CHAPTER II
REVIEW LITERATURES

1. General characteristics and pathogenesis of *E. coli*

E. coli is the type species of the genus *Escherichia*, gram-negative rod (Figure 1) size 1-2 μm within the family *Enterobacteriaceae* and the tribe *Escherichia*, classified as coliform group which contains motile peritrichous flagella. *E. coli* is normal flora in intestinal tract of human and animal gut. *E. coli* can be recovered easily from clinical specimens on general or selective media at 37°C under aerobic conditions. *E.coli* in stool is most often recovered on MacConkey or eosin methylene-blue agar, which selectively grow members of the *Enterobacteriaceae* and permit differentiation of enteric organisms on the basis of morphology, ferment glucose and lactose. And for fermentation of sorbitol exhibit glucuronidase. According to the modified Kauffman scheme, *E. coli* is serotyped on the basis of their O (somatic antigen), H (flagella antigen), and K (capsular antigen) surface antigen profiles. A total of 170 different O antigens, each defining a serogroup, are recognized currently. The presence of K antigens was determined originally by means of bacterial agglutination tests: an *E. coli* strain that was inagglutinable by O antiserum but became agglutinable when the culture was heated was considered to have a K antigen.

The discovery that several different molecular structures, including fimbriae, conferred the K phenotype led experts to suggest restructuring the K antigen designation to include only acidic polysaccharides. Proteinaceous fimbrial antigens have therefore been removed from the K series and have been given F designations. A specific combination of O and H antigens defines the "serotype" of an isolate. *E. coli* of specific serogroups can be associated reproducibly with certain clinical syndromes, but it is not in general the serologic antigens themselves that confer virulence. Rather, the serotypes and serogroups serve as readily identifiable chromosomal markers that correlate with specific virulent clones (Hayes et al., 1995; Nataro and Kaper, 1998).

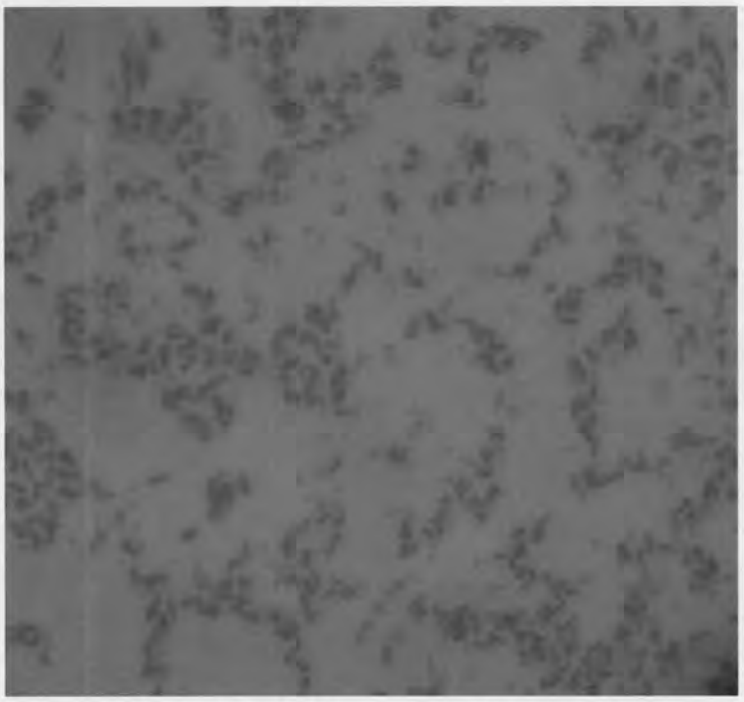

Figure 1: The rod-shaped bacterium Gram stain *E. coli*
(Courtesy of Mr. Datsakorn Kharuram and Ms. Boontharika Naknam, Kasetsart University, Bangkok, Thailand)

