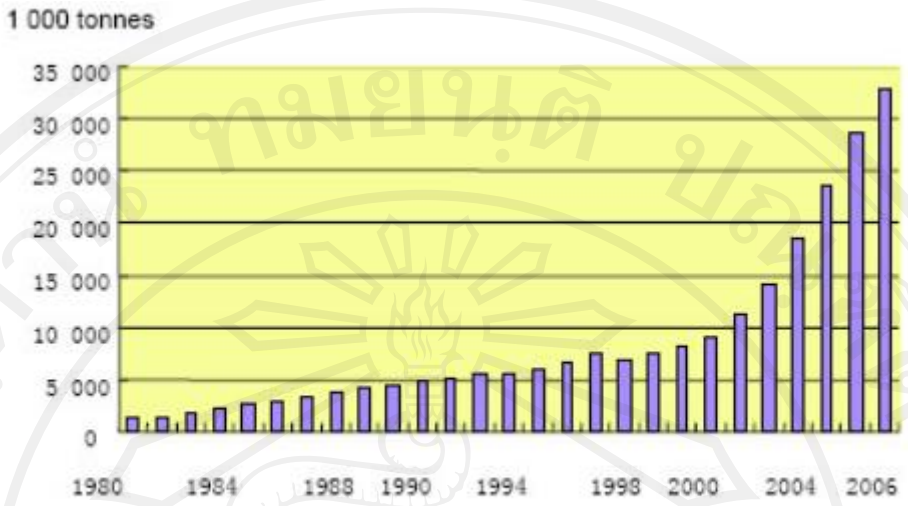
CHAPTER II

LITERATURE REVIEW

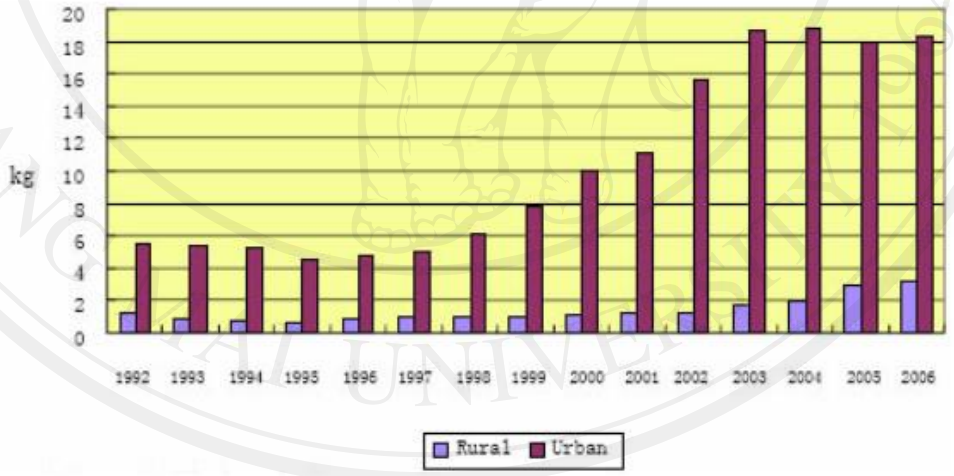
2.1 Dairy farming industry in China

As the most populous country, China represents one-fifth of the global population, but the total dairy product output accounts only 4% of world production (Hu, 2009). There are huge demands on dairy products increasing also dairy industry growing. In China, dairy product production jumped dramatically since the last decades (Figure 1) (Gao, 2006, CDSY, 2003-2009, Hu, 2009, Ma et al., 2012). In 1980, total milk output was 1.4 million tonnes; by 2006, it had soared to 33 million tonnes, with per capita consumption of milk rising from 1 kg to 25 kg over the same period (Figure 2) (Hu, 2009). Nevertheless, the per capita consumption of dairy products is lower than the global average level, especially in rural households (Figure 2). However, China's net imports of dairy products have also expanded at a growth rate in excess of 30% during year 2003 to 2008 (Ma et al., 2012).



