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Prevalence and Antimicrobial Resistance of *Escherichia coli* and *Salmonella* spp. Isolated from Wild Animals, Northeast Thailand

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Abstract: This study was determined to prevalence and antimicrobial resistance of *E. coli* and *Salmonella* isolated from wild animals at Khon Kaen Zoo, Northeast of Thailand. The 140 samples were collect from reptile (34), birds (46) and mammals (60) by rectal swab technique during August-October 2016. Wild animals infected *E. coli* and *Salmonella* were 66.4 and 10.7%, respectively. All isolations were tested for antimicrobial sensitivity against ampicillin, ceftazidine, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfamethoxazole/trimethoprim and tetracycline. *E. coli* and *Salmonella* isolates were resistant to 40.9, 6.5, 9.7, 2.2, 4.3, 2.2, 32.3, 17.2, 36.6% and 13.3, 6.7, 13.3, 20.0, 13.3, 6.7, 73.3, 13.3, 6.7%, respectively. Infection of *E. coli* and *Salmonella* in wild animals was impact to animal health, especially, infant animals besides infected animals were carriers and can spread to other animals, environment and their keepers. The infection can be minimized by good management and good quality of feed.

Key words: Prevalence, antimicrobial resistance, *E. coli*, *Salmonella*, wild animals, quality of feed

INTRODUCTION

Nowadays, antimicrobial resistance has become an extremely important problem that threat the effectivity of antimicrobial therapy, increase patient morbidity and mortality and treatment costs. Antimicrobial resistance on *E. coli* and *Salmonella* spp. are often influenced directlyby antimicrobial using. They play an important role in development of resistance in the population because they are ubiquitous. In fact, *E. coli* is considered as an important "indicator bacteria" that is used to investigate about the current trend of antimicrobials susceptibility in human and animals (Van de Bogaard and Stobberingh, 2000). The resistance of *E. coli* is also, stimulated by the use of antimicrobials for therapy and growth promotion in animals (Alexander *et al*., 2008). Antimicrobial resistance, including resistance to multiple antimicrobial classes of *Salmonella* spp. has increased coincided with the increase of antibacterial drugs using in both humans and animals (Foley and Lynne, 2008). The constantly increasing drug resistance of bacteria has become a global concern, since, infection of resistance strains may lead to ineffective treatment. Furthermore, *E. coli* and *Salmonella* spp. can pass their resistance ability to other pathogenic bacteria, making one of the most serious threats to public health as bacteria that have origin from animals may pass their resistance to human bacteria.

The first report about antimicrobial resistance of *E. coli* in wildlife was published in 1978 (Sato *et al*., 1978). Since, then antibiotic resistance of *E. coli* in wild animals had been detected all around the world (Santos *et al*., 2013; Dias *et al*., 2015). Antibiotic resistance for *Salmonella* spp. had also been recorded in both wild animals live in both wild and captivity (Koochakzadeh *et al*., 2015).

Many studies had pointed out that humans and other animals can be infected of *E. coli* and *Salmonella* spp. from wild animals (Silva *et al*., 2010). Therefore, the important of wild animals in transmission of zoonotic pathogens and antibiotic resistance should not be underestimated. This study was performed to keep trackt of the epidemiological situation and to determine the antimicrobial resistance pattern of *Salmonella* spp. and *E. coli* isolated from wild animals at Khon Kaen Zoo.

MATERIALS AND METHODS

Sample collection: During August-October 2015, all samples were collected from 140 wild animals by rectal

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Table 1: Type of wild animals

Wild animals	Types
Reptiles	Iguana, Python, Turtle
Birds	Emu, Peacock, Golden pheasant, Silver pheasant, Red jungle fowl, chicken, White pigeon, other bird (Class Aves)
Mammals	Springbok, Rhino, Wallaby, Hair Less Cavy, Nyala, Goat, Rusa deer, Donkey, Leopard cat, Capybara, Horse, Rabbit, Loris

swab at Khon Kaen Zoo, Northeast Thailand kept in an ice box and transferred to laboratory for analysis (Table 1).