E. coli infective diseases such as Colibacillosis usually occurs during the sucking and post-weaning period. For example, Edema disease can affect sucking pigs, 1-2 days after birth and after weaning but not over than 6 weeks old. Affected pigs develop nervous symptoms or die suddenly. Edema is an excessive accumulation of fluid in body tissues. The morbidity rate usually is about 15%, but 50% or more of some groups are affected. Diarrheagenic symptoms may be found yellow-brownish creamy fecal matter, accumulation of excess fluid in the walls of the stomach and intestine and, in severe cases, exhibit vomiting. At the same time, one usually finds pigs with nervous symptoms, including staggering, head tilting, stumbling and falling, assuming a "dog sitting" position, lying on the sternum, or lying on the side and making continuous kicking movements. Edema disease-producing strains of *E. coli* produce one or more toxins that are absorbed from the intestine into the blood. These toxins damage blood vessels and affect blood pressure, which in turn causes fluid to leak from vessels and accumulate in many body tissues. This fluid accumulation is most important in the brain, where swelling can result in destruction of some brain tissue and, in many cases, death

of the animal. Enterotoxigenic *Escherichia coli* (ETEC)-associated diarrhea causes morbidity and mortality in young pigs. Porcine post-weaning diarrhea (PWD) caused by ETEC is economically one of the most important diseases for the swine industry. The key virulence factors of ETEC in diarrhea are enterotoxins and fimbrial adhesions (Zhang, 2007).

In human, diarrhea continues to be a major cause of mortality and morbidity in third world countries as well as a major symptomatic complaint in the primary care setting in the United States. *Escherichia coli* is the predominant facultative anaerobe of the human colonic flora. The organism typically colonizes the infant gastrointestinal tract within hours of life and thereafter *E. coli* and the host derive mutual benefit *E. coli* usually remains harmlessly confined to the intestinal lumen; however in the debilitated or immunosuppressed host or when gastrointestinal barriers are violated, even normal "nonpathogenic" strains of *E. coli* can cause infection. Moreover, even the most robust members of our species may be susceptible to infection by one of several highly adapted *E. coli* clones which have evolved the ability to cause a broad spectrum of human diseases. For patients presenting with fever, bloody stools, abdominal pain, tenesmus, or appearing toxic, a selective diagnostic workup is indicated.

Infections due to pathogenic *E. coli* may be limited to the mucosal surfaces or can disseminate throughout the body. Three general clinical syndromes result from infection with inherently pathogenic *E. coli* strains:

(i) urinary tract infection,

(ii) sepsis/meningitis and

(iii) enteric/diarrhea disease.

Base on the mechanisms of diarrheagenic disease development and focus on pathogens afflicting humans, six recognized categories of diarrheagenic *E. coli* are:

1. Enteropathogenic *E. coli* (EPEC),
2. Enterotoxigenic *E. coli* (ETEC),
3. Enteroinvasive *E. coli* (EIEC),
4. Enterohemorrhagic *E. coli* (EHEC),
5. Enteroaggregative *E. coli* (EAEC)

6. Diffusely adhearing *E. coli* (DAEC)

(Cheney and Wong, 1993, Nataro and Kaper, 1998).

Food-borne illness has become an increasing problem in the U.S. Each year, the Centers for Disease Control and Prevention (CDC) said, a strain of *Escherichia coli* O157:H7 has emerged that kills 50 to 100 people each year (Bette, 2001). EHEC O157:H7 has been recognized as the leading illness causes in the United States, Canada, Europe, Japan, Central and South Africa, The Middle East and Far East. The most frequent mode of transmission for EHEC O157:H7 infection is through consumption of contaminated food and water. Outbreak was traced to undercooked hamburgers from fast-food restaurant chain, associated with unchlorinated municipal water and swimming water (Besser et al., 1999).

2. Occurrence and epidemiology of antibiotic resistance in of *E. coli*

Food-producing animals, raised in modern intensive production systems frequently receive antibiotics for therapy, prevention and control of diseases. Furthermore, most food animals receive antibiotics as feed additives for growth promotion at sub therapeutic doses. This, often imprudent, use of antibiotics, promotes antibiotic resistant bacterial which can adversely affect positive clinical outcome as result of the decreased susceptibility of bacteria in the animal herds (Limpoka, 1997).

