2. LITERATURE REVIEW

2.1 BIOLOGICAL AND BIOCHEMICAL ASPECTS OF *SALMONELLA*

The genus *Salmonella* belongs to the family *Enterobactericeae*. There are two species in this genus: *Salmonella enterica* and *Salmonella bongori* (Doyle *et al*., 2001). In addition, six important subspecies have been classified into *Salmonella enterica* species namely, *S. enterica* ssp. enterica, *S. enterica* ssp. salamae, *S. enterica* ssp. arizona, *S. enterica* ssp. diarizonae, *S. enterica* ssp. houtenae, and *S. enterica* ssp. indica.

Classification and detection of these bacteria are based on serology and phage susceptibility assays (Bell and Kyriakides, 2002). According to the Kaufman-White classification scheme, there are more than 2,500 serotypes isolated and more than 2,400 named serotypes, as shown in Table 1.

Table 1: *Salmonella* species and subspecies (Popoff *et al*., 2004)

***Salmonella* species and subspecies**	**Number of serotypes**
Salmonella enterica	
- *S. enterica* subspecies *enterica*	1, 504
- *S. enterica* subspecies *salamae*	502
- *S. enterica* subspecies *arizonae*	95
- *S. enterica* subspecies *diarizonae*	333
- *S. enterica* subspecies *houtenae*	72
- *S. enterica* subspecies *indica*	13
Salmonella bongori	22
TOTAL	**2,541**

Salmonellae are chemo-organotrophic, with the ability to metabolize nutrients by both respiratory and fermentative pathways, the so-called **facultative anaerobic bacteria** (Doyle *et al*., 2001). Thus, they can ordinarily metabolize when oxygen is present (aerobic metabolism), but they are able to shift to anaerobic metabolism (Black, 2002). Because they are able to adjust themselves to and tolerate different environmental conditions, *Salmonellae* are widespread in natural settings, including soil and water, in which they do not usually multiply significantly but may survive for long periods (Bell and Kyriakides, 2002).

Salmonellae are **mesophiles** and prefer room temperature (35°C) as the optimum growth temperature. Nevertheless this group has a temperature range of 10°C minimum and 48°C maximum but grow optimally at 37°C (range between 5.2 – 46.2°C). However, most serotypes will not grow at temperatures less than 7°C (Bell and Kyriakides, 2002).

The optimum pH for growth is between 6.5 and 7.5 (Holt *et al*., 2000). *Salmonella* sometimes can grow under different pH levels (range 3.8 to 9.5) but most serotypes will not grow below 4.5 (Bell and Kyriakides, 2002). In addition, *Salmonellae* are able to catabolize D-glucose and other carbohydrates with the production of acid and gas, which can be used for biochemical identification. They are oxidase negative and catalase positive and grow on citrate as a sole carbon source. They generally produce hydrogen sulfide, decarboxylate lysine and ornithine, and do not hydrolyze urea. Many of these characteristics form the basis for the presumptive biochemical identification of *Salmonella* isolates (Table 2).

Table 2: Biochemical characteristics of *Salmonella* (Bell and Kyriakides, 2002)

Characteristic	Usual reaction
Catalase	+
Oxidase	-
Acid produced from lactose	-
Gas produced from glucose	+
Indole	-
Urease produced	-
Hydrogen sulphide produced from triple-sugar iron agar	+
Citrate utilized as sole carbon source*	+
Methyl red	+
Voges-Proskauer	-
Lysine decarboxylase	+
Ornithine decarboxylase	+

+ = Positive reaction; - = negative reaction

* *S*. Typhi is negative in this test

2.2 MORPHOLOGICAL BASE FOR SEROTYPING

2.2.1 *Salmonella* morphology and basic structure

Salmonella are **gram-negative**, **straight**, **small** (0.7 – 1.5 X 2.0 – 5.0 μm) **rods**, which are usually motile with **peritrichous flagella** (Bell and Kyriakides, 2002). The morphology of *Salmonella* and its internal structure are shown in Figure 1.