Source: *Chinese statistical yearbook*

Figure 1. Milk output in China, 1980–2006(adopted (Hu, 2009))



Source: *Chinese statistical yearbook*

Figure 2. Per capita consumption of dairy products in rural and urban households (adopted(Hu, 2009))

According to the Dairy Association of China, there were approximately 1.37 million dairy cattle farms in 2002. Of them, 83% owned 1–5 cows (Hu, 2009). In accordance with a former report, 4 levels of dairy farms were defined. For the dairy farms whose herd is ≤10 heads defined as backyard dairy farm, more than 10 and less

than 50 defined as small dairy farm, more than 50 and less than 500 defined as medium dairy farm and more than 500 heads defined as large dairy farm (Ma et al., 2012). However, China's dairy farm structure has experienced fundamental changes in both production structure and farm sizes since 2003. As a result of this change, the number of backyard dairy farms has dramatically declined and the herd numbers of larger dairy farms have increased largely. Particularly, the share of dairy cow numbers from small dairy farms has risen by 18.8%, from 22.9% in 2003 to 27.2 % in 2008; the share of dairy cow numbers from medium dairy farms has risen by 22.2%, from 16.2% in 2003 to 19.8 % in 2008; but the share of dairy cow numbers from large dairy farms has risen by 80.8%, from 5.6% in 2003 to 10.1 % in 2008 (CDSY, 2003-2009, Hu, 2009, Ma et al., 2012).

2.2 Antimicrobials and antimicrobial resistance

Antimicrobials are substances that kill or inhibit the growth of microorganisms such as bacteria, fungi or protozoans. Antimicrobial agents are substances of natural, semi-synthetic, or synthetic origin that at in vivo concentrations kill or inhibit the growth of micro-organisms by interacting with a specific target but cause little or no damage to the host (FAO/WHO/OIE., 2008). Antimicrobial drugs fight against microbial by either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Antimicrobial drugs play a very important role for the disease controlling, preventing and eliminating, especially for the bacterial diseases, no matter for human health or animal health.

Use of antimicrobials in animal production is done mainly for the purpose of preventing and controlling infections and for growth promotion (Schwarz and Chaslus-Dancla, 2001, Aarestrup, 2005). Although since 2006, European Union (EU) banned the use of antimicrobials as growth promotion (EU, 2005), some antimicrobials still be used as growth promoter in other area. To prevent and control infections: Antimicrobials have been widely used for the treatment of animals suffering from bacterial infections. In food producing animals these antimicrobial agents have been used for therapeutic, prophylactic and metaphylactic reasons

(Schwarz and Chaslus-Dancla, 2001, Schwarz et al., 2001, Guardabassi and Kruse, 2009).

Antimicrobial resistance is a resistance of a microorganism to an antimicrobial agent to which it was previously sensitive. Resistant organisms (they include bacteria, viruses and some parasites) are able to withstand attack by antimicrobial medicines, such as antibiotics, antivirals, and antimalarials, so that standard treatments become ineffective and infections persist and may spread to others (WHO, 2012). AMR is a consequence of the use, particularly the misuse, of antimicrobial medicines and develops when a microorganism mutates or acquires a resistance gene (WHO, 2012). Study founded that AMR would not develop in animals if antimicrobials were not used in animals (McKellar, 1998).

Many bacterial diseases of animals are potentially fatal or cause pain and distress which dramatically affects the animal husbandry industry and the animal welfare. Antimicrobial chemotherapy is important for treating and in some cases preventing bacterial diseases, although they may be controlled by eradication, maintenance of animals of good health status, vaccination, or good hygiene (McKellar, 1998). Antimicrobials have been widely used for a long history in human medicine and animal industry since they are discovered. Nevertheless, there are certain risks when use antimicrobials in humans and animals. Any use of antibiotics in human and veterinary medicine may lead to the development of resistant microorganisms (Kreausukon, 2011). World Health Organization (WHO, 2011) stated “People infected with resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness and greater risk of death”.

Appropriate use of antimicrobials will cure some sick animals and speed the recovery of others, and may improve the welfare of treated animals and reduce the spread of infection to other animals or, in the case of zoonotic disease, to humans (McKellar, 1998). The challenge is to use antimicrobials wisely, minimizing the risk of resistance. Lowering the number of pathogens resistant to antibiotics is a global concern in the animal livestock industry(Aarestrup, 2004).

