

ความชุกของเชื้อซาลโมเนลลาที่ดื้อต่อยาต้านจุลชีพหลายชนิดที่แยกได้จากเนื้อสุกร
จากตลาดสดในจังหวัดพัทลุง

Prevalence of Multiple Antimicrobial Resistant in Salmonella Isolated
from Retail Pork at Phatthalung Province

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บทคัดย่อ

ทำการเก็บตัวอย่างเนื้อสุกรทั้งหมด 41 ตัวอย่างจากจังหวัดพัทลุง เพื่อสำรวจหาความชุกและการดื้อยาต้านจุลชีพของเชื้อ Salmonella ผลการศึกษาพบว่าตัวอย่างเนื้อสุกรมีการปนเปื้อนทั้งหมด 34 ตัวอย่าง จากทั้งหมด 41 ตัวอย่าง คิดเป็น 83 % โดยแยกเชื้อ Salmonella ได้ทั้งหมด 117 ไอโซเลต แบ่งออกเป็น sero-group ต่างๆ ดังนี้คือ group E จำนวน 46 ไอโซเลต (39%) group C จำนวน 32 ไอโซเลต (28%) group B จำนวน 17 ไอโซเลต (14%) group G 17 ไอโซเลต (14%) และ group I จำนวน 5 ไอโซเลต (3.5%) ตามลำดับ การทดสอบความไวของเชื้อ Salmonella ที่แยกได้ทั้งหมด 117 ไอโซเลต ต่อยาต้านจุลชีพจำนวน 10 ชนิด พบว่าเชื้อ Salmonella จำนวน 99 ไอโซเลต (85%) ดื้อต่อยาต้านจุลชีพที่นำมาทดสอบ โดยเชื้อมีการดื้อต่อยา tetracycline และ streptomycin มากที่สุด คือ 61.5 % เท่ากัน นอกจากนี้พบว่าเชื้อ Salmonella จำนวน 62 (53%) ไอโซเลต ที่แยกได้เป็นเชื้อที่ดื้อต่อยาต้านจุลชีพหลายๆ ชนิด

คำสำคัญ : Salmonella เนื้อสุกร ยาต้านจุลชีพ การดื้อยาต้านจุลชีพ

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Abstract

Total of 41 retail pork samples from Phatthalung Province were investigated for the prevalence and antimicrobial resistance profile of Salmonella. The results demonstrated Salmonella were isolated from 34 of 41 samples (83%), and a total 117 Salmonella were isolated from 34 contaminated samples. Most of them were identified as group E 46 isolates (39%), followed by group C 32 isolates (28%), group B 17 isolates (14%), group G 17 isolates (14%) and group I 5 isolates (3.5%). Antimicrobial susceptibility testing to 10 antimicrobials of all 117 isolates were carried out according to CLSI recommendations. These results indicated that 99 (85%) isolates were resistant to all 10 antimicrobials. Most of isolates were resistant to streptomycin (61.5%) and tetracycline (61.5%). In addition, sixty-two isolates (53%) were multiple antimicrobial resistant (MDR) strain which resistant to two antimicrobials or more in different combinations.

Keywords : Salmonella, Pork, Antimicrobial Drugs, Antimicrobial Drug Resistance

Introduction

Salmonella is an important bacteria causing of food poisoning and gastroenteritis in human and is growing world-wide public health problem. In developing countries, such as Thailand, non-typhoidal Salmonella is the most common cause of diarrheal illness that can be found spread annually in all part of Thailand [1]. Since Salmonella are able to colonize and shed from animal intestine in many species, mainly swine, therefore contamination of these pathogenic bacteria in pork is unavoidable in the human food supply chain [2-6]. Illness usually transmitted to human through ingestion of contaminated pork [7, 8]. Contamination of Salmonella is not only the problem for consumer's health, but also resistance to a range of antimicrobial agents and multidrug resistant (MDR) Salmonella are becoming significant problems of global dimensions. Selective pressure by antimicrobials use as growth promoter in animal husbandry are now believe as a driving force behind the emergence of resistance [9-11]. In a global context these days, food safety issues associated with the food animal products has become a growing concern for consumers. Since foods from animal products are traded in globally, thus emergence of antimicrobial resistance Salmonella from one country can have serious health problem in other countries. Furthermore, these issues are now becoming a barrier to trade for exporter countries including Thailand. In order to ensure safety for consumers and exportation, many ways to reduce contamination have implemented into a large animal farm system. Whereas, in the countryside, livestock was produced primarily for domestic consumption, thus the issue of food safety and Salmonella contamination for local people may have insufficient attention. In addition, there are few researches on prevalence and antimicrobial resistance profile of Salmonella from food animal products particularly retail pork at Phatthalung Province. Therefore, the purpose of this study was to investigate the prevalence and antimicrobial resistance profile against Salmonella contaminated in retail pork at Phatthalung Province.