There is evidence use in animals selects for resistance in both pathogenic and commensal organisms. A commensal organism of interest, *E. coli* may serve as a reservoir of transferable antimicrobial resistance genetic elements (Wagner et al., 2008).

In Thailand, the antibiotics increasingly used for growth promotion have been tylosin, penicillin and gentamicin. These three drugs represented 51.5% of total administrated antibiotics on Thai commercial pig farms (Kanarat and Nijthavorn, 1998).

In a study of antibiotic sensitivity for *E. coli* isolated from pigs and submitted to KPS Veterinary Diagnostic Laboratory between 1995 and 1998 were resistant to penicillin G, streptomycin, tetracycline and sulfa-trimethoprim.

It has been observed that *E. coli* had tendency to become more resistant to norfloxacin and gentamicin (Jirawattnapong et al., 1999) than other microbes tested.

Pig cases submitted to National Institute of Animal Health (NIAH) during 1994 to 1996 were reviewed to monitor the antimicrobial drug resistance of 72 *E. coli* isolates to the 14 antimicrobial drugs used on the Thai farms. The greater resistance was observed to oxytetracycline, tetracycline, streptomycin, trimethoprim and sulfamethoxazole (91-99% of isolates). Resistance to ampicillin, neomycin, chloramphenicol and kanamycin were high among *E. coli* isolates (60% - 80%). Multidrug resistance, ranging from 2 to 14 antimicrobial drugs was 99%. The records indicated that twenty antimicrobial drugs were used on swine farms of which the predominant drugs were tylosin, penicillin and gentamicin. The present study revealed that the problem of antimicrobial drug resistant bacteria in pig is at a serious stage among *E.coli* isolates (Pathanasophon et al., 1998). The prevalence and antibiotic sensitivity of *E. coli* and hemolytic *E. coli* $F4^+$ and hemolytic *E. coli* $F4^-$ ($E/F4^+$, $E/F4^-$, $HE/F4^+$ and $HE/F4^-$) was investigated in 530, randomly collected, rectal swabs from pig herds in five areas of Thailand between the dates June 2000 and January 2001. The antibiotic susceptibility patterns of $E/F4^+$ from each area were similar, respectively increasing resistant to chloramphenicol, kanamycin, furazolidone, sulfamethoxazole, trimethoprim and neomycin. When a comparison was made between *E. coli* $F4^+$ and $F4^-$, the antibiotic sensitivity patterns were similar. However, some herds were found to be different from the majority, due to different antibiotic usage in each herd (Assavacheep et al., 2003).

Thirty-six of hemolytic *E. coli* were isolated from pigs with watery diarrhea and 27 *E. coli* and two hemolytic *E. coli* were isolated from healthy pigs. The MIC values and their resistant pattern of the two groups were compared. However, *E. coli* isolated from pig with diarrhea trend to be resistant to colistin and enrofloxacin than that of healthy pig. Interestingly, all isolates were resistant to amoxycillin, berberline, chlortetracycline, lincomycin, nalidixic acid, penicillin and tylosin (MIC_{90} > 512 µg/ml). For doxycycline, erythromycin, Halquinol, streptomycin, tetracycline and tiamulin had MIC values ranging from 0.125 to 512 µg/ml and had MIC_{50} in resistant level (Tripipat et al., 2005).

Previous studies have identified antimicrobial resistance in *E. coli* that have been continuously exposed to colistin for a period of 6 weeks. This *In vitro* model was

designed to determine the characteristics of antimicrobial resistance in *E. coli* isolated from pigs when exposed to low levels of colistin ($\leq$ 4 µg/ml). The results demonstrated a rise in MIC values following increasing colistin concentration and passage levels.

These results prove the induction of antimicrobial resistance in *E. coli* isolates following the administration of low-dose colistin. Therefore, antimicrobials given as feed additives should be reduced to minimal concentrations and exposure to avoid the development of antimicrobial resistance (Prapasarakul et al., 2005).

Sporadic cases of hemorrhagic colitis over the past 3 years have been referred to the Centers for Disease Control (CDC) for testing. *E. coli* isolated from patients with hemorrhagic colitis were susceptible to antimicrobial agents, ampicillin, trimethoprim-sulfamethoxazole, tetracycline and quinolones but resistant to erythromycin, metronidazole and vancomycin (Bopp et al., 1987).

