4. RESULTS

4.1 Descriptive data

4.1.1 Results of the questionnaire surveys

Results of the questionnaire survey of selected factors related to the pig husbandry practice

The detailed results are shown as in appendix 3a. The overall percentages are illustrated as in figure 2.

In reference to the pig rearing model there were only 13.3 % of the farms where farmers used the entire feed-stuff. The most common rearing model (47.5 %) consisted of salvaging remaining food (waste food, by-products of some traditional food processing). 39.2 % of the farms used a model which was a combination of food salvage and feed stuff.

In terms of the farm sizes, the sizes of < five and five to 10 animals predominated by the rates 29.2 % and 28.3 %, respectively. The groups of farms with sizes 11-15, 16-20 and 21-25 amounted to 10.0 %, 12.5 % and 9.2 %, respectively, followed by groups keeping 26–50 and 51–100 with 4.2 % and 5.8 %, respectively. Farms which kept more than 100 pigs represented only 0.8 %.

The use of antimicrobials in pig husbandry was very common in there 97.5 % of farmers used them at least one time in a pig life. However, the use of drugs for growth promotion was very rare. The most common purposes of antimicrobial use were disease treatment (54.7 %) and a combination of disease treatment and prevention (44.4 %).
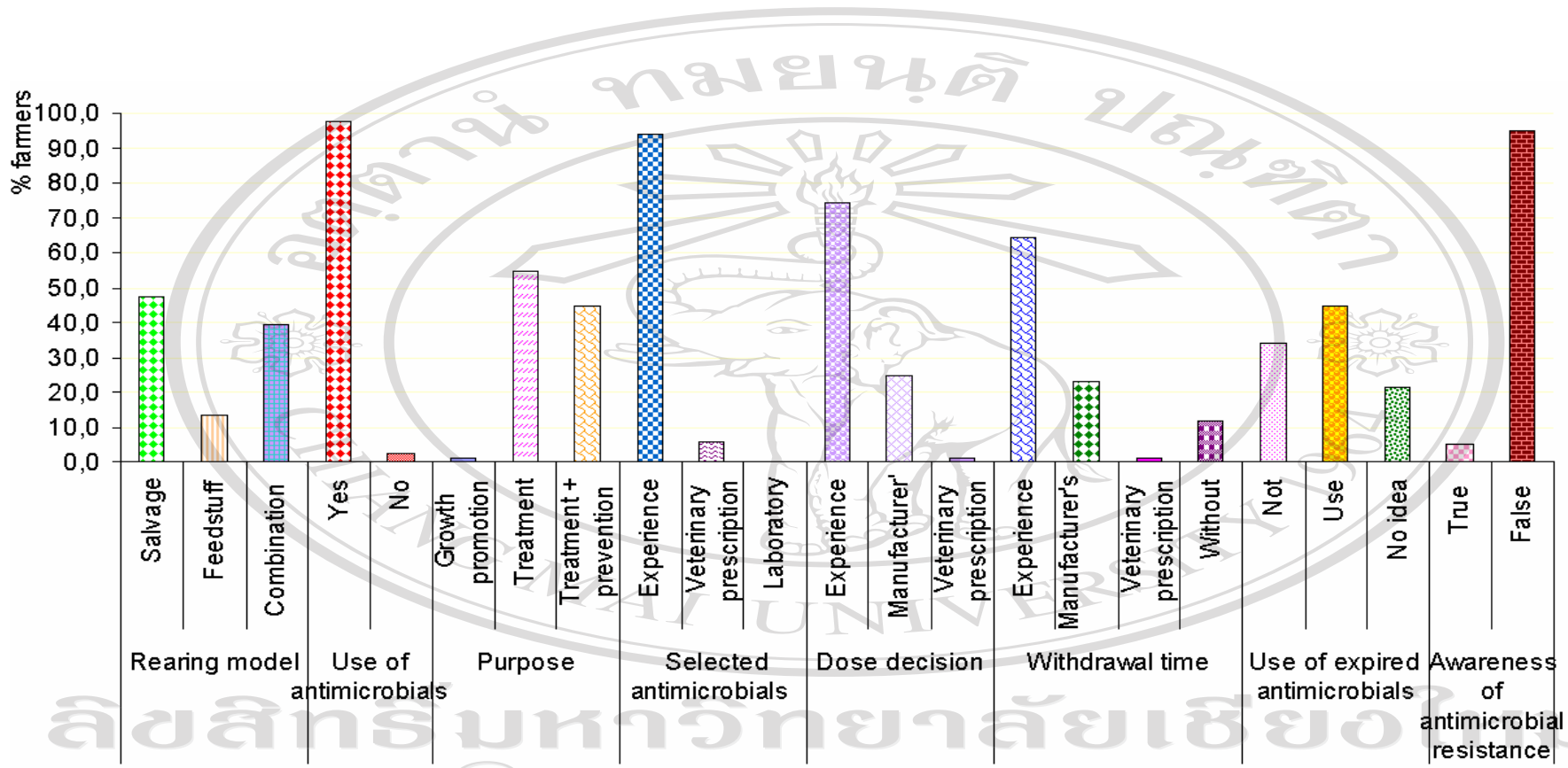
Figure 2: Distribution of selected factors related to the pig husbandry practice (n = 120)

The choice of using antimicrobials, the decision about dosage and withdrawal time was based mainly on the farmer's experience (that represented 94.0 %, 74.4 % and 64.2 %, respectively). No farmers, who selected antimicrobials in pig husbandry, based their decision on laboratory diagnostic results.