2.3 Mechanisms of Action of Antimicrobial Agents

The mechanisms of different antimicrobial agents act in different ways. However, the mechanism of action of antimicrobial agents can be categorized based on the structure of the bacteria or the function that is affected by the agents. These include generally the following 5 groups (Rollins and Joseph, 2000, Byarugaba, 2009):

1) Inhibition of the cell wall synthesis (most common mechanism).
2) Inhibition of protein synthesis (Translation) or ribosome function (second largest class).
3) Inhibition of nucleic acid synthesis.
4) Inhibition of folate metabolism.
5) Inhibition of cell membrane function.

The Mechanism of Action of Antimicrobial Agents is summarized as Table 1 (Anonymous).

Table 1. Specific Mechanism of Action of Antimicrobial Agents

Mechanism of Action		Antimicrobials
Inhibition of Cell Wall Synthesis	Inhibit cross-linking of peptidoglycan by inactivating transpeptidases (PBPs)	Penicillins, Cephalosporins, Aztreonam,Imipenem
	Bind to terminal D-ala-D-ala & prevent incorporation into growing peptidoglycan	Vancomycin, Teicoplanin
	Inhibition of transglycosylation	Oritavancin, Teicoplanin, lipophilic vancomycin analogs, ramiplanin
	Inhibit dephosphorylation of phospholipid carrier in peptidoglycan structure	Bacitracin
	Prevents incorporation of D-alanine into peptidoglycan	Cycloserine
Inhibition of Protein Synthesis	Bind to 50S ribosomal subunit	Macrolides, Chloramphenicol, Clindamycin
	Bind to 30S ribosomal subunit	Aminoglycosides, Tetracyclines
Inhibition of Nucleic acid synthesis	Inhibition of DNA gyrase & topoisomerase	Quinolones
	Inhibition of nucleic acid biosynthesis	Flucytosine, Griseofulvin
	Inhibition of mRNA synthesis	Rifampin, Rifabutin, Rifapentine
Alteration of Cell Membrane Function	Inhibition of ergosterol biosynthesis	Imidazole antifungals
	Bind to membrane sterols	Polymyxins, Amphotericin B, Nystatin
Alteration of Cell Metabolism	Inhibition of tetrahydrofolic acid production (cofactor for nucleotide synthesis)	Sulfonamides, Trimethoprim, Trimetrexate Pyrimethamine
	Inhibition of mycolic acid biosynthesis	Isoniazid
	Interference with ubiquinone biosynthesis & cell respiration	Atovaquone
	Bind to macromolecules	Metronidazole, Nitrofurantoin

2.4 Mechanism of antimicrobial resistance

There are several types of genetic structures in bacteria that may confer resistance such as chromosomes, mobile elements (including plasmids), and transposons. The ability to exchange genetic material has resulted in the development of resistance to antimicrobials by many animal bacteria (McKellar, 1998). In principle, bacteria have access to a large selection of resistance genes scattered throughout the bacterial kingdom and mechanisms have evolved to reassort these genes, moving them genetically from one DNA molecule to another and physically from one bacterial cell to another (Bennett, 1999). Antimicrobial resistance mechanisms include intrinsic (or innate) to the micro-organism and acquired through forced mutations or through the acquisition of mobile genetic elements (Poole, 2002, SCENIHR, 2009, Byarugaba, 2009). Transformation, transduction, and conjugation are the three possible approaches by which plasmids may migrate from one bacterium to another (Bennett, 1999, Tenover, 2006). Antimicrobial resistance may be developed by vertical or horizontal evolution. Resistance that develops due to chromosomal mutation and selection is termed vertical evolution, and bacteria may also develop resistance by acquiring from other bacteria the genetic information encoding for resistance termed horizontal evolution, and may occur between strains of the same species or between different bacteria species or genera (Kreausukon, 2011). Previous studies indicated that antimicrobial use is the single most important factor responsible for increased antimicrobial resistance (Aarestrup et al., 2001, Byarugaba, 2004). Generally, microbe resistance to antimicrobial agents evolves by the following mechanisms (White and McDermott, 2001, Byarugaba, 2009, Kreausukon, 2011):

