CHAPTER 2
LITERATURE REVIEW

2.1 Systematic classification of Enterobacteriaceae

Enterobacteriaceae are a large family of bacteria, including many pathogens, such as *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli, Klebsiella* spp., *Morganella morganii, Proteus* spp., *Providencia* spp., *Salmonella* spp., *Serratia* spp., *Shigella* spp., and *Yersinia* spp. (Engelkirk, 2007). They are the causative agents of such diseases as meningitis, bacillary dysentery, typhoid.

Genetic studies place the Enterobacteriaceae family among the Proteobacteria phylum, Gamma Proteobacteria class, and they are given their own order Enterobacteriales (Rahn, 1937).

The Enterobacteriaceae family is subdivided into eight tribes ***(Table 1)***. These tribes are further divided into genera, each with a number of species (World of Microbiology and Immunology 2003).

Table 1 Tribes and genera in the family of Enterobacteriaceae

Tribe I – Escherichieae	*Escherichia* *Shigella*
Tribe II – Edwardsieleae	*Edwardsiella*
Tribe III – Salmonellaceae	*Salmonella*
Tribe IV – Citrobactereae	*Citrobacter*
Tribe V – Klebsiellaceae	*Klebsiella* *Enterobacter* *Hafnia* *Serratia*
Tribe VI – Proteeae	*Morganella* *Proteus* *Providencia*
Tribe VII – Yersinieae	*Yersinia*
Tribe VIII – Erwinieae	*Erwinia*

2.2 Isolation and identification of Enterobacteriaceae

Many members of the Enterobacteriaceae family are a normal part of the gut flora found in the intestines of humans and many animals, while others are found in water or soil, or are parasites on a variety of different animals and plants. They are often isolated from fecal material (Allaby, 1998).

Members of the Enterobacteriaceae are rod-shaped, with a Gram-negative staining, and are typically 0.3 to 1.0 μm in width and 0.6 to 6.0 μm in length (Engelkirk, 2007). They are facultative anaerobes, fermenting sugars to produce acid and various other end products. Most also reduce nitrate to nitrite, Enterobacteriaceae generally lack cytochrome oxidase and are referred to as oxidase-negative. Most have many flagella used to move about, but a few genera are non-motile. They are non-spore forming, and except for the *Shigella dysenteriae* strains, they are catalase-positive (Mac Faddin, 2000).

Important serotypes can be differentiated by their O (Ohne Hauch) antigens constructed by lipopolysaccharides, H (Hauch) antigens from flagella which are proteins and K (Capsular) antigens from polysaccharides ***(Figure 1)***.

The cell wall or somatic antigen (called O antigens or O polysaccharide) attaches to the core polysaccharide. It consists of repeating oligosaccharide subunits made up of 3 to 5 sugars. A major antigenic determinant (antibody combining site) of the Gram-negative cell wall resides in the O polysaccharide. Variations in the sugar content of the O polysaccharide contribute to the wide variety of antigenic types of *Salmonella* and *E. coli* and presumably other strains of Gram-negative species. In *E. coli* and *Salmonella*, loss of the O antigens results in partial loss of virulence (Todar, 2008).

Flagellar antigen is constructed from proteins (Iino, 1977). Flagella are locomotive organelles, propagating bacteria by helical waves and by pushing bacterial bodies forward (Berg and Anderson, 1973; Larsen et al., 1974). The flagellar filament is composed of a single protein, flagellin (Mimori-Kiyosue et al., 1998). Molecular weights of flagellin are reported to range from 33,000 to 60,000 among different bacterial species (Joys and Rankis, 1972; Kanto et al., 1991). In some bacterial genera, such as *Salmonella*, many varieties of flagellar antigen have been identified, and the difference of amino acid composition among different *Salmonella* serotypes has been demonstrated (Iino, 1969).

Capsular antigen, composed from polysaccharides, is found on the cell surface of a broad range of bacterial species. The polysaccharide capsule often constitutes the outermost layer of the cell; as such, it may mediate direct interactions between the bacterium and its immediate environment and has been implicated as an important factor in the virulence of many animal pathogens (Cross, 1990; Moxon and Kroll, 1990; Roberts, 1995). Capsular polysaccharides are linked to the cell surface of the bacterium via covalent attachment to either phospholipid or lipid-A molecules (Whitfield and Valvano, 1993). Bacterial capsular polysaccharides play pivotal roles in mediating a number of biological processes, particularly in affecting microbe-host interactions (Roberts, 1996).