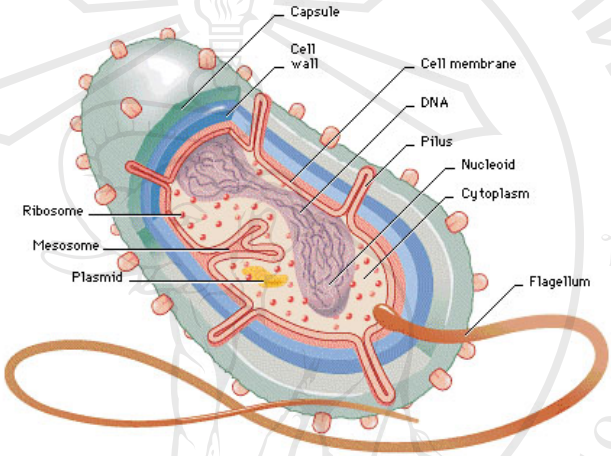

Figure 1: The micrographic structure of *Salmonella*
(Source: http://science.nasa.gov/.../ yeast/salmonella_sm.jpg)

2.2.2 Flagella and outer membrane

The term flagella (singular--flagellum), in its conventional and historical sense, suggest a helical filament extending from the cell surface. A flagellum consists of three structural parts: the filament, the hook and the basal body (Sussman *et al*., 2002a). The general structure of a typical flagellum is shown in Figure 2.

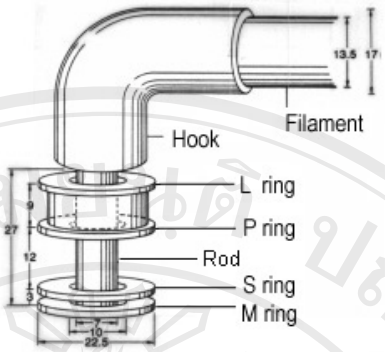

Figure 2: General structure of flagella
(Source: www.tnau.ac.in/.../ UGMicro/AGM151_201/theory.htm)

The genes, termed *hag* (from H antigen), were encoded at the building block of flagella filament (so-called flagellin) with the different sequences of its central region. The sequences are not only variable among different bacterial species but also among *Salmonella* serotypes. These differences impart H antigenic specificity on *Salmonella*.

Similar to those in other gram-negative bacteria, *Salmonella* has a distinguished outer membrane, which is bi-layered, forms the outmost layer of the cell wall, and is attached to the thin layer of peptidoglycan. The latter is almost a continuous layer of small lipoprotein molecules. The structural arrangement of the outer layer is shown in Figure 3.

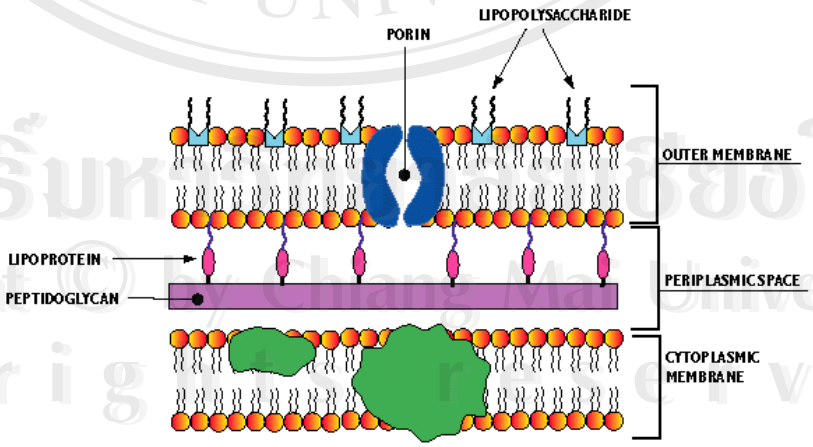

Figure 3: Structural arrangement of the outer layer of *Salmonella*
(Source: www.tnau.ac.in/.../ UGMicro/AGM151_201/theory.htm)

At the outer membrane lie lipopolysaccharides or endotoxins. Endotoxins in gram-negative bacteria such as those in *Salmonella*, *Shigella*, and *Escherichia*, can cause toxic and pathogenic symptoms in humans and mammals (Tamil Nadu Agricultural University, India, 2005). The components of lipopolysaccharides are shown in Figure 4.

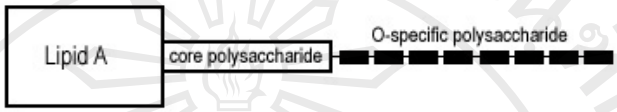

Figure 4: The lipopolysaccharide components
(Source: www.tnau.ac.in/.../ UGMicro/AGM151_201/theory.htm)

Lipopolysaccharides can be divided into three regions from a functional and a biosynthetic standpoint (Cary *et al*., 2000). Those components are so-called (1) Lipid A, (2) Carbohydrate core polysaccharides and (3) the O-side chains (O antigens). The latter is used to differentiate *Salmonella* serotypes.