- the presence of an enzyme that inactivates the antimicrobial agent
- the presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent
- a mutation in the antimicrobial agent's target, which reduces the binding of the antimicrobial agent

- post-transcriptional or post-translational modification of the antimicrobial agent's target, which reduces binding of the antimicrobial agent
- reduced uptake of the antimicrobial agent
- active efflux of the antimicrobial agent
- overproduction of the target of the antimicrobial agent
- expression or suppression of a gene in vivo in contrast to the situation in vitro
- previously unrecognized mechanisms

2.5 The ESBLs

Extended-Spectrum β-Lactamases (ESBLs) are enzymes that can be produced by bacteria making them resistant to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics - which are the most widely used antimicrobials in curing human or animals, and which are inhibited by β-lactamase inhibitors such as clavulanic acid (Paterson and Bonomo, 2005). Among these ESBL-producing bacteria, most of them are multidrug resistant (Hunter et al., 2010).

ESBL-producing bacteria were first discovered in Western Europe in mid 1980s and subsequently in the US in the late 1980s (Nathisuwan et al., 2001, Chaudhary and Aggarwal, 2004). ESBLs can be found in a variety of *Entrerobacteriaceae* species, the majority of ESBL producing strains are *K. pneumoniae, K. oxytoca* and *E. coli* (Chaudhary and Aggarwal, 2004).

There are a number of mutations of ESBLs that have the ability to hydrolyze expanded-spectrum β-lactam antibiotics (Bradford, 2001). The majority of ESBLs are derived from the widespread broad-spectrum β-lactamases TEM-1 and SHV-1 and new class of ESBLs including the CTX-M and OXA-type enzymes as well as novel, unrelated β-lactamases have emerged (Bradford, 2001) and the CTX-M enzymes have been widely detected among *Escherichia coli* (*E. coli*) bacteria.

ESBL-producing *E. coli* may acquire their resistance from farm animals, which are increasingly treated with modern cephalosporin and these ESBL-producing strains

are then transmitted to humans via food or materials contaminated by ESBLs, where the bacteria can persist for a period of time and then cause infection, or transmit their ESBLs resistance genes to other *E. coli* in the human gut, which subsequently infect humans (Nunan and Young, 2012).

2.6 ESBL-producing *E. coli* in humans and in farm animals

The scope of the farm contribution to the ESBL-producing *E. coli* problem is now a major area of investigation. There is very strong evidence that farm animals are important reservoirs of human ESBL *E.coli*, or of their resistance genes (Nunan and Young, 2012). In the UK, the main epidemic strain, which accounts for about 45% of all infections, appears to be circulating among humans, although a farm-animal link cannot yet be ruled out entirely because it has now been found once in cattle (Snow et al., 2011).

However, the most common CTX-M resistance genes found in humans, CTX-M-14 and CTX-M-15, are also the most common CTX-M genes found in cattle. These resistance genes are on 'plasmids', small loops of DNA which can often readily be transmitted between bacteria. The same CTX-M plasmids have been found in ESBL *E. coli* from cattle and humans in the UK (Nunan and Young, 2012).

The first case of ESBLs *E. coli* in British farm animals was found by the Veterinary Laboratories Agency in the autumn of 2004, in scouring calves from a dairy farm in Wales. Testing showed that 56% (27 of 48) calves and 3% (2 of 60) cows were positive for the bacteria (Teale et al., 2005) .

Further research carried out in 2008/2009 and first published in 2010, found that 37.5% of randomly selected dairy herds in the North-West of England were found to have CTX-M ESBLs, and farms which had used modern cephalosporin in the previous year were four times more likely to be positive. A study carried out on a single positive farm showed that nearly all the calves were positive at 2 days of age,

but that there appeared to be a window between 53 and 177 days where the probability of a calf testing positive fell from 90% to 10% (Snow et al., 2011).

In China, the trends in the prevalence of the predominant antimicrobial resistant bacteria in China from 2000 to 2009 (Figure 3) showed that the prevalence of ESBL producing *E. coli* increased quickly.

