

Original Research Article

Antibiotic susceptibilities, molecular Characterization of

Salmonella from Cattle faeces in Chiang Mai, Thailand and their **genetic**
relatedness to **clinical** isolates

Abstract

Background

Salmonella are the most common pathogenic bacteria causing food borne and zoonotic diseases in humans. The primary sources of Salmonellosis are considered to be food-producing animal. Cattle is also the production animal slaughtered for human consumption which have been implicated as a source of human infection with antimicrobial resistant *Salmonella*. The purpose of this study was to determine the prevalence of *Salmonella* in the faeces of apparently healthy slaughtered cattle and characterize the circulating non-typhoidal *Salmonella* (NTS) serovars in cattle in Chiang Mai, Thailand as well as determine their antibiotic resistance profiles and genetic relatedness to human isolates.

Methodology

A cross-sectional study was conducted from June to September 2017 at the municipal abattoir of Chiang Mai, Thailand. One hundred fifty fecal samples were collected from apparently healthy slaughtered cattle for *Salmonella* isolation. One hundred and ten blood samples were also collected from febrile patients at Chiang Mai provincial hospital, prior to any antimicrobial treatment. The identification of the circulating *Salmonella* serovars using culture techniques and DNA sequencing, antimicrobial sensitivity testing using disc diffusion method and phylogenic relationships using 16S rRNA were determined.

Results The result showed that the prevalence of *Salmonella* from cattle was 22.6%. According to the NCBI blast, three serovars were identified; S.Typhimurium (50.0%), S.Enteritidis (41.2%) and S.Weltevreden (11.8%) and these three serovars formed distinct clades on the phylogenetic tree. The

27 S.Typhimurium and S.Enteritidis isolates from cattle were closely related to human isolates. All
28 isolates from cattle were susceptible to 10 selected commonly prescribed antimicrobials.

29 Conclusion

30 Our study revealed that the healthy slaughtered cattle in Chiang Mai carried Salmonella with a
31 high prevalence. The isolation of S.Typhimurium and S.Enteritidis from cattle and humans were
32 closely related. The finding suggested that slaughtered cattle is a source of human infected
33 Salmonella. This poses a major public health threat since slaughtered cattle might be a potential
34 source of food borne salmonellosis. Therefore, the local administration should have regular
35 systematic surveillance for the infectious sources.

36 Keywords: *Salmonella*, Antimicrobial susceptibility testing, DNA sequencing, Phylogenic tree analysis

37 Introduction

38 *Salmonella* is an enteric gram-negative bacteria which is one of the major foodborne
39 pathogens worldwide. It can cause a variety of clinical manifestations from mild gastroenteritis to
40 bacteremia and typhoid fever. The global burden of non-typhoidal *Salmonella* gastroenteritis has
41 been estimated to be 93.8 million cases of gastroenteritis each year and causes approximately
42 155,000 deaths(1). Salmonellosis is an important public health problem in both developing and
43 developed countries, although incidence rates vary according to the country (2). In Africa, Non
44 Typhoidal Salmonellosis (NTS) has been consistently reported as leading cause of bacteremia among
45 immune-compromised people. While in Thailand, NTS are common bloodstream isolates in febrile
46 patient (3, 4). However, the sources and transmission routes of *Salmonella* in developing countries
47 are poorly understood due to the lack of coordinated national epidemiological surveillance systems(5).
48 From previous study, the primary sources of salmonellosis are considered to be food-producing
49 animals such as cattle, poultry and swine (6). The pathogen are mainly disseminated by trade in
50 animals and uncooked animal food products (7). Also the fecal wastes from infected animals and
51 humans are important sources of bacterial contamination of environment and the food chain (8). In
52 human, *Salmonella* infections can lead to invasive and focal infections that can be severe(9).
53 Treatment with antimicrobials is crucial for the proper management of bacterial infections, severe