2.3 SEROLOGICAL ASPECTS OF *SALMONELLA*

Serological analysis of *Salmonella* has identified three general antigens: H antigens, which are related to motility and flagella antigens, K or Vi antigens, which are present on the surface layer. These can be removed by extraction with mild solvents, such as saline or hot water. The third antigens are referred to as somatic or O antigen reference(s) (Sussman *et al*., 2002a).

The O antigens

The O antigens are the most dominant and express their activity as endotoxin (Sussman *et al*., 2002a). The term endotoxin refers to certain common features of all lipopolysaccharide (LPS) molecules, which bind to specific receptors and elicit a broad range of host defenses, including activation of various components of the immune systems of the hosts (Sussman *et al*., 2002a). Alteration in the sugar moiety of the O antigen results in a change in the immunological specificity (Botteldoorn *et al*., 2004). The sugar found in the O antigen region can occur in a wide variety of combinations, accounting for tremendous antigenic diversity and many hundreds of chemical types or serotypes of *Salmonella* and other *Enterobateriaceae* (Moat and Foster, 1995).

H antigens and their phase variation

Salmonella species have two flagellin genes, *fliC* and *fljB*, at separate locations on the chromosome (Sussman *et al*., 2002b). These can be expressed as the major flagellins, but not at the same time in any given cell. The two flagellins, H1 and H2, have significantly different antigenic specificity, resulting in two types of cells with completely different flagella antigens. This alternative expression of two different flagella with different antigenic specificities, a phenomenon known as phase variation, allows the *Salmonella* cells to escape attack by antibodies in hosts (Sussman *et al*., 2002b).

K or Vi antigens

Another antigen represented in *Salmonella* serotypes is the virulent **(Vi)** or capsular antigen. This occurs in *Salmonella* serotypes Typhi, Paratyphi C and Dublin (Selander *et al*., 1992, Morris *et al*., 2003). This antigen is located in an external polysaccharide microcapsule and is associated with **virulence** in particular hosts (Krieg and Holt, 1984).

Most laboratories perform agglutination reactions based on specific O antigens, designating *Salmonella* serogroups A, B, C1, C2, D and E. Examples for some *Salmonella* species are (WHO, 2001):

Serogroup A – *S*. Paratyphi A

Serogroup B – *S*. Paratyphi B, *S*. Typhimurium

Serogroup C1 – *S*. Paratyphi C, *S*. Choleraesuis, *S*. Enteritidis

Serogroup C2-C3) – *S*. Utah, *S*. Paris

Serogruop D – *S*. Typhi, *S*. Enteritidis

Serogruop E – *S*. Anatum, *S*. London

2.4 DISTRIBUTION OF *SALMONELLA* SEROTYPES IN THAILAND

The most common *Salmonella* serotype causing human salmonellosis found in Thailand between 1993 and 2002 was *Salmonella enterica* Weltevreden (Bangtrakulnonth *et al*., 2004). This investigation serotyped *Salmonella* from all diagnostic laboratories in Thailand, using both direct plating and enrichment broth. A total of 70,235 isolates received was confirmed as *S. enterica* and serotyped. All strains identified as *S. enterica* were serotyped according to the Kauffman-White Serotyping Scheme. *Salmonella* antisera (S and A Reagent Laboratory LMT, Bangkok, Thailand) were used in that serotyping.

A total of 118 serotypes were identified among the 44,087 isolates from humans (Table 3). The 25 prevalent serotypes accounted for 86% of the isolates, followed by 10 serotypes (64.7%), and the 5 (44.3%) most other serotypes (*S*. Weltevreden, *S*. Enteritidis, *S*. Anatum, *S*. Derby, *S*. 1,4,5,12:i) of the isolates.

The distributions of *Salmonella* serotypes in Thailand during 1993 – 2002 by different reservoirs are shown in Table 4. Samples have not been systematically taken from the different sources for *Salmonella* infections in humans. However, data from samples were available from chicken, seafood, other food products, and water for 10 years (1993-2002). Data from ducks were only available from 1998 to 2002.

Table 3: Common serotypes of *Salmonella* isolates from humans in 1993 to 2002, Thailand (Bangtrakulnonth *et al*., 2004).