The compliance with the descriptions of the veterinary practitioners concerning the choice of drugs was 6.0 %. As far as the decision about the dosage of drugs or the withdrawal time was concerned the compliance represented 0.9 %. There were 24.8 % and 23.1 % of the farmers who complied with veterinary pharmaceutical manufacturers' guidelines in drug dosage and withdrawal time. In case of expired antimicrobials 44.4 % of the farmers would have clearly used them for pig disease prevention and treatment while 34.2 % of them would not have done. 21.4 % would have been equivocal in such a situation.

To assess the real awareness of antimicrobial resistance of farmers, groups of three questions were used to check the accuracy and consistency of the answers (appendix 2a). The results indicate that 37 (30.8 %) out of 120 of the farmers said that they were aware of antimicrobial resistance of bacteria from the use of antimicrobials (Q1). With these farmers, they continued answering the Q2 and Q3. However, only 2 (5.4 %) out of them agreed that was a phenomenon in where a patient (e.g. tuberculosis patient) would not recover when he was treated by the same antimicrobial which used to be effective to that disease at the acceptable dose. And 7 (18.9 %) out of them agreed that inappropriate use of antimicrobials could lead to resistance in pathogens (Q3). The real awareness of antimicrobial resistance of bacteria of farmers who said "Yes" in Q1 and gave the true answers with both Q2 and Q3 was only 2.7 %.

With regards to individual profiles factors like age, sex, experience and training in pig husbandry were taken into account. As in appendix 3a there was an approximate ratio between two genders of people who supplied information for this questionnaire survey. Among them, 40–50 year old farmers were highest, occupying

35.8 % or 43 out of 120, followed by groups of 30–40 and > 50 with 26.7 % and 29.2 %, respectively. There was only 10 (8.3 %) out of 120 persons aging up to 30.

In terms of the number of years in experience in pig husbandry, about half of the farmers had more than 20. One third of the farmers had between of 10-20 and one fifth of them had less than 10 (years in experience). Although 55 (45.8 %) out of 120 of the farmers said that they had some basic knowledge of pig rearing, none of them had ever participated in any professional training course on Good Husbandry Practice (GHP) and/or Good Veterinary Practice (GVP).

The results of the questionnaire survey of selected factors related to the meat-selling practice

The results are illustrated in figure 3 and detailed in appendix 3b.

100 % of the meat for sale was preserved in the natural condition. With regard to the wrapping during the transport most meat (97.9 %) was not wrapped. 2.1 % of the meat was wrapped during the transport but materials used for the wrapping often were old rain coats or sack-cloth, which was often washed only perfunctorily or even barely.

In relation to the origin of the meat 79 out of 141 meat shops or 56.0 % of meat in all shops originated from the suburban districts of Hanoi. The rest, 44.0 % of the shops obtained meat from the neighboring provinces. The meat shops which sold only pork were 65.3 % whereas 34.8 % of meat shops sold not only pork but also beef, chicken meat and even internal organs.