Microbial analyses

***E. coli* isolation and identification:** Samples were processed to isolate *E. coli* as described by the Bacteriological Analytical Manual (BAM), US Food and Drug Administration (USFDA) (7). The samples were inoculated into MacConkey broth for enrichment at 37°C for 24 h, the enrichments were streaked on MacConkey agar and inoculated for 24 h at 37°C. Pink colored colonies were sub cultured on Eosin Methylene Blue (EMB) agar. Colonies producing greenish metallic sheen on EMB agar were considered as having *E. coli*. In addition, various biochemical tests were done for the confirmation of *E. coli* as proposed by Edward and Ewing (1972).

***Salmonella* isolation and identification:** *Salmonella* was performed according to ISO. (2002) recommendations. After incubated in BPW, three loops full were transferred to modified semi-solid rappaport vassiliadis medium (Difco) and then were streaked on Xylose lysine deoxycholate agar (Difco) and hektoen enteric agar (Difco). To confirm *Salmonella* spp., suspected colonies had to biochemical tests including triple sugar iron (Difco) and motility, indole, lysine (Difco). The antisera polyvalent A-67 (Biotechnical, Bangkok, Thailand) was also used for final confirmation of the presence of *Salmonella*. Finally, each *Salmonella* isolate was cultured on nutrient agar (Difco) and sent to Center for Antimicrobial Resistance Monitoring in Foodborne Pathogens (in cooperation with WHO), Faculty of Veterinary Science, Chulalongkorn University, Thailand for serotyping.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed using disk diffusion method and following the guideline of the Clinical and Laboratory Standards Institute (CLSI., 2015). The 9 antimicrobial agents (Oxoid; Basingstoke, Hampshire England) were Ampicillin 10 μg (AMP), Ceftazidine 30 μg (CAZ), Chloramphenicol 30 μg (C), Ciprofloxacin 5 μg (CIP), gentamicin 10 μg (CN), Nalidixic Acid 30 μg (NA), Streptomycin 10 μg (S), Sulfamethoxazole/Trimethoprim 25 μg (SXT) and Tetracycline 30 μg (TE).

Statistical analysis: Percentage of antibiotic resistance of each type of bacterial isolate were calculated. The 95% confidence intervals of these proportions were constructed for each type of animal species. Fisher's exact tests were used to compare these proportion using online statistical tools (Graph Pad Software). Statistically significant difference was defined if the value of $p<0.05$.

RESULTS AND DISCUSSION

Prevalence of *E. coli* and *Salmonella* spp.: Prevalence of *E. coli* isolated from the total of 140 samples was 66.4%. The specific prevalence of *E. coli* in reptiles was 35.3% while those of birds and mammals were 84.8% and 70.0%, respectively. The differences in prevalence of *E. coli* infection between three groups were statistically significant ($p<0.05$). Among 6 samples collected from iguana, none have showed positive result this was the only species that had negative result for *E. coli*. Noticeably, 28 white pigeons were just entering the zoo at the time that samples were collected; The prevalence of these pigeons (89.3%) was higher than the prevalence of the other birds (77.8%) that had lived in the zoo for a longer time ($p<0.05$).

The fifteen isolates of *Salmonella* spp. were isolated from all samples (10.7%), seven of which originated from reptiles, one from birds and seven from mammals. Since, there was only one isolates which was obtained from an unidentified birds, the prevalence of *Salmonella* in birds in this study is only 2.2% while the prevalence in reptiles and mammals was 20.6 and 11.7%, respectively. The association between the prevalence of birds and reptiles was statistically significant ($p<0.05$), however, the prevalence between birds and mammals and between reptiles and mammals were not significant ($p>0.05$). The highest prevalence was observed from iguana with 4 isolates of *Salmonella* were yield out of 66.7% (Table 2).

The result of serotyping showed that fifteen *Salmonella* isolates are belonged to thirteen serotypes. There were three strains isolated from iguana belonged to serotype *S. suelldorf* (20.0%), the rest of serotypes were *S. typhimurium*, *S. rubislaw*, *S. bovismorbificans*, *S. amager*, *S. arhus*, *S. paratyphi* B, *S. gaminara*, *S. eastbourne*, *S. saintpaul*, *S. rissen*, *S. yalding* and *S. stanley*. Table 3 shows the prevalence of *E. coli* and *Salmonella* spp. obtained in each species or group of animals at Khon Kaen Zoo as well as the serotypes of *Salmonella* strains.