Materials and Methods

Samples

Forty one of retail pork from retail markets of 7 districts at Phatthalung Province were randomly selected during October to December 2010. Samples were stored in the cooler box and transported to laboratory for *Salmonella* isolation.

Salmonella isolation and identification

Twenty five grams of collected pork were cut in to small pieces with sterile scissors before put into the stomacher bag containing 225 ml of buffered peptone water (BPW). Samples were incubated at 37 °C for 24 h. Then 100 µl of BPW were separately dropped on to the Modified Semi-solid Rappaport-Vassiliadis (MSRV) Medium (Criterion, U.S.A). After that MSRV were incubated at 42 °C for 24 h. The selective enrichment media were streaked on the Xylose Lysine Desoxycholate (XLD) agar (Himedia, India). Then petri-dishes were incubated at 37 °C for 18-24 h. At least 5 single of *Salmonella* typical colonies were randomly picked up and stabbed into Triple Sugar Iron (TSI) agar (Criterion, U.S.A) and Lysine Indole Motile (LIM) medium (Himedia, India) then media were incubated at 37°C for 18- 24 h. The typical characteristics of *Salmonella* that exhibited in the TSI were selected and sub-culture again onto TSA agar. Considered *Salmonella* colony was confirmed by slide agglutination with *Salmonella* O antigen antisera.

Antimicrobial susceptibility Testing

All isolates were tested for 10 antimicrobial drugs (oxid); tetracycline (30 µg), ciprofloxacin (5µg), chloramphenicol (30 µg), ampicillin (10 µg), norfloxacin (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), trimethoprim/sulfamethoxazole (25 µg), cephalothin (30 µg), and gentamicin (10 µg). Susceptibility testing was performed according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) [12]. To standardized, the bacterial colonies suspension (in 0.8% NaCl) turbidity were adjusted to 0.5 McFarland, then the suspensions were spread with a sterile cotton swab over the entire surface of Mueller Hinton agar (MHA; Himedia, India)). After that antimicrobial disks were applied on to the MHA and incubated at 37 °C for 24 h. Inhibition zone were measured by Venire Caliper, zones were interpreted accordingly to the CLSI breakpoint, and the results were analyzed by WHONET 5 program.

Results

Salmonella identification

Total of 41 retail pork were collected in Phatthalung Province to investigate the prevalent of *Salmonella* and antimicrobials resistant. The results demonstrated that 34 (83%) samples were contaminated with *Salmonella*. Among of the positive samples, 117 *Salmonella* were isolated. The most predominant sero-groups were group E46 isolates (39%), followed by group C 32 isolates (28%), group B 17 isolates (14%), group G 17 isolates (14%) and group I 5 isolates (3.5%), respectively.

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Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for all 117 isolates. This result revealed that 99 (85%) isolates were resistant to all 10 antimicrobials. Most isolated Salmonella were resistant to streptomycin (61%) and tetracycline (61%), followed by ampicillin (46%), trimethoprim/sulfamethoxazole (31%), chloramphenicol (12%), gentamicin (4%), cephalothin (2%), nalidixic acid and ciprofloxacin (0.9%) and there was no Salmonella isolates resistant to norfloxacin (Table 1).

Table 1. Number and percentages of antimicrobial resistant Salmonella isolated from retail pork at Phatthalung Province.

Antibiotic name	Breakpoints	Number	%R	%I	%S	%R; 95%C.I.
Tetracycline	12 - 14	117	61.5	0	38.5	52.0-70.2
Streptomycin	12 - 14	117	61.5	21.4	17.1	52.0-70.2
Chloramphenicol	13 - 17	117	12	0.9	87.2	7.0-19.6
Ciprofloxacin	16 - 20	117	0.9	1.7	97.4	0.1-5.4
Ampicillin	14 - 16	117	46.2	0	53.8	37.0-55.6
Trimethoprim/Sulfamethoxazole	11 - 15	117	31.6	0.9	67.5	23.5-40.9
Gentamicin	13 - 14	117	4.3	2.6	93.2	1.6-10.2
Cephalothin	15 - 17	117	2.6	12.8	84.6	0.7-7.9
Nalidixic acid	14 - 18	117	0.9	2.6	96.6	0.1-5.4
Norfloxacin	13 - 16	117	0	0	100	0.0-4.0

