rate with salmonellae was noted in children living in an area with sewage water irrigation practices (Melloul and Hassani, 1999; Melloul et al., 2002).

Livestock manure may disposed on agricultural land and/or widely used as fertilizer, which often contains high concentrations of different types of human pathogens, including

Salmonella. The presence and the levels of any given pathogen in livestock manure depends on (i) source animal, (ii) animal's health state and (iii) the storage and treatment methods of the manure (Venglovsky et al., 2006). Unfortunately, treatment of animal waste does not receive the required attention by public health authorities as in the case of human waste (Murray, 2000), thus the direct disposal of manure or slurry to agricultural lands or discharge to aquatic environments may constitute potential risk for the spread of salmonellae infections to human and animals. In this respect, special attention should be paid to the disinfection of contaminated waste of livestock to prevent the spread of infective agents (e.g. *Salmonella*) in the environment (Venglovsky et al., 2006).

2.3 Free-living wild birds

The intestinal carriage of various salmonellae serovars, including multidrug-resistant strains, by free-living wild birds and their role in the spread of the bacterium in the environment is well documented. These birds include, ducks and geese, pigeons, sea gulls and other species belonging to a wide range of different genera (Kapperud & Rosef, 1983; Palmgren et al., 1997; Hernandez et al., 2003; Tsai & Hsiang, 2005; Kobayashi et al., 2007; Čížek et al., 2007; Abulreesh, 2011) (Table 4). The majority of these birds seem to carry *Salmonella* spp. without obvious symptoms of infection, which suggests that salmonellae inhibiting the intestinal tract of free-living wild birds are commensal (Tizard, 2004; Abulreesh et al. 2005, 2007). Nonetheless, *Salmonella* spp. are also common cause of salmonellosis and other various serious infections in wild birds (Henry, 2000; Poppe, 2000; Tizard, 2004). Although various salmonellae serovars have been isolated from apparently healthy free-living birds, the incidence of the bacterium tends to be low (Table 4). Indeed Fallacara et al. (2001) found only one *Salmonella* isolate in 82 faecal droppings of mallard, while the bacterium was completely absent from 375 faceal samples of Canadian geese. Low incidence or complete absence of salmonellae carriage was also observed in other wild birds such as gulls, passerines, owls, pigeons, thrushes and eagles (Brittingham et al., 1988; Palmgren et al., 1997; Kirk et al., 2002; Hernandez et al., 2003; Reche et al., 2003; Dovč et al., 2004; Abulreesh, 2011).

Healthy free-living wild birds that live well away from pollution may not harbour *Salmonella* serovars (Čížek et al., 1994; Tizard, 2004). Indeed, when Hernandez et al. (2003) sampled Palearctic birds migrating southwards and which were likely to have had no recent experience of areas with domestic animals, they found only one *Salmonella*-positive bird, a mistle thrush (*Turdus viscivorus*), amongst 2,377 samples from 110 bird species. In the same way, a total of 233 faecal samples from eight penguins were all *Salmonella*-negative, suggesting that tourism has not yet introduced human-associated enteric pathogens to the Antarctic (Bonnedahl et al., 2005). Results obtained from different studies suggest that free-living wild birds may acquire salmonellae after exposure to human-contaminated environments or after scavenging on refuse tips and sewage sludge (Fricker, 1984; Ferns & Mudge, 2000; Tizard, 2004; Abulreesh et al., 2005).

Free-living and migratory wild birds are recognized as a potential reservoir for the transmission of human-associated *Salmonella* spp., including multidrug-resistant strains, through the contamination of water, farms and other environments. Therefore, it was concluded that free-living wild birds may play a significant role in the epidemiology of human salmonellosis (Tizard, 2004; Abulreesh et al., 2007; Literák et al., 2007; Tsiodras et al., 2008).

Bird species	Location	p:n (%)	*Salmonella* serovar	Reference
Black-headed Gull (*Larus ridibundus*)	Czech Republic	38:154 (25)	Typhimurium, Enteritidis, Panama, Anatum	Hubálek et al. (1995)
	Sweden	28:1047 (3)	Typhimurium	Palmgren et al. (2006)
Waterfowl (ducks and geese)	USA	8:450 (2)	Typhimurium	Fallacara et al. (2004)
	Taiwan	91:2000 (5)	ND	Tsai & Hsiang (2005)
Pigeon (*Columba livia*)	Japan	17:436 (4)	ND	Tanaka et al. (2005)
	Norway	3:72 (4)	Typhimurium	Refsum et al. (2002)
	Croatia	2:14 (14)	Typhimurium	Vlahović et al. (2004)
	Saudi Arabia	8:400 (2)	ND	Abulreesh (2011)
Coot (*Fulica atra*)	Czech Republic	1:3 (33)	Typhimurium	Hubálek et al. (1995)
House Sparrow (*Passer domesticus*)	USA	14:451 (3)	Montevideo, Meleagridis	Kirk et al. (2002)
	Norway	7:31 (23)	Typhimurium	Refsum et al. (2002)
Starling (*Sturnus vulgaris*)	USA	1:80 (1)	Typhimurium	Kirk et al. (2002)
	Czech Republic	4 isolates	ND	Čížek et al. (1994)
Magpie (*Pica pica*)	Norway	1:40 (3)	Typhimurium	Refsum et al. (2002)
Great Tit (*Parus major*)	Norway	6:87 (7)	Typhimurium	Refsum et al. (2002)
	Czech Republic	1 isolate	ND	Čížek et al. (1994)
Brown-headed Cowbird (*Molothrus ater*)	USA	3:95 (3)	Meleagiridis, Muenster	Kirk et al. (2002)
Rook (*Corvus frugilegus*)	Croatia	2:13 (15)	Typhimurium, Enteriditis	Vlahović et al. (2004)
Crow (*Corvus corone*)	Norway	1:52 (2)	Paratyphi B	Refsum et al. (2002)

