

ANTIMICROBIAL RESISTANCE *SALMONELLA* ISOLATED FROM BEEF IN UPPER NORTHEASTERN THAILAND

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ABSTRACT: This study investigates the distribution of *Salmonella* serotypes, antimicrobial susceptibility and patterns of multidrug resistance of *Salmonella* spp. isolated from beef, received from different slaughterhouses and butcher shops in the upper northeastern region of Thailand. Amongst the beef samples, one hundred and forty-five isolates were detected. There are three serogroups of salmonella which can be classified into eight serovars; five serovars belonging in three serogroups were contaminated on meat from slaughterhouses, likewise, seven serovars which included two serogroups were detected from butcher shops; by which, 12.50 % (32/145) of the salmonella strains was resistant to antimicrobial agents and almost 6.89 % (10/145) were multidrug resistant. The most multiresistant serotype was *S. Derby*, with a pattern of multi-resistance to six antibiotics, followed by *S. Rissen*, *S. Anatum*, and *S. Muenchen*, respectively. Moreover, most of the salmonella strains were resistant to ampicillin, oxytetracycline and sulfamethoxazole/trimethoprim, whilst, colistin, gentamicin, kanamycin, nalidixic acid, and norfloxacin are considered highly susceptible drugs. In addition, these strains have been shown resistant to chloramphenicol also, despite it has been banned use in food-producing animals. In summary, beef from the local slaughterhouses and retail butcher shops in upper northeastern Thailand was found to be contaminated with *Salmonella* spp., notably, from the serogroups B, C and E, which are multidrug resistant. For this reason, veterinarian and all relevant authorities need to strictly regulate the use of antibiotics in animal production, whether for use as therapy, prophylaxis, or growth promotion.

Keywords: Antibiotics, Contamination, Drug-resistance, *Salmonella* spp.

1. INTRODUCTION

Antimicrobial resistance is a public health problem throughout the world and causes increased morbidity and mortality among humans and animals. Various studies were found a commensal bacterium in livestock contaminated on fresh meat frequency and may serve as reservoirs for resistant genes that could potentially be transferred to pathogenic organisms in humans [1]. The evidence is accumulating to support the hypothesis that antibiotic-resistant bacteria from cattle enter the food supply, can be found in human food [2]. Furthermore, the transfer of drug resistance within the gastrointestinal tract is still possible; thus, if not strictly regulated the application of antibiotics delivered to animals, for instance, therapy, prophylaxis, or growth promotion in domestic livestock can potentially lead to widespread dissemination of antimicrobial-resistant bacteria. Therefore, the antibiotics resident in animal products may be harmful or provide an antibiotic-resistant bacteria from animals to the consumer, and lead to increase a pathogenic resistance to antibiotics, becoming a reservoir of resistance genes for pathogenic transmission in and between microorganisms, and spread into humans through various routes [3, 4].

Salmonella as a foodborne pathogen in animal products is a major cause of human salmonellosis and food poisoning. They are common inhabitants on the intestinal tract of humans and mammals, which can be distributed in the environment and contaminate the food chain in the process of food production. Normally, humans are infected by eating or touching an infected animal or their feces, in particular, during the processing of meat production [5]. The processes during the pre-slaughter, slaughter and the storage period after slaughter are suitable for contamination of pathogenic salmonella, which can cause serious diseases in humans and animals. Thus, the purpose of this study was to investigate the distribution of *Salmonella* serotypes that are resistant to antibiotics, which has contaminated beef from difference slaughterhouses and which available for purchase in retail butcher shops in upper northeastern Thailand.

2. MATERIALS AND METHODS

A total of 145 beef samples were taken for analysis of *Salmonella* serotypes contamination and resistance to antimicrobial drugs. Of these, 64 samples were local slaughterhouse meat samples and 81 were retail butcher shop beef meats within 12 provinces of the northeastern region of Thailand. All experiments were performed in the Veterinary

Research and Development Centre (upper northeastern region) laboratory, Thailand.