Antimicrobial susceptibility test of *E. coli* isolates: The ninty three *E. coli* isolated from reptiles, birds and mammals resistant to antimicrobial agent were 12.9, 41.9 and 45.2%, respectively. All isolates highest resistant to ampicillin were antimicrobial agents that had highest resistant rate 40.9% while ciprofloxacin, gentamicin and nalidixic acid were the most susceptible agents (Table 4).

Antimicrobial susceptibility test of *Salmonella* isolates: The fifteen *Salmonella* spp. isolated from reptiles, birds and mammals resistant to antimicrobial agent were 46.7, 46.7 and 6.7%, respectively. All isolates highest resistant to streptomycin were antimicrobial agents that had highest resistant rate 73.3% while ceftazidine, nalidixic acid and tetracycline were the most susceptible agents (Table 5).

Table 2: Prevalence of *E. coli* and *Salmonella* spp. isolated from wild animals

		Number of positive (%)	
Type of animals	Number	*E. coli*	*Salmonella* spp.
Reptiles	34	12 (35.3)	7 (20.6)
Birds	46	39 (84.8)	1 (2.2)
Mammals	60	42 (70.0)	7 (11.7)
Total	140	93 (66.4)	15 (10.7)

Table 3: Serotype of *Salmonella* isolated from wild animals

Animals	*Salmonella* positive	Serotype of *Salmonella* (number)
Reptiles		
Iguana	4	Suelldorf (3)
		Typhimurium (1)
Turtle	3	Amager (1)
		Bovismorbificans (1)
		Rubislaw (1)
Birds		
Other bird (Class Aves)	1	Aarhus (1)
Mammals		
Leopard cat	2	Rissen (1)
		Saintpaul (1)
Capybara	1	Yalding (1)
Hairless Cavy	1	Gaminara (1)
Horse	1	Stanley (1)
Loris	1	Eastbourne (1)
Rhino	1	Paratyphi B (1)
Total	15	

In this study, 93 strains of *E. coli* were isolated from 140 samples, the prevalence was 66.4%. In comparison with other studies about *E. coli* prevalence in captive wild animals this prevalence is higher than the one of 52.6% that had been observed in Asa Zoological Park, Japan (Ahmed *et al*., 2007) and 27.5% observed in a study at Kuwait Zoo, Kuwait (Mahmoud, 2015). However, the percentage of *E. coli* presence in this study is significantly similar to the rate of 67% that had been discovered at the Emperor Valley Zoo (Adesiyun, 1999). The prevalence of reptiles and amphibians, birds and mammals from the study at the Emperor Valley Zoo were 37, 78 and 83%, respectively, quite close to the results of the current study with 35.3% for reptiles, 84.8% for birds and 70.0% for mammals. Since, none of the iguanas in this study carried *E. coli*, the rate in this species was 0%, unmatched with the prevalence of 40% that was found in an iguana study perform in West Indies. The difference may be due to the low samples size of iguana in our study (6 compared to 62) or it may suggest a difference in epidemiology of *E. coli* in iguanas between two regions. The prevalence of *Salmonella* spp. from Khon Kaen Zoo was 10.7% much higher than the 5.8% rate in the study at Seoul Grand Park, Korea (Jang *et al*., 2008). More specifically, the percentages of *Salmonella* spp. positive samples isolated from reptiles and birds were 20.6 and 2.2%, lower than 30.4 and 6.7% of Jang's study; Conversely, the isolation rate of *Salmonella* spp. from mammals was 11.7%, remarkably higher than 0.9% in the study of Jang. The prevalence of *Salmonella* spp. isolated from iguanas was notably high (66.7%) compared to other species. The

Table 4: Antimicrobial resistance of *E. coli* isolates

		Antimicrobial resistant (%)								
Animals	No. of sample	AMP	CAZ	C	CIP	CN	NA	S	SXT	TE
Reptiles	12	9	0	0	0	0	0	9	3	7
Birds	39	16	0	3	1	1	1	11	9	16
Mammals	42	13	6	6	1	3	1	10	4	11
Total	93	38 (40.9)	6 (6.5)	9 (9.7)	2 (2.2)	4 (4.3)	2 (2.2)	30 (32.3)	16 (17.2)	34 (36.6)

