5. DISCUSSION AND CONCLUSION

5.1 Discussion

5.1.1 The proportion of *E. coli* positive in retail fresh pork in Hanoi

The overall *E. coli* detection rate on retail pork in this study was 82.4 %. As the presence of *E. coli* in the pork is regarded as an indicator of fecal contamination (Tortorello, 2003), the result reflects a high degree of contamination. The isolation rate was higher than that of a previous study which had used the same method and found 76.7 % of the fresh pork marketed in Hanoi being contaminated with *E. coli* (Do *et al.*, 2006).

The presence of *E. coli* in the pork was an indicator of fecal contamination (Tortorello, 2003). The overall *E. coli* detection rate in this study was 82.4 %. That gives an indication of the high degree of contamination. The isolation rate was higher than that of a previous study which had found 76.7 % of the fresh pork marketed in Hanoi being contaminated with *E. coli* (Do *et al.,* 2006).

The rate of *E. coli* contamination in retail fresh pork in the present study is very high in comparison to other reports such as a study in Washington, D.C. which found 16.3 % retail pork samples being positive for *E. coli* (Zhao *et al*, 2001). According to the annual meat report of the USA FDA, the percentage of positive pork samples with *E. coli* contamination were different from this site to others but an average prevalence of *E. coli* on pork samples was 47.2 % in 2002 (FDA, 2002) and 45.5 % in 2003 (FDA, 2003).

In other developed countries such as Switzerland, the proportion of *E. coli* contamination was generally much lower because *Enterobacteriaceae* were detected on only 20.2 % of 650 pig carcasses collected by swab sampling from 5 abattoirs (Zweifel *et al.,* 2004). A prospective survey of 1,648 food items from 10 retail markets in the Minneapolis–St. Paul (America) area during 2001–2003 the prevalence of *E. coli* contamination in pork was 69 % (Johnson *et al*, 2005). However, a survey on the microbiological quality of pork pies, a ready-to-eat dish, within the frame of the Western Australian food monitoring program reported that 16 out of over 19 samples were found to be contaminated with *E. coli*. The survey concluded that pork pies are a high risk product concerning bacteria (Wilson, 2005).

Many factors can contribute to the contamination *E. coli* in fresh meat during the slaughtering, the storage, the transport, the processing and the displaying for sale. In this connection slaughtering is a potential stage for bacterial contamination of meat (Warriner *et al.,* 2002; Pearce *et al.,* 2006; Namvar and Warriner, 2006). Wegener (1999b), a consultant on the hygiene inspection system of the veterinary services in Vietnam, indicated that at slaughter points or slaughterhouses almost every part of the carcass comes into contact with the dirty floor or other contaminated structures. Workers were squatting in between carcasses. Intestinal tracts and bones were all lying on the floor. Therefore, most fresh meat was contaminated with micro-organism, especially, intestinal bacteria. Besides, the practice in meat selling also contributes to the contamination with micro-organisms (Hanashiro *et al.,* 2005). The results of the questionnaire survey of selected factors related to the meat selling practice revealed that meat was preserved and transported in a very poor sanitary condition. As mentioned above *E. coli* can be transferred to meat surfaces from many sources and then getting multiplied. As a result of that, the retail fresh pork had a high degree of *E. coli* contamination.

The sample number of the study met the statistical requirements. We believe that the overall *E. coli* detection rate was valid and that the actual proportion was representative.

Procedures and laboratory practices are complied with the ISO guide 17025. The determination of the presence of presumptive *E. coli* was performed following conventional method described in the ISO 7251: 2005. The isolated colonies were identified by the confirmed test for *E. coli* (FAO, 1992). This method is valid (Todar, 2005). However, this method might still miss some, in particular, pathogenic strains of *E. coli*. But it is the detection of possible fecal contamination that is important in the test rather than the presence of specific types of *E. coli* (Todar, 2005). Thus, the proportion of *E. coli* contamination in retail fresh pork in the Hanoi market might be a little higher.

5.1.2 The prevalence of the antimicrobial resistant *E. coli* isolates

The percentage of the overall resistance of *E. coli* isolated from retail fresh pork in this study is over 93 %. A study in Austria found 76.0 % commensal *E. coli* isolates from retail pork being resistant to antimicrobial drugs (Mayrhofer *et al.,* 2006). In an another study in the area of Minneapolis–St. Paul 85 % of *E. coli*–positive pork samples for antimicrobial resistance were detected (Johnson *et al*, 2005).