human salmonellosis in particular. In addition, the overuse of antimicrobials in the treatment of bacterial infection in humans and animals has led to antimicrobial resistance (10). Resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on- farm selection of antimicrobial- resistant strains (11, 12). Thus, food –producing animals are considered one of the main routes of transmission of antimicrobial- resistant bacteria especially pathogenic bacteria between animal and human population (13). The lacking regular surveillance programs in Thailand, as in the most of developing countries, information on the prevalence of *Salmonella* in food –producing animals is limited. Critically, little is known about the prevalence of *Salmonella* antimicrobial- resistant from food –producing animals and the genetic relatedness of the isolates from animal and human salmonellosis. Cattle have been implicated as a source of human infection with antimicrobial- resistant *Salmonella* through direct contact with livestock. Improper condition of slaughtering process was also considered as a factor enhancing resistant ability of *Salmonella* (14, 15). The purpose of this study was to determine the prevalence, serotype, antibiotic susceptibilities and genetic relatedness to clinical isolates, of *Salmonella* from the faeces of healthy slaughtered cattle in Chiang Mai, Thailand.

Method

Sample collection

One hundred fifty fecal samples from healthy cattle, collected from June to September 2017, were obtained from Chiang Mai municipal slaughter house complex. Approximately 50 g of fresh sample were collected in a clean sterile air tight stool cup directly from the rectum immediately after the animals were slaughtered. The sample was determined using prevalence rate of 3.0% from previous study, with a 99% confidence interval covering an error of 5 percentage points from the true value and the following formula was employed (16,17).

$$n = \frac{Z^2 P(1 - P)}{d^2}$$

Where n= sample size, Z= Z statistic for a level of confidence, p= expected prevalence or proportion, d= precision

80 Based on the above formula the calculated sample size was 78. A total of 150 cattle were randomly
81 selected in this study.

82 One hundred and ten blood samples were also collected from febrile patients at Chiang Mai provincial
83 hospital, prior to treatment with any antimicrobial agents during June to September 2017. The
84 samples were transported using an ice box and culture within 24 hours and analyzed at Regional
85 Medical Sciences center, Chiang Mai, Department of Medical Sciences, Ministry of Public Health,
86 Thailand.

87 Isolation of Salmonella from Cattle

88 The isolation was conducted according to ISO 6579/AMD 1:2007 standard protocol(18). The
89 isolation involves three steps as follows;

- 90 1) One g of fecal sample was pre-enriched with 9 ml of buffered peptone water (BPW) (Oxoid,
91 England) and incubated for 24 hr at 37°C.
- 92 2) A portion (0.1 ml) of the pre-enriched cultured was transferred to 10 ml of selenite
93 cysteine(SC) broth (Himedia, India) and another 0.1 ml portion was transferred to 10 ml of
94 Rappaport and Vassilidis (RV) broth (Merck, Germany) broth and incubated at 37°C and 42°C
95 for 24 hr, respectively.
- 96 3) Sample from the selective enrichment media was inoculated on to Xylose Lysine Deoxycholate
97 (XLD) (Oxoid, England) agar and incubated at 37°C for 24 hr and the incubation was prolonged to
98 48 hr for those that did not show any growth during the 24 hr incubation.
99 Characteristic *Salmonella* colonies, having a slightly transparent zone of reddish color and a black
100 center, were sub-cultured on nutrient agar (Oxoid, England) and confirmed biochemically using
101 triple sugar iron agar (TSI) (Oxoid, England), Christensen's urea agar (Oxoid, England), lysine iron
102 agar (LIA) (Oxoid, England), Voges Proskauer (VP), methyl red (MR) (Micromaster, India), and
103 Indole tests (Becton Dickinson, USA) (19).

104 Isolation of Salmonella from human

105 Blood samples of 5 to 10 ml and 2 to 5 ml were collected from adults and children, respectively.
106 Bacteria was isolated from each blood sample following standard procedure by using the BacT/Alert
107 3D automated system (BioMerieux, Inc., Durham, N.C.) according to the manufacturer's instructions.
108 All isolates were identified using standard diagnostic techniques (20).

109

110 Identification of Salmonella

111 **Extraction of Genomic DNA** Pure colonies of Salmonella cultured in nutrient broth (Oxoid),UK) was
112 used for DNA extraction using the Nucleospin Tissue (Machrey-Nagel, Germany) according to the
113 manufacturer's instructions.