Recent study has reported the emergence of community-acquired infections caused by Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* in Canada, France, Israel, Spain, Italy and the United Kingdom. In the prevalence and the susceptibility patterns study of ESBL-producing *E. coli* isolated from patients with community-acquired urinary tract infection (UTI) at Songklanagarind Hospital, Hat Yai, southern Thailand from July 2003 to January 2004. ESBL-producing *E. coli* were detected in six of 107 (6%) urine isolates. All of these isolates (6) were resistant to ampicillin, cefazolin and cefuroxime while 67%, 50% and 50% were resistant to gentamicin, cefotaxime and norfloxacin respectively. ESBL-non producing *E. coli* isolates were detected in 101 of 107 (94%) isolates in which 76%, 30%, 6%, 8%, 0% and 3% were resistant to ampicillin, norfloxacin, cefazolin, cefuroxime, cefotaxime and gentamicin respectively. The minimal inhibitory concentration 50 (MIC_{50}) of cefazolin, cefuroxime, cefotaxime, gentamicin and norfloxacin against ESBL-producing *E. coli* isolates was 32 to 256 times higher than in ESBL-non producing *E. coli* isolates. In addition, the community-acquired ESBL-producing *E. coli* isolates were resistant to several commonly used antimicrobials for the treatment of UTI.

From many previous studies, multi-drug resistant bacteria have emerged as a major clinical problem and can arise through a number of mechanisms (Walsh, 2003).

A variety of antimicrobial growth promoters (AGPs) are used in animal husbandry at sub therapeutic levels as previous studies have shown that the use of AGPs may select for resistant bacteria in the normal intestinal flora of animals. For instance, avoparcin, a member of the glycopeptide family and tylosin, a member of the macrolide family, have been widely used in Europe as AGPs. These two substances have consequently been associated with a high prevalence of vancomycin-resistant enterococci (VRE) and of macrolide-resistant enterococci in the intestinal flora of pigs.

The transmission of bacteria between animals and humans is not limited to agents of zoonotic diseases. Therefore, the selection of a reservoir of resistant opportunistic human pathogens and of possibly transmissible resistance determinants through the use of AGPs may have undesirable consequences for human health. The epidemiology of antimicrobial resistance in commensal bacteria from the animal reservoir and the effects of the use of AGPs should therefore be examined, not only at the local level but also, at a more global and international level (Boerlin et al., 2001).

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Antimicrobial resistance of animals *E. coli* can be directly related to veterinary and management uses of antimicrobial agents. In a study of faecal specimens of a representative number of animals from five farms in the United States, 555 isolates of *E. coli* were obtained. The incidence of multiple resistance in the *E. coli* isolates was higher in those herds exposed to continuous feeding of antimicrobial agents (84.8%) than in a herd not receiving antimicrobials (15.7%). The most common resistance configuration observed was the triple pattern of dihydrostreptomycin, sulfonamide and tetracycline (Mercer et al., 1971). A summary of these resistance mechanisms is presented in Figure 2 (Threfall et al., 1994).