Bird species	Location	p:n (%)	*Salmonella* serovar	Reference
Peregrine Falcon (*Falco peregtinus*)	Sweden	2:69 (3)	Amager	Palmgren et al. (2004)
Long-eared Owl (*Asio otus*)	Spain	1:7 (14)	Typhimurium DT104	Reche et al. (2003)
Kestrel (*Falco naumanni*)		3:59 (5)	Enteritidis	
Buzzard (*Buteo buteo*)		1:17 (6)	Typhimurium DT104	

p = number of positive samples, n = number of samples tested, (%) percentage of positive samples
ND = not determined

Table 4. Examples of the incidence of *Salmonella* spp. in fresh faeces or cloacal swabs of various free-living wild birds.

2.4 Domestic and wild animals

Salmonellae serovar have long been associated with diseases in animals, and there are reports suggested that salmonellae are wide spread in the intestinal tract of domestic and wild animals of different taxa (Simpsons, 2002; Angulo et al., 2004; Schlundt et al., 2004). Domestic pets such as dogs and cats that live in close proximity to humans have been responsible for a wide range of bacterial and parasitic zoonoses. For instance, Brucellosis (*Brucella canis*) and septic animal bite (*Pasteurella multocida*) were associated with dogs, whereas, cat-scratch disease (*Bartonella henselae*) and abortion and stillbirth (*Toxoplasma gondii*) were linked with cats (Timbury et al., 2002). These animals have also been found to carry different *Salmonella* serovars in their guts; both healthy and diseased individuals (Carter & Quinn, 2000; Sato et al., 2000; Van Immerseel et al., 2004). In Japan, a 4-month-old infant manifested with diarrhea and *Salmonella* Virchow was detected in his stool. The same serovar (Virchow), was also detected in faecal samples from two out of three household dogs that were living in close proximity with the infected infant. This finding lead to the conclusion that *Salmonella* Virchow infection in the infant was transmitted by the household dogs (Sato et al., 2000). In order to determine whether cats can present a potential risk for the transmission of salmonellae to humans, rectal swabs were taken from 278 healthy house cats, 58 dead cats, and 35 group-house cats were examined in Belgium (Van Immerseel et al., 2004). The results showed that 51.4% of the group-housed cats, 8.6% of diseased cats, and 0.36% of the healthy house cats excreted *Salmonella*. Most of the serovars recovered were human-pathogenic and resistant to multiple antibiotics, such as Typhimurium, Enteritidis. Thus, it was concluded that cats that shed salmonellae can pose health hazards to highly susceptible individuals, such as children, the elderly and immunocompromised people (Van Immerseel et al., 2004). Dog and cats can easily acquire *Salmonella* spp., either directly or indirectly via the faecal-oral route. Dogs and cats are allowed to roam, and hunt and thus have access to diverse sources of *Salmonella* serovars. Salmonellae can be transmitted to cats and dogs via contaminated dry pet's food, uncooked offal and bones, raw chicken and unchlorinated water. Scavenging on wildlife carcasses, households rubbish and/or hunting rodents or wild birds are also potential routes of transmission of salmonellae serovars to cats and dogs (Carter & Quinn, 2000).

Cold-blooded animals harbour a wide range of *Salmonella* serovars in their intestinal tract. *S. bongori* and *enterica* subsp. II, IIIa, IIIb, IV and VI are commonly isolated from reptiles,

however, isolation of *S. enterica* subsp. I from captive or free-living reptiles is common (Briones et al., 2004). *S. enterica* subsp. I is common in warm-blooded animals, the presence of this subsp. in the faeces of reptiles is probably due to the fact that reptiles usually fed on rodents, rats or mice and other small warm-blooded animals that seem to carry salmonellae (Pfleger et al., 2003). It seems that there is no specific serovar associated with specific reptilian species, yet subsp. III was observed to be predominant in snakes, while subsp. IV was found to be common in iguana lizards (de Sá & Solari, 2001; Pfleger et al., 2003). Serovar Typhimurium and Enteritidis were rarely detected from reptiles (Warwick et al., 2001; Seepersadsingh & Adesiyun, 2003), nevertheless, the carriage of other human-associated salmonellae serovars, particularly multidrug-resistant strains usually occur without obvious symptoms of diarrhea, thus salmonellae seem to be essentially normal component of reptilian intestinal flora (Warwick et al., 2001; Ebani et al., 2005). Cases of reptile-associated human salmonellosis were reported in the United States, Canada and Europe since the 1960's (Weinstein et al., 1995; Woodward et al., 1997; Olsen et al., 2001; Warwick et al., 2001). Transmission of salmonellae from pet reptiles to humans may occur directly (i.e. faecal contamination of food and water) or indirectly (i.e. contamination of hands and other body parts, or households fomites). A number of formal recommendations from the UK Communicable Disease Surveillance Centre and the Department of Health in the USA were issued to advise pet store owners and pet keepers of good code of practice to prevent, or at least minimize, reptiles-related salmonellosis (Warwick et al., 2001). Some of these recommendations include; informing pet owners to wash their hands after handling pet reptiles or their cages, pet reptiles should not be allowed to roam freely throughout the house or living area and other measures (Warwick et al., 2001). Unlike reptiles, the incidence of salmonellae in amphibians seems to be very low (Pfleger et al., 2003) and sometimes totally absent (Briones et al., 2004). *Salmonella* Abidjan and Wandsworth were detected, with low numbers, in the faeces of horned frog (*Ceratophrys cranwelli*). These serovars have not been implicated in human salmonellosis. Apparently, amphibians may not represent an important reservoir of *Salmonella* spp. in nature and may not have potential implications for public health (Briones et al. 2004).