Serotype	Year and Numbers of Isolates (%)										Total
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	
1.Weltevreden	443 (13.5)	574 (9.9)	816 (12.3)	337 (9.3)	335 (9.7)	485 (11.6)	862 (18.0)	660 (16.1)	657 (15.9)	322 (7.9)	5,491 (12.5)
2. Enteritidis	471 (14.3)	833 (14.4)	877 (13.2)	489 (13.4)	365 (10.5)	396 (9.5)	401 (8.4)	306 (7.5)	357 (8.6)	515 (12.6)	5,010 (11.4)
3. Anatum	146 (4.4)	397 (6.9)	568 (8.5)	229 (6.3)	298 (8.6)	320 (7.6)	235 (4.9)	412 (10.1)	340 (8.2)	318 (7.8)	3,263 (7.4)
4. Derby	368 (11.2)	650 (11.3)	576 (8.7)	277 (7.6)	252 (7.3)	251 (6.0)	141 (3.0)	156 (3.8)	111 (2.7)	107 (2.6)	2,889 (6.6)
5. 1, 4, 5, 12:i:-ssp.I	193 (5.9)	272 (4.7)	422 (6.3)	355 (9.8)	212 (6.1)	228 (5.4)	248 (5.2)	248 (6.1)	336 (8.1)	290 (7.1)	2,804 (6.4)
6. Typhimurium	154 (4.7)	216 (3.7)	326 (4.9)	238 (6.5)	305 (8.8)	278 (6.6)	258 (5.4)	205 (5.0)	175 (4.2)	167 (4.1)	2,322 (5.3)
7. Rissen	54 (1.6)	162 (2.8)	222 (3.3)	143 (3.9)	295 (8.5)	246 (5.9)	317 (6.6)	287 (7.0)	259 (6.3)	334 (8.2)	2,319 (5.3)
8. Stanley	64 (1.9)	147 (2.5)	186 (2.8)	85 (2.3)	99 (2.9)	147 (3.5)	245 (5.1)	210 (5.1)	242 (5.9)	263 (6.4)	1,688 (3.8)
9. Panama	31 (0.9)	64 (1.1)	9 (1.4)	80 (2.2)	173 (5.0)	172 (4.1)	264 (5.5)	209 (5.1)	160 (3.9)	230 (5.6)	1,474 (3.3)
10. Agona	118 (3.6)	215 (3.7)	236 (3.6)	103 (2.8)	102 (2.9)	76 (1.8)	95 (2.0)	76 (1.9)	75 (1.8)	90 (2.2)	1,096 (2.7)
11. Choleraesuis	99 (3.0)	87 (1.5)	139 (2.1)	122 (3.4)	68 (2.0)	118 (2.8)	92 (1.9)	69 (1.7)	85 (2.1)	186 (4.5)	1,065 (2.4)
12. Hadar	64 (1.9)	8 (1.4)	198 (3.0)	67 (1.8)	80 (2.3)	8 (2.0)	96 (2.0)	106 (2.6)	136 (3.3)	112 (2.7)	1,023 (2.3)
13. Paratyphi A	76 (2.3)	107 (1.9)	134 (2.0)	330 (9.1)	47 (1.4)	157 (3.8)	108 (2.3)	—	15 (0.4)	7 (1.7)	981 (2.2)

Continued

Table 3: Continued

Serotype	Year and Numbers of Isolates (%)										Total
	1993	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	
14. Krefeld	149 (4.5)	129 (2.2)	135 (2.0)	52 (1.4)	74 (2.1)	67 (1.6)	72 (1.5)	36 (0.9)	32 (0.8)	39 (1.0)	785 (1.8)
15. Paratyphi B Java	31 (0.9)	40 (0.7)	66 (1.0)	46 (1.3)	61 (1.8)	56 (1.3)	113 (2.4)	120 (2.9)	117 (2.8)	48 (1.2)	698 (1.6)
16. Typhi	61 (1.9)	53 (0.9)	41 (0.6)	42 (1.2)	43 (1.2)	64 (1.5)	68 (1.4)	—	213 (5.2)	82 (2.0)	667 (1.5)
17. Virchow	52 (1.6)	69 (1.2)	77 (1.2)	28 (0.7)	35 (1.0)	45 (1.1)	89 (1.9)	70 (1.7)	102 (2.5)	79 (1.9)	646 (1.5)
18. Lexington	40 (1.2)	67 (1.2)	66 (1.0)	35 (1.0)	45 (1.3)	60 (1.4)	68 (1.4)	56 (1.4)	88 (2.1)	52 (1.3)	577 (1.3)
19. Blockley	82 (2.5)	78 (1.4)	53 (0.8)	27 (0.7)	20 (0.6)	49 (1.2)	45 (0.9)	56 (1.4)	47 (1.1)	41 (1.0)	498 (1.1)
20. Hvittingfoss	12 (0.4)	94 (1.6)	125 (1.9)	27 (0.7)	12 (0.3)	16 (0.4)	66 (1.4)	41 (1.0)	33 (0.8)	35 (0.9)	461 (1.0)
21. Senftenberg	62 (1.9)	126 (2.2)	64 (1.0)	16 (0.4)	28 (0.8)	37 (0.9)	29 (0.6)	20 (0.5)	26 (0.6)	44 (1.1)	452 (1.0)
22.Bovismorbificans	32 (1.0)	54 (0.9)	87 (1.3)	16 (0.4)	37 (1.1)	42 (1.0)	56 (1.2)	30 (0.7)	29 (0.7)	56 (1.4)	439 (1.0)
23. London	27 (0.8)	92 (1.6)	72 (1.1)	45 (1.2)	67 (1.9)	71 (1.7)	24 (0.5)	15 (0.4)	8 (0.2)	0 (0.0)	421 (1.0)
24.Schwarzengrund	0 (0.0)	9 (0.2)	3 (0.0)	3 (0.1)	6 (0.2)	26 (0.6)	76 (1.6)	99 (2.4)	98 (2.4)	52 (1.3)	372 (0.8)
25. Emek	31 (0.9)	38 (0.7)	56 (0.8)	29 (0.8)	29 (0.8)	51 (1.2)	30 (0.6)	26 (0.7)	27 (0.7)	30 (0.7)	347 (0.8)
Other	424 (12.9)	1,116 (19.3)	1,011 (15.2)	415 (11.4)	380 (11.0)	643 (15.4)	679 (14.2)	577 (14.1)	366 (8.9)	598 (14.6)	6,299 (14.3)
Total	**3,284**	**5,770**	**6,647**	**3,636**	**3,468**	**4,184**	**4,777**	**4,090**	**4,134**	**4,097**	**44,087**