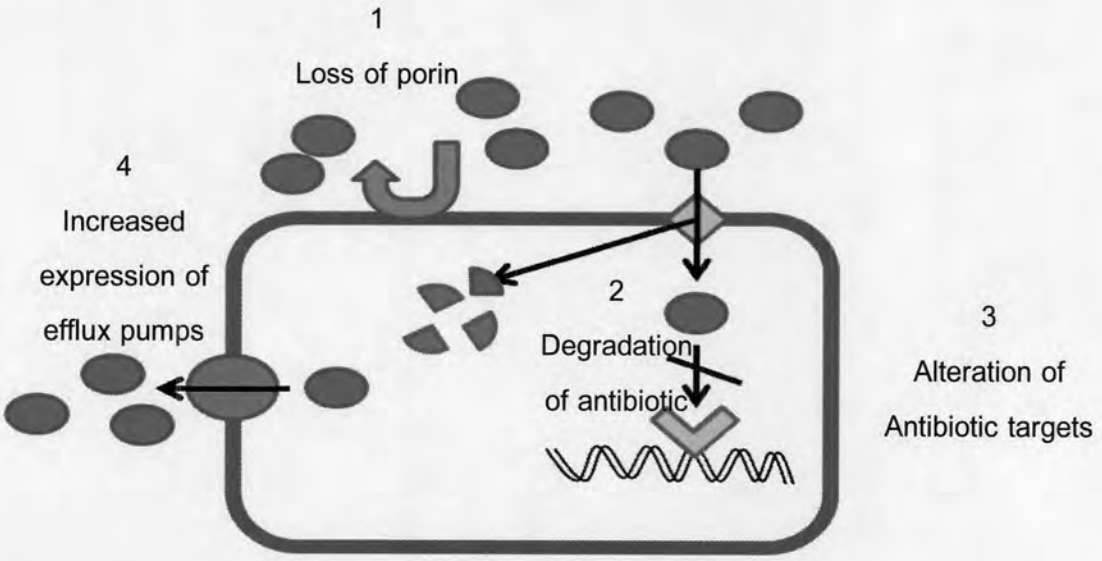

Figure 2: Mechanisms of antibiotic resistance in bacteria. In general, resistance mechanisms in bacteria include: (1) reduction of permeability by loss of porins in Gram-negative bacteria, (2) physical degradation of the antibiotic (3) alteration of the target for the antibiotic to prevent antibiotic binding, and (4) increased efflux of antibiotic resulting from over-expression of efflux pumps

3. Antibiotics cross-resistance of *E. coli*

The minimum inhibitory concentration against faecal *E.coli* isolated from pigs in southern part of Thailand during 1979-1983 was determined by using agar dilution

method. A total of 292 (96%) of 303 isolates from pigs used in this study were resistant to one or more drugs that had been used. The most common resistant pattern was multiple against streptomycin, oxytetracycline and sulfadimethoxine (Ekpanethanpong et al., 1990) Bacterial resistance to antibiotics can arise either from new mutations in the bacterial chromosome or through the acquisition of genes coding for resistance on plasmids which can be transferred from strain to strain and between bacterial species. This phenomenon is capable of promoting cross-resistance against antibiotics as well (Woodford et al., 1995).

Gentamicin is commonly used in pig production units. Importantly, our findings demonstrate that food-producing animals are an important reservoir for gentamicin-resistant enterococci and are, therefore, a source of such bacteria for humans. We found that when gentamicin-resistant genes are present in the resistant enterococci isolated from animals, they are also present in the enterococci isolated from food products of the same animal species The results of this study show a commonality of gentamicin resistant determinants and gentamicin resistant enterococcal isolates among humans, food and food-producing animals (Donabedian et al., 2003). Antibiotic resistant microorganisms have become a major worldwide health issue as many of the currently available antibiotics are becoming ineffective in the treatment of infectious diseases. Multi-drug resistance in bacteria typically occurs as a result of accumulation of multiple mutations and/or the horizontal transfer of resistance genes on mobile genetic elements such as plasmids and transposons along with novel genetic elements such as integrons and resistance islands from strain to strain and different strains. These resistant organisms can be transmitted to humans via the food chain.

A plasmid transferability study established successful plasmid transference, *in vivo* and *in vitro*, of resistance from 7 *Salmonella* donors to *E.coli* recipient strains (Threfall et al., 1994; Purushothaman and Venkatesan.,1993)

Demonstration of R plasmid in faecal *E. coli* isolated from a pig farm in Nakhorn Si Thammarat was studied. A total of 281 resistant *E. coli* strains isolated from pigs, was resistant to one or more antibiotics that had been used on the farm. Conjugative R plasmid were detected in 107 (38%) of pig isolates, including 23 (8%) thermo-sensitive

(ts) R plasmid from pig isolates. These results suggested that the high frequency of resistant *E. coli* carried conjugative R plasmid was present in faecal *E. coli* isolated from pigs (Ekpanethanpong et al., 1991).