114 PCR Amplification

115 PCR was performed as described previously (21). Except for 25 µl of Quick Taq HS DyeMix
116 (TOYOBO, Japan), 18 µl of nuclease free water, 1 µl of each primer and 5 µl of extracted DNA were
117 used. Forward and reverse PCR primers were synthesized by Bio Basic Inc., Canada.

118 **DNA sequencing** The PCR products obtained were extracted using QIAquick gel extraction kit
119 (Qiagen, USA). Thirty microliters of the purified DNA of each sample was sequenced by Bio Basic
120 Inc., Canada.

121 Phylogenetic analysis

122 The 16S rRNA sequences obtained were compared with known 16S rRNA sequences at
123 National Center for Biotechnology Information (NCBI) database using BLASTn (Basic Local Alignment
124 Search Tool) (22). Identification of the sequences at both the genus and species level was defined as
125 a 16S rRNA sequence similarity of $\geq 99\%$ with that of the prototype strain sequence in GenBank. The
126 sequences together with reference sequences derived from the Genbank were aligned using
127 CLUSTAL OMEGA. The probability of phylogenetic tree were determined using MEGA software
128 version 6.0 (23).

129 *Salmonella* antimicrobial susceptibility tests

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (24). Briefly, pure colonies, bacterial suspension were placed in test tubes and their turbidity adjusted to 0.5 McFarland turbidity standards. The diluted bacterial suspensions were then transferred onto Mueller-Hinton agar plates using a sterile cotton swab and seeded uniformly. Antibiotic impregnated discs were then placed to the plate surfaces using sterile forceps. The plates were incubated aerobically at 37 °C for 24 h and *E.coli* ATCC 25922 was used as a quality control organism for the antimicrobial susceptibility test. A total of 10 selected antibiotic disks were used which contained the commonly used antibiotics namely; amoxycillin/clavulanic acid(30 µg), ceftazidime (30 µg), ceftriaxone, (30 µg) ciprofloxacin(30 µg), colistin(10 µg), imipenem (30 µg), sulbactam/cefoperazone(30 µg), ertapenem(10 µg), piperacillin/tazobactam(10 µg) and tigecycline (15 µg) (Oxoid, , England). Zones of inhibitions were measured to determine whether the bacteria were susceptible, intermediate or resistant in comparison to CLSI critical points.

Data Analysis

Data was described and presented in tables and figure in antimicrobial resistance bacteria and Phylogenetic analysis tree. The 16S rRNA sequences of *Salmonella* isolates from human and cattle's feces were compared with known 16S rRNA sequences at National Center for Biotechnology Information (NCBI) database using BLASTn (Basic Local Alignment Search Tool) algorithm obtained from; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. Identification of the sequences at both the genus and species level was defined as a 16S rRNA sequence similarity of $\geq 99\%$ with that of the prototype strain sequence in GenBank. The probability of phylogenetic tree was determined. The topological robustness of the trees was evaluated by a bootstrap analysis involving 10,000 replications.

Result

Salmonella prevalence and serotypes

Salmonella was isolated from 34 (22.6%) out of the total 150 fecal samples of healthy slaughtered cattle. The bacteria was also isolated from seven of one hundred and ten blood samples obtained from febrile patients. The PCR product obtained was 572 bp (Figure 1). According to the NCBI blast, 3 serovars of *Salmonella* were identified: 17 (50%) out of 34 of cattle samples and 5 (71.4%) out of 7 of human samples were identified to be *S. Typhimurium*. Thirteen out of 34 (41.2%) of cattle samples and 2 out of 7 (28.6%) of human samples were identified to be *S. Enteritidis*. Four out of 34 (11.8%) of cattle

samples were identified to be *S. Weltevreden*. None of *S. Weltevreden* was isolated from human samples (Table 1).

Table 1 The percentage of *Salmonella* positive samples isolated from cattle (n=150) and human (n=110) in Chiangmai, Thailand.