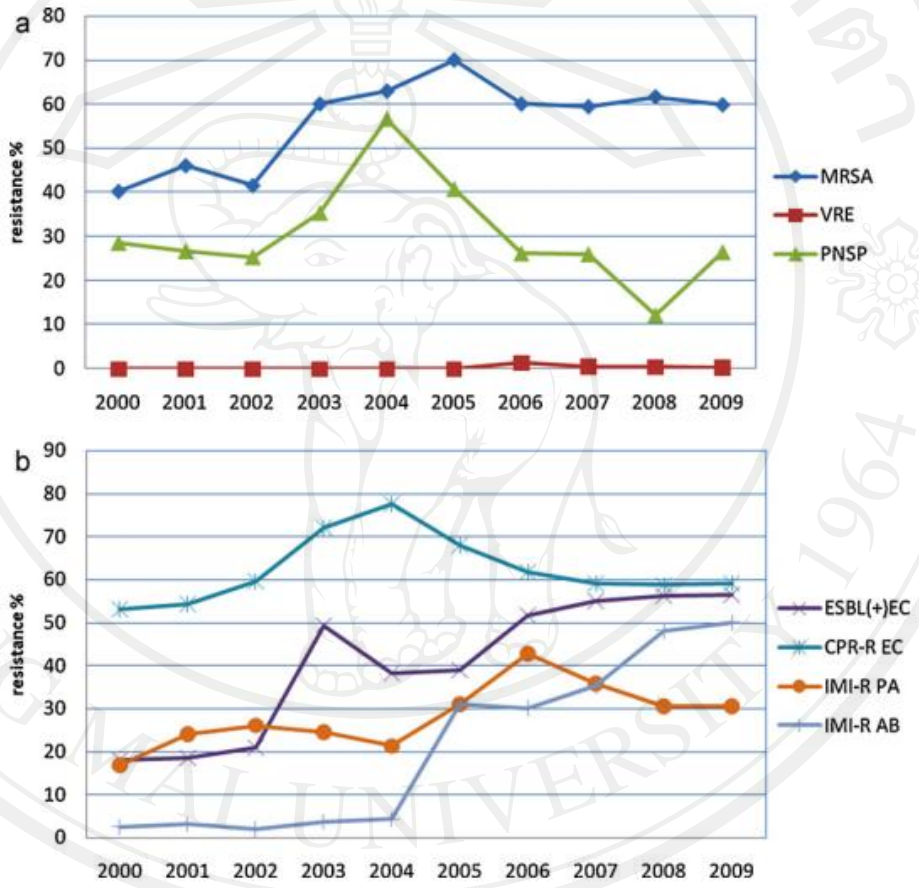


Figure 3. Trends in the prevalence of the predominant antimicrobial resistant bacteria in China from 2000 to 2009 (Xiao et al., 2011). (a) Gram-positive bacteria; (b) Gram-negative bacteria. Note: MRSA: methicillin-resistant S. aureus; VRE: vancomycin-resistant enterococcus; PNSP: penicillin non-susceptible S. pneumoniae; ESBL (+) EC: extended-spectrum-lactamase-producing *E. coli*; CPR-REC: ciprofloxacin-resistant *E. coli*; IMI-R PA: imipenem-resistant P. aeruginosa; IMI-R AB: imipenem-resistant A. baumannii.

In veterinary section, previous study carried out in pig farms in Sichuan Province, China, between August 2002 and February 2007 demonstrated the prevalence of ESBL-producing *E. coli* increased dramatically from 2.2% to 10.7% during this period, and this increase appeared mostly related to dissemination of CTX-M-type ESBLs among *E. coli* isolates (Tian et al., 2009).