2.1 Isolation and Identification

Salmonella was isolated from beef samples by standard methods according to [6]. Briefly, a beef sample was minced, a sample weight of 25 g was homogenized with 225 ml of sterile Buffered peptone water (BPW) for 2 min. The mixture was then incubated at $37\pm1^{\circ}\text{C}$ overnight. After that, 1 ml each of the pre-enriched cultures was added to two enrichment media; Rappaport Vassiliadis (RV) was incubated at $41.5\pm1^{\circ}\text{C}$ for 24 ± 3 h and Muller-Kauffmann tetrathionate novobiocin broth (MKTn) was incubated at $37\pm1^{\circ}\text{C}$ for 24 ± 3 h. Subsequently, the enriched cultures from RVS and MKTn were transferred to Xylose lysine desoxycholate agar (XLD) and Brilliant green agar modified (BGM). All inoculated were incubated at $37\pm1^{\circ}\text{C}$ for 24 ± 3 h. After that, a pink colony with or without black centers are select, then the isolated strains were grown at $37\pm1^{\circ}\text{C}$ overnight in Nutrient agar (NA). Subsequently, the isolates were biochemically tested on Triple sugar iron agar (TSI), L-lysine decarboxylation medium (LIM), Urea agar, VP medium and ONPG to identify the species of Salmonella resign as serotypes. The slide agglutination test was carried out by slurry agglutination test somatic-antiserum specific for O-antigen on slides, read reaction results, sedimentation and serotyping by culture on swarm agar. The flagella-antiserum is specific for H-antigen on slides and reads the precipitation reaction. The results of the tests were compared with antigenic formulas of the *Salmonella* serovars according to the Kauffmann-White Scheme [7, 8].

2.2 Antimicrobial Susceptibility Testing

Salmonella isolates were tested for antimicrobial susceptibility by disk diffusion method as described by [9]. Overnight-grown cultures were spread on Mueller Hinton agar. The antibiotic disks were placed on it then incubated at $37\pm1^{\circ}\text{C}$ for 16-18 h. An interpretation result was guided according to standards established by the Clinical and Laboratory Standards Institute (CLSI) [10], *E. coli* ATCC 25922 was used as a quality control strain. The following antimicrobial were tested: amoxicillin/clavulanic acid (AMC) 20/10 $\mu\text{g/mL}$, ampicillin (AMP) 10 $\mu\text{g/mL}$, cephalothin (KF) 30 $\mu\text{g/mL}$, chloramphenicol (C) 30 $\mu\text{g/mL}$, ciprofloxacin (CIP) 5 $\mu\text{g/mL}$, colistin (CT) 10 $\mu\text{g/mL}$, enrofloxacin (ENR) 5 $\mu\text{g/mL}$, gentamicin (CN) 10 $\mu\text{g/mL}$, kanamycin (K) 30 $\mu\text{g/mL}$, nalidixic acid (NA) 30 $\mu\text{g/mL}$, neomycin (N) 30 $\mu\text{g/mL}$, norfloxacin (NOR) 10 $\mu\text{g/mL}$, oxytetracycline (OT) 30 $\mu\text{g/mL}$, streptomycin (S) 10 $\mu\text{g/mL}$, sulfamethoxazole/ trimethoprim (SXT) 1.25/23.75

$\mu\text{g/mL}$ and tetracycline (TE) 30 $\mu\text{g/mL}$. The diameter zone of growth inhibition surrounding the paper disk is measured and compared to the standard inhibition diameter [10].

3. RESULTS AND DISCUSSION

3.1 Prevalence of Salmonella-Contaminated in Meat from Slaughterhouses and Butcher Shops.

One hundred and forty-five samples that originated from beef carcasses at sixty-four slaughterhouses and eighty-one butcher shops, in the upper northeastern region of Thailand were included for determination of *Salmonella* spp. prevalence.