<i>Salmonella</i> (S.) serovars	No. of cattle (%)	No. of humans (%)
<i>S. Typhimurium</i>	17 (50)	5 (71.4)
<i>S. Enteritidis</i>	13 (41.2)	2 (28.6)
<i>S. Weltevreden</i>	4 (11.8)	0 (0)
Total	34 (22.6)	7 (6.3)

Phylogenetic analysis

S. Typhimurium and *S. Enteritidis* and *S. Weltevreden* were identified and formed three clades in the phylogenetic tree. *S. Weltevreden* and *S. Enteritidis* clades showed 58% and 62% probabilities from the *S. Typhimurium* clades. Genetic relatedness of the isolates belonging to the same serotype ranged from approximately 70% to 100%. *S. Typhimurium* and *S. Enteritidis* from cattle and humans were closely related whereas *S. Weltevreden* isolates from cattle had no relationship to human. The branch length of *S. Enteritidis* and *S. Weltevreden* were more than *S. Typhimurium* (Figure 2).

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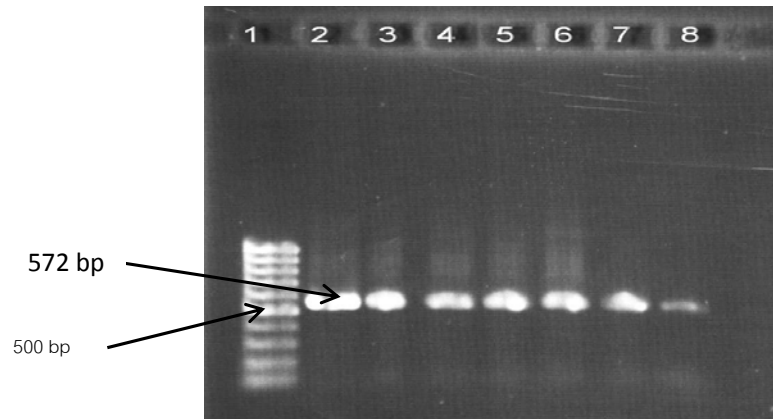
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Figure 1 Agarose gel analysis of 16S rRNA gene of *Salmonella* isolates demonstrating the pattern of positive results. Lane 1: 100 bp, Lane 2: Positive control Lane 3,4 : *S. Typhimurium* Lane 5,6 : *S. Enteritidis* Lane 7,8 : *S. S. Weltevreden*