The definition of the size of a meat shop based on its daily average amount of sold meat with a minimum level of 50 kg/day up to more than 200 kg/day. A group
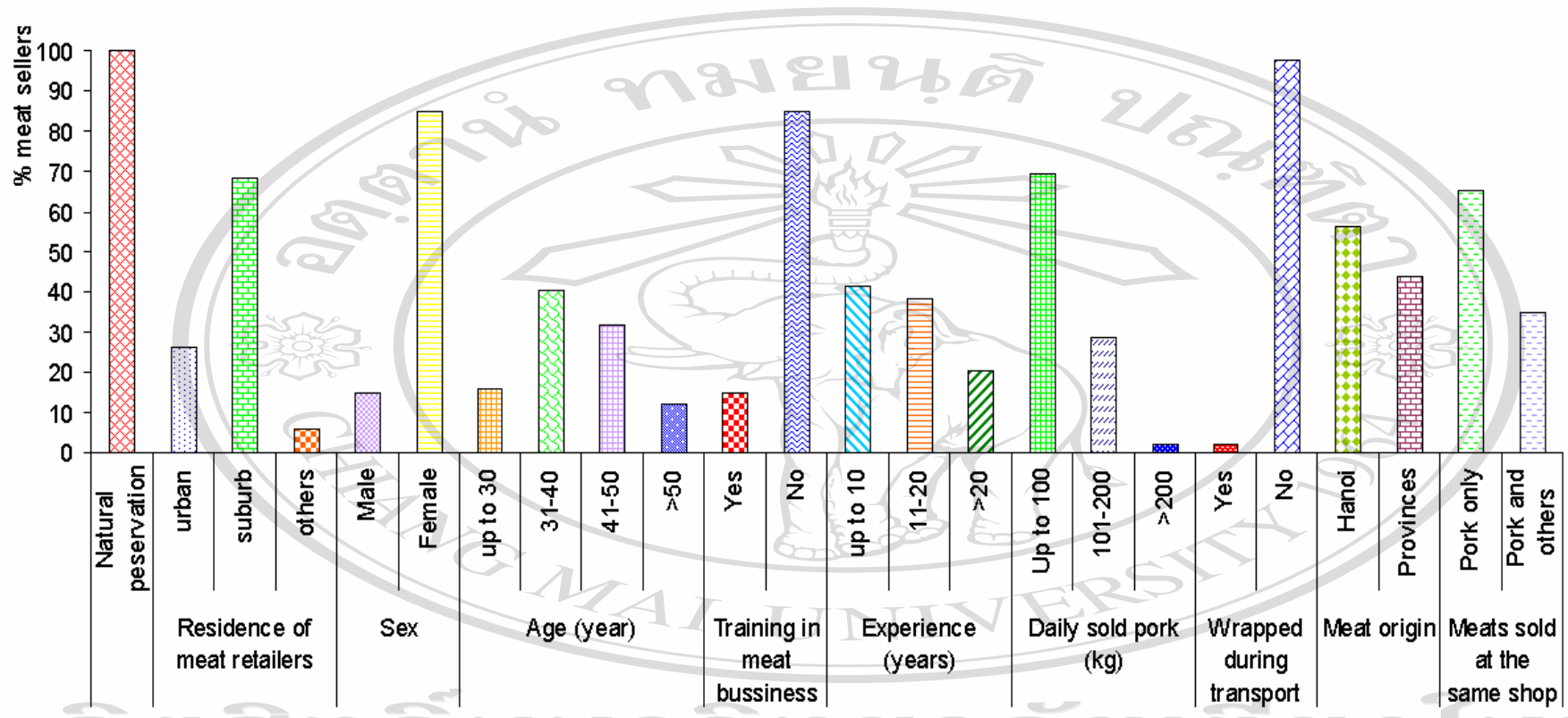
Figure 3: Distribution of selected factors related the meat selling practice (n = 141)

of shops which sold an average amount of 50 kg to 100 kg every day represented a rate of 69.5 %. The following group was shops which sold more, namely, 100 to 200 kg/day, which represented a rate of 28.4 %. Besides, there were very few meat shops which sold more than 200 kg/day, representing only 2.1 %.

With regards to the meat-retailers' individual factors like residence, age, sex, experience and professional training in the meat business were dealt with. As in appendix 3b the meat retailers, who resided in the suburban areas, predominated with 96 (68.1 %) out of 141, followed by a group of people living in the urban areas with 26.2 %. The rest, 5.7 % of them came from the surrounding provinces.

Among the meat retailers, 120 (85.1 %) out of 141 were female and the rest was male (14.9 %). Most of them (120 out of 141 or 95.0 %) had never taken part in a professional training course on hygiene and food safety in the meat business except 7 (5 %) out of 141.

In reference to the age of the meat-retailers it can be stated that the biggest age group is the one of the 31–40 year old (40.4 % or 57 out of 141), followed by the age group of the 41–50 (31,9 %) and the age group of the up to 30 (15.6 %). Only 17 (12.1 %) out of 141 persons were older than 50.

Regarding the years of experience in the meat business 41.1 % of the meat retailers had less than 10 and 38.3 % had more than 10 up to 20. The group having more than 20 years of experience was the lowest (20.6 %).

