PREVALENCE OF *SALMONELLA* AND *E. COLI*, AND THEIR RESISTANCE TO ANTIMICROBIAL AGENTS, IN FARMING COMMUNITIES IN NORTHERN THAILAND

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Abstract. A cross-sectional pilot study was conducted in Chiang Mai, Thailand, to determine the prevalence of *Salmonella* and *Escherichia coli* in swine, broiler chickens and human workers from farms and abattoirs in northern Thailand, and compare their antimicrobial resistance profiles. Fecal samples and cloacal swabs were collected from 150 swine and 150 chickens at the farm. Fecal samples from swine, cloacal swabs from chickens, and carcass swabs from both animals were collected from 100 swine and 100 chickens at the abattoir. Stool samples were collected from 15 swine farm workers and seven chicken farm workers. Primary isolation and identification of *Salmonella* and *E. coli* were conducted using standard methods. *In vitro* susceptibility testing of *Salmonella* and *E. coli* was conducted using the broth microdilution method, based on the United States National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

The prevalence of *Salmonella* from swine and chicken samples ranged from 2% to 25%. The prevalence of *E. coli* in chickens and swine ranged from 36.8% to 47.6%. In humans, the prevalence of *Salmonella* was 15%, and the prevalence of *E. coli* ranged from 51% to 53%. Resistance in *Salmonella* was found for tetracycline (84.7%), nalidixic acid (27.1%), florfenicol (18.6%), ampicillin (13.6%), and ceftiofur (3.4%), and in *E. coli* for tetracycline (91.5%), nalidixic acid (67.4%), ampicillin (61.6%), florfenicol (51.8%), enrofloxacin (28.7%), ciprofloxacin (12.5%), ceftiofur (4.9%) and ceftriaxone (1.5%).

INTRODUCTION

The progressive increase in antimicrobial resistance among enteric pathogens in developed and developing countries has become a critical area of concern (Levy, 1998). Previous studies have shown that food-borne pathogens, such as *Escherichia coli* and *Salmonella,* are highly prevalent, and have been isolated in stool samples from humans affected by food-borne illnesses, as well as in the meat and poultry products processed for human consumption (Sunthadvanich *et al*, 1990; Suthienkul *et al,* 1990; Sasipreeyajan *et al*, 1996; Boonmar *et al*, 1997). Two of the most common etiologic bacterial organisms responsible for causing gastroenteritis, a major public health concern in most regions of Thailand, are *Salmonella* and *E. coli* (Rasrinual *et al*, 1988; Varavithya *et al*, 1990).

It is increasingly important to address the issues of antimicrobial resistance produced by the overuse of antimicrobial agents utilized by farming communities throughout the world. Antimicrobial agents are given as prophylaxis to farm animals destined for human consumption, to improve their overall health and robustness at market time. In Thailand, increasing resistance of *Salmonella* and *E. coli* to antimicrobial agents has been reported (Boonmar *et al*, 1998; Hoge *et al*, 1998). The emergence of resistance in enteric pathogens to different antimicrobial agents in farming communities will adversely affect the availability of antimicrobial therapies available for use in human clinical medical practice (Witte, 1998; Wagener *et al*, 1999; Witte *et al,* 2000).

The objectives of this study were to: 1) determine the prevalence of *Salmonella* and *E. coli* in animals processed for human consumption and humans working on farms and in abattoirs in northern Thailand; and 2) compare antimicrobial resistance patterns of *Salmonella* and *E. coli* isolated from meat-producing farms and abattoirs, and humans working on these farms and abattoirs.

MATERIALS AND METHODS

Sample collection

Two abattoirs and six farms (three swine farms and three chicken farms) within a 80-km radius from Chiang Mai University were selected for inclusion in the study, based on their willingness to participate. A government-operated abattoir, located in Lamphun Province, provided swine slaughter specimens, and a private broiler chicken processing plant in Chiang Mai Province provided poultry specimens. Samples were collected once from each farm, samples from the swine

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abattoir were gathered over one three-day period, and chicken abattoir samples were collected over one two-night period.

On each swine farm, proportional sampling was used to select individual pigs from each housing unit, so that the samples collected would be representative of all fattening pigs in that housing unit. On each chicken farm, proportional sampling was also used to select 6-week old chickens from each housing unit, so that the samples collected would be representative of all 6-week old chickens in that housing unit. Samples were collected from all pigs during the abattoir sample collection period, and a systematic sample of every tenth chicken processed during the abattoir sample collection period was taken.