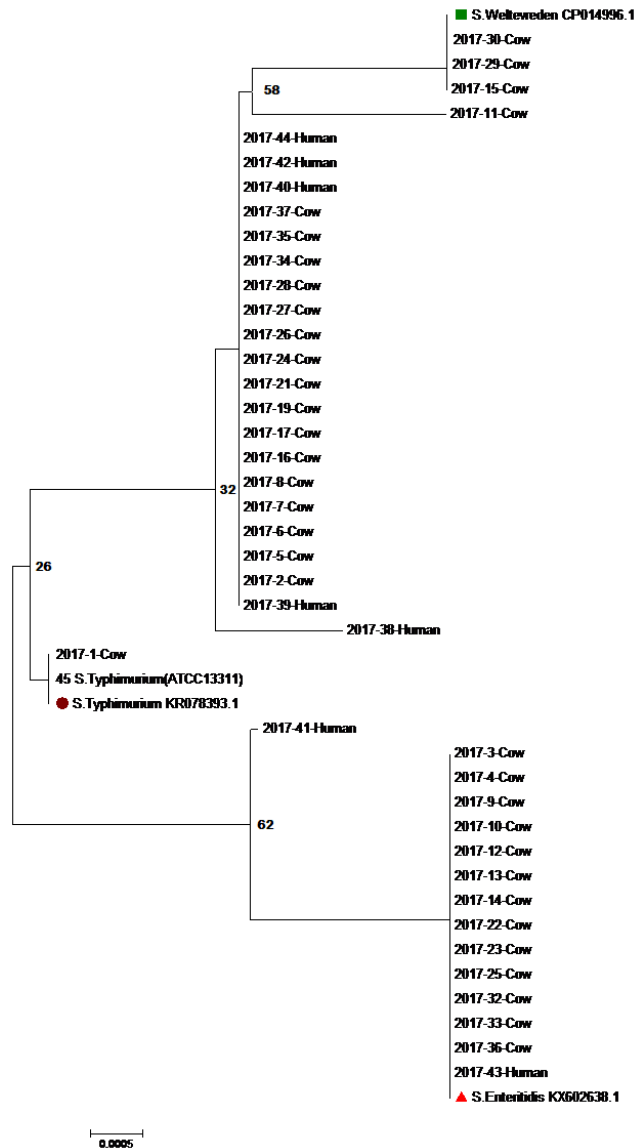


Figure 2 Phylogenetic tree based on 16S rRNA sequences of *Salmonella* isolates. The phylogeny was inferred by CLUSTAL OMEGA using MEGA software version 6.0.

Antimicrobial resistance profiles of *Salmonella* isolates

Antibiotic resistance was determined in all of *Salmonella* isolates. All isolates from cattles were susceptible to amoxycillin/clavulanic acid, ceftazidime, ceftriaxone, ciprofloxacin, colistin, imipenem, sulbactam/cefoperazone, ertapenem, tigecycline and piperacillin/tazobactam. Six of

7(85.7%) isolates from human showed susceptible pattern the same as cattle. One of 7(14.3%) isolates from human was resistant to amoxycillin/clavulanic acid, ceftazidime ceftriaxone and ciprofloxacin (Table 2).

Table 2 *Salmonella* Isolated from Cattles (n=150) and patients (n=110) and their antibiotic resistance patterns

Salmonella serovars	Cattle	Antibiotic resistance patterns		Patients	Antibiotic resistance patterns	
		Resistance	Susceptible		Resistance	Susceptible
<i>S.Typhimurium</i>	17	0	17	5	0	5
<i>S.Enteritidis</i>	13	0	13	2	1(amp,ftz,ftx,cip)	1
<i>S.Weltevreden</i>	4	0	4	0	0	0
Total	34	0	34	7	1	6

Abbreviations: amp, amoxycillin/clavulanic acid; ftz, ceftazidime; ftx, ceftriaxone; cip, ciprofloxacin

205

206 Discussion

207 The *Salmonella* prevalence of healthy slaughtered cattle reported in this study was higher than
 208 that reported in a previous study in northern Thailand (3.0%) (16). In addition, prevalence of
 209 *Salmonella* found in this study was higher than a study in Australia (14.4%) but lower than a previous
 210 study in Cameroon (27%) (25, 26). This difference may be attributed to the difference in time of study,
 211 traditional of living condition, like housing conditions, feeding habits, types of feed given for the cattle
 212 including animal management system; for example, in Thailand the humans and cattle often live in
 213 close vicinity whereas in Australia the human house and the ranch are strictly separate (16, 27). It is
 214 interesting to note that *Salmonella* was detected in the fecal matter of healthy slaughtered cattle
 215 although the animals are slaughtered a certified veterinarian inspects the health status of the animal
 216 by checking the skin fur, fecal matter, physical appearance and other clinical parameters. According
 217 to nearly closed ecosystem of slaughterhouse, the process of slaughtering animals must be a
 218 concern. In addition, factor that may affect prevalence that affect detection of the organism, such as
 219 sampling procedures, bacterial isolation, identification methods and cross contamination. The low
 220 prevalence of *Salmonella* from Australian cattle probably, therefore, represents a result of strict
 221 regulation of animal management systems (26). Thus, the high prevalence of *Salmonella* may be a

sign of considerable threat in the human health from seeming healthy animal slaughtered for human consumption.

In this study, *S. Typhimurium*, *S. Enteritidis* and *S. Weltevreden*, were detected from healthy cattle in Chiang Mai (northern Thailand) whereas only *S. Brunei* was currently reported in Sakon Nakorn (north-Eastern Thailand)(28). In addition, previous studies have reported *S. Rissen* and *S. Weltevreden* in Chiang Mai and Lamphung (16). As observed elsewhere, *S. Anatum*, *S. Weltevreden* and *S. Lexington* were detected in Vietnam while *S. Typhimurium* and *S. Newport* were the frequently isolates in Ethiopia(29, 30). The results highlight the complexity of global epidemiology of *Salmonella* as the frequency and occurrence of different serovars changes overtime in countries and regions. Serotyping is phenotypic characteristics providing useful epidemiological markers so it may assist in the recognition and tracing of new emerging pathogens (31).