Rodents, rats and mice are common commensal pests and usually regarded as an indicator of unsatisfactory sanitation (Murray, 2000). They are responsible for considerable damage to various stored products and buildings, as well as they can be a source of serious bacterial zoonosis (Healing, 1991; Timbury et al., 2002). Rats and mice are regarded as a potential reservoir of different salmonellae serovars, accordingly, they are considered as a major public health hazard (Murray, 2000). *Salmonella enterica* serovar Typhimurium definitive phage type 104 (DT 104) was recovered from the faeces of house rats (*Rattus rattus* and *Rattus norvegicus*) in Japan. This finding highlights the important role of mice and rats in the dissemination of serovar Typhimurium (DT104), which regarded as one of the emerging zoonotic agent in Europe and the United States because this strain has acquired multiple drug resistance (Yokoyama et al., 2007). In the UK, a total of 100 faecal samples, 50 recatal swabs and 25 swabs taken from the fur, paws and tail of wild urban brown rats (*Rattus norvegicus*) were examined for the presence of *Salmonella* spp (Hilton et al., 2002). The results showed that *Salmonella enterica* was recovered from 8% of the faecal droppings, and 10% of the rectal swabs. No salmonellae were recovered from the fur, paws and tail of the rats. These data suggest that physical spread of *Salmonella* from the body of the animal may not be possible and rat faeces are still the most likely source of *Salmonella* contamination (Hilton et al., 2002). The meat of the African great cane rat (*Thryonomys swinderianus*) is a valued and expensive food delicacy in Nigeria (Oboegbulem

& Okoronkwo, 1990). Almost ten salmonellae serovar were detected from liver and spleen samples of great cane rat that were caught and/or purchased from restaurant in Nsukka, Nigeria. These serovars were Agama, Poona and Ajiobo. Incidentally, these serovars have been isolated from the stool of diarrhea patients in Nigeria. Although no incidence of human salmonellosis attributed to eating cane rat has yet been reported, the consumption of undercooked rat meat and/or eating sugar cane that are contaminated with excretion of carrier rats may constitute potential health hazards (Oboegbulem & Okoronkwo, 1990).

Human-associated *salmonella* serovars such as Typhimurium and Enteritidis were found to be predominant in clinically and subclinically infected hedgehogs. The intestinal tract of hedgehogs seem to be the natural habitat of serovar Enteritidis phage type 11, which may infect humans and dogs, but not livestock. *Salmonella* Agama usually causes infection in cattle; it was detected in the faeces of badgers, which may be the natural reservoir of that serovar (Murray, 2000; Simpson, 2002).

2.5 Farm animals and farm environment

The incidence of salmonellosis in the community is related to the consumption of contaminated food and water. There is significant historical evidence suggesting that poultry products serve as a major source of *Salmonella* infections in humans. At the beginning of this century, *Salmonella pullorum* was one of the most recognized species as it was responsible for pullorum disease, which infects poultry intended for human consumption. Later, fowl typhoid (*S. gallinarum*) was noticed in almost all poultry-producing areas worldwide. With regard to human-associated salmonellae infection that are traced back to the consumption of poultry products, typhoid fever (*S. typhi*) was highly prevalent in the first five to six decades of the 1900's. This serovar was sharply declined in Europe and North America during the late 1940's and was replaced with *S. typhimurium*, which was declined in the last 20 years, particularly with the emergence of *S. enteritidis* as the commenst serovar in poultry in many countries around the world (Poppe, 2000).