Fecal samples and cloacal swabs were collected from 150 swine and 150 chickens at the farm, and fecal samples, cloacal swabs and carcass swabs from both animals were collected from 100 swine and 100 chickens at the abattoir. Additionally, fecal samples were collected from all workers on the farm (15 swine farm and seven chicken farm workers). Based on previously documented prevalence values (Jerngklinchan *et al*, 1994), the number of samples collected were adequate to ensure isolation of *Salmonella* and *E. coli* with 10% error and 80% power.

The swine fecal samples consisted of approximately five grams of fecal material, which were collected from finisher pigs on the farm and rectal contents of carcasses at slaughter. Surface swabs from swine carcasses consisted of two samples collected near the rectum of each carcass. Chicken carcass swab samples consisted of two swabs collected from under the wings, where *Salmonella* were thought to be concentrated with the accumulation of water during the slaughter process. Human stool samples were also collected from farm and abattoir workers.

After collection, each specimen was labeled with a specimen ID, the animal species, the abattoir or farm ID, and the date and location of collection. All samples were kept on ice during the transportation to the laboratory at Chiang Mai University for same-day or next-day processing. All samples were processed within 15 hours of collection. Additional information on risk factors that may affect bacterial shedding, or the development of antimicrobial resistance by these bacteria, were collected through a pre-tested questionnaire administered at the time of sample collection.

Laboratory methods: *Salmonella*

For isolation of *Salmonella*, fecal specimens and swab samples were directly inoculated into RVS (Rappaport and Vasiliadis) broth for selective enrichment. The RVS broth was incubated overnight at 42˚C under aerobic conditions. A loop of inoculum from the RVS broth was streaked onto Brilliant Green Agar (BG) and incubated for 24 hours at 37˚C in an aerobic environment. Two colonies were selected from each plate, based on their appearance. *Salmonella* organisms are lactose and sucrose negative, and their colonies appeared pinkish-white on the red agar background. The selected colonies were inoculated onto TSI (triple-sugar iron) agar, and those which exhibited an alkaline slant, an acid butt, and H_2S production were subjected to further biochemical testing. Colonies that demonstrated positive motility, and were decarboxylase positive and indole negative, were considered to be *Salmonella*, and were subcultured and stored on TSA (tryptic soy agar) slants at 4˚C (Quin *et al*, 1994).

Laboratory methods: *E. coli*

For isolation of *E. coli,* the samples collected were streaked onto MacConkey (MAC) agar and incubated for 24 hours at 37˚C in an aerobic environment. MAC agar is a selective media for *Enterobacteriaceae* organisms, which are lactose fermenting and produce a pink hue on the media, and three colonies from each sample that matched this description were subjected to biochemical testing. Colonies that exhibited an acid slant, an acid butt, and no H_2S production on TSI were subjected to further biochemical testing. Colonies that were indole and decarboxylase positive, regardless of motility, were considered to be *E. coli*, and were subcultured and stored on TSA slants at 4˚C (Quin *et al*, 1994).

***In vitro* antimicrobial susceptibility testing**

The *Salmonella* and *E. coli* organisms isolated from the fecal samples and carcass swabs were analyzed for their antimicrobial resistance patterns using the broth microdilution method based on guidelines established by the US National Committee on Clinical Laboratory Standards (NCCLS, 1999). The panel of antimicrobial agents tested was recommended by the US National Antimicrobial Resistance Monitoring System (NARMS), and included ampicillin, ceftiofur, ceftriaxone, nalidixic acid, florfenicol, tetracycline, ciprofloxacin and enrofloxacin. The interpretive categories described by NCCLS were used to categorize the bacteria as either resistant or not resistant, based on their minimum inhibitory concentration (MIC) values.

Statistical analysis

The chi-square test for independence was used to analyze the data.

RESULTS

Salmonella prevalence and antimicrobial resistance patterns

The prevalence of *Salmonella* isolated from swine, chickens, and workers from farms and abattoirs are shown in Fig 1. The overall prevalence of *Salmonella* was 8%, and the prevalence rate in swine (15%) was significantly higher than in chickens (1%) ($p < 0.01$). No *Salmonella* were isolated from chickens at the abattoir or farm workers at chicken farms. The largest proportion of *Salmonella* recovered came from all samples from abattoirs (12%), followed by all farm workers (9%) and all farm samples (2%). More *Salmonella* were isolated from swine at the abattoir (25%) than from swine at the farm (2.1%) ($p < 0.01$).

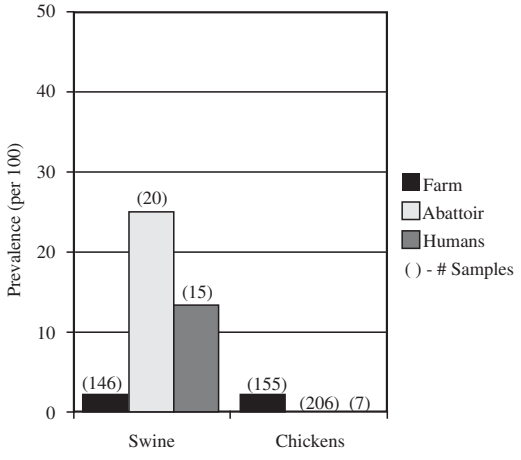

Fig 1- Prevalence of *Salmonella* isolated from swine, chickens, and workers from farms and abattoirs in northern Thailand.

Antimicrobial resistance to tetracycline, nalidixic acid, florfenicol, ampicillin and ceftiofur was seen in *Salmonella* isolates in this study (Table 1). No resistance to ceftriaxone, ciprofloxacin or enrofloxacin was found. Multi-resistant *Salmonella*, isolates with resistance to more than one antimicrobial drug, were isolated from farm and abattoir samples (Fig 2).

E. coli prevalence and antimicrobial resistance patterns

The prevalence of *E. coli* isolated from swine, chickens, and workers from farms and abattoirs are shown in Fig 3. The prevalence in swine from both the farm and abattoir (47%) was significantly higher than in all chickens (39%) ($p = 0.04$). The overall prevalence at the abattoir for both swine and chickens (44%) was not significantly different from the animal at the farms (41%) ($p = 0.49$).

Table 2 shows the proportions of *E. coli* resistant to the antimicrobial agents tested in this study. The highest levels of resistance in *E. coli* were to tetracycline, followed by nalidixic acid, ampicillin, florfenicol and enrofloxacin, while relatively few isolates were resistant to ciprofloxacin, ceftiofur and ceftriaxone. Isolates from farm workers showed the highest proportions of resistance to tetracycline, florfenicol, nalidixic acid, ampicillin and enrofloxacin, despite the fact that florfenicol and enrofloxacin are not drugs approved for human use. Multi-resistant *E. coli* were found in comparable levels on the farm and at abattoirs (Fig 4) ($p = 0.46$). The levels of multi-resistant *E. coli* were higher in samples from swine

Table 1
Percentage of *Salmonella* isolates exhibiting resistance to antimicrobial agents[a], from swine, chickens, and workers from farms and abattoirs in northern Thailand.

Source	No.	Ampicillin	Ceftiofur	Florfenicol	Nalidixic acid	Tetracycline
Swine:						
Farm	3	0.0	0.0	33.3	0.0	66.7
Abattoir	50	14.0	4.0	16.0	22.0	86.0
Workers	2	50.0	0.0	100.0	50.0	50.0
Chickens:						
Farm	4	0.0	0.0	0.0	100.0	100.0
Total	59	13.6	3.4	18.6	27.1	84.7

[a] No antimicrobial resistance was seen in any samples to ceftriaxone, ciprofloxacin, or enrofloxacin.

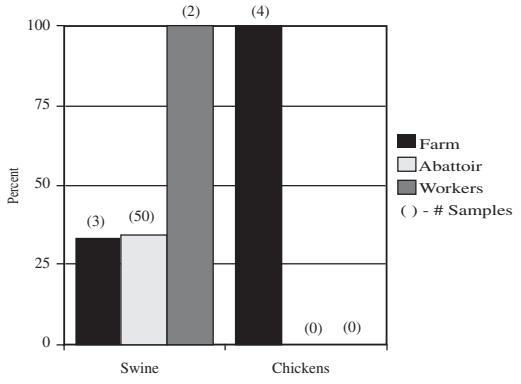

Fig 2- Proportion of antimicrobial-resistant *Salmonella* demonstrating resistance to more than one antimicrobial drug, isolated from swine, chickens, and workers from farms and abattoirs in northern Thailand.

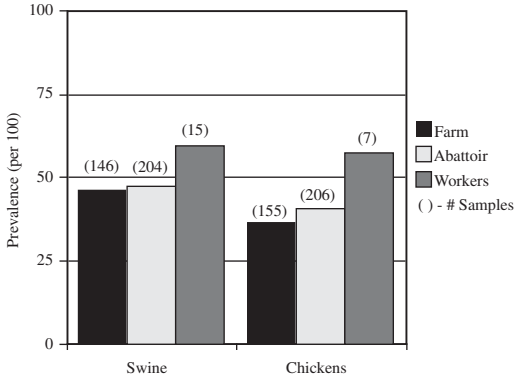

Fig 3- Prevalence of *E. coli* isolated from swine, chickens, and workers from farms and abattoirs in northern Thailand.

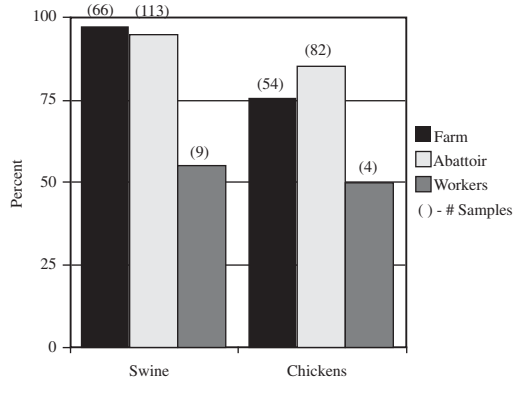

Fig 4- Proportion of antimicrobial-resistant *E. coli* demonstrating resistance to more than one antimicrobial drug, isolated from swine, chickens, and workers from farms and abattoirs in northern Thailand.

than from chickens ($p < 0.01$). On both swine and chicken farms, the proportions of multi-resistant *E. coli* were higher in animals than in humans ($p < 0.01$).

Comparison of sampling methods at the abattoir

Two different types of samples taken from animals at the abattoir: fecal material from fecal samples or cloacal swabs, and carcass swabs. In swine, the prevalence of *Salmonella* and *E. coli* were higher for abattoir fecal samples in comparison to carcass swabs (Fig 5).

DISCUSSION

The recovery rate of *Salmonella* from swine farms in this study (2%) was lower than previously reported rates of 25% (Davies *et al*, 1997) and 42% (Sasipreeyajan *et al*, 1996). The prevalence rate of *Salmonella* from chickens in our study (2%) was lower than reported rates of 57% of fecal and 25% of cloacal samples from chickens in Thailand (Sasipreeyajan *et al*, 1996). Specimen collection methods in this study were different from the aforementioned study. We employed a sterile gel transport medium for the sample swabs, whereas the Sasipreeyajan group (Sasipreeyajan *et al*, 1996) employed a buffered peptone water medium to transport the sample swabs to the laboratory. This difference in the method of specimen transport may have affected the overall recovery of *Salmonella* organisms, resulting in our lower prevalence rate.

A higher percentage of *Salmonella* organisms were isolated from swine abattoir samples than from swine farm samples. In particular, more *Salmonella* were found in the unfinished pig carcass fecal samples than in the finished pig carcass swab samples (Fig 5). This may reflect the differences in the sensitivities of the direct fecal and surface swab sampling methods. In chickens, there were no *Salmonella* isolated from abattoir samples or finished carcasses. One possible conclusion to draw is that the chickens were not contaminated with *Salmonella*; however, based on results of previous studies (Jerngklinchan *et al*, 1994), it is highly unlikely that the chicken samples tested were *Salmonella*-free.

The results of the *in-vitro* antimicrobial susceptibility testing have revealed that *Salmonella* species isolated from swine and chicken at the farms and abattoirs exhibited different antimicrobial resistance patterns. Higher numbers of resistant isolates were seen in *Salmonella* from the swine abattoir than from the swine farms. As previously mentioned, this may reflect the sensitivity levels of the different sampling method.

The levels of resistance to specific antimicrobials

Table 2
Percentage of *E. coli* isolates exhibiting resistance to antimicrobial agents, from swine, chickens, and workers from farms and abattoirs in northern Thailand.

Source	No.	Ampi	Ceft	Ceftri	Florf	NA	Tetra	Cipro	Enrof
Swine									
Farm	66	68.2	1.5	0.0	63.6	80.3	97.0	19.7	40.9
Abattoir	113	73.5	1.8	0.0	61.9	55.8	94.7	4.4	14.2
Workers	9	44.4	0.0	0.0	55.6	33.3	66.7	0.0	11.1
Chickens									
Farm	54	38.9	14.8	1.9	50.0	61.1	77.8	7.4	9.3
Abattoir	82	59.8	61.0	4.9	29.3	82.9	93.9	23.2	54.9
Workers	4	0.0	0.0	0.0	50.0	25.0	100.0	0.0	0.0
Total	328	61.6	4.9	1.5	51.8	67.4	91.5	12.5	28.7

Ampi = Ampicillin, Ceft = Ceftiofur, Ceftri = Cefriaxone, Florf = Florfenicol, NA = Nalidixic acid, Tetra = Tetracycline, Cipro = Ciprofloxacin, Enrof = Enrofloxacin

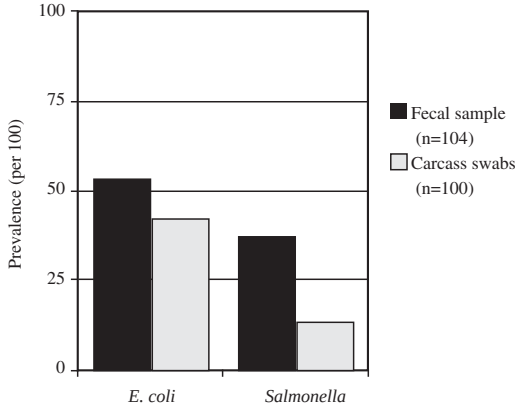

Fig 5- Prevalence of *E. coli* and *Salmonella* isolated from swine at the abattoir in northern Thailand, by type of sample taken.

in this study are significantly different from other reports in the literature. In our study, the only chicken samples that yielded *Salmonella* organisms were chickens on the farms, and all of these samples were resistant to only nalidixic acid. In a study by Hoge *et al* (1998), resistance to nalidixic acid in isolates from humans with diarrhea from 1991-1992 was 2%, and increased to 4% in 1993-1994 and 9% in 1995 (Hoge *et al*, 1998). The same study saw resistance to ciprofloxacin increase from 0% in 1991-1994 to 0.3% in 1995. The differences in levels of antimicrobial susceptibility seen by the two studies may be attributed to the different methods used for antimicrobial susceptibility testing. Our laboratory used the broth microdilution method, while the Hoge group used the disk diffusion and agar dilution methods (Hoge *et al*, 1998). Direct comparison of antimicrobial resistance patterns between these studies should only be done with thoughtful scientific scrutiny. Also, our study was conducted as a short-term, cross-sectional study with a limited number of samples, compared to the 15 year prospective, longitudinal study involving 1,879 *E. coli* and 2,718 *Salmonella* samples (Hoge *et al*, 1998).

This study shows a higher prevalence of resistance of *E. coli* on the pig farms as compared to the pig abattoirs, which may be the result of the different types of samples collected at the different locations.

The patterns related to the antimicrobial susceptibility testing in our study indicated the *E. coli* isolated from swine on the farm displayed more resistance to antimicrobial agents than those isolates recovered at swine abattoirs, while there was more resistance found at the chicken abattoirs than chickens on the farm. This increase in levels of resistant isolates may be due to the mixing of chicken carcasses in defeathering machine, which would take resistant *E. coli* from one carcass and spread it to several others. The proportion of *E. coli* with resistance to ciprofloxacin was relatively lower when compared to nalidixic acid, tetracycline and enrofloxacin.

The levels of antimicrobial resistance in *E. coli* isolates from humans in this study differed from levels reported in the literature. A study conducted in the Netherlands looked at 797 *E. coli* isolates from humans, and reported resistance levels of 84% for ampicillin, 29% for tetracycline, and 9% for nalidixic

acid (Bonten *et al*, 1990). In our study, 318 *E. coli* isolates were tested, and the 13 isolates from farm workers in our study showed 45% of isolates were resistant to ampicillin, 10% to tetracycline and 30% to nalidixic acid. Even though both groups used similar broth microdilution testing methods, the Netherlands study used the Dutch Working Party on Antimicrobial Susceptibility Testing breakpoints for their MIC determinations (Bonten *et al*, 1990), while this study utilized the NCCLS Antimicrobial Susceptibility Testing breakpoints to determine MIC values. The two agencies have established distinct and different antimicrobial MIC breakpoints, and direct comparison of results should be done with caution.

As a pilot study, we were able to generate prevalence values to support the design of a more extensive study on *E. coli* and *Salmonella* in food chain in northern Thailand. Work on this study also provided valuable experience in the collection of samples in the field, and allowed our study group to refine bacterial isolation and identification techniques, and antimicrobial susceptibility testing. In conclusion, this study also leads to the hypothesis that bacteria with resistance to antimicrobial agents may travel from the farm, through carcass processing, to meats for human consumption. The serious nature of the human health implications of hypothesis warrants further investigation in the future.

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