Phylogenetic tree analysis is emerging as an alternative or complement to established phenotypic identification procedures. For bacteria, 16S rRNA gene sequence analysis is a widely accepted tool for molecular identification (32). From the phylogenetic tree (Figure 2), the three serovars: *S. Typhimurium*, *S. Enteritidis* and *S. Weltevreden* formed three distinct clades. This study demonstrated that *S. Typhimurium*, *S. Enteritidis* and *S. Weltevreden* were the most common observed in healthy cattle whereas *S. Typhimurium* and *S. Enteritidis* were the most common *Salmonella* serovars found from human isolates. According to the branch length, it appears that more variation has occurred in *S. Enteritidis* and *S. Weltevreden* than the *S. Typhimurium*. Particularly, *S. Enteritidis* isolated from human blood sample no. 201741 shows bacterial genetic change in the clade of *S. Enteritidis*. Interestingly, this study reveals the relationship of *Salmonella* from cattle to human Salmonellosis. However, phenotypic finding was provided only primary investigation and the 16S rRNA sequences are not enough to conclude into the same strain. Thus, studies of molecular epidemiology such as Multi Locus Sequence typing and Pulse Field Gel Electrophoresis should be conducted in further studies.

All *Salmonella* isolates from cattle in this study were susceptible to the commonly used antimicrobials whereas one of *Salmonella* isolates from human conferred resistance to amoxycillin/clavulanic acid, ceftazidime ceftriaxone and ciprofloxacin. As the *Salmonella* from human isolates were resistant to commonly used antimicrobials so this may be a considerable threat in the treatment of clinical cases in future. Especially, fluoroquinolones and third generation cephalosporins are now commonly used for empiric treatment and are listed by the World health Organization as

critically important antimicrobials for human health (33,34). The antimicrobial resistance is the evolutionary response by bacteria to the strong selective pressure that results from exposure to antimicrobials, the detection of resistance of *S. Enteritidis* to third generation cephalosporins from human isolate no. 201741 might cause by bacterial genetic change and could be an indicator of the increased use of the antimicrobials in Salmonellosis treatment which may promote on acquired selection of antimicrobial resistant strains(35). Even though, the *Salmonella* isolated from cattle in this study were not antimicrobial resistance strain, the cattle was found to be reservoirs of *Salmonella* infection and could serve as vehicle for its transmission to human. Therefore, these cattle might be implicated as a source of human *Salmonella* infection.

Conclusion

This study helps to update the information on the occurrence, serotype distribution and antibiogram of *Salmonella* in slaughtered cattle in Chiang Mai, Thailand. Its result shows molecular characteristic of *Salmonella* spp. which isolated from healthy slaughtered cattle and human. The information should be beneficial in *Salmonella* surveillance. Interestingly, prevalence of *Salmonella* from cattle in this study is significantly high to be a potential source of food borne salmonellosis. *S. Typhimurium* and *S. Enteritidis* isolated from cattle and human were closely related. Even though, all *Salmonella* spp. from cattle isolates were susceptible to commonly used antimicrobials but *Salmonella* isolate from human was multidrug resistance that are common used in public health set up. This may pose a considerable threat in human health especially difficulties in treatment of human clinical cases in future.

CONSENT

It is not applicable.

ETHICAL APPROVAL: The study was approved by the Institutional Ethics Board of Chiang Mai provincial hospital, Ministry of public Health, Thailand.

REFERENCES

1. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, et al. The global Burden of Nontyphoidal *Salmonella* Gastroenteritis. Clin Infect Dis 2010;50:882-9.