The incidence of various salmonellae serovars in the intestinal tract of chickens is well documented; serovars Infantis, Thompson, Agona and Enteritidis were frequently detected from the faces of chickens in Japan (Ahmed et al., 2009). A total of 550 faecal samples were collected from healthy (500) and diseased (50) chicken from different farms in Shanghai, China. The occurrence of salmonellae was (3.8%) from healthy chickens and (12%) from diseased ones (Liu et al., 2010). Among the serovars that were identified from the faeces of healthy chickens; *S. pullorum* and *S. typhimurium* were prevalent, whereas *S. paratyphi* B and *S. senftenberg* were most commonly recovered from the faeces of diseased chickens (Liu et al., 2010). *Salmonella* spp. are not one of the normal common components of the intestinal flora of chickens (Guard-Petter, 2001). Accordingly, colonization of chicken by salmonellae is most likely due to contamination of broiler houses (Guard-Petter, 2001) (Table 5). Contamination of broilers was noted to take horizontal routs that include; contaminated water and feed, fluff, dust, insects, equipments and other fomites inside broilers environment. Transmission by direct contact with other chicks, rodents, wild and domestic birds and animals and personnel contaminated by salmonella serovar is also common (Al-Nakhli et al., 1999; Poppe, 2000; Guard-Petter, 2001; Garber et al., 2003; Skov et al., 2004; Kinde et al., 2005; Padungtod & Kaneene, 2006). On the other hand, vertical transmission of salmonellae in chicken is also common, it occurs when follicles in the ovary are infected or the developing of eggs become infected in the oviduct (Poppe, 2000; Guard-Petter, 2001).

Production	Box liners	Litter	Drag swab	Faecal droppings	Mice	Feed
Broilers	Enteritidis	Java Chester Virchow	Java Sofia Livingstone Concord Mbandaka Virchow Albany Enteritidis PT4	Java Eppendorf Sofia Livingstone Virchow Stockholm		Java Livingstone Concord Mbandaka Virchow
Layers			Enteritidis PT4	Livingstone		
Broilers breeders	Enteritidis PT4	Enteritidis PT4				
Layers breeders					Enteritidis PT4 Java	

Table 5. Isolation of *Salmoenlla* serovars from poultry farm environments in Saudi Arabia, 1998-1997. (Adapted from Al-Nakhli et al., 1999).

Chicken carcasses obtained from slaughterhouses, broilers, retail shops and supermarkets were found to be highly contaminated with various salmonellae serovars (Cogan & Humphrey, 2003; Humphrey & Jørgensen, 2006). Capita et al. (2003) examined the incidence of salmonellae on chicken carcasses, chicken parts (wings, legs, livers and hearts) and processed chicken products (sausage and hamburgers) in Spain. The study revealed that higher detection rates of salmonellae serovars were obtained from carcasses (55%) compared to hamburgers (20%), in addition, chicken carcasses sold in supermarkets were more contamined (75%) than those from poulterers shops (25%). The most frequent serovars encountered were Enteritidis, Poona and Paratyphi B. Likewise, in Portugal chicken carcasses available for consumers in local butcher shops were found to be heavily contaminated with 10 different multidrug-resistant *Salmonella* serovars. Again serovar Enteritidis was predominant together with serovar Hadar (Antunes et al., 2003). Serovar Enteritidis was also reported as the most commonly isolated salmonellae serovar from chicken carcasses and eggs in Brazil, Europe and the United States (Guard-Petter, 2001; de Oliveira et al., 2005; Capita et al., 2007). By contrast, serovar Thompson followed by serovar Enteritidis were the most frequently isolated salmonellae in chicken meat in Iran (Dalla et al., 2010). While in Japan, serovars Infantis, Typhimurium and Haifa were frequently present on chicken carcasses, yet serovar Enteritidis was frequently encountered in unpasteurized liquid eggs (Murakami et al., 2001). In general, serovar Enteritidis has received a considerable attention by public health authorities, as for the last two decades there has been a pandemic of *Salmonella enteritidis* infection worldwide, and this has been largely associated with the consumption of contaminated eggs and undercooked chicken meat (Cogan & Humphrey, 2003; Humphrey & Jørgensen, 2006). Apparently, the sources of

carcasses contamination is the intestinal tract of the birds, nevertheless, processing and packing at slaughterhouses and other equipments in broilers could be another major source of contamination. Improved competitive exclusion measures and vaccination of flocks were found to play an important role in the prevention of *Salmonella* serovars to colonize the intestinal tract of chickens and their products (e.g. eggs). Improved processing procedures at slaughterhouses may minimize the rate of contamination on carcasses; refrigeration and reducing the available water on carcasses by drying may also reduce the rate of contamination (Plummer et al., 1995; Poppe, 2000; Cogan & Humphrey, 2003; Humphrey & Jørgensen, 2006).

As mentioned earlier, salmonellae is strongly linked with infection in livestock; species of veterinary significance include *S. typhimurium* and *S. dublin* that are responsible for acute salmonellosis in cattle and calves (Wray & Davies, 2000); serovars Typhimurium and Choleraesuis are predominant in porcine salmonellosis, other serovars such as Enteritidis and Dublin may also cause diseases in pigs (Fedorka-Cray et al., 2000); *S. typhimurium* and *S. abortusovis* are the commonest cause of various salmonellae infections in sheep (Wray & Linklater, 2000). It can be noted that some of the above-mentioned serovars are also commonly involved in human-associated salmonellae infections, in this respect, it is of great importance to ascertain whether or not livestock serve as a reservoir of salmonellae of clinical importance and therefore play a role in their transmission in the community. Several investigations were carried out worldwide, and reported the carriage of various human-associated, multiple drug-resistant *Salmonella* serovars in the intestinal tract of healthy livestock (Bywater et al., 2004; Guerin et al., 2005). Moreover, livestock meat at salughterhouses and retail shops was also found to be contaminated with various salmonellae serovars worldwide (Davies et al., 2004; Mølbak, 2004; Dallal et al., 2010). Livestock may acquire salmonellae through contaminated water and feed, aerosol and poor farm management practices were also identified as risk factors for the transmission of the bacterium to livestock (Wray & Davies, 2000; Suk-Kyung et al., 2011). Reports of salmonellae outbreaks in the community associated with the consumption of unpasteurized milk, undercooked red meat are well documented. An outbreak of multidrug-reisistant *S. typhimurium* in Denmark was traced back to a Danish pig farm; in the UK, outbreak of *S. typhimurium* DT104 was associated with contaminated milk from dairy farm (Mølbak, 2004), between 1993 and 1997, there were 13 reported outbreaks of salmonellosis associated with the consumption of undercooked beef, 16 outbreaks associated with the consumption of pork and 10 outbreaks related to ice cream made of unpasteurized milk in the United States (Schlundt et al., 2004). These reports suggest that livestock represent a potiental source of human salmonellosis (Khakhria et al., 1997; Fedorka-Cray et al., 2000; Wray & Davies, 2000; Wray & Linklater, 2000; Bywater et al., 2004; Mølbak, 2004; Guerin et al., 2005).