Table 4: Distribution of the 10 most common serotypes from different sources in Thailand (Bangtrakulnonth *et al*., 2004).

Serotype	Sources and Numbers of Isolates (%)					
	Humans	**Frozen chicken**	**Frozen seafood**	**Frozen duck**	**Other food products**	**Water**
1. Weltevreden	5,491 (12.5)	—	265 (26.3)	320 (12.0)	457 (6.6)	143 (14.5)
2. Enteritidis	5,010 (11.4)	2,901 (19.9)	14 (1.4)	—	309 (4.5)	22 (2.2)
3. Anatum	3,263 (7.4)	423 (2.9)	20 (2.0)	—	1,177 (17.0)	113 (11.5)
4. Derby	2,889 (6.6)	—	20 (2.0)	—	370 (5.3)	71 (7.2)
5. 1, 4, 5, 12:i:- ssp.I	2,804 (6.4)	—	—	—	—	—
6. Typhimurium	2,322 (5.3)	—	12 (1.2)	—	198 (2.9)	—
7. Rissen	2,319 (5.3)	—	21 (2.1)	—	712 (10.3)	93 (9.5)
8. Stanley	1,688 (3.8)	—	20 (2.0)	279 (10.4)	—	—
9. Panama	1,474 (3.3)	—	—	41 (1.5)	254 (3.7)	47 (4.8)
10. Agona	1,096 (2.7)	452 (3.1)	—	80 (3.0)	273 (3.9)	39 (4.0)
11.Paratyphi B var Java	—	1037 (7.1)	—	—	—	—
12. Hadar	—	1,357 (9.3)	21 (2.1)	263 (9.9)	439 (6.3)	—
13. Virchow	—	863 (5.9)	—	—	249 (3.6)	27 (2.7)
14. Schwarzengrund	—	565 (3.9)	—	—	—	—
15. Emek	—	359 (2.5)	—	—	—	—
16. Blockley	—	676 (4.6)	—	—	—	—
17. Amsterdam	—	368 (2.5)	—	103 (3.9)	—	—
18. Seftenberg	—	—	49 (4.9)	86 (3.2)	—	—
19. Lexington	—	—	47 (4.7)	—	—	35 (3.6)
20. Newport	—	—	—	100 (3.7)	—	—
21. Tennessee	—	—	—	77 (2.9)	—	—
22. Chester	—	—	—	171 (6.4)	—	—
23. London	—	—	—	—	—	22 (2.2)
Other	15,824 (35.9)	5,558 (38.2)	518 (51.4)	1,150 (43.1)	2,490 (35.9)	372 (37.8)
Total	**44,087**	**14,559**	**1,007**	**2,670**	**6,928**	**984**

2.5 LABORATORY IDENTIFICATION AND SEROTYPING

2.5.1 Conventional *Salmonella* isolation

In general, the detection of *Salmonella* consists of four successive steps, namely pre-enrichment, selective enrichment, plating out, and confirmation using media (Table 5).