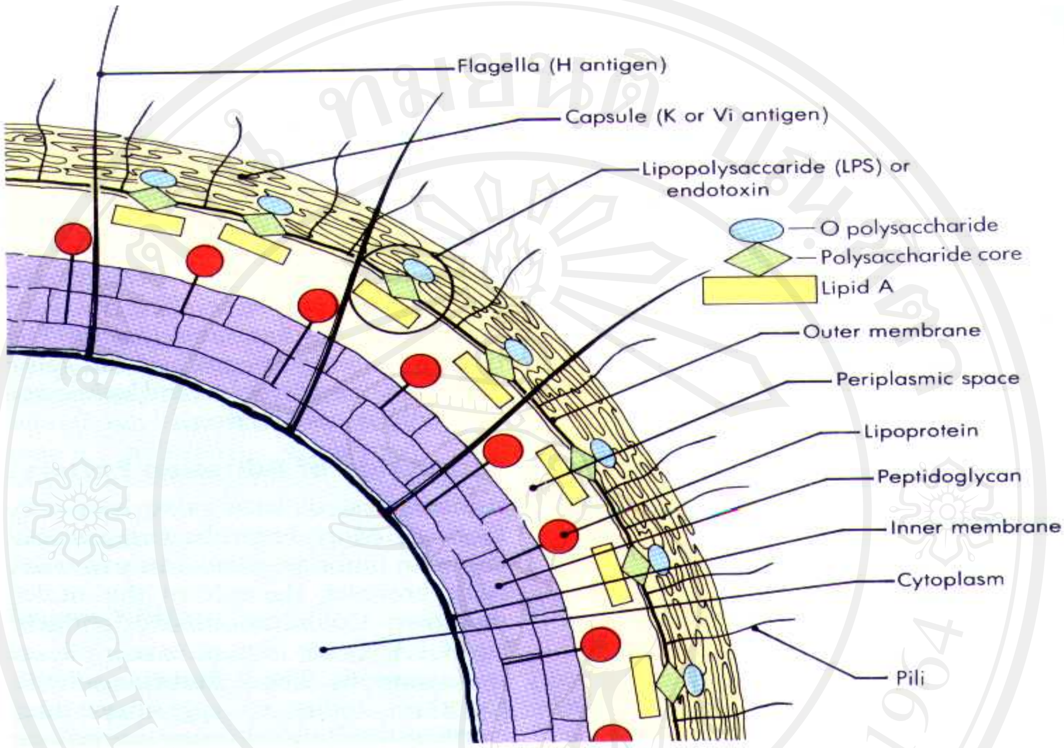

Figure 1 O, H and K antigens of Enterobacteriaceae (Source: CMU lecture 2008)

2.3 General characteristics of pig slaughterhouses

Basically, there are two kinds of pig slaughterhouses. Modern slaughterhouses have a complete slaughter line starting with stunning, then hoisting the unconscious animals onto an overhead rail, sticking and bleeding done by vacuum aspirators into blood collecting barrels, pre-washing the carcasses with portable water, scalding at 60 to 64^0C and dehairing by machine, singeing, polishing, evisceration, splitting the carcasses, chilling and further processing of the carcasses. The advantage of a modern slaughter line is minimization of cross-contamination during the process. Modern slaughterhouses are common in developed countries - they exist in some meat-exporting companies in developing countries as well ***(Figure 2)***.