3. Environmental factors associated with *Salmonella* infections

Salmonella serovars are ubiquitos and widely distributed in the environment. Climate and other environmental factors play a significant role in the incidence of salmonellae in various ecological niches. Environmental conditions may significantly affect the ability of salmonellae to persist in nature, in particular when these serovars are subject to hostile conditions and their ability to acquire multidrug-resistance.

3.1 Seasonal variation

The presence of *Salmonella* spp. in polluted aquatic environemtns is marked with strong seasonal trend. Various serovars were readily isolated from contaminated rivers in Italy during summer and autumn (Pianetti et al., 1998). In freshwater lakes in India, high density of *Salmonella* spp. was recorded during summer season over a two-year study. The apparent high levels of salmonellae recovered during summer seasons was influenced by discharges of human and animals excreta, and the ability of salmonellae to persist in contaminated fresh waters. Water temperature, Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) had influential roles in the presence of salmonellae in high numbers during warmer months in fresh water lakes (Sharma & Rajput, 1996). Similarly, Statistically significant high densities of salmonellae serovars were detected in Salmon River, British Columbia in Canada during the warm seasons (spring and summer) (Jokinen et al., 2010). Positive correlations were found between high rainfall and temperature with the presence of high densities of various salmonellae serovars in August in River Little, Georgia, USA (Haley et al., 2009). Conflicting reports on salmonellae seasonality in natural waters have, however, come from Spain, where various salmonellae serovars were detected in coastal water during warmer (July-September), but were present in even higher densities during colder (October-December) months (Martinez-Urtaza et al., 2004a). Interestingly, serovar Senftenberg and other serovars were found to peak during winter season, by contrast serovar Typhimurium was always present in high densities during summer seasons in marine environments in Spain (Martinez-Urtaza et al., 2004a, b). In general, the seasonal presence of salmonellae serovars in aquatic environments is associated with storm-generated flow, torrential rains and monsoon seasons in temperate and tropical regions, highlighting the washing effect of torrential rainfall as one of the principal environmental drivers of *Salmonella* contamination in aquatic environments (Martinez-Urtaza et al., 2004a, b; Semintal & Martinez-Urtaza, 2008; Haley et al., 2009; Setti et al., 2009).

In faecally contaminated village ponds, salmonellae can be readily detected in bottom sediments during warm months (June-August) but was not detected in colder months (Abulreesh et al., 2004). Seasonal incidence of salmonellae serovars in the faecal droppings of small passerines in Norway (e.g. *Salmonella typhymurium)* were found to peak in Feburary and March (cold months). Interstingly, the incidence of the same serovar (Typhimurium) in other bird species (e.g. gulls, ducks, crows and other species) in Norway was recorded without any apparent seasonality (Refsum et al., 2002). No statistical significant seasonal trends were recorded in the carriage of salmonellae by apparently healthy rock pigeons in Saudi Arabia (Abulreesh, 2011). In the United States, Pedersen et al. (2006) did not find any seasonal patterns with regards to the presence of salmonellae in the faeces of pigeons. Likewise, a temporal study of various salmonellae serovars in animals (chickens, turkeys, cattle and pigs) in Alberta, Canada between 1990 and 2001, showed that a seasonal pattern was not apparent, as the months with highest number of isolates differed from year to year (Guerin et al., 2005).

The incidence of salmonellosis in humans was found to show strong seasonal patterns worldwide. The peak of nontyphoidal salmonellae infection in the community was recorded during summer months (June-September) in the majority of Eropean, Mediterranean countries and North America. The summer seasonality was more marked in

northern/western Europe than in the Mediterranean region (Ekdahl et al., 2005; Naumova et al., 2007). Similarly, in Korea the highst peak of nontyphidal salmonellae infection in the community was recorded during the summer (June-August) (Cho et al., 2008). Seasonal trends of human nontyphoidal salmonellosis (Enteritidis) in East Asia and (Typhimurium) in India and nighboring countries were found to peak in colder months (November-December) (Ekdahl et al., 2005). In the case of typhoid infection in the Indian community (serovar Typhi), the maximum number of cases occurred during April-June (dry season) followed by July-September (monsoon season) (Mohanty et al., 2006). Although these cases were coincided with the peak of annual temperature and rainfall, it is not clearly understood which factor drives this seasonality regionally, or how this pattern might relate to the overall presence of salmonellae in the environment (Haley et al., 2009).