- 281 2. Stevens A, Kabore Y, Perrier-Gros-Claude JD, Millermann Y, Brisaboris A, Catterau M, et al.
282 Prevalence and antibiotic-resistance of *Salmonella* isolated from beef sampled from the
283 slaughterhouse and retailers in Dakar (Senegal). *Int J food Microbiol* 2006;110:178-86.
- 284 3. Kagambega A, Lienemann T, Aulu L, Traore A, Barro N, Siitonen A, et al. Prevalence and
285 Characterization of *Salmoella enteritica* from the feces of cattle, poultry, swine and hedgehogs in
286 Burkina Faso and their comparison to human *Salmonella* isolates. *BMC Microbiology*
287 2013;13:253.
- 288 4. Department of Medical Sciences, Ministry of Public Health, Thailand. Thai NIH annual report, 2016 Top
289 5 *Salmonella* serotype list from each sample. 2016 [cited 11 september 2017] .Available form:
290 http://webapp1.dmsc.moph.go.th/annual_report/pdf/2559/59-nih.
291
- 292 5. Kariuki S, Revathi G, Kariuki N, Kiiru J, Mwituria J, Githinji JW, et al. Invasive multidrug-resistance
293 non-typhoidal *Salmonella* infections in Africa: Zoonotic or anthroponic transmission? *J. Med*
294 *Microbiol.* 2006;55:585-91.
- 295 6. Thrans CJ. Bacterial food –borne zoonoses. *Rev Sci Tech* 2000;19:226-39.
- 296 7. Gillespie IA, O'Brien SJ, Adak, Ward LR, Smith HR. Foodborne general outbreaks of *Salmonella*
297 *Enteritidis* phage type 4 infection, England and Wales, 1992-2002: where are the risks? *Epidemiol*
298 *Infect* 2005;133:795-801.
- 299 8. Ponce E, Khan AA, Cheng CM, Summage WC, Cerniglia CE. Prevalence and characterization of
300 *Salmonella enteric* serovar *Weltevreden* from Imported seafood. *Food Microbiol.* 2008;25:29-35.
- 301 9. Hohnmann EL. Nontyphoidal salmonellosis. *Clin Infect Dis* 2001;32:263-9.
- 302 10. Patel A.R, Shah N.P, Prajapati J.B. .Antibiotic resistance profile of lactic acid bacteria and their
303 implications in food chain, *World J of Dairy and Food Sci* 2012;7;202-11.
- 304 11. Molla W, Molla B, Alemayehu D, Muckle A, Cole L, Wikie E. Occurrence and antimicrobial
305 resistance of *Salmonella* serovars in apparently healthy slaughtered sheep and goats of central
306 Ethiopia. *Trop Anim Health Prod* 2006;38:455-62.
- 307 12. Zewdu E, Cornelius P. Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food
308 items and personnel in Addis Ababa, Ethiopia. *Trop Anim Health Prod* 2009;41:241-9.

13. Thamlikitkul V, Rattanaumpawan P, Boonyasiri A, Pumsuwan V, Judaeng T, Tiengrim S., et al. Thailand antimicrobial resistance containment and prevention program. *J Glob Antimicrob Resist*. 2015;3:290-4.
14. Alexander KA, Warnick LD, Wiedmann M. Antimicrobial resistant *Salmonella* in dairy cattle in the United States. *Vet Res Com*. 2009;33:191-209.
15. Boonkhot P, Tadee P, Patchanee P. Serodiversity and Antimicrobial resistance Profiles of Detected *Salmonella* on swine Production Chian in Chiangmai and Lamphun, Thailand. *Acta. Sc.Vet*. 2015;43:1263.
16. Padungtod P, Kaneene JB. *Salmonella* in food animals and human in northern Thailand. *International J food Microbiol*. 2006;108(3):346-54.
17. Daniel WW. *Biostatistics: a foundation for analysis in the health sciences*. 7th ed. New York: John Willey&son. 1999.
18. International Organization for Standardization, ISO 6579:2002/AMD 1:2007. Detection of *Salmonella spp.* in animal feces and in environment sample from primary production stage, amendment 1, annex D. In *microbiology of food and animal feeding stuffs*. Horizontal method for the detection of *Salmonella spp.* (3): Geneva, Switzerland: International Organization for standardization; 2007.
19. Hendriksen RS. A global surveillance and laboratory support project of the world Health Organization: Laboratory protocols (Isolation of *Salmonella*)(3); 2003.
20. Cruickshank R, Duguid JP, Mamion BP, Swainr HA. *The practice of Medical Microbiology*. Edinburgh. Churchill Livingstone. 1975;171-91.
21. Nyabundi D, Onkoba N, Kimathi A, Nyachio A, Juma G, Kinyanjui P, et al. Molecular characterization and antibiotic resistance profiles of *Salmonella* isolated from faecal matter of domestic animals and animal products in Nairobi. *Trop Dis, Travel Medicine and Vaccines*. 2017;3:2.
22. Basic Local Alignment Search Tool. [cited 12 October 2017] Available from: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>
23. Molecular Evolutionary Genetics Analysis. [cited 12 October 2017] Available from: <http://www.megasoftware.net/>