4.1.2 The results of the detection and identification of E. *coli* in retail fresh pork

A total of 332 *E. coli* isolates were obtained from 403 meat samples collected throughout the Hanoi market. As in figure 4 the proportion of *E. coli* positive in retail fresh pork is 82.4 % (95 % CI: 78.2 % – 85.9 %).
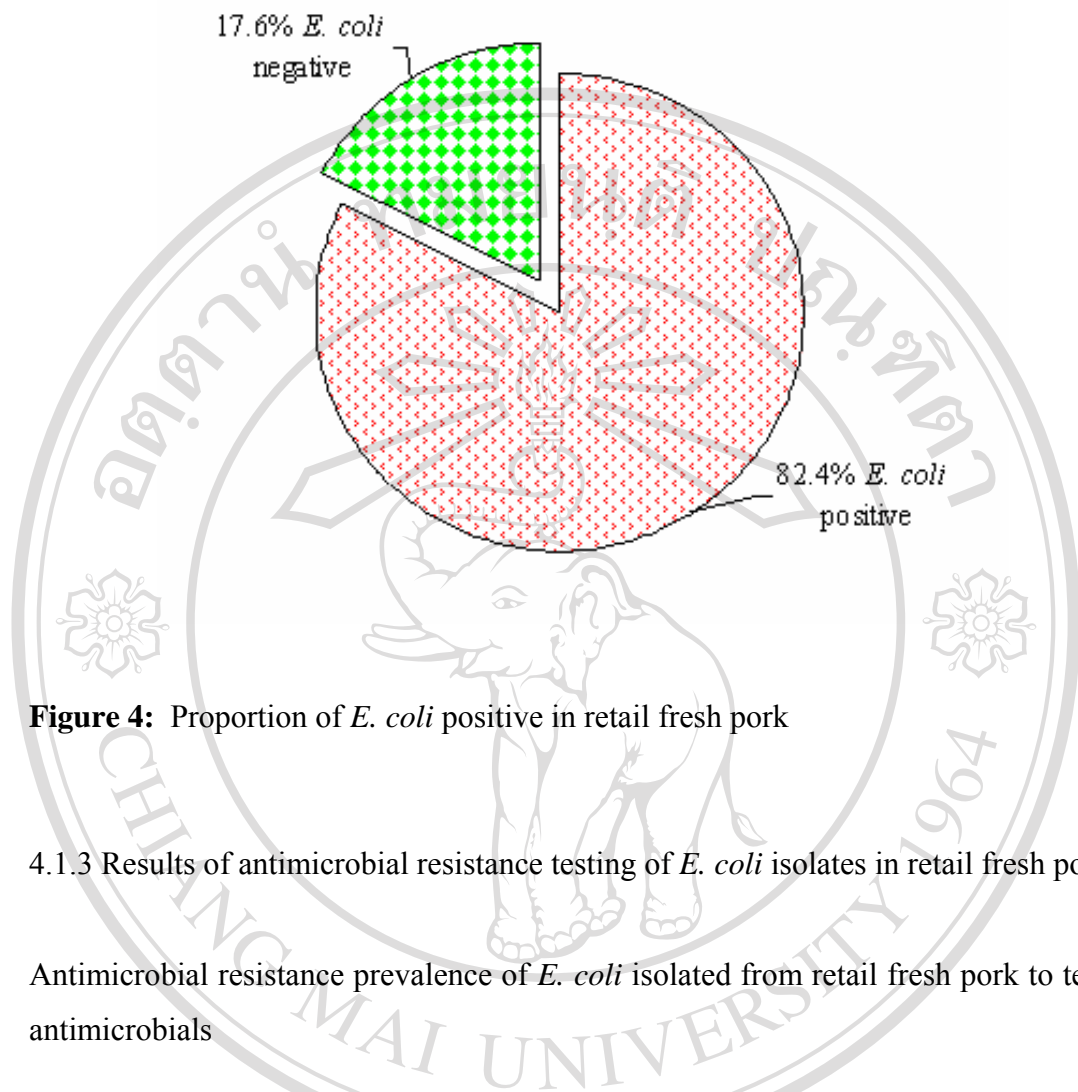
17.6% *E. coli* negative

82.4% *E. coli* positive

Figure 4: Proportion of *E. coli* positive in retail fresh pork

4.1.3 Results of antimicrobial resistance testing of *E. coli* isolates in retail fresh pork

Antimicrobial resistance prevalence of *E. coli* isolated from retail fresh pork to tested antimicrobials

In overall, 93.1 % of the *E. coli* isolates were resistant to at least one of 12 antimicrobials tested as shown in figure 5.
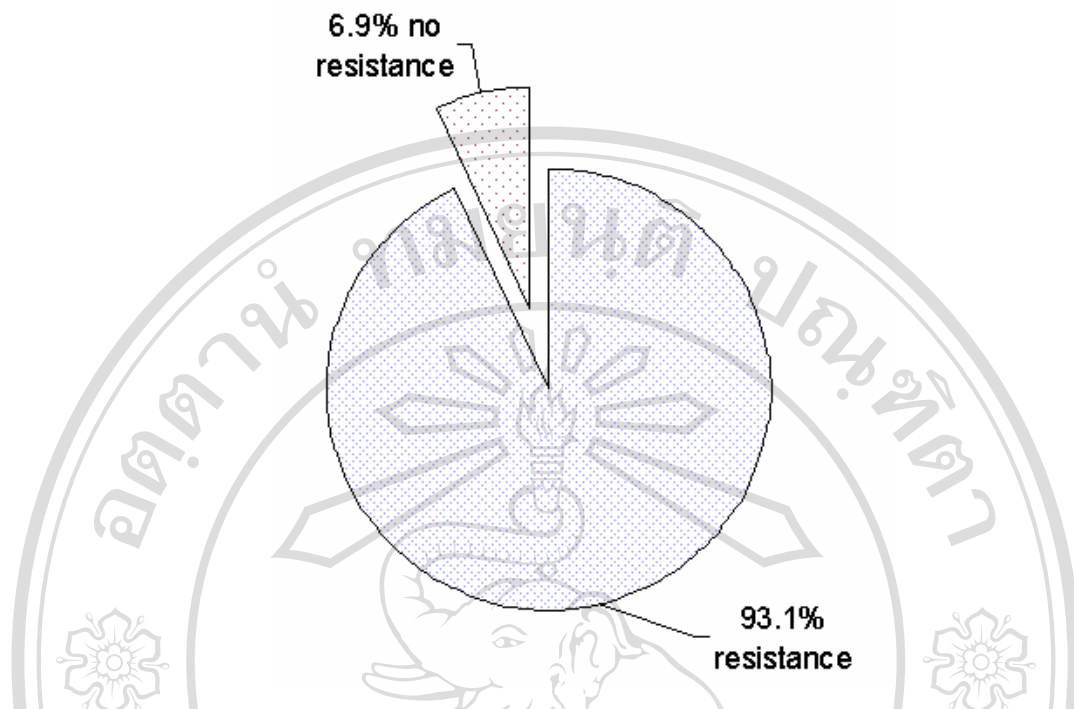
Figure 5: Overall antimicrobial resistance of *E. coli* isolates from retail fresh pork