3.2 Survival in nature

Salmonella spp. are exclusively of faecal origin, they are therefore, allochthonous to aquatic environments. Once the bacteria are released into the environment (aquatic and terrestrial), they confront a wide range of drastic, stressful conditions, that make their persistence rather difficult and they may die rapidly. Thus, one would expect that salmonellae may not survive for long periods in the environment. This assumption started to change when Pokorný (1988) showed that *Salmonella enteritidis* survived for up to 30 days in seeded pollution-free drinking water microcosms held at 4 °C. In laboratory experiments using sterile water, seeded cells of serovar Typhi was found to remain viable for up to 65 days when microcosms were incubated in the refrigerator (4-6 °C). When microcosms were incubated at room temperature (18-24 °C) and at 37 °C, survival of serovar Typhi was decreased to 25 and 5 days respectively (Uyanik et al., 2008). The apparent long survival of enteric bacteria (e.g. *Campylobacter* and *Salmonella*) at low temperature perhaps explained by increased metabolism and more rapid substrate utilization at higher temperature at the expense of culturability (Abulreesh et al., 2006; Uyanik et al., 2008). In addition, light (particularly U. V. radiation) is potentially lethal to salmonellae in aquatic environments. In laboratory experiments, culturable populations of *Salmonella Typhimurium* in seawater microcosms were decreased rapidly after exposure to direct natural sunlight (Davies & Evison, 1991). The lethat effect of sunlight is probably aggravated by the high salinity of seawater, therefore, the U. V. component of sunlight and the high salinity may act synergistically in causing decrease in the numbers of culturable *S. Typhimurium* (Davies & Evison, 1991). The same conclusion was drawn with regard to the combined effect of sunlight and salinity on the survival of *Salmonella enterica* in seeded *in situ* seawater chambers (Sinton et al., 2007). In freshwaters however, the survival of salmonellae may be extended for periods longer than in seawater, as a result of the presence of humic materials that may absrobe U. V. radiation, therefore, protecting the cells from possible damage to the DNA by radiation (Davies & Evison, 1991). Generally, natural light affects the uptake of nutrients and inhibits active transport and biosynthesis in *Escherichia coli* (Barcina et al., 1990); its effect on salmonellae serovars may require further investigation (Sinton et al., 2007).

Nutrient availability and predation are also important factors in the survival of enteric bacteria, in aquatic environments (Flint, 1987). Using *in situ* membrane diffusion chambers, serovar Typhimurium remained viable for 5 days in tropical rainforest

watershed (Jiménez et al., 1989). Thus, it was concluded that salmonellae may not survive for long period in oligotrophic natural waters (Domingo et al., 2000). The densities of viable salmonellae serovars were decreased rapidly, as measured by plate counts, in spiked filtered river and seawater. Filtration may removes valuable biological agents, while filterable substances such as heavy metals, antibiotics and small preadtors (e.g. protozoa) may then be responsible for the rapid decrease of bacteria in filtered natural waters (Cornax et al., 1990; Domingo et al., 2000). High densities of salmonellae were found to survive for as long as 50 days in sediment-containing microcosms compared with the overlying water (Fish & Pettibone, 1995). Aquatic sediments were culture-positive of serovar Typhimurium up to 119 days, while this serovar was viable in water column in the same microcosm for only 54 days (Moore et al., 2003). The observed extended survival of salmonellae in aquatic sediments is probably due concentration of these bacteria by sedimentation of bacteria-bound particles. Sediments may protect enteric bacteria from certain stressful conditions associated with aquatic environments; it also provides enteric bacteria with nutrients and protection against grazing protozoans, thus supports the growth of these bacteria (Lim & Flint, 1989; Fish & Pettibone, 1995).

In terristerial ecosystems, salmonellae were found to multiply and survive in soil for up to 12 months, as soil ecosystems were believed to act as microecological niches where enteric bacteria can survive and even grow. The ability of salmonellae to attach to soil particles was related to cell surface hydrophobicity by which the bacteria can undergo morphological changes in order to adapt to this new environment (Stenstrom, 1989; Davies & Wray, 1996). The ability of salmonellae to survive and grow in soil environments may explain, in part, the detection of *Salmonella* spp. from pasture 2 months, and from soil 8 months after the application of contaminated pig slurry, and highlight the need of proper sanitation of contaminated animal waste before its disposal on agricultural land (Venglovsky et al., 2006). Virtually, *Salmonella* serovars can persist in different environments. On clean laminated surfaces (i.e. utensils), salmonellae was found to survive for less than 4 hours, however when these utensils (stainless steel bowl) were covered with soil, *Salmonella* spp. survived for up to 4 hours (Scott & Bloomfield, 1990). Survival of serovars Enteritidis, Heidelberg and Enteritidis phage type 4 on surfaces was extended to up to 24 hours under dark conditions, but when these surfaces exposed to direct sunlight, the numbers of salmonellae serovars decreased rapidly (Nyeleti et al., 2004). Thus it is not surprising that *Salmonella* spp. can persist and survive in domestic kitechens for a year or even longer (Humphrey, 2001). *Salmonella* spp. persisted in the biofilm material found under the recess of the toilet bowl rim which was difficult to remove with household toilet cleaner. Serovar Enteritidis remained in toilet bowl for up to 4 weeks despite the use of cleaning fluids. Flushing the toilet may contaminate the toilet seat and its lid with salmonellae that were found in biofilm formation in toilet bowl (Barker & Bloomfield, 2000). In conclusion, it was suggested that the ubiquitous nature of *Salmonella* may facilitate a cyclic lifestyle consisting of passage through a host into the environment and back into a new host (e.g. from human intestine to the water then to birds), therefore, the long-term survival of *Salmonella* in the secondary habitat (i.e. water, soil) ensures its passage to the next host (Winfield & Groisman, 2003).