2.7 Resistance of *E. coli* to antimicrobials

Escherichia coli (*E. coli*) was first reported as *Bacterium coli commune* by the German pediatrician and bacteriologist Theodor Escherich, who observed a rod-shaped bacterium in the fecal flora of infants (Waghela, 2004). *E. coli* belongs to the family of *Enterobacteriaceae* and is common in the gastrointestinal microflora of warm-blooded animals (Meng and Schroeder, 2007). *Enterobacteriaceae* is composed of about 30 genera and 100 species, including some natural pathogens such as *Salmonella* spp., *Yersinia* spp., *Shigella* spp., and others like *Escherichia*, *Klebsiella*, *Citrobacter*, *Proteus*, and *Klebsiella*, which are usually commensals (Waghela, 2004). *E. coli* bacteria normally live in the intestines of people and animals. As commensal bacterial, most *E. coli* are harmless and actually are an important part of a healthy human and animal intestinal tract. However, only a few of the several hundred strains of *E. coli* that inhabit the gut may be capable of causing disease, meaning they can cause illness, either diarrhea or other serious illnesses. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons (CDC, 2013). Pathogenic strains are differentiated from nonpathogenic strains in that they have virulence genes located either extrachromosomally or chromsomally (Waghela, 2004).

E. coli is defined as a gram-negative, fermentative, non-spore forming, rod-shaped (1.1-1.5 μm x 2.0-6.0 μm) microorganism, which can grow in aerobic or anaerobic conditions (Bell and Kyriakides, 2002). *E. coli* strains may be either motile, by means of flagella, or non-motile. Some biotype characteristics of *E. coli* are shown in Table 2. One reason that *E. coli* is a good indicator of other bacteria is that they are capable of surviving in poor media such as water, floors, and inanimate surfaces

(Martin et al., 2005). *E. coli* is transmitted between humans, animals, the environment, and food, through air, water, soil, and equipment routes (Meng and Schroeder, 2007). One unique feature of *E. coli* is its ability to acquire and disseminate resistance to other pathogenic or zoonotic bacteria (Martin et al., 2005).

Although *E. coli* is mainly a gut commensal in human and other warm blooded hosts, there are also pathogenic strains and strains with resistance to widely-used antimicrobials, which have the potential to transfer their resistance to other organisms, including pathogens (Mead, 2007). Free-living microorganisms provide a large reservoir for resistance genes that could transfer to pathogens and under specific conditions, commensal *E. coli* strains might serve as a reservoir of resistance genes that could be acquired by pathogenic bacteria (Blake et al., 2003, Angulo et al., 2004, Ajiboye et al., 2009, Marshall et al., 2009). Commensal bacterial strains are exposed to the same selective pressures as pathogenic strains, which is why commensal bacterial strains can serve as indicators of trends in antimicrobial resistance (Bywater et al., 2004).

Table 2. Biotype characteristics of *E. coli*.

Characteristic	***Reaction***
Gram stain	Negative
Cell morphology	Non-sporing straight rod, 1.1-1.5 x 2.0-6.0 µm
Motility	+ by peritrichous flagellae or non motile
Aerobic growth	+
Anaerobic growth	+
Optimum growth temperature	37 ºC
Catalase	+
Oxidase	–
D-Mannitol fermentation	≥90% +
Lactose 37 ºC and 44 ºC	≥90% +
D-Adonitol	≥90% –
Indole 37 ºC and 44 ºC	≥90% +
Methyl red reaction	≥90% +
Voges-Proskauer reaction	≥90% –
Growth in simmons' citrate	≥90% –
Urease, Christensens'	≥90% –
Phenylalanine deamination	≥90% –
Lysine decarboxylase	76 – 89 % strains +
H_2S on TSI (triple sugar iron) medium	≥90% –
Growth in KCN (Potassium cyanide)medium	≥90% –
Gelatine liquefaction (at 22 ºC)	≥90% –

+ = positive reaction; – = negative reaction; Source: Bell and Kyriakides (2002).

2.8 Application of Judicious and Prudent Microbial Use Principles by Dairy Cattle Practitioners

Antimicrobials are an extremely important for protecting animal health and improving productivity in livestock production. There are concerns that the use of antimicrobial agents in food animals creates a selective pressure on bacteria populations and contributes to the development of antimicrobial resistance in foodborne pathogens which can be transferred to humans and presents a threat to human health (Barlow, 2011). The prudent use of antimicrobial agents in livestock production such as dairy farms will continue to provide benefits to society and will help ensure high standards of welfare for those animals in our care (McKellar, 1998). FDA defined there are three responsibilities for veterinarians treating cattle with antimicrobials: first, to diagnose, prevent and treat disease; second, to optimize the production and health maintenance; and third, to meet the expectations regarding the safety of food animal production (FDA, 2012).