Multiple antimicrobials resistance (MDR)

To determine the multiple antimicrobials resistant profiles, All 117 of Salmonella isolates were analyzed using WHONET 5.1 program. Among of the 117 isolates, 62 isolates (53%) were MDR strains, which resistance to two or more antimicrobials in different combinations. Fifteen phenotypic patterns of antimicrobial resistance were demonstrated among of 62 isolates. Further analysis demonstrated that the most of MDR strain were resistant to ampicillin and streptomycin (23%) (Table 2).

Table 2. Number and percentages of multiple antimicrobial resistant Salmonella isolates from retail pork.

Resistance profile	Number of isolates	%Isolates
STR CHL	1	0.9
GEN STR	4	3.4
CEP STR	2	1.7
AMP STR	27	23.1
CEP STR CHL	1	0.9
AMP STR CHL	9	7.7
AMP STR NAL	1	0.9
AMP CEP STR	9	7.7
AMP STR CIP CHL	1	0.9
AMP GEN STR CHL	1	0.9
AMP CEP STR CHL	1	0.9
AMP CEP STR CIP	1	0.9
AMP CEP GEN STR	2	1.7
AMP STR CIP NAL CHL	1	0.9
AMP CEP GEN STR NAL	1	0.9

AMP=Ampicillin, CEP=Cephalothin, GEN=Gentamicin, STR= Streptomycin, NAL=Nalidixic acid, CIP=Ciprofloxacin, NOR=Norfloxacin, SXT=Trimethoprim/sulfamethoxazole, CHL=Chloranphenicol

Discussions

It is well recognized that the emergence of antimicrobial resistant in Salmonella is continuing to be a serious public health problem. Since this pathogen is highly involved in contamination of food animal products, which not only involve the loss of product shelf life but also cause food borne illness and probably death. Therefore, the epidemiological information that monitor trends over time is necessary to declare to public health sector to prevention of this pathogens. In this study, it was demonstrated that approximately 83% (Table 2) of retail pork were highly contaminated with Salmonella. The findings of the current study are consistent with several previous reports in both Thailand and oversea, which indicated that food of animal origins are usually contaminated with Salmonella [6, 13-16]. This result probably implies that the hygienic performance of carcass production processes in abattoirs or during transportation, and at the retail market perhaps insufficiently. Contamination of Salmonella in retail pork is health

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hazards further, be higher if the pork are consumed undercooked or cross contamination during the meal preparation.

Antimicrobial susceptibility testing revealed that most of isolates (85%) were resistant to tetracycline and streptomycin. Highly resistant to both antimicrobials of this study is similar to previous reports from various countries [17-20]. Since, tetracycline, streptomycin and derivatives have been widely attributed as a feed additive in livestock for times; therefore, it could enhance spread of tetracycline and streptomycin resistant encoding gene. This was supported by many observations revealed that sub-therapeutic doses of antimicrobial drugs in livestock production responsible for the emergence and maintenance of multiple antimicrobial resistant pathogenic bacteria [21-23]. According to other reports, our results demonstrated that the fluoroquinolones, ciprofloxacin and norfloxacin are still the most effective drugs to treat Salmonella infection [24-26]. Decreasing susceptibility to fluoroquinolones is growing as a special attention issue, since fluoroquinolones are good drug for clinical activity against Salmonella and usually selected as treatment of choice in case of life threatening [27]. In these observations demonstrated that 53% of the Salmonella resistance strain exhibited multiple resistant to two antimicrobials or more in different combinations (Table 2). At the present day, the prevalence of multiple antimicrobial resistant Salmonella in different countries continues to be a significant public health concern. This phenomenon is associated with the transferable of drug resistant determinants, mediated by mobile genetic elements, such as integrons, transposons and plasmid [28-30].

In conclusion, these results revealed evidence that the high prevalence of MDR Salmonella in retail pork reflects a reservoir of resistance in animal that able to transmit to humans. Therefore, an extensive of epidemiological and molecular study on the frequency, sources of Salmonella and other food borne pathogens contamination at various levels of swine and other livestock as well as the sources of acquisition of resistant genes are essential to ensure the effectiveness to control Salmonella infection.

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