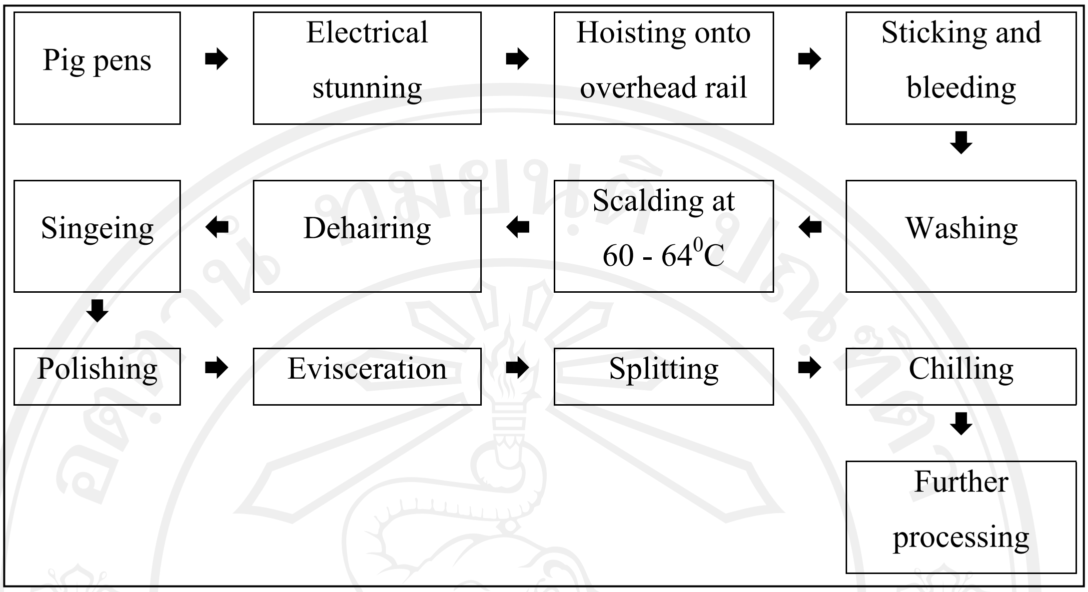

Figure 2 Procedures in modern pig slaughterhouses

So-called traditional slaughterhouses are popular in developing countries. Infrastructure is frequently old and obsolete, especially cold chain storage of the carcasses is rarely available. The process of slaughtering animals can be summarized as: stunning animals by head-hitting or even without stunning, bleeding with knives, scalding by pouring boiled water on carcasses or dipping carcasses into a hot water vat, dehairing with knives, pre-evisceration washing of carcasses with tap water, evisceration, then washing carcasses again, transporting carcasses or cut meat directly to markets without chilling. All stages of pig slaughtering are done on the ground (floor-dressing), products are dragged across the floor from one area to another for further processing. Parallel processes of slaughtering are done simultaneously by a large number of butchers and meat handlers. Butchers would use the same knife for bleedings, scalding and eviscerations. This kind of pig slaughterhouse, as indicated in Figure 3, does possess a high risk of cross-contamination.

Pens	➡	Stunning or not	➡	Bleeding	➡	Dehairing
						⬇
Markets	⬅	Second washing	⬅	Evisceration	⬅	First washing

Figure 3 Procedures in traditional pig slaughterhouses

The term “slaughter point” or slab implies that pig slaughtering is done in the house of meat traders or on pig farms. The slaughter area can be the house yard, nearby water wells or somewhere inside the pig farms. The area for slaughtering is normally narrow. The method to slaughter the animals is obviously floor-dressing. Amounts of pork supplied to domestic market outlets from slaughter points are often even much higher than from traditional slaughterhouses in some developing countries, where rural areas make up most of the country and small-scale pig production is dominant.

2.4 Pig slaughterhouses in Southeast Asian countries

In Thailand, most of the pig slaughterhouses are small (Simachaya, 2003), and only 18.68 % of the domestic slaughterhouses do possess the license from a veterinary authority (Kueylaw et al., 2007). In Lao PDR, the biggest slaughterhouse (named Dorn Du) has a capacity of only 60 – 80 pigs slaughtered per day (Inthavong, 2005). A multitude of small to medium-sized slaughterhouses, mostly for pigs, supply to cities in the Philippines, but many of them do not meet the standards (FAO 2008). In Phnom Penh, the capital of Cambodia, there are 4 pig slaughterhouses with a capacity of 100 – 600 slaughtered animals per day (Sovyra, 2005).

In Vietnam, except for one large-scale pig abattoir which was designed in the 1980s in Hai Phong city, and one with a continuous slaughter line of pigs (the VISSAN abattoir) in Ho Chi Minh city built in 1974, there are predominantly semi-line pig slaughtering systems with traditional slaughtering (FAO 2008).

2.5 Enterobacteriaceae in pig slaughterhouses

The contamination with microorganisms in pig slaughterhouses originates from the animals going to slaughter.

The first carcasses slaughtered in the beginning of a shift more or less "clean up" the slaughter equipment. Cross-contamination from infected herds to free herds during lairage, or cross-contamination between herds via slaughter equipment have been reported (Snijders, 1976). In herds with *Salmonella* carriers, the usual slaughter procedures and hygiene cannot avoid contamination of surfaces of carcasses or organs during processing. Consequently, different methods for decontamination are used and have been shown to be effective in reducing numbers of pathogenic bacteria on pork. Therefore, it can be useful to implement a decontamination step after slaughter (Swanenburg et al., 2001).