Bacterial antimicrobial resistance is a growing problem as global dimension. Multidrug efflux systems (MES) have currently emerged as a predominant cause of antimicrobial resistance across a broad spectrum of, resistance of a single bacterial isolate to more than one structurally–unrelated antimicrobials called antimicrobial drug multi-drug resistance (MDR). Efflux-mediated resistance is promoted by the exclusion of agents from the systems, resulting in reduced intracellular concentration of drugs. MES occurs in many gram-positive and gram-negative bacteria, including medically important pathogens, therefore they are of significant concern. Expression of the systems is governed by genes located on either a chromosome or a plasmid (Chuanchuen and Herbert, 2004).

Antimicrobial resistance has been recognized as an emerging worldwide problem in both human and veterinary medicine with a seriously effect on public health and economics. A study found more than 21% of all *E. coli* isolates exhibited resistance to more than one antimicrobial agents. The ability of *E. coli* to transfer antimicrobial resistance is well known and the dissemination of antimicrobial resistant bacteria-acquired resistant genes between pathogenic animal bacteria and humans via the human food chain is of considerable concern. Vigilance in studying the antimicrobial resistance in food-producing animals is required to monitor the prevalence profiles of antimicrobial resistant bacteria which can possess a risk to the public health (Raida et al., 2005).

4. Prudent use of Halquinol in pigs

Biocides are molecules, generally of synthetic or semi-synthetic origin, that above certain critical concentrations and under defined conditions will kill living cells within a specified time. They may also be formulations containing germicidal, microbicidal or bactericidal agents such as antiseptics or disinfectants.

Antiseptics are formulated safe for application to living things while disinfectants are for application to inanimate surfaces. Antiseptics such as copper sulfate ($CuSO_4$) have been extensively used in food-producing animals to inhibit some of the bacterial populations in the intestinal tract and thereby improve the health and/or feed utilization of the animals.

Copper sulfate is commonly used as a feed supplement in food animal production, especially on pig farms. In Denmark the use is most pronounced in weaning pigs (<35 Kg) receiving concentrations of copper sulfate 165 ppm (Hasman and Aarestrup, 2002).

Haquinol is an antiseptic which is chemically classified in the group of Chloro-8-Hydroxyquinoline; CHQ (British Medical Journal,1961), Chlorohydroxyquinoline is prepared by the chlorination of 8-Hydroxyquinoline under controlled conditions and is effective for *Shigella dysenteriae* infection treatment (Approved Names, British Medical Journal, Aug 19, 1961).

Halquinol is a chemically standardized mixture of controlled and reproducible composition, containing the following three chlorinated quinoline in a ratio which provides optimal antimicrobial activity, 5,7-dichloro-8-hydroxyquinoline (65%), 5-chloro-8-hydroxyquinoline and 7-chloro-8-hydroxyquinoline as shown in Figure 3. (Weinberg, 1957; Fraser and Creanor, 1975).

8-Hydroxyquinoline (CAS 148-24-3; synonyms: quinoline-8-ol, 8-hydroxy benzopyridine, 8-quinolinol, oxyquinol, oxine) is a heterocyclic phenol which readily forms stable metal chelates. Its bacteriostatic and fungistatic action is believed due to the chelating of essential trace minerals on the surface of bacteria and fungi. Halquinol is used in human medicine as skin disinfectant and in hair-shampoos at low concentrations (EMEA, 1998). Halquinol is a broad spectrum antimicrobial agent with pronounced activity against; gram-negative and gram-positive bacteria, fungi and protozoa.