Table 5: Principles and media for conventional culturing of *Salmonella* (modified from ISO 6579 (2002))

Steps	Commonly used components
1. Non-selective pre-enrichment	- Buffered Peptone Water (BPW)
2. Selective enrichment	- Rappaport Vasiliadis broth (RV) - Rappaport Vasiliadis Soya broth (RVS) - Modified Semi-solid Rappaport Vasiliadis (MSRV) - Selenite broth - Selenite Brilliant Green broth - Tetrathionate broth - Tetrathionate Brilliant Green broth
3. Plating on solid agars	- Brilliant Green agar (BGA) - Desoxy Cholate Citrate agar (DCA) - Rambach agar - Brilliant Green Phenol Red Lactose Sucrose (BPLS) - Xylose Lysine Deoxycholate (XLD) - Xylose-lysine-tergitol 4 (XLT4)
4. Verification	- Biochemistry
5. Further identification steps	- Serotyping

Non-selective pre-enrichment

Buffered Peptone Water (BPW) is the commonly used medium for *Salmonella* pre-enrichment. It is a non-selective medium that allows for the repair of cell damage and aids in the recovery of *Salmonella*. The recommended incubation temperature for pre-enrichment is 35-37°C for 18-24 hrs.

Selective enrichments

Various media are used for the selective enrichments of *Salmonella* prior to isolation. The temperatures and times for incubation are different, depending on the different types of media. The incubation temperature at 42°C, for 24-48 hrs, is recommended for *Salmonella* culture in Rappaport Vasiliadis (RV) broth, whereas in selective culturing in Tetrathionate (TT) broth, the recommended conditions are 37°C for 18-24 hrs for *Salmonellae*.

Plating solid agar

The selection of suitable nutrients in plating solid agar allows optimal growth of *Salmonellae*. At the same time, the surfactant, Tergitol-4/Sodiumtetradecylsulfate in Xylose-lysine-tergitol 4 (XLT4) agar for instance, largely inhibits the accompanying flora, so that the *Salmonella* organisms have the ability to form a unique, pure colony.

Salmonella colonies are presented as the different forms or colors after culture in various types of solid agars. For instance, colony appearance on Rambach agar is pink salmon, while red and translucent colonies grow on both the Brilliant Green Phenol Red Lactose Sucrose (BPLS) agar. Appearance on Xylose Lysine Deoxycholate (XLD) and Xylose-lysine-tergitol4 (XLT4) agar is black due to H_2S-production or mauve-gray with a central black, "bull's eye", on MCLB agar.

2.5.2 Biochemical identification

Based on key biochemical characteristics of *Salmonella* (Table 2), testing is performed in order to identify the particular characteristics of *Salmonella*. All biochemical tests are recommended to incubate at 37±1 °C for 18 to 24 hrs (WHO, 2001b).

Triple Sugar Iron agar (TSI) is used as a differential medium for gram-negative bacteria, based on their fermentation of lactose, dextrose and sucrose and on the production of hydrogen sulfide. Phenol red in the agar is used as an indicator when these carbohydrates are fermented. The medium changes color due to the pH. A change from red (original color) to yellow indicates the acid pH. A constant color of red indicates alkaline pH. The hydrogen sulfide produced by *Salmonella* reacts with an iron salt to yield black iron sulfide. Agar contained in TSI is the solidifier of the medium.

From Motile-Indole-Lysine (MIL) medium, *Salmonella* can be identified by its motilile characteristics. Lysine decarboxylase and Indole reaction are performed through an overnight incubation. A purple color due to Lysine reaction indicates the positive results for *Salmonella*, while a yellow/brown color indicates the negative results. In addition, Kovacs reagent is added to the medium for the detection of Indole reaction. The formation of a red ring indicates a positive- and a yellow-brown ring indicates a negative reaction.

Voges-Proskaur (VP) reaction is tested for Acetoin produced by *Salmonella*. Four drops of creatine solution, six drops of ethanolic solution of 1-naphthol and four drops of potassium hydroxide solution are added in the VP broth after incubation. A pink/red color indicates a positive reaction and a negative reaction is indicated by a colorless reaction.

Urea agar is used as a solid agar medium for the differentiation of enteric bacilli, which differentiates between *Salmonella* and urea-positive *Proteus* species or other urea-positive members of the *Enterobacteriaceae*. *Salmonella* cannot use urea agar, hence the color of test agar remains the same (yellow/brown color). More details for biochemical interpretation of *Salmonella* are shown in Table 6.