In developed countries, at large abattoirs where high hygienic standards are used for animals to enter slaughter, pathogenic microorganisms nevertheless are detected. For instance, in Italy, *Salmonella* were isolated from the intestinal content, tonsils, carcasses in 36.7%, 5.3%, 6.0% of cases, respectively (Bonardi et al., 2003). The prevalence of *Salmonella* in environmental samples in pig slaughterhouses in The Netherlands also was found to be high in drain water samples (61%) and on the carcass splitter (33%) (Swanenburg et al., 2001). In Norway, *Yersinia enterocolitica* was detected from 5.2% to 12.5% in submaxillary lymph nodes, and 8.3% in mesenteric lymph nodes, indicating that the compulsory procedures of incisions for meat inspection represent a cross-contamination risk for virulent *Yersinia* (Nesbakken et al., 2003). For Verotoxin-producing *Escherichia coli* in France, faecal carriage was 31%. Carcass contamination decreased from 46% to 15% with the slaughter process, whereas environmental contamination increased from 7% to 29% (Bouvet et al., 2002).

In developing countries, the microbial load on carcasses as well as in the environment often is higher. For example, in Thailand, the prevalence of pre-slaughter *Salmonella* infection at the slaughterhouse level was 82.5% (Patchanee, 2003). A longitudinal study of *Salmonella* in pigs showed that the incidences of *Salmonella* isolations at the farm and subsequently at the slaughterhouse were 7%, 50%, respectively (Padungtod et al., 2006). In Lao PDR, the frequency of *Salmonella* isolation from mesenteric lymph nodes of slaughter pigs was 53.2% (Inthavong et al., 2006).

In Vietnam, the prevalence of *Salmonella* in pigs was investigated by a survey of small abattoirs in the capital of Hanoi. Prevalence rates did exceed 50% in pig samples and 62% in water samples (Cedric et al., 2006). In a big commercial slaughterhouse in Hanoi, the prevalence of *Salmonella* in pig carcass swabs and in lymph nodes were 50% and 34.38%, respectively (Thai, 2007). In an epidemiological analysis for *Salmonella* spp. in specimens collected from a pork production chain in Hue city, seven serovars of *Salmonella* spp. were detected in retail pork, in slaughterhouse carcasses, on floors, weighing bowls, cooking boards and in tank water samples at rates of 32.8%, 15.5%, 47.4%, 38.1%, 28.6% and 16.7%, respectively (Takeshi et al., 2009).

Slaughterhouse sludge also was found heavily contaminated with Enterobacteriaceae (6.3–10.0 in $\log_{10}$ cfu/*gram* dry matter) (Fransen et al., 1996). Enterobacteriaceae counts on pig carcasses prior and post evisceration were 2.81 $\log_{10}$ cfu/cm^2 and 2.98 $\log_{10}$ cfu/cm^2, respectively (Inthavong et al., 2006).

2.6 Theory of cleaning and sanitation in slaughterhouses

In pig slaughterhouses, cleaning and sanitation of rooms, machines, and equipment are important to achieve or maintain acceptable hygienic conditions. Measures should be combined with processing hygiene, personal hygiene and environmental hygiene.

For cleaning, it is necessary to eliminate waste from surfaces with water or/and detergents. The most common types of waste in slaughterhouses are fat, protein, carbohydrates, and salts. They can be removed by water (for carbohydrates), addition of alkali (for fat, protein) or of acid (for salts).

Disinfection is the next step after cleaning to decrease microorganisms (but not usually bacterial spores) to a tolerable level. There are two groups of disinfection methods, which are non-chemical disinfection by heating or steam, and chemical disinfection with chlorine and chlorine-releasing compounds, quarternary ammonium compounds, amphotheric (ampholytic) compounds or peracetic acid.

An ideal cleaning and sanitation program can be achieved by following steps:

- Preparation: clearance of all items which may retard performance and preparation of solutions of detergents, disinfectants, cleaning materials and equipment
- Removal of solid waste
- Pre-rinsing with water: to moisten the soil and the surfaces and to loosen and collect a small amount of solid waste
- Application of detergent
- Rinsing with water: to decompose and suspend soil and detergent residues
- Disinfection: to destroy microorganisms by use of chemical or physical agents or processes
- Post-rinsing: to avoid residues of disinfectants on surfaces and to counteract corrosion
- Equipment maintenance: cleaning equipment should be maintained and stored in accordance with instructions from the suppliers and the management of the slaughterhouse

The frequency of cleaning and sanitation depends on the particular slaughterhouse management. In principle, a cleaning and sanitation program has to be carried out at least once a day after the working shift.

In fact, cleaning and sanitation in traditional pig slaughterhouses is carried out less often, as well as less efficient than in modern slaughterhouses where management, planning, and discipline are better.