The prevalence of the resistance in *E. coli* isolates was observed at high levels with the older antimicrobials (tetracycline, oxytetracycline, doxycycline, sulphonamides, ampicillin); at low levels with newer antimicrobials (cephalothin, norfloxacin and orfloxacin) and at the intermediate levels with streptomycin, gentamicin and neomycin. Compared to a study on retail meat *E. coli* isolates in the USA (Schroeder *et al.,* 2003b) the levels of resistance in this study are higher with respect to tetracycline, ampicillin, sulphonamides, gentamicin but lower with respect to cephalothin and streptomycin.

Most *E. coli* derive, directly or indirectly, from the contents of the intestinal tract (FSA, 2002). Results of the studies on resistance in *E. coli* isolates from pig faces or pigs in slaughterhouses can be considered as antimicrobial resistant *E. coli* prevalence. A study in Korea found that the prevalent resistance of *E. coli* to tetracyclines (96.3 %), streptomycin (66.8 %), ampicillin (66.1 %) was higher those in this study while the prevalent resistance of *E. coli* to sulfonamides (38.8 %) was lower (Lim *et al.*, 2007). Bywater (2004) showed some findings from a survey on antimicrobial susceptibility of commensal bacteria isolated from food-producing animals in European countries which state that the resistance prevalence varied among the countries. In Sweden, for example, *E. coli* isolates from pigs showed at low levels of resistance to ampicillin, chloramphenicol, streptomycin, tetracycline, trimethoprim and sulfamethoxazole whereas among isolates from pigs in Spain, resistance to these antimicrobials were at high level. In the UK, France, the Netherlands, Germany, Italy and Denmark, those rates were at intermediate levels. Guerra *et al.* (2003) also showed the prevalence of resistance to tetracycline, streptomycin, ampicillin and sulfamethoxazole at low levels in swine at slaughter in Germany. In Portugal, a higher degree of *E. coli* resistant to tetracyclines, sulphonamides were observed (Pena *et al*, 2004). *E. coli* isolates from pigs in Australia were moderately resistant to ampicillin and sulphadiazine but had a widespread resistance to tetracycline (Hart *et al.*, 2004). Bywater (2004) found that *E. coli* isolated from food-producing animals in European countries resistant to older compounds (ampicillin, streptomycin, tetracycline and trimethoprim and sulfamethoxazole except gentamicin) was at high levels.

The prevalence of resistant *E. coli* isolates to antimicrobials tested in the present study were higher than stated in previous studies in Vietnam (using the disk diffusion method on Muller-Hinton agar following the NCCLS 2000 guidelines) where *E. coli* isolated from fresh pork had a low level of resistance to tetracycline, doxycycline, streptomycin, orfloxacin (To, 2004, 2006). However, *E. coli* isolates which were collected some years ago in the earlier study originated from a larger area including many provinces in the Red River Delta, while the focus of the present study was directed on a smaller study site. We suppose that the prevalence of resistance to tetracycline, oxytetracycline, doxycycline, trimethoprim, sulphonamides and ampicillin in the present study is higher than that of other antimicrobials. That might be due to the fact that these drugs have already been used widely in food animals, which includes pig husbandry in Vietnam. Besides the animal populations have already had a long history of exposure to them (Dinh *et al.,* 2003; Alhaj *et al.,* 2007). A series of studies agree that the levels of antimicrobial resistance in bacteria gradually increase together with the time of used antimicrobials (Ha *et al.,* 1999; Kang *et al.,* 2005; Lim *et al.,* 2007). So antimicrobials such as norfloxacin, gentamicin and neomycin were effective to *E. coli* isolates in the previous study (To, 2004) but in this study, *E. coli* proved to be resistant.

There are many factors which can contribute to antimicrobial resistance in bacteria such as the pig rearing model, the technique and knowledge of the farmers on irrational utilization of antimicrobials (Dinh *et al.,* 2003; Do *et al.,* 2003). The survey results of selected factors related to the pig husbandry practice showed factors which were far more likely to cause failure of antimicrobial use than the adequate use of them in food animal production. Vo *et al*. (2007) also showed that it is easy to buy antimicrobials in Vietnam and, abuse of antimicrobials in the veterinary practice might have an important influence on antimicrobial resistance.

As demonstrated in table 4a, almost all farmers had a wrong awareness of antimicrobial resistance of bacteria. As a result, the selected antimicrobials mostly based on the farmers' acquired experience which may result in an inappropriate choice of antimicrobials in treating sick animals because of a clinical misdiagnosis without any laboratory tests. Others factors such as the dosage of the antimicrobials and the withdrawal time were also mainly based on the farmers' experience. Even antimicrobials which had already expired were still used.