For the purpose of maintaining the longstanding effectiveness of antimicrobials for animal and human use and to increase the possibility of future antimicrobial agents approvals for the treatment of animals, the American Veterinary Medical Association (AVMA) and the American Association of Bovine Practitioners (AABP) are committed to judicious and prudent use of antimicrobials by veterinarians for the prevention, control, and treatment of animal diseases. The AVMA Executive Board approved a general set of judicious use principles are (Kreausukon, 2011, FDA, 2012):

1) Preventive strategies, such as appropriate husbandry and hygiene, routine health monitoring, and immunizations, should be emphasized.

2) Other therapeutic options should be considered prior to antimicrobial therapy.

3) Judicious use of antimicrobials, when under the direction of a veterinarian, should meet all the requirements of a valid veterinarian-client-patient relationship.A veterinarian is required to direct the use of prescription antimicrobials or antimicrobials being used in an extralabel manner. This direction may only take place within the context of a valid veterinary-client-patient relationship (VCPR).

4) Prescription, Veterinary Feed Directive, and extralabel use of antimicrobials must meet all the requirements of a valid veterinarian-client-patient relationship.

5) Extralabel antimicrobial therapy must be prescribed only in accordance with the Animal Medicinal Drug Use Clarification Act amendments to the Food, Drug, and Cosmetic Act and its regulations.

6) Veterinarians should work with those responsible for the care of animals to use antimicrobials judiciously regardless of the distribution system through which the antimicrobial was obtained.

7) Regimens for therapeutic antimicrobial use should be optimized using current pharmacological information and principles.

8) Antimicrobials considered important in treating refractory infections in human or veterinary medicine should be used in animals only after careful review and reasonable justification. Consider using other antimicrobials for initial therapy.

9) Use narrow spectrum antimicrobials whenever appropriate.

10) Utilize culture and susceptibility results to aid in the selection of antimicrobials when clinically relevant.

11) Therapeutic antimicrobial use should be confined to appropriate clinical indications. Inappropriate uses such as for uncomplicated viral infections should be avoided.

12) Therapeutic exposure to antimicrobials should be minimized by treating only for as long as needed for the desired clinical response.

13) Limit therapeutic antimicrobial treatment to ill or at risk animals, treating the fewest animals indicated.

14) Minimize environmental contamination with antimicrobials whenever possible.

15) Accurate records of treatment and outcome should be used to evaluate therapeutic regimens.

In addition, to improve the animal welfare, increase economic benefit and protect human health, the AABP Board of Directors approved following specific recommendations for the prudent and judicious use of antimicrobials in dairy cattle (Kreausukon, 2011, FDA, 2012):

1) The veterinarian should accept responsibility for helping clients design management, immunization, housing, and nutritional programs that will reduce the incidence of disease and the need for antimicrobials.
2) The use of antimicrobials only within the confines of a valid veterinarian-client-patient relationship. In addition, extralabel usage should be within the provisions contained within the AMDUCA regulations.
3) Veterinarians should properly select and use antimicrobial drugs and participate in continuing education programs that include therapeutics and emergence and/or development of antimicrobial resistance, should have strong clinical evidence of identity of the pathogen causing the disease before making a recommendation for antimicrobial use and giving an appropriate dosage and duration.
   - The antimicrobial product choices and regimens should be based on approved parameters and documents such as product label. Antimicrobials should be used with specific clinical outcome(s) in mind and be used at a dosage appropriate for the condition treated and for as short a period of time as reasonable.
   - Periodically monitor herd pathogen susceptibility and therapeutic response and use products that have the narrowest spectrum of activity and known efficacy in vivo against the pathogen causing the disease problem.
   - Therapy should be discontinued when it is apparent that the immune system can manage the disease, reduce pathogen shedding and minimize recurrence of clinical disease or development of the carrier state.
   - When possible, antimicrobials of lesser importance in human medicine should be chosen before choosing a newer generation animal antimicrobial that may be in the same class as a human antimicrobial that may be used as the primary or sole treatment for a human infection. An antimicrobial for which emergence of resistance is expected to be in an advanced stage, should also not be chosen.
   - Antimicrobials labeled for use for treating the condition diagnosed should be used whenever possible. When appropriate, local therapy (e.g. intramammary, intrauterine, and topical) is preferred over systemic therapy.