Regarding the individual antimicrobials, the *E. coli* resistance observed for all antimicrobial agents tested to different degrees (Figure 6). Many of the isolates showed high levels of resistance (> 50 %) for tetracycline (79.8 %), oxytetracycline (74.4 %), sulphonamides (69.3 %), trimethoprim (61.1 %), doxycycline (58.4 %) and ampicillin (54.0 %). The antimicrobials detected at low levels of resistance (< 10 %) were cephalothin (6.3 %), norfloxacin (4.2 %) and orfloxacin (3.9 %). The middle levels within 10 % to 50 % resistance observed for streptomycin (43.0 %), neomycin (19.9 %) and gentamicin (12.1 %).

Antimicrobial resistance levels of *E. coli* isolates to the tetracycline group (58.4 %–79.8 %) and the sulphonamide group (61.1 %-69.3 %) dominated over other groups while their antimicrobial susceptible level to the fluroquinolon group was still high (resistance levels were less than five percents). With antimicrobials in the aminoglycoside and β-lactam groups, levels of resistance of *E. coli* isolates were higher in the 'older' antimicrobials (ampicillin, streptomycin) than those in the 'newer' antimicrobials (cephalothin, neomycin, and gentamicin). For more detail, see appendix 4.
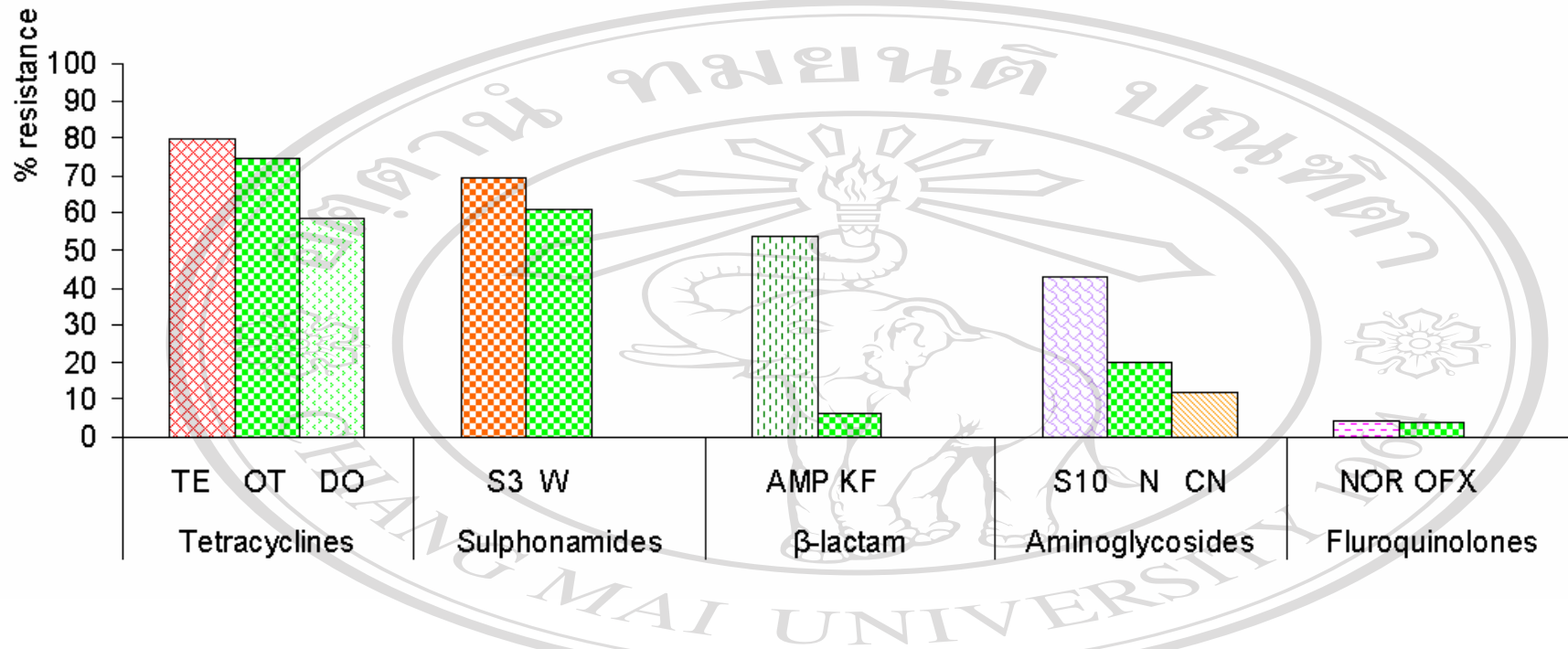
Figure 6: Antimicrobial resistance prevalence of *E. coli* isolated from retail fresh pork