These survey results are in agreement with the study of Dinh *et al.* (2003) which also showed that the predominant causes which contribute to antimicrobial resistance in bacteria were the unreasonable use of antimicrobials in the pig husbandry practice. Besides there is a consultant's report (Boiseau, 2002) which showed that veterinary medicine containing products were used by farmers without veterinary supervision or the support of a professional diagnosis. Such a behavior led the farmers to the use of preferably wide-spectrum antimicrobials or any combination of them, to the increase of the dosage and to the reduction of the duration of the treatment for the purpose of saving money. The misuse of antibiotics may result in the development of antimicrobial resistance (Donovan, 2002). All these different mistakes favor the emergence and the development of resistance in bacteria.

Norfloxacin and orfloxacin should be discussed, specifically. Both belong to the fluoroquinolone class, a new group with a potent wide spectrum against micro-organisms both gram-negative and gram-positive bacteria, which is very important to treat a variety of serious infections in humans when pathogens are resistant to other antibiotic agents (Jennifer, 2007). Series of reports on the effects of quinolones in food animals showed that the use of fluoroquinolone in food animals causes resistance of micro-organism to fluoroquinolones, which can transfer to humans through the food chain, rapidly (FDA, 1996; Wegener, 1999a). Cross-resistance can occur throughout this entire class of drugs (Hakanen *et al.,* 2003; Price *et al.,* 2005; Luo *et al.,* 2005) and resistance to one drug of the fluoroquinolone class can compromise the effectiveness of other fluoroquinolone drugs (Bren, 2001). Therefore, in many countries there are strict regulations concerning the application of these drugs (Bren, 2001; Wallace, 2002).

In Vietnam, fluoroquinolones have been already widely been used in the food animal production (Boiseau, 2002). Both norfloxacin and orfloxacin are used in combinations with other antimicrobials for the application via both the parenteral and the oral route. Such inappropriate use of antimicrobials can lead to a quick increase of resistant animal bacteria to this class.

Multi-resistance in bacteria has become common in many countries (Bell and Turnidge, 2002). In this study around 90 % of *E. coli* isolates, including 105 different multi-resistance patterns, were exhibited to the tested drugs (appendix 5) whereas resistance with six, seven, five antimicrobials were observed popularly. *E. coli* isolates from retail meat purchased in the USA found that two and three antimicrobials resisting together to the same isolate were the most common patterns of multi-resistance.

The rate of multi-resistance in this study is very high compared to the data on multi-resistance in *E. coli* of the Asia-Pacific surveillance program on resistance which found rates of multi-resistance being around 15 % in many countries in this region (Bell and Turnidge, 2002). Prescott and Baggot (1988) supposed that under conditions of animal husbandry where antimicrobials are used, animals are often kept in contact with their feces and thus with an enormous reservoir of intestinal bacteria. This can lead to multi-resistance in bacteria carrying R plasmids with multi-resistant genes. Worldwide, there has been a rapid increase of multi-resistant bacterial strains (AstraZeneca, 2007).

All combinations of multi-resistance shown in the present study always include resistance to tetracycline. This suggests that *E. coli* isolates which are resistant to tetracycline are in an enhanced emergence to resist to other additional antimicrobial drugs (Sayah *et al.,* 2005). Some recent studies have also suggested that the use of tetracycline is a major factor in the emergence and dissemination of antimicrobial-resistant *E. coli* (Teshager *et al.,* 2000; Schroeder *et al*, 2003a).

One problem should be considered if there is a simultaneous development of resistance to different agents because of an increasing order of combinations of antimicrobials in such classes? Since the high frequency of multi-resistance among *E. coli* isolates in this study may be due to the linkage of resistant mediated genes on plasmids (Petrocheilou *et al.,* 1979; Oppegaard *et al.,* 2001). Moreover, there is evidence of the increase of the most commonly identified antimicrobial combinations in order of classes, starting with the tetracycline group, followed by sulphonamides, then β-lactam class and the last one was aminoglycosides. That may be a trend of co-resistance to several drugs that was observed, apparently, in Europe (Tiemersma *et al.,* 2005). Further studies are needed to explore genetic characteristics of multi-resistance *E. coli* together with combinations of agents of antimicrobial classes.

The factor of meat origin was identified as a risk factor associated with the resistance of *E. coli* isolated from pork to ampicillin, streptomycin, doxycycline and trimethoprim. The factor of origin of meat (to the other antimicrobials left) and other factors were substantial but not statistically significant. They were determined as potential risk factors. This suggests that it might be significantly related to the different usage of antimicrobials in the pig husbandry practice between the Hanoi suburban area and the neighboring provinces. The farmers in the neighboring provinces might have already abused these drugs more much. Wegener *et al.* (2004) also supposed that the volumes of the used antimicrobials and the development of the resistance exist together. The proportion of avoparcin resistant enterococci, for example, dropped from 73 % to 5 % in a five year period after having a ban avoparcin as a growth promoter in food animals in Denmark (WHO, 2003).