337 24. Clinical and Laboratory Standards Institute (CLSI) 2011. Performance standards for antimicrobial
338 susceptibility testing. CLSI document M02-A10. CLSI, Wayne, PA. 10th ed. (3);2011.

339 25. Akoachere JF, Tanih NF, Ndip LM, Ndip RN. Phenotypic characterization of *Salmonella*
340 typhimurium isolates from food-animals and abattoir drains in Buea, Cameroon. J. Health. Popul
341 Nutr. 2009;27:1-7.

342 26. Barlow RS, McMillan KE, Duffy LL, Fegan N, Jordan D, Mellor GE. Prevalence and Antimicrobial
343 Resistance of *Salmonella* and *Escherichia coli* from Australian Cattle Populations at Slaughter. J
344 Food Prod 2015;78(5):912-20.

345 27. Callaway TR, Keen JE, Edrington TS, Baumgard LH, Spicer L, Fonda ES, et al. Fecal prevalence
346 and diversity of *Salmonella* species in lactating dairy cattle in four states. J Dairy Sci
347 2005;88:3603-8.

348 28. Jitjak T, Sornplang P, Noppon B. Prevalence and antimicrobial resistance of *Salmonella* spp.
349 isolated from fattening beef cattle at the slaughterhouse in Sakon Nakhon Province. SNRU J of Sci
350 and Tech. 2016;8(3):338 – 43.

351 29. Vo AT, van Duikeren E, Fluit AC, Heck ME, Verbruggen A, Maas HM, et al. Distribution
352 of *Salmonella enterica* Serovars from humans, livestock and meat in Vietnam and the Dominance
353 of *Salmonella* Typhimurium Phage Type 90. Vet Microbiol 2006;113:153-8.

354 30. Alemu S, Zewde MB. Prevalence and antimicrobial resistance profiles of *Salmonella enterica*
355 serovars isolated from slaughtered cattle in Bahir Dar, Ethiopia. Trop Anim Health and Prod. 2012 ;
356 44:595-600.

357 31. Hendriksen RS, Vieira AR, Karlsmose S, Lo Fo Wong DM, Jensen AB, Wegener HC, et al. Global
358 monitoring of *Salmonella* serovar distribution from the World Health Organization Global
359 Foodborne Infections Network Country Data Bank: results of quality assured laboratories from
360 2001 to 2007. 2011;8(8):887-900.

361 32. Bosshard PP, Zbinden R, Abels S, Böddinghaus B, Altwegg M, Böttger EC. 16S rRNA gene
362 sequencing versus the API 20 NE system and the VITEK 2 ID-GNB card for identification of
363 nonfermenting Gram-negative bacteria in the clinical laboratory. J Clin Microbiol 2006
364 ;44(4):1359-66.

365 33. Sirichote P, Bangtrakulnonth A, Tianmanee K, Unahalekhaka A, Oulai A, Chittaphithakchai P, et al.
366 Serotypes and antimicrobial resistance of *Salmonella enterica* spp in central Thailand, 2001-2006.
367 Southeast Asian J Trop Med Public Health. 2010;41(6):1405-15.

- 368 34. World Health Organization: Critically Important Antimicrobials for Human Medicine, 3rd Revision
369 2011.[cited 10 Sep 2017] Available from:
370 http://apps.who.int/iris/bitstream/10665/77376/1/9789241504485_eng.pdf
371 35. Wright GD. Antibiotic resistance: Where does it come from and what can we do about it? BMC
372 Biology 2010;8:123.

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