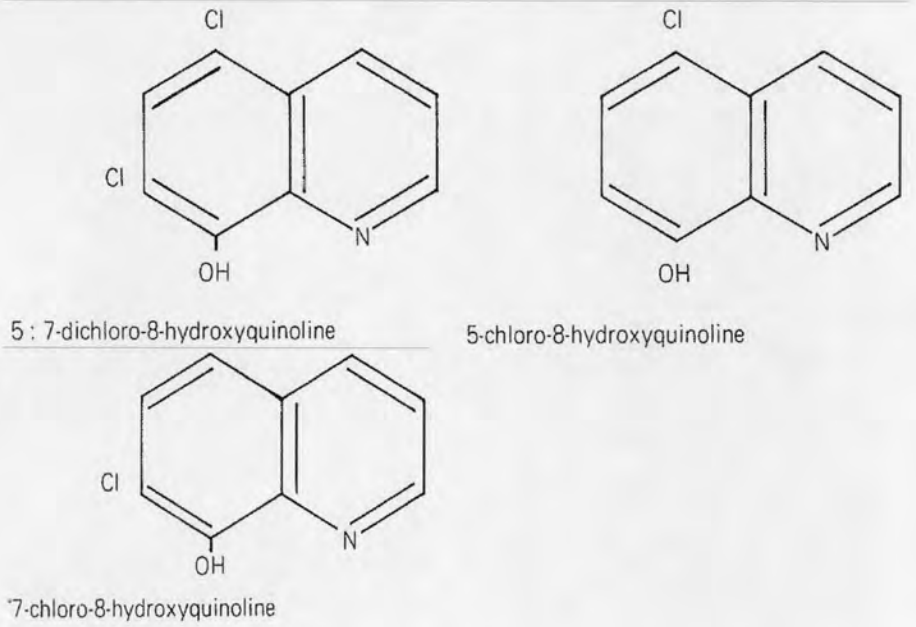

Figure 3: Halquinol in chemically standardized mixture of controlled and reproducible composition, containing the following three chlorinated quinoline in a ratio which provides optimal antimicrobial activity, 5,7-dichloro-8-hydroxyquinoline (65%), 5-chloro-8-hydroxyquinoline and 7-chloro-8-hydroxyquinoline. (Courtesy of Novartis (Thailand) Ltd., Animal Health Business Unit).

Although Halquinol has been used for more than fifty years as a metal reagent and as an antiseptic active in suppressing the action of the drugs, it may be blocking that the compound might be biologically active by virtue of its chelating ability, the stability constants of Halquinol, 8-hydroxyquinoline (oxine) as chelating agent binding with metallic bivalent cation, Mn^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+} and Cu^{2+} as shown in figure 4 (Weinberg , 1957).

RNA polymerase is metaloenzyme which requires bivalent cations, Mn^{2+}, $Mg^{2+}$and Zn^{2+} for its normal function. Demonstration of the mode of action of an inhibitor in a simplified system *in vitro* provides a strong indication, though not complete proof, of the way it acts in the intact cell.

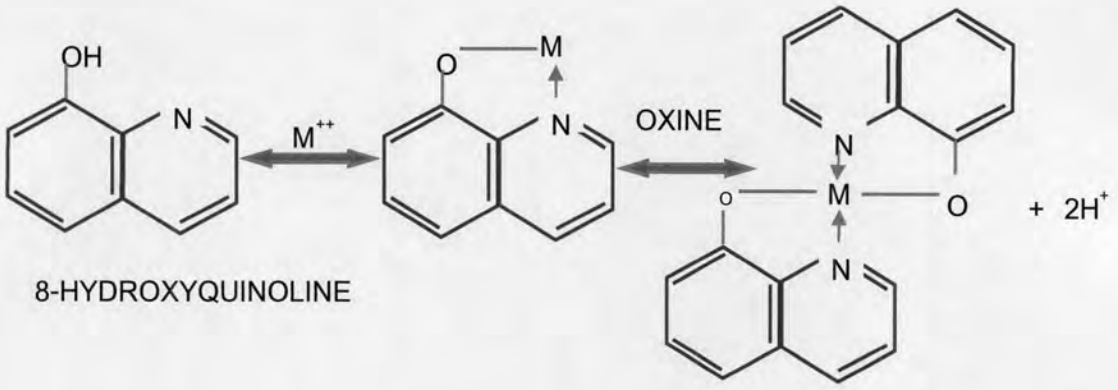

Figure 4: The stability constants of 8-hydroxyquinoline (oxine) molecular structure and affinity for metallic bivalent cations (M^{++}). (Courtesy of Novartis (Thailand) Ltd., Animal Health Business Unit)

Halquinol is an antiseptic, widely used for killing *E. coli* and *Salmonella* since it has never been resistant report against both organism from previous study (Foster and Duggan, 1974).