The viable but nonculturable (VBNC) state in the context of enteric bacteria refers to the ability of bacterial cells to remain viable (i.e. retaining basal metabolic activities) yet unable to grow in artificial media in the laboratory (Barer et al., 1993; Oliver, 2005, 2010). This state is believed to be a survival strategy of enteric bacteria that are released into the

environment and suffer prolonged exposure to environmental stressors such as suboptimal temperature; U. V. irradiation; nutrient deprivation and biological interactions (McKay, 1992; Barer & Harwood, 1999). Xu et al. (1982) were the first to address the VBNC phenomenon in enteric bacteria when they examined the survival of *Vibrio cholerae* and *Escherichia coli* in estuarine and marine water microcosms. It was found later on that the VBNC state is exhibited by other enteric bacterial species such as *Campylobacter*, *Shigella*, *Ligeonella* and *Salmonella* (McKay, 1992; Barer et al., 1993). Nonculturability in salmonellae has been attributed, in part, to nutrient depletion. As a result of nutrient limitations viable cells of serovar Enteritidis lost culturablity within 48 hours at 25 °C when they were incubated in sterile river water (Roszak et al., 1984). Other relevance factors for the possible VBNC state in salmonellae may include exposure to elevated temperatures, U. V. irradiation, salinity, exposure to antibiotics and chlorination (Caro et al., 1999; Oliver, 2000; Smith et al., 2002; Oliver et al., 2005). VBNC forms of salmonellae may undergo morphological transition in response to stress, as they form a shorter rods shape (Roszak et al., 1984). Resuscetation of VBNC *Salmonella* cells may be possible (Roszak et al., 1984; Smith et al., 2002; Dhiaf et al., 2010), yet these resuscitated cells may lose their virulence or ability to cause infection (Caro et al., 1999) as serovar Typhimurium in VBNC forms and active but nonculturable (ABNC) (i.e. resuscitated cells) were unable to infect, nor colonize laboratory animals (Smith et al., 2002).

In contrast, other studies have rejected the whole VBNC concept. It is suggested that VBNC cells are actually dead and that apparent resuscitation represents the growth of surviving culturable cells (Morgan et al., 1991; Weichart et al., 1992; Bogosian et al., 1998). This suggestion was supported by failure of nonresuscitated forms of VBNC *Salmonella typhimurium* to colonize mouse model (Caro et al., 1999). Although the VBNC phenomenon is not fully elucidated, it has a major relevance to public health authorities in two areas: (i) the ability to cause infection, and (ii) the monitoring of enteric pathogens in the environment using conventional culture methods (McKay, 1992; Barer et al., 1993).

3.3 Antibiotic resistance

The emergence of anitmicrbial resistance in pathogenic bacteria, particularly in enteric bacteria is a major public health issue. The over use of antibiotics in food-producing animals, mass treatment and long-term administration of antimicrobial growth promoters may lead to the emergence of multidrug-resistant strains of enteric bacteria, including *Salmonella* spp. These bacteria may become reservoirs of highly transferable drug-resistance genes, and as they spread widely in the environment, they may cause serious infections as they spread within the food chain. Consequently, therapeutic failure of these infections may occur and complications due to these infections may result in increase in morbidity and mortality (Mølbak 2004, 2005).

The implications of infections caused by drug-resistant *Salmonella typhimurium* DT29 was addressed by Anderson (1968). Infections in calves in the UK due to this serovar were proven difficult to treat even after using a range of antibiotics. Instead, this use of multidrug resulted in the acquisition of serovar Typhimurium DT29 transferable multiple drug-resistance, that later caused many infections in humans (Anderson, 1968). In his report Anderson (1968) suggested that infections due multidrug-resistant salmonellae can be eliminated not by the massive use of antibiotics, but by improvement in conditions of

animal husbandry and reduction in the opportunities for the initiation and spread of the disease. Unfortunately, these suggestions were not taken well into consideration in many parts of the world and as a result, different serovars of salmonellae have acquired multiple-drug resistance and became ubiquitous in the environment. Various salmonellae serovars resistant to wide range of antimicrobial drugs were isolated from the faeces of diseased and apparently healthy livestock and poultry (Ahmed et al., 2009), food (dairy products; meat; poultry products) (Antunes et al., 2003; Zhao et al., 2003; Dallal et al., 2010), free-living wild animals and birds (Palmgren et al., 1997; Čížek et al., 2007; Abulreesh, 2011), domesticated animals (Seepersadsingh & Adesiyun, 2003; Van Immerseel et al., 2004; Ebani et al., 2005), natural waters (fresh and marine) (Morinigo et al., 1990; Harakeh et al., 2006), sewage effluents and sludge (Berge et al., 2006; Espigares et al., 2006) and from diarrhea patients (Ling et al., 1998; Graziani et al., 2008) worldwide. In general, a well established link between the use of antibiotics in food-producing animals and drug-resistant salmonellae is described and well understood for food-borne nontyphoidal salmonellae (Mølbak, 2004).