The results as shown in Table 1, indicated that a total of sixteen samples (11.03 %) detected *Salmonella* spp., nine samples (14.06 %) were from slaughterhouses and the other seven samples (8.64 %) were from butcher shops. There were five serovars from three serogroups in contaminated meat from slaughterhouses, and seven serovars which included two serogroups were detected from butcher shops. Notably, only *S. Derby* was found in beef from a slaughterhouse in Roi-Et province. In addition, *S. Kouka* and *S. Okefoko* were detected in beef from a butcher shop in Sakon Nakhon province only. The serovar *S. Lexington* was found in beef from the butcher shop in Nakhon Phanom only. Furthermore, the most prevalent serovars were *S. Weltevreden* (4.68 %), followed by *S. Rissen* (2.07 %), *S. Anatum* (2.07 %) and *S. Muenchen* (1.38 %), which is same serotypes as [11]. *Salmonella* spp. are commonly found in the animal gastrointestinal tract, wherein the presence of *Salmonella* spp. in beef suggests poor hygiene management, therefore food contamination with this pathogen could happen throughout the food chain. This study was found in *Salmonella* spp. contamination in beef carcasses (11.03 %) much less than in previous reported [11] which found a prevalence of 52 % (28/54) in beef samples and included Thai self-service style restaurants in Khon Kaen municipality, Thailand. Therefore, the production of Thai beef meat is in the process of developing into a standard, including prescription and control measures in accordance with the slaughterhouse strictly so that most slaughterhouses now operate to a good standard of practice. Furthermore, most slaughtering is carried out in local municipal slaughterhouses or private slaughterhouses, which are certified by the Department of Livestock Development. As a result, the prevalence rate of *Salmonella* spp. contained on beef in these studies were decreased when compared with a previous report that studies in the same area.

When comparing the prevalence rate of *Salmonella* spp. contamination in the beef between a slaughterhouse and retail butcher shop, there are no statistically significant differences. This shows that the

transportation from the local slaughterhouse to the butcher shop, including the preservation and deliver to the butchers' shop, are carried out hygienically. However, some local slaughterhouses still operate with poor hygiene and meat is usually delivered in open buckets in a car. Typically, meat in butcher shops are sold at ambient temperatures, this indicates the application of strict hygiene practices is therefore essential. The regulatory authorities should control

policy for a good manufacturing practice for cattle abattoir, together with stringent sanitation in fresh meat production. Moreover, the presence of *Salmonella* from food product reaffirms the importance of the need for strengthening collaboration between veterinary and public health sectors on the regulation, particularly on a food animal production.

Table 1 Prevalence of *Salmonella* serotypes obtained from beef carcasses in slaughterhouse and butcher shop

<i>Salmonella</i> spp.	Number of positive samples (%)			Provinces*
	Slaughterhouse (n=64)	Butcher shop (n=81)	Total (n=145)	
Group B	1 (1.56)	0 (0.00)	1 (0.69)	
<i>S. Derby</i>	1 (1.56)	0 (0.00)	1 (0.69)	Roi Et(Sh:1)
Group C	3 (4.69)	2 (2.47)	5 (3.45)	
<i>S. Muenchen</i>	1 (1.56)	1 (1.23)	2 (1.38)	Roi Et(Bs:1), Sakon Nakhon(Sh:1)
<i>S. Rissen</i>	2 (3.13)	1 (1.23)	3 (2.07)	Roi Et(Sh:1), Sakon Nakhon(Bs:1), Khon Kaen(Sh:1)
Group E	5 (7.81)	5 (6.17)	10 (6.90)	
<i>S. Anatum</i>	2 (3.13)	1 (1.23)	3 (2.07)	Kalasin(Sh:1), Maha Sarakham(Bs:1), Roi Et(Sh:1)
<i>S. Kouka</i>	0 (0.00)	1 (1.23)	1 (0.69)	Sakon Nakhon(Bs:1)
<i>S. Lexington</i>	0 (0.00)	1 (1.23)	1 (0.69)	Maha Sarakham(Bs:1)
<i>S. Okefoko</i>	0 (0.00)	1 (1.23)	1 (0.69)	Sakon Nakhon(Bs:1)
<i>S. Weltevreden</i>	3 (4.68)	1 (1.23)	4 (2.76)	Kalasin(Sh:1), Maha Sarakham(Bs:1), Sakon Nakhon(Sh:2)
Total	9 (14.06)	7 (8.64)	16 (11.03)	Kalasin, Khon Kaen, Maha Sarakham, Sakon Nakhon, Roi Et