A study of fluctuations in *E. coli* sensitivity patterns was conducted using pigs fed a Halquinol - supplemented diet containing 120 ppm Halquinol over a 6-week period. *E. coli* isolated from these pigs had not developed any resistance to this addition to their medicated diet. Sensitivity patterns of the *E. coli* isolates to eight antimicrobial substances fluctuated slightly during the test period (but no more than a control group), but did not significantly alter. However, the patterns did change significantly when for 17 days after the completion of the Halquinol trial the pigs were fed a normal commercial ration medicated with a commonly used feed additive containing chlortetracycline- hydrochloride, procaine penicillin and sulphadimidine (Cosgrove et al., 19881).

Several enzymes and cell functions in *E. coli* require trace metals and chelating ions. One study model assumes that, *in vivo,* chelators bind to (and inactivate) the Zn^{2+}-RNA polymerase complex resulting in an inactivated complex. Alternatively, chelators

could indirectly inhibit RNA synthesis by competing for a trace metal that is required for RNA synthesis (Collins et al., 1979; Fraser and Creanor, 1975).

Halquinol was introduced to Thai pig herds many years ago and is registered by the Thai Food and Drug Administration for the prevention and treatment of scour in weaning and fattening pigs which may be infected with *E. coli* and *Salmonella* . Halquinol is added to finished feed to provide a concentration level of 120 ppm or 600 ppm and given to nursery pigs consecutively for 7 or 7-10 days respectively

In 2007, the Livestock Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University, Nakorn Pathom conducted a Halquinol susceptibility test. The findings showed that 92% *E. coli* and 100% haemolytic *E. coli* were susceptible to Halquinol (Khanda, 2007). Thirty-six of haemolytic *E. coli* were isolated from pigs with watery diarrhea (Group 1) and 27 *E. coli* and two haemolytic *E. coli* were isolated from healthy pigs (Group 2) age 4-10 weeks old in Nakorn Pathom, Ratchaburi, Chon Buri and Chachengsao. The MIC values and the resistant pattern of the two groups were also compared. The MIC values to enrofloxacin, colistin, and Halquinol of *E. coli* in the pigs with diarrhoea were higher than those from the healthy pigs. Interestingly, all isolates were resistant to amoxycillin, berberline, chlortetracycline, lincomycin, nalidixic acid, penicillin and tylosin (MIC_{90} > 512 µg/ml). For doxycycline, erythromycin, Halquinol, streptomycin, tetracycline and tiamulin the MIC values ranged from 0.125 to 512 µg/ml and had MIC_{50} in resistance.

The MICs of Halquinol, colistin and enrofloxacin for isolates from the watery diarrhoea pigs were double the values for isolated from healthy pigs. This was because the ill pigs had already been treated with three antimicrobial beforehand (Tripipat et al., 2005).

A newly discovered on antiseptic, copper sulfate was cross-resistance to avilamycin, bacitracin, chloramphenicol, erythromycin, gentamicin, kanamycin, penicillin, quinupristin-dalfopristin, streptomycin, tetracycline, vancomycin and virginiamycin *Enterococcus faecium* conferred to gene, designated *tcrB*, which is located on a conjugative plasmid conferring acquired copper resistance in *E. faecium* was identified in an isolate from a pig. Furthermore, the *tcrB* gene is genetically linked

to genes encoding resistance to macrolides [*erm(B)*] and glycopeptides (*vanA*) in the plasmids originating from pig isolates. Copper resistance, and therefore the presence of the *tcrB* gene, was strongly correlated to macrolide and glycopeptide resistance in isolates from pigs and the *tcrB* gene was shown to be located on the same conjugative plasmid as the genes responsible for resistance to these two antimicrobial agents. The frequent occurrence of this new copper resistance gene in isolates from pigs, where copper sulfate is being used in large amounts as feed additive suggests that the use of copper has selected for resistance (Hasman and Aarestrup, 2002). However, there has never been report and published on cross-resistance between an antiseptic Halquinol and antibiotics in *E. coli* isolated from pig.