The testing of antimicrobial resistance of *E. coli* isolates in this study is based on the Kirby-Bauer disk diffusion method, following the guidelines by the NCCLS, 2000. Although this is qualitative or semi-qualitative method it is suitable for this study because it is designed to distinguish between susceptible and resistant members of a bacterial population and it applies well to fast growing aerobic bacteria (Lalitha, 2004). Thus, the quality of the collected data in terms of accuracy and precision can be guaranteed.

The number of isolates meets the statistically required amount of samples. We believe that the prevalence of resistance of *E. coli* isolated from retail fresh pork in Hanoi to the tested antimicrobials is valid and representative. However, the findings of this present study reflect the problem of antimicrobial resistance of retail fresh pork *E. coli* only for the Hanoi area. They do not represent antimicrobial resistance of pork *E. coli* for the whole country. Further studies are needed to gain a comprehensive view on this matter.

Besides, it is not possible to come to a conclusion regarding the quantitative contributions of antimicrobial resistance of fresh pork *E. coli* in this study. An antimicrobial resistance surveillance system in food of animal origin should be done in order to detect profiles, patterns and trends in antimicrobial resistance in bacteria isolated from food of animal origin, especially pathogens such as *Salmonella, Campylobacter, Shigella* etc.

5.2 Conclusion

1) The proportion of *E. coli* positive in retail fresh pork was 82.4 % (95 % CI: 78.2-95.9).
2) A total of 332 *E. coli* isolates were tested for susceptibility to 12 commonly used antimicrobial agents (tetracycline, oxytetracycline, doxycycline, trimethoprim, sulphonamides, streptomycin, neomycin, gentamicin, ampicillin, cephalothin, norfloxacin and orfloxacin) through the disk diffusion method on Muller-Hinton agar following the NCCLS 2000 guidelines for interpretation. Overall, 93.1 % of the *E. coli* isolates were resistant to at least one of 12 antimicrobials tested. Resistance was observed for all tested antimicrobial agents to different degrees.
3) Many of the isolates showed high levels of resistance (> 50 %) for tetracycline (79.8 %), oxytetracycline (74.4 %) compound sulphonamides (69.3 %), trimethoprim (61.1 %), doxycycline hydrochloride (58.4 %) ampicillin (54.0 %). The antimicrobials detected with low levels of resistance (< 10 %) were cephalothin (6.3 %), norfloxacin (4.2 %) and orfloxacin (3.9 %). Within 10 % to 50 % resistance was observed for streptomycin (43.0 %), neomycin (19.9 %) and gentamicin (12.1 %).
4) The isolates revealed multi-resistance pattern (89.6 %) and up to 11 antimicrobials had shown resistance. The multi-resistance pattern

observed was 17.2 % to 6 antimicrobial agents, followed by 16.3 % to seven, 12.1 % to five, 10.5 % to four, 10.2 % to eight, 7.8 % to three, 4.8 % to two, 3.6 % to nine, 0.6 % to 11 and 0.3 % to ten antimicrobials.

5) Out of a total of 113 observed resistance patterns 105 were different multi-resistance patterns. However, the combination of tetracycline and oxytetracycline was the most frequently observed resistance pattern from pork *E. coli* isolates. There is evidence of an increase of the most commonly identified antimicrobial combinations in order of classes, from the tetracycline group, followed by sulphonamides, then β-lactam class and the last one was aminoglycosides.
6) The Odds ratios of antimicrobials were 1.73 (95 % CI: 1.08-2.77) for ampicillin, 1.23 (95 % CI: 1.15-2.91) for streptomycin, 1.67 (95 % CI: 1.04- 2.70) for doxycycline and 1.70 (95 % CI: 1.05-2.79) for trimethoprim estimated for the neighboring provinces against the Hanoi suburban area. The risk factor associated with the resistance of *E. coli* was significant ($p \leq 0.05$) for the origin of meat with the antimocrobials ampilicilin, streptomycin, doxycycline and trimethoprim.
7) The drug abuse by the farmers in pig husbandry might be an established fact fact in Vietnam.

In summary, the findings from this study demonstrated that the retail fresh pork in Hanoi market, Vietnam may often be contaminated frequently with the antimicrobial resistant *E. coli*.