Table 6: Biochemical test for *Salmonella* (WHO, 2001b)

Medium	**Reactions/enzymes**	**Results**	
		Negative	**Positive**
TSI	Acid production from glucose	Butt red	Butt yellow
TSI	Acid production from lactose and/or sucrose	Surface red	Surface yellow
TSI	Gas production	No air bubble in butt	Air bubble in butt
TSI	Hydrogen sulfide production	No black colour	Black colour
Urea agar	Urease	Yellow	Rosa pink-deep cerise
LDC test	Lysine decarboxylase	A yellow/brown color	A purple color & yellow/brown color
ONPG	β-galactosidase	Remain colourless	Yellow
Voges-Prokauer	Acetoin production	Remain colourless	A pink/red colour
Indole	Indole production	Yellow ring	Red/pink ring

2.5.3 Serological testing (slide agglutination)

Besides biochemical identification, serological tests are used for *Salmonella* confirmation. Serotyping is based on the somatic (O) and flagella (H) antigens. The slide agglutination test is used for this purpose. Suspicious colonies could be roughly tested using commercial polyvalent antisera, I/II/III (Behring®) or antisera I/II (Sifin®). The test could be designed for further serotype identification, which could be performed by use of commercial antisera. A drop of the serum on the slide would be rubbed into a suspicious colony. The object would be moved by slight rotation as shown in Figure 5.

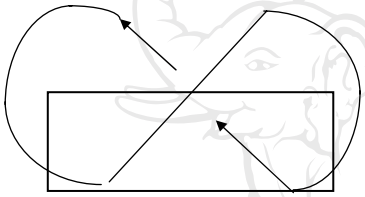

Figure 5: Slide movement

The test must firstly be performed with physiological NaCl-solution and material from the suspicious colony; in case of agglutination, the strain is untypable. Holding the object slide against a dark pad or a mirror could perform the result assessment. The positive result could be macroscopically detectable by the white agglutinated particles in the drop. Homogeneous, cloudy liquid indicates negative results of the agglutination (Figure 6).

Positive reaction Negative reaction

Figure 6: Slide agglutination: positive and negative reaction

2.6 *SALMONELLA* AND SALMONELLOSIS IN PIGS

The primary sources of *Salmonella* are the gastrointestinal tracts of humans and of domestic and wild animals. Consequently they are widespread in the natural environments including soil and water (Bell and Kyriakides, 2002). The ubiquitous *Salmonella* spp. can enter the pork production chain at any point, for example, via feeds at the farm production, at the slaughterhouse, in post-slaughter processing, or at the moment of food catering and preparation (Lo Fo Wong and Hald, 2000).

The epidemiology of salmonellosis in pigs must be regarded as two relatively separate problems: salmonellosis as a disease of pigs and *Salmonella* infection or contamination of pork carcasses and products.

The clinical signs of salmonellosis in pigs vary from case to case depending on serotype virulence, host resistance, and on the route and size of the infectious dose. However, the most common clinical signs may be the result of either septicemia caused by *S. choleraesuis* and/or enterocolitis mainly caused by *S. typhimurium* (Wilcock and Schwartz, 1999). Both forms of disease occur in intensively kept pigs, reared and weaned in less than five months, but may be seen occasionally in finishing pigs or adult breeding stock (Wilcock and Schwartz, 1999).

Infections in the affected adult pigs are unapparent or may be present with a wide range of severity, from mild fever to sudden death without diarrhea in case of septicemic salmonellosis. Watery diarrhea with a low mortality rate may be found in the case of enterocolitis. Most pigs recover completely but remain carriers and intermittent shedders for several months (Swanenburg *et al*., 2001, Hurd *et al*., 2002). The disease could be easily transmitted to others in the same herds via pig-to-pig contact and, most importantly, by the introduction of an infected carrier animal (Dickson *et al*., 2003).

2.7 DISTRIBUTION OF *SALMONELLA* IN PIGS AND PORK

Various studies have indicated that *Salmonellae* can be present either in pigs or pork at different contamination rates.

A study in the U.S.A., conducted by Morrow and Funk (2001), found out that *Salmonella* contamination was on 0-48% of pig carcasses after slaughtering. In the Netherlands, Swanenburg *et al*. (2001) revealed that 25% of carcass samples from slaughter pigs delivered from **sero-positive** herds were *Salmonella* positive, while 5% of such samples from **sero-negative** herds were positive. Based on these findings, Swanenburg *et al*. postulated that at least 5% *Salmonella* occurrence could be present during slaughter, even in those slaughter pigs that come from *Salmonella*-free herds.