Salmonella spp. can acquire resistance to antimicrobial drugs via different mechanisms such as (i) transferable resistance genes; (ii) excessive use of antibiotics in treatment "selective pressure"; and (iii) as a response to exposure to environmental drastic conditions. The resistance of aminoglycosides, β-lactams, chloramphenicol, macrolides, quaternary ammonium and trimethoprime in salmonellae serovars is attributed to the acquisition of foreign genes that encoded enzymes to destroy, chemically inactive, or "pump" the noxious drug out of the bacterial cell or provide an alternative pathway to the one targeted by the antibiotic (D'Aoust & Maurer, 2007). These antibiotic-resistance genes usually reside on mobile genetic elements such as plasmids; transposons and integrons that can potentially transfer resistance from commensal to pathogenic bacteria (Mølbak, 2005; D'Aoust & Maurer, 2007). Integron in particular, described as a genetic material that is capable of capturing, combining, or swapping a large assortment of antibiotic-resistance genes, then integrating the captured genes into a resident integration site. This genetic element (i.e. integron) can create tandem antibiotic-resistant genes. In salmonellae serovars, tetracycline resistance genes are the only resistance genes that have not identified among the integron gene cassettes, nonetheless they are carried on plasmids (Ling et al., 1998; D'Aoust & Maurer, 2007).

Overuse of antibiotics in the treatment clinical or veterinary cases together with environmental stresses (e.g. detergents, dyes, food components, preservatives) can induce the *mar* (multiple antibiotic resistance) operon. This operon regulates the expression of a large number of genes, including those coding for at least one broad-specificity efflux pump (i.e. *arcAB* efflux pump), which are more strongly expressed under drastic conditions of environmental stress (McMahon et al., 2007). There seem to be a role of antimicrobial drug intake for acquiring infections with drug-resistant pathogenic bacteria (Threlfall, 2002; Mølbak, 2004). This was confirmed by Glynn et al. (2004) where patients infected with serovar Typhimurium resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline were most likely to have treated by antibiotics to which that serovar was resistant. This may shed some light on the reasons why outbreaks of drug-resistant pathogens are particularly frequent in hospitals and other environments where antibiotics are commonly used, furthermore, it may explains the fact that drug-resistant bacteria may be more virulent than susceptible ones (Threlfall, 2002; Mølbak, 2004, 2005). In addition, it was found that food preservation processes (e.g. low pH, high NaCl) can lead to

the development of populations or subpopulation of salmonellae with decreased susceptibility to a range of currently used antibiotics (e.g. amikacin, ceftriaxone, trimethoprim). Such decreases in antibiotic susceptibility are maintained as long as the food preservation stress is maintained, and in some cases, even after the food preservation stress is removed (McMahon et al., 2007). In general, it is well established that the likelihood that pathogenic bacteria could develop resistance, persist and spread involves more than selective pressure for antibiotics use alone (overuse of antibiotics in treatment of human and animal infections), and could also result from a complex of interactions of genes, ecosystems, and the environment (D'Aoust & Maurer, 2007).

4. Concluding remarks

Despite the decline of typhoidal salmonellae infections in major parts of the world, nontyphoidal salmonellae is the second leading cause of water-and food-borne infections worldwide. *Salmonella* serovars are ubiquitous in natural waters and sediments. The presence of salmonellae in aquatic environments is related to one or a combination of sewage effluents; agricultural run-off and direct faecal contamination from natural fauna. *Salmonella* serovars cause major infections in domestic and wild animals, nonetheless, a wide range of domesticated and free-living animals appears to carry salmonellae without obvious symptoms. *Salmonella* seovars have unique seasonal trends. In the environment, generally they peak during wet seasons and are more associated with rainfall and monsoon seasons. Some serovars peak in aquatic environments during warmer months, while other peak in colder months. Although no seasonal trends were found in the carriage of salmonellae by domestic and wild animals, the presence of salmonellae infection in the community is season-dependent. Untreated water, contaminated raw milk, poultry products and undercooked meat are the major sources of infection. Handling of doestic animals and wild fauna may also poses a risk for acquiring salmonellosis. Salmonellae serovars may survive and remain virulent for long periods in the environment, they may undergo a viable but nonculturable stage as a survival strategy, their ability to cause infection in the VBNC state, however, remains questionable. *Salmonella* spp. can easily acquire drug-resistance genes via transferable genetic elements. In addition, overuse of antibiotics in the treatment of human and veterinary diseases is another mechanism in which salmonellae serovars can aquire resistance to antimicrobial drugs. The ability of faecal indicators to predict the presence of salmonellae in polluted environmental waters remains questionable, and the absence of faecal indicators is not always a reliable indication of the absence of *Salmonella* spp.

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