(TE - tetracycline, OT - oxytetracycline, DO - doxycycline, W - trimethoprim, S3 - sulphonamides, S10 - streptomycin, N - neomycin, CN - gentamicin, AMP - ampicillin, KF - cephalothin, NOR - norfloxacin, OFX - orfloxacin)

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Antimicrobial resistance patterns of *E. coli* isolated from retail fresh pork to tested antimicrobials

Out of a total of 113 resistance patterns there were 8 (9.6 %) patterns of resistance against one antimicrobial in which the most frequently encountered antimicrobial agent was ampicillin (15 times observed of all 32 isolates of resistance against one antimicrobial). Multi-resistance was observed in 277 (89.6 %) of 309 resistant isolates, for up to 11 out of 12 antimicrobials tested (0.6 % of *E. coli* isolates), with 105 out of 113 different resistance patterns. The most common multi-resistance patterns were observed to 6 antimicrobial agents (17.2 %), 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, and 3.6 % to nine. The lowest frequent prevalence of multi-resistance was 0.3 %, encountered 10 antimicrobial agents (Figure 7). The observed patterns in detail of antimicrobial resistance of *E. coli* isolates to antimicrobials tested showed in as appendix 5. The most frequently encountered antimicrobials in 113 different resistance patterns are described as in table 7.
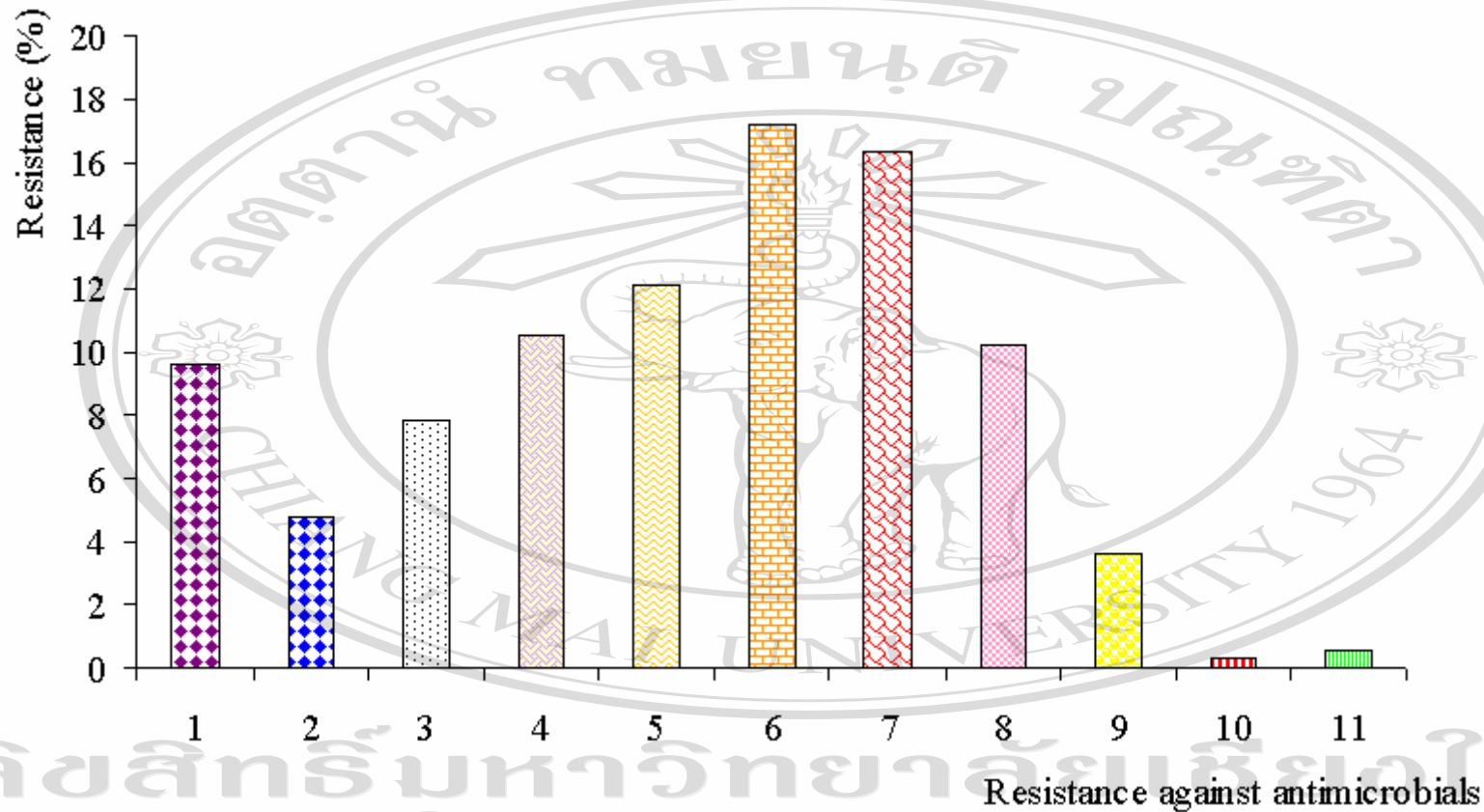
Figure 7: Antimicrobial resistance types of *E. coli* isolated from retail fresh pork