Some studies performed in Italy and Belgium demonstrated different prevalence magnitudes and distributions of *Salmonella* in pigs and pork. In northern Italy, fecal material, carcass swabs, and tonsils were collected and examined for *Salmonella*. A prevalence of 36.7% was found in fecal content, 5.3% was found in tonsils, and 6.0% in carcasses. The serotypes found in that study were *S.* Derby, *S.* Bredeney, and *S.* Typhimurium (Bonardi *et al*., 2002). In Belgium, *Salmonella* was isolated from carcasses, colon contents, and mesenteric lymph nodes. The serotypes identified were *S*. Typhimurium and *S*. Derby (Botteldoorn *et al*., 2004)

In Chiang Mai, Thailand, the prevalence of *Salmonella* in pre-slaughter pigs increased from 69.5% at the farm level to 82.5% at two local slaughterhouses. This increased *Salmonella* contamination rate was considered to be due to stress before slaughtering and the hygienic aspects of slaughtering (Patchanee *et al*., 2002). The stress precipitates *Salmonella* shedding by pig carriers, which in turn increases the probability of contamination at the slaughter level.

2.8 SLAUGHTERING PROCESS AND *SALMONELLA* CONTAMINATION

Pig slaughtering is an open process with many opportunities for contamination with *Salmonella* and other potentially pathogenic bacteria, e.g. *Aeromonas*, *Campylobacter*, *Listeria*, *Staphylococcus* and *Yersinia* (Borch *et al*., 1996). Major risk factors for contamination during the slaughter process are feces, tonsil or cross-contamination from tools, machinery, workers or other slaughterhouse environments.

During slaughter, *Salmonella* may spread from infected to non-infected pigs. Scalding would be carried out either by hanging the pigs or in vats using stream or circular water. The scalding and dehairing procedures take 2-3 minutes and water temperature ranges from 61- 62 °C. That temperature can eliminate *Salmonella*, but not completely. In general, there are two forms of dehairing, combining with scalding in vats or separate scalding and then, dehairing. Whatever the scalding forms are, all could lead to *Salmonella* contamination on the carcasses, in which fecal material can easily spread on the surface. Flaming/singeing is usually conducted after dehairing but it is not sufficient to eliminate the bacterial contamination on the carcass surface. However, it has a significant effect in reducing the contamination level (Borch *et al*., 1996).

The workers or machinery normally perform further scalding and polishing. Both of them can contribute to the spread of bacteria that survive the previous procedures. Because of the difficulty of cleaning these machines during the slaughtering day, *Salmonella* may become established on scalding vats and the surfaces of the scrapers that may be sources of contamination.

When the intestines are removed, there is a risk of spilling their contents so that fecal matter is spread over the carcass. The tongue and the tonsils are removed along with the pluck set. Spread of pathogenic bacteria from the tonsils and pharynx to the carcass and the pluck must be expected.

Splitting of the carcasses is done using splitting saws. There is a risk that the machines will come into contact with the intestinal content or head, which can cause spread of pathogenic bacteria. Knives, cutters and other tools used are likely to become contaminated by *Salmonella* and other pathogenic bacteria that will subsequently be transferred to the carcasses.

During the operation following slaughtering, e.g. cutting, de-boning, and further processing, a further spread of pathogenic bacteria might be extensive.

According to the HACCP system, Borch *et al*. (1996) had specified the microbiological hazards in pig slaughterhouses at each step together with Control Points (CP) and Critical Control Points (CCP), hygienic aspects and preventive actions (Table 7).

Table 7: Hygienic aspects and preventive actions (Borch *et al*., 1996)

Process step	Hygienic aspect	Preventive action	CP/CCP
Lairage	Contamination between animals	Cleaning and disinfection	CP
Stunning			
Bleeding (killing)	Contamination from tools	Cleaning and disinfection	CP
Scalding	Reduction of bacterial levels	Time/Temperature	CP
Dehairing	Contamination from machine	Cleaning and disinfection	CP
Singeing/ flaming	Reduction of bacterial levels	Time/Temperature	CP
Polishing	Contamination from machine	Cleaning and disinfection	CP
Evisceration	Contamination from intestine, tongue, pharynx and tonsils Contamination from tools	Enclosure of rectum Working instruction Disinfection of tools	CCP
Splitting	Contamination via splitter/saw	Line-speed Water temperature	CP
Meat inspection	Contamination from inspection	Disinfection of tools	CCP
Chilling	Bacterial growth at improper temperature	Time/Temperature	CCP
Processing	Contamination from personnel and tools	Working instruction Tool disinfection	CCP