- Combination antimicrobial therapy should be discouraged unless there is information to show increase in efficacy or suppression of resistance development for the target organism. Compounding of antimicrobial formulations should be avoided. Treatment of chronic cases or those with a poor chance of recovery should be avoided. Prophylactic or metaphylactic use of antimicrobials should be based on a group, source or production unit evaluation rather than being utilized as standard practice.

4) Veterinarians should endeavor to ensure proper on-farm drug use. Drug integrity should be protected through proper handling, storage and observa-tion of the expiration date.

- Prescription or dispensed drug quantities should be appropriate to the production-unit size and expected need so that stockpiling of antimicrobials on the farm is avoided.
- The veterinarian should train farm personnel who use antimicrobials on indications, dosages, and withdrawal times, route of administration, injection site precautions, storage, handling, record keeping, and accurate diagnosis of common diseases.
- Veterinarians are encouraged to provide written, updated protocols for diagnosis and treatment to clients whenever possible. Those protocols should describe conditions and provide instructions for antimicrobial use at a farm or unit when a veterinarian is unavailable.

2.9 Commonly Used Antimicrobials on Dairies

A previous survey (Zwald et al., 2004, Kirk, 2011) of dairy farms in some states of the USA studied the antibiotic use strategies found that 71% of the dairies kept antibiotic treatment records for lactating dairy cows. They discovered that slightly over half of the dairies kept records of treatment of dry-cows and only a third kept records of antibiotic treatment in replacement heifers, and the organic dairy farms kept even fewer records than the conventional dairy farms. In the USA, some reports found that the most common used antimicrobials on conventional dairy cows were

penicillin, cephalosporin and tetracyclines (Zwald et al., 2004, USDA, 2008, Kirk, 2011).

A study (Raymond et al., 2006) carried out in Washington State, USA to evaluate the effectiveness of a collaborative approach to promoting judicious antibiotic use on dairy farms showed that the most commonly cited drugs used for disease treatment were penicillin, ceftiofur, and oxytetracycline. The study indicated that intramammary infusion at dry-off was a treatment most producers appeared to consider rather than a preventative practice. According to this study, there was twenty-three percent of initial respondents indicated at least one extra-label use of antimicrobials, yet only half routinely consulted with a veterinarian when doing so. Meanwhile, most of the respondents agreed that using written protocols for disease treatment could reduce errors, but less than one-third had protocols.

2.10 Antimicrobials application in China

In China, Ministry of Agriculture (MOA), People's Republic of China in charge of the administration of veterinary drugs in the whole country and bureau of agriculture in local government in province, city and county level in charge of the administration of veterinary drugs. According to the Regulations on Administration of Veterinary Drugs, the entire veterinary drug including antimicrobials should be registered and approved by MOA, only veterinarians can make a prescription and use antimicrobials according to the approved label of the products. The target animal of each antimicrobial should be registered and approved by MOA also.

According to an investigation (BSNABC, 2013), animal husbandry is the biggest application sector for antibiotics in China, and the total drug expenditure of hog, broiler, layer and cow farms is estimated around 28-30 billion RMB (by farms expenditures) in 2012. Of which, Hog farm expenditure ranks highest, covering 43%, broiler expenditure accounts for 33%, and drug costs of layer and dairy farms occupies 13% and 10%, respectively.

In the last decades, China gradually improved the laws, regulations, and technical standards for veterinary drug, and strengthened the veterinary drug supervision system. In 2002, the MOA released in the Ministry announcements No.176, No.193 and No.1519 and published a total of 82 drugs and substances including antimicrobials such as Chloramphenicol and its salts, esters, Nitrofurans, Nitromidazoles, Vancomycin, that were banned in feed, animal drinking water, and animal production. In dairy cows, some antimicrobials such as gentamicin has also been excluded from approved drugs (Tan et al., 2009).