Table 7: Most frequent resistance patterns of *E. coli* isolates

Resistance against antimicrobials	No. of resistant *E. coli* isolates	Most frequent resistance patterns (No. of isolates)
1	32	AMP (15)
2	16	TE, OT (9)
3	26	TE, OT, DO (14)
4	35	TE, OT, S3, W (8)
		TE, OT, DO, S3 (8)
5	40	TE, OT, DO, S3, W (15)
6	57	TE, OT, S3, W, AMP, S10 (13)
		TE, OT, DO, S3, W, S10 (13)
7	54	TE, OT, DO, S3, W, AMP, S10 (31)
8	34	TE, OT, DO, S3, W, AMP, S10, CN (9)
9	12	TE, OT, DO, S3, W, AMP, S10, CN, N (5)
10	1	TE, OT, DO, S3, W, AMP, S10, CN, NOR, OFX (1)
11	2	TE, OT, DO, S3, W, AMP, S10, CN, N, NOR, OFX (2)

(TE - tetracycline, OT - oxytetracycline, DO - doxycycline, W - trimethoprim, S3 - sulphonamides, S10 - streptomycin, N - neomycin, CN - gentamicin, AMP - ampicillin, KF - cephalothin, NOR - norfloxacin, OFX - orfloxacin)

Many combinations of antimicrobial agents in multi-resistant *E. coli* isolates were observed among them most commonly identified combinations of antimicrobials agents in multi-resistant *E. coli* isolates are showed in table 8. The combination of tetracycline resistance and oxytetracycline resistance was found in all of the most frequent multi-drug resistance patterns of *E. coli* isolates (resistance against two to 11 antimicrobials). Moreover, an increase in the class order of the most commonly identified antimicrobial combinations, starting with the tetracycline group, followed by sulphonamides, then β-lactam and the last one was aminoglycosides.
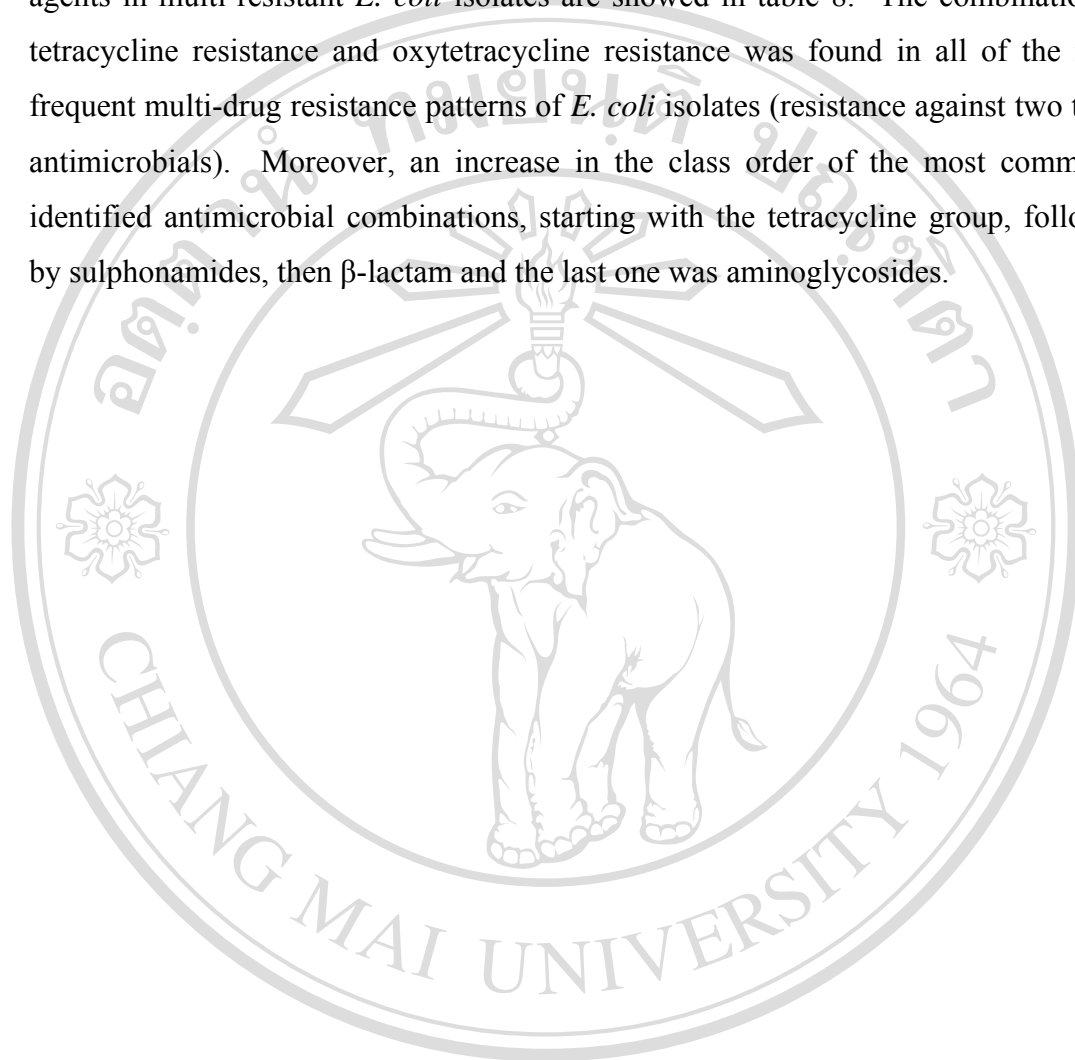
Table 8: Most commonly identified combinations of antimicrobials agents in multi-resistant *E. coli* isolates