Table 5: Antimicrobial resistance of *Salmonella* isolates

		Antimicrobial resistant (%)								
Animals	No. of sample	AMP	CAZ	C	CIP	CN	NA	S	SXT	TE
Reptiles	7	0	0	0	1	1	0	6	0	0
Mammal	7	1	1	1	1	1	1	4	1	1
Bird	1	1	0	1	1	0	0	1	1	0
Total	15	2 (13.3)	1 (6.7)	2 (13.3)	3 (20.0)	2 (13.3)	1 (6.7)	11 (73.3)	2 (13.3)	1 (6.7)

AMP: Ampicillin; CAZ: Ceftazidine; C: Chloramphenicol; CIP: Ciprofloxacin; CN: gentamicin; NA: Nalidixic Acid; S: Streptomycin; SXT: Sulfamethoxazole/trimethoprim; TE: Tetracycline result is unsurprising, since, there are others studies showed that the presence of *Salmonella* spp. in iguana species can be very high such as a report of 12 iguanas which all were found to be shedding *Salmonella* at least once during a 10-weeks study (Burnham *et al*., 1998). The infection rates of reptiles in this study and studies performed in other zoos was higher than that of birds and mammals this result is comparable with other studies, showing that reptiles have higher prevalence than mammals and birds, hence, they are an important reservoir of *Salmonella* (Gopee *et al*., 2000). The study could not find serotypes Weltevreden, Enteritidis and Anatum which cause the majority of salmonellosis cases in humans in Thailand (Bangtrakulnonth *et al*., 2004). The number of 13 serotypes were found with eight of them absented in local food animals suggest that wild animals may be a rich reservoir for *Salmonella* serotypes diversity.

In all *E. coli* isolates that were tested, 43.6% showed single or multiple antibiotic resistance. It was not surprising that the most resistant agents were AMP, S and TE as these drugs are older and commonly used. The prevalence of AMP, NA and TE resistances in this study are remarkably lower than ones from a study performed in swine, chickens and farm workers in Northern of Thailand (61.6 for AMP, 67.4 for NA and 91.5% for TE) (Hanson *et al*., 2003). Another research on *E. coli* isolated from food in Khon Kaen municipality also showed very high rate of resistance with 76 for AMP, 44 for NA and 70% for TE (Chomvarin *et al*., 2005).

Multi drug resistance was detected in 26 out of 34 isolates that showed resistance to at least one drug (76.5%). The most common resistance phenotypes in *E. coli* isolates were against AMP, C, S, SXT and TE. Similar phenotypes have been reported in many *E. coli* studies in other countries but the prevalence rates are various between phenotypes (Wasyl *et al*., 2013). As the resistance patterns are similar yet still diverse, the answer perhaps due to the different trends of using antibiotics between different areas.

The 4 out of 15 *Salmonella* spp. isolates exhibited resistance to one or more antimicrobial drugs. In general, the resistant prevalence was 26.7%, remarkably lower than the rate that were found in the Emperor Valley Zoo, Trinidad (Gopee *et al*., 2000). The highest resistance of *Salmonella* spp. isolates from Khon Kaen Zoo was against S (20.0%), followed by AMP, CN and C (13.3%); None of the isolates were resistant to CIP. The patterns of resistance in current study is different from the patterns that were observed in *Salmonella* spp. isolated from pork, chicken meat and humans in Khon Kaen (Angkititrakul *et al*., 2005) as the previous study showed that the level of resistance were significantly high in S, SXT and TE. The dissimilarity may come from the differences of using antibiotics in humans and farm animals versus wild animals.

CONCLUSION

In summary, this study indicates that wild captivity animals can be is an important reservoir for zoonotic pathogens. Wild animals are not only the preserving source resistance genes but also, the important vehicles for antibiotic resistance spreading. Antibiotic-resistant bacteria with multi-drug resistance were observed, therefore, more attention should be paid for antimicrobial usage in wild animals.

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