* Sh=Slaughterhouse; Bs=Butcher shop

3.2 Antimicrobial Susceptibility and Patterns of Multidrug Resistance of the *Salmonella* Isolates

All *Salmonella* isolates have been tested for antimicrobial susceptibility; the results showed that 12.50 % of isolates were resistant to at least one antimicrobial agent and 25.00 % were multi-drug resistant. Moreover, sixteen isolates were multi-drug resistant (MDR) based on the categorization of non-susceptible to at least one antimicrobial agent in three or more antimicrobial categories [12] (Fig. 1.) The resistance rate to amoxicillin/clavulanic acid, ampicillin, cephalothin, chloramphenicol, ciprofloxacin, oxytetracycline, streptomycin, sulfamethoxazole/trimethoprim and tetracycline were 6.25 %, 43.75 %, 6.25 %, 6.25 %, 6.25 %, 43.75 %, 12.50 %, 43.75 % and 31.25 %, respectively (Table 2); by which found these drugs had been used in human medicine also [13]. Although farmed animals being treated under veterinary control, drugs residue

in meat remained. Therefore, the presence of these antibiotic residues in meat may be due to the short withdrawal period before slaughtering or prolonged courses of antibiotics. The misuse of antibiotics may lead to the development of drug-resistant *salmonella*. Besides this, drug-resistant *salmonella* present in meat, maybe transfer of resistance to other human pathogens and be the cause of poor response of treatment with human medicines, according to a recent report [14].

It is astonishing to observe the isolates resistant to chloramphenicol, in spite of the fact that the drug has been forbidden use in food-producing animals. It was suggested that this phenomenon may be generated by other antibiotics on co-selection or cross-resistance [15]. This finding even recommends that the removal of certain antimicrobial selections may not completely eliminate AMR and be a transfer of resistance to other important human pathogens.

Table 2 Antimicrobial susceptibility of *Salmonella* spp. isolated from beef by disk diffusion methods

Antimicrobial disk	Numbers of antimicrobial susceptibility*											
	Group B (n=1)			Group C (n=5)			Group E (n=10)			Total (%) (n=16)		
	S	I	R	S	I	R	S	I	R	S	I	R
AMC (20/10 µg)	1	0	0	3	1	1	10	0	0	14 (87.50)	1 (6.25)	1 (6.25)
AMP (10 µg)	0	0	1	1	0	4	8	0	2	9 (56.25)	0 (0.00)	7 (43.75)
KF (30 µg)	1	0	0	3	1	1	10	0	0	14 (87.50)	1 (6.25)	1 (6.25)
C (30 µg)	0	0	1	5	0	0	10	0	0	15 (93.75)	0 (0.00)	1 (6.25)
CIP (5 µg)	0	0	1	5	0	0	10	0	0	15 (93.75)	0 (0.00)	1 (6.25)
CT (10 µg)	1	0	0	5	0	0	10	0	0	16 (100.00)	0 (0.00)	0 (0.00)
ENR (5 µg)	0	1	0	5	0	0	10	0	0	15 (93.75)	1 (6.25)	0 (0.00)
CN (10 µg)	1	0	0	5	0	0	10	0	0	16 (100.00)	0 (0.00)	0 (0.00)
K (30 µg)	1	0	0	5	0	0	10	0	0	16 (100.00)	0 (0.00)	0 (0.00)
NA (30 µg)	1	0	0	5	0	0	10	0	0	16 (100.00)	0 (0.00)	0 (0.00)
N (30 µg)	0	1	0	2	3	0	7	3	0	9 (56.25)	7 (43.75)	0 (0.00)
NOR (10 µg)	1	0	0	5	0	0	10	0	0	16 (100.00)	0 (0.00)	0 (0.00)
OT (30 µg)	0	0	1	2	0	3	7	0	3	9 (56.25)	0 (0.00)	7 (43.75)
S (10 µg)	0	1	0	2	1	2	8	2	0	10 (62.50)	4 (25.00)	2 (12.50)
SXT (1.25/23.75 µg)	0	0	1	2	0	3	7	0	3	9 (56.25)	0 (0.00)	7 (43.75)
TE (30 µg)	0	0	1	4	0	1	7	0	3	11 (68.75)	0 (0.00)	5 (31.25)
Total	7	3	6	59	6	15	144	5	11	210 (82.03)	14 (35.84)	32 (12.50)

* S = sensitive, I = intermediate, R = resistant

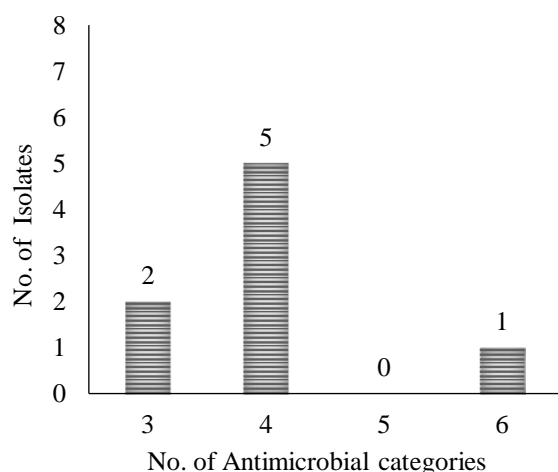


Fig. 1 Proportion of isolates that were multi-drug resistant and number of antimicrobial categories

As shown in Table 3, eight salmonella isolates were classified with six antimicrobial resistance patterns, which were resistant to three, four and six antimicrobial agents. The most common resistance patterns were AMP-OT-S-TE and AMP-OT-TE-SXT, whereas the highest number antimicrobial resistance pattern is AMP-C-CIP-OT-SXT-TE. Thus, AMP, TE and SXT are belonging to most multidrug patterns. Indicated that the β -Lactams, Tetracyclines and Sulfonamides are widespread drugs used in this area for administrating to cattle. Similar results have been reported recently too [16]. The previous survey showed that the resistance pattern of isolated salmonella contaminated with pork, identified as multidrug resistant and the most resistance pattern was AMP-TE-SXT. Whereas chloramphenicol is the one antibiotic agent found in the resistance pattern, like in accordance with those of this study. For this reason, veterinarian and relevant authorities need to regulate the use of antibiotics in animal production strictly, whether for disease treatment, prevention, or growth promotion.

Table 3 Patterns of multidrug resistance of *Salmonella* spp. isolated from beef receiving difference slaughterhouse and butcher shop in upper northeastern part of Thailand

Number of drugs showing resistance	The pattern of <i>Salmonella</i> isolate showed multidrug resistance (n)			
	Group B	Group C		Group E
	S. Derby	S. Muenchen	S. Rissen	S. Anatum
3	(0)	AMC-AMP-C(1)	(0)	OT-TE-SXT(1)
4	(0)	(0)	AMP-OT-S-TE(2) AMP-OT-TE-SXT(1)	AMP-OT-TE-SXT(2)
6	AMP-C-CIP-OT-SXT-TE(1)	(0)	(0)	(0)

4. CONCLUSIONS

In conclusion, the results demonstrate the low contamination rate of MDR *Salmonella* spp. in beef carcasses in local slaughterhouses and retail butcher shops of northeastern Thailand. These findings indicated a low level of antibiotics residues in beef were an important source for exposure of human to antibiotics. Evidently, antimicrobial use in beef production is controlled more strictly and under supervision by veterinarians. This confirms that slaughterhouses and butcher shops in Thailand generally have a good manufacturing practice and are developing international standards.

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