(n = 332)

Multi-resistance against antimicrobials	Antimicrobial combinations	No. of identified combinations	% all isolates
2	TE, OT	241	72.6
3	TE, OT, S3	225	67.8
4	TE, OT, S3, W	171	51.5
5	TE,OT,DO, S3, W	131	39.5
6	TE,OT,DO, S3, W, AMP	87	26.2
7	TE,OT,DO, S3, W, S10, AMP	64	19.3
8	TE,OT,DO, S3, W, S10, AMP, CN	20	6.0
9	TE,OT,DO, S3, W, S10, AMP, CN, N	7	2.1
10	TE,OT,DO, S3, W, S10, AMP, CN, NOR, OFX	3	0.9
11	TE, OT, DO, S3, W, AMP, S10, CN, N, NOR, OFX	2	0.6

(TE - tetracycline, OT - oxytetracycline, DO - doxycycline, W - trimethoprim, S3 - sulphonamides, S10 - streptomycin, N - neomycin, CN - gentamicin, AMP - ampicillin, KF - cephalothin, NOR - norfloxacin, OFX - orfloxacin)

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4.2 Inferential analysis

The resistance percentage of *E. coli* isolates from pork samples originating from the neighboring provinces was statistically significantly ($p \leq 0.05$) different from those originating from the Hanoi area to ampicillin (63.0 % and 49.6 %, p-value: 0.029), streptomycin (52.8 % and 38.0 %, p-value: 0.015) , doxycycline (66.7 % and 54.5 %, p-value: 0.046) and trimethoprim (69.4 % and 57.1 %, p-value: 0.042) (appendix 6c, d, f, I, respectively). The Odds ratios for antimicrobial resistance of *E. coli* isolates to ampicillin, streptomycin, doxycycline and trimethoprim was 1.73 (95 % CI: 1.08, 2.77), 1.23 (95 % CI: 1.15, 2.91), 1.67 (95 % CI: 1.04, 2.70) and 1.70 (95 % CI: 1.05, 2.79), respectively which were far more likely in *E. coli* from samples originating from the neighboring provinces as compared with Hanoi. Thus the factor of origin of meat was identified as a risk factor associated with the resistance of *E. coli* isolated from fresh pork to ampicillin, streptomycin, doxycycline and trimethoprim.

Also with the same factor of meat origin, the difference in resistance percentage of *E. coli* isolates to the other antimicrobials such as tetracycline, oxytetracycline, sulphonamides, neomycin, gentamicin, cephalothin, norfloxacin and orfloxacin was not statistically significant ($p > 0.05$) (appendix 6a, b, e, g, h, k, m, l respectively). Thus the factor of meat origin was identified as a non-risk factor associated with antimicrobial resistance of *E. coli* isoalates from fresh pork to these antimicrobials.

The other factors such the types of meat which were sold at the same shop or with the different daily amount of sold meat or with whether the meat was wrapped during the transport to the markets or not, the difference in resistance proportion of *E. coli* isolates to 12 antimicrobials tested within each factor was not statistically significant ($p > 0.05$) (appendix 6). These factors were